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(54) Title: COMPOSITIONS FOR SACCHARIFICATION OF CELLULOSIC MATERIAL

(57) Abstract: The present invention relates to enzyme compositions comprising components selected from the group consisting of a cellobiohydrolase I, a cellobiohydrolase II, an endoglucanase I, an endoglucanase II, and a beta-glucosidase. The present invention also relates to methods for degrading or converting a cellulosic material, comprising: treating the cellulosic material with such an enzyme composition. The present invention also relates to methods method for producing a fermentation product, comprising: (a) saccharifying a cellulosic material with such an enzyme composition; (b) fermenting the saccharified cellulosic material with one or more (several) fermenting microorganisms to produce the fermentation product; and (c) recovering the fermentation product from the fermentation. The present invention further relates to methods of fermenting a cellulosic material, comprising: fermenting the cellulosic material with one or more (several) fermenting microorganisms, wherein the cellulosic material is saccharified with such an enzyme composition.

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COMPOSITIONS FOR SACCHARIFICATION OF CELLULOSIC MATERIAL

Statement as to Rights to Inventions Made Under Federally Sponsored Research and Development

This invention was made in part with Government support under Cooperative Agreement DE-FC36-08GO18080 awarded by the Department of Energy. The government has certain rights in this invention.

Reference to a Sequence Listing

This application contains a Sequence Listing in computer readable form. The computer readable form is incorporated herein by reference.

Reference to Deposits of Biological Material

This application contains a reference to deposits of biological material, which deposits are incorporated herein by reference.

Background of the Invention

Field of the Invention

The present invention relates to enzyme compositions for high temperature saccharification of cellulosic material and to uses thereof.

Description of the Related Art

Cellulose is a polymer of the simple sugar glucose linked by beta-1,4 bonds. Many microorganisms produce enzymes that hydrolyze beta-linked glucans. These enzymes include endoglucanases, cellobiohydrolases, and beta-glucosidases. Endoglucanases digest the cellulose polymer at random locations, opening it to attack by cellobiohydrolases. Cellobiohydrolases sequentially release molecules of cellobiose from the ends of the cellulose polymer. Cellobiose is a water-soluble beta-1,4-linked dimer of glucose. Beta-glucosidases hydrolyze cellobiose to glucose.

The conversion of lignocellulosic feedstocks into ethanol has the advantages of the ready availability of large amounts of feedstock, the desirability of avoiding burning or land filling the materials, and the cleanliness of the ethanol fuel. Wood, agricultural residues, herbaceous crops, and municipal solid wastes have been considered as feedstocks for

ethanol production. These materials primarily consist of cellulose, hemicellulose, and lignin. Once the cellulose is converted to glucose, the glucose is easily fermented by yeast into ethanol.

There is a need in the art for new enzyme compositions to increase efficiency and to provide cost-effective enzyme solutions for high temperature saccharification of cellulosic material.

The present invention provides compositions for high temperature saccharification of cellulosic material and to uses thereof

Summary of the Invention

The present invention relates to enzyme compositions, comprising two or more (several) components selected from the group consisting of:

(I) a polypeptide having cellobiohydrolase I activity selected from the group consisting of:

(A) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 2; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 1, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 1, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 1;

(B) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 4; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 3, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 3, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even

more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 3;

(C) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 6; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 5, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 5, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 5;

(D) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 8; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 7, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 7, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 7;

(E) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 158; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 157, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 157, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and

most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 157;

(F) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 160; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 159, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 159, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 159;

(G) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 162; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 161, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 161, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 161;

(H) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 164; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 163, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 163, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide

comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 163; and

(I) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 166; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 165, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 165, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 165;

(II) a polypeptide having cellobiohydrolase II activity selected from the group consisting of:

(A) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 10; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 9, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 9, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 9;

(B) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 12; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature

polypeptide coding sequence of SEQ ID NO: 11, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 11, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 11;

(C) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 14; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 13, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 13, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 13;

(D) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 16; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 15, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 15, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 15;

(E) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 18; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high

stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 17, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 17, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 17;

(F) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 168; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 167, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 167, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 167;

(G) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 170; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 169, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 169, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 169; and

(H) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 172; (b) a polypeptide encoded by a polynucleotide that

hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 172, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 172, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 172;

(III) a polypeptide having endoglucanase I activity selected from the group consisting of: (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 20; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 19, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 19, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 19;

(IV) a polypeptide having endoglucanase II activity selected from the group consisting of:

(A) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 22; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 21, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 21, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 21;

(B) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 24; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 23, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 23, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 23;

(C) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 26; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 25, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 25, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 25;

(D) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 174; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 173, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 173, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID

NO: 173; and

(E) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 176; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 175, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 175, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 175; and

(V) a polypeptide having beta-glucosidase activity selected from the group consisting of:

(A) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 28; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 27, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 27, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 27;

(B) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 30; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 29, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 29, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide

sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 29;

(C) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 32; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 31, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 31, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 31;

(D) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 178; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 177, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 177, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 177;

(E) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 180; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 179, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 179, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a

nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 179;

(F) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 182; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 181, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 181, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 181;

(G) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 184; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 183, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 183, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 183;

(H) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 186; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 185, (ii) the cDNA sequence of the

mature polypeptide coding sequence of SEQ ID NO: 185, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 185;

(I) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 188; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 187, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 187, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 187; and

(J) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 190; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 189, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 189, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 189.

The present invention also relates to host cells encoding such an enzyme composition and methods of producing such an enzyme composition.

The present invention also relates to methods for degrading or converting a cellulosic material, comprising: treating the cellulosic material with such an enzyme composition.

The present invention also relates to methods for producing a fermentation product,

comprising:

(a) saccharifying a cellulosic material with such an enzyme composition;

(b) fermenting the saccharified cellulosic material with one or more (several) fermenting microorganisms to produce the fermentation product; and

(c) recovering the fermentation product from the fermentation.

The present invention also relates to methods of fermenting a cellulosic material, comprising: fermenting the cellulosic material with one or more (several) fermenting microorganisms, wherein the cellulosic material is saccharified with such an enzyme composition.

Brief Description of the Figures

Figure 1 shows a comparison of two enzyme compositions with a *Trichoderma reesei*–based composition in hydrolysis of milled washed PCS at 50°C, 55°C, and 60°C.

Figure 2 shows the effect of *Thermoascus aurantiacus* GH61A or *Thielavia terrestris* GH61E GH61 polypeptides having cellulolytic enhancing activity on PCS-hydrolysing activity of a high-temperature enzyme composition at 50°C, 55°C, and 60°C..

Figure 3 shows the boosting performance of a binary composition comprising equal amounts of *Thermoascus aurantiacus* GH61A and *Thielavia terrestris* GH61E GH61 polypeptides having cellulolytic enhancing activity in comparison with the boosting performance of the individual GH61 polypeptides in hydrolysis of milled washed PCS at 50°C, 55°C, and 60°C.

Figure 4 shows the effect of compositions containing different ratios of *Thermoascus aurantiacus* GH61A and *Thielavia terrestris* GH61E polypeptides on PCS-hydrolysing activity of a high-temperature enzyme composition at 60°C.

Figure 5 shows the effect of different levels of individual *Thermoascus aurantiacus* GH61A and *Thielavia terrestris* GH61E GH61 polypeptides having cellulolytic enhancing activity and their binary 1:1 composition on PCS-hydrolyzing activity of a high-temperature enzyme composition at 60°C.

Figure 6 shows the effect of a *Thermobifida fusca* GH11 xylanase on hydrolysis of milled washed PCS by a high-temperature enzyme composition at 50-65°C.

Figure 7 shows the effect of replacing *Chaetomium thermophilum* Cel7A cellobiohydrolase I in a high-temperature enzyme composition with various thermostable cellobiohydrolase I proteins on hydrolysis of milled washed PCS at 50-65°C.

Figure 8 shows a comparison of *Aspergillus fumigatus* Cel7A- and *Chaetomium thermophilum* Cel7A-based high-temperature enzyme compositions with *Trichoderma reesei*-based cellulase XCL-533 at 50°C and 60°C in hydrolysis of milled washed PCS.

Figure 9 shows the hydrolysis time-course for *Aspergillus fumigatus* Cel7A-based

high-temperature enzyme composition in comparison with *Trichoderma reesei*-based cellulase XCL-533 at 50°C and 60°C (2 mg protein / g cellulose).

Figure 10 shows an evaluation of *Aspergillus aculeatus* GH10 xylanase II, *Aspergillus fumigatus* GH10 xyn3 xylanase, *Trichophaea saccata* GH10 xylanase, and *Thermobifida fusca* GH11 xylanase at 10% addition (0.35 mg protein / g cellulose) to a high-temperature enzyme composition (3.5 mg protein / g cellulose) in hydrolysis of milled washed PCS at 50°C, 55°C, and 60°C.

Figure 11 shows an evaluation of *Aspergillus fumigatus* GH10 xyn3 xylanase, *Trichophaea saccata* GH10 xylanase, and *Thermobifida fusca* GH11 xylanase for synergy with a high-temperature enzyme composition in hydrolysis of milled washed PCS at 50°C, 55°C, and 60°C. Each xylanase was added at different levels (1.25%, 2.5%, 5%, 10%, and 20%) to a constant loading of the high-temperature enzyme composition (3 mg protein per g cellulose).

Figure 12 shows a comparison of an improved high-temperature enzyme composition containing *Aspergillus fumigatus* GH10 xyn3 xylanase at 60°C with *Trichoderma reesei*-based cellulase XCL-533 at 50°C in hydrolysis of milled washed PCS.

Figure 13A and 13B show a comparison of improved high-temperature enzyme compositions containing *Aspergillus fumigatus* GH10 xyn3 xylanase or *Trichophaea saccata* GH10 xylanase (60°C) with *Trichoderma reesei*-based cellulase XCL-533 (50°C) in hydrolysis of washed (A) and unwashed (B) PCS.

Figures 14A and 14B show the effect of replacement of protein in a high-temperature enzyme composition (3 mg protein per g cellulose) with GH3 beta-xylosidases from *Trichoderma reesei* and *Talaromyces emersonii* at 60°C.

Figure 15 shows a comparison of *Trichoderma reesei* Cel7A CBHI, *Chaetomium thermophilum* Cel7A CBHI, *Aspergillus fumigatus* Cel7A CBHI, and *Thermoascus aurantiacus* Cel7A CBHI replacing a CBHI component in a high-temperature enzyme composition in hydrolysis of milled unwashed PCS at 50-65°C.

Figure 16 shows a comparison of *Myceliophthora thermophila* Cel6A CBHII, *Thielavia terrestris* Cel6A CBHII, *Aspergillus fumigatus* Cel6A CBHII, and *Trichophaea saccata* Cel6A CBHII replacing a CBHII component in a high-temperature enzyme composition in hydrolysis of milled unwashed PCS at 50-65°C.

Figure 17 shows a comparison of *Trichoderma reesei* Cel7B EGI and *Aspergillus terreus* Cel7 EGI replacing an endoglucanase component in a high-temperature enzyme composition in hydrolysis of milled unwashed PCS at 50-65°C.

Figure 18 shows a comparison of *Trichoderma reesei* Cel5A EGII, *Myceliophthora thermophila* Cel5A EGII, and *Thermoascus aurantiacus* Cel5A EGII replacing an endoglucanase component in a high-temperature enzyme composition in hydrolysis of milled

unwashed PCS at 50-65°C.

Figure 19 shows a comparison of *Aspergillus fumigatus* Cel3A beta-glucosidase, *Penicillium brasilianum* Cel3A beta-glucosidase, and *Aspergillus niger* Cel3 beta-glucosidase in a high-temperature enzyme composition at 50-60°C using milled unwashed PCS.

Figure 20 shows a comparison of *Aspergillus fumigatus* Cel3A beta-glucosidase, *Penicillium brasilianum* Cel3A beta-glucosidase, and *Aspergillus niger* Cel3 beta-glucosidase in a high-temperature enzyme composition at 50-65°C using milled unwashed PCS.

Figure 21 shows a comparison of *Aspergillus aculeatus* GH10 xyn II xylanase, *Aspergillus fumigatus* GH10 xyn3, *Trichophaea saccata* GH10 xylanase, *Thermobifida fusca* GH11 xylanase, *Penicillium pinophilum* GH10 xylanase, and *Thielavia terrestris* GH10E xylanase replacing a xylanase component in a high-temperature enzyme composition in hydrolysis of milled unwashed PCS at 50-65°C.

Figure 22 shows a comparison of the cellulase-enhancing activity of *Thermoascus aurantiacus* GH61A, *Thielavia terrestris* GH61E, *Penicillium pinophilum* GH61, and *Aspergillus fumigatus* GH61B polypeptides replacing a GH61 component in a high-temperature enzyme composition in hydrolysis of milled unwashed PCS at 50-65°C.

Figure 23 shows a comparison of the cellulase-enhancing activity of *Thermoascus aurantiacus* GH61A, *Thielavia terrestris* GH61N, and *Penicillium sp* GH61A polypeptides replacing a GH61 component in a high-temperature enzyme composition in hydrolysis of milled unwashed PCS at 50-65°C.

Figure 24 shows the effect of *Trichoderma reesei*-based XCL-602 cellulase replacement by *Aspergillus fumigatus* Cel7A cellobiohydrolase I and/or *Myceliophthora thermophila* Cel6A cellobiohydrolase II on saccharification of milled unwashed PCS at 50-60°C.

Figures 25A and 25B show the hydrolysis of milled unwashed PCS by *Trichoderma reesei*-based XCL-602 cellulase compositions containing *Aspergillus fumigatus* Cel7A cellobiohydrolase I and *Myceliophthora thermophila* Cel6A cellobiohydrolase II (3 mg total protein per g cellulose) and additionally supplemented by 5% *Aspergillus fumigatus* GH10 xyn 3 and/or 5% *Thielavia terrestris* GH61E at 50-60°C.

Figure 26 shows the hydrolysis of milled unwashed PCS by *Trichoderma reesei*-based XCL-602 compositions containing different replacement levels of *Trichoderma reesei*-based XCL-592 cellulase at 50-60°C.

Figures 27A and 27B show a comparison of *Thermoascus aurantiacus* GH61A and *Thielavia terrestris* GH61E polypeptides replacing 5% of protein in *Trichoderma reesei*-based XCL-602 cellulase or XCL-602-based enzyme composition in hydrolysis of milled

unwashed PCS at 50-60°C.

Figures 28A and 28B show the hydrolysis of milled unwashed PCS by non-replaced *Trichoderma reesei*-based XCL-602 cellulase and various XCL-602-based enzyme compositions (3 mg protein per g cellulose) in comparison with *Trichoderma reesei*-based XCL-533 cellulase (4.5 mg protein per g cellulose) at 50-60°C.

Figure 29 shows a comparison of *Aspergillus fumigatus* Cel7A CBHI and *Penicillium emersonii* Cel7 CBHI in a high-temperature enzyme composition in hydrolysis of milled unwashed PCS at 50-65°C.

Figure 30 shows an evaluation of *Aspergillus fumigatus* Cel7A CBHI and *Penicillium pinophilum* Cel7A CBHI in a high-temperature enzyme composition in hydrolysis of milled unwashed PCS at 50-65°C.

Figure 31 shows an evaluation of *Aspergillus fumigatus* Cel7A CBHI and *Aspergillus terreus* Cel7A CBHI in a high-temperature enzyme composition in hydrolysis of milled unwashed PCS at 50-65°C.

Figure 32 shows an evaluation of *Aspergillus fumigatus* Cel7A CBHI, *Neosartorya fischeri* Cel7A CBHI, and *Aspergillus nidulans* Cel7A CBHI in a high-temperature enzyme composition in hydrolysis of milled unwashed PCS at 50-60°C.

Figure 33 shows an evaluation of *Aspergillus fumigatus* Cel6A CBHII and *Finnellia nivea* Cel6A CBHII in a high-temperature enzyme composition in hydrolysis of milled unwashed PCS at 50-65°C.

Figure 34 shows an evaluation of *Aspergillus fumigatus* Cel6A CBHII, *Penicillium emersonii* Cel6A CBHII, and *Penicillium pinophilum* Cel6A CBHII proteins replacing a CBHII component in a high-temperature enzyme composition in hydrolysis of milled unwashed PCS at 50-65°C.

Figure 35 shows an evaluation of *Aspergillus fumigatus* Cel5A EGII, *Neosartorya fischeri* Cel5A EGII, and *Myceliophthora thermophila* Cel5A EGII proteins replacing a EG component in a high-temperature enzyme composition in hydrolysis of milled unwashed PCS at 50-65°C.

Figure 36 shows an evaluation of *Aspergillus fumigatus* Cel3A beta-glucosidase and *Aspergillus aculeatus* beta-glucosidase in a high-temperature enzyme composition in hydrolysis of milled unwashed PCS at 50-65°C.

Figure 37 shows an evaluation of *Aspergillus fumigatus* Cel3A beta-glucosidase, *Aspergillus kawashii* Cel3A beta-glucosidase, *Aspergillus clavatus* Cel3 beta-glucosidase, and *Talaromyces emersonii* Cel3A beta-glucosidase in a high-temperature enzyme composition in hydrolysis of milled unwashed PCS at 50-60°C.

Figure 38 shows an evaluation of *Aspergillus fumigatus* Cel3A beta-glucosidase, *Penicillium oxalicum* Cel3A beta-glucosidase (Example 77) and *Penicillium oxalicum* Cel3A

beta-glucosidase (Example 78) in a high-temperature enzyme composition in hydrolysis of milled unwashed PCS at 50-65°C.

Figure 39 shows an evaluation of three GH61 polypeptides having cellulolytic enhancing activity in a high-temperature enzyme composition in hydrolysis of milled washed PCS at 50-65°C.

Figure 40 shows an evaluation of three xylanases in a high-temperature enzyme composition in hydrolysis of milled unwashed PCS at 50-65°C.

Figure 41 shows an evaluation of three xylanases in a high-temperature enzyme composition in hydrolysis of milled unwashed PCS at 50-65°C.

Figure 42 shows the hydrolysis of milled unwashed PCS by non-replaced *Trichoderma reesei*-based XCL-602 cellulase and various XCL-602-based enzyme compositions containing different cellobiohydrolases and xylanases (3 mg protein per g cellulose) at 50-60°C.

Definitions

Cellulolytic enzyme or cellulase: The term "cellulolytic enzyme" or "cellulase" means one or more (several) enzymes that hydrolyze a cellulosic material. Such enzymes include endoglucanase(s), cellobiohydrolase(s), beta-glucosidase(s), or combinations thereof. The two basic approaches for measuring cellulolytic activity include: (1) measuring the total cellulolytic activity, and (2) measuring the individual cellulolytic activities (endoglucanases, cellobiohydrolases, and beta-glucosidases) as reviewed in Zhang *et al.*, Outlook for cellulase improvement: Screening and selection strategies, 2006, *Biotechnology Advances* 24: 452-481. Total cellulolytic activity is usually measured using insoluble substrates, including Whatman №1 filter paper, microcrystalline cellulose, bacterial cellulose, algal cellulose, cotton, pretreated lignocellulose, *etc.* The most common total cellulolytic activity assay is the filter paper assay using Whatman №1 filter paper as the substrate. The assay was established by the International Union of Pure and Applied Chemistry (IUPAC) (Ghose, 1987, Measurement of cellulase activities, *Pure Appl. Chem.* 59: 257-68).

For purposes of the present invention, cellulolytic enzyme activity is determined by measuring the increase in hydrolysis of a cellulosic material by cellulolytic enzyme(s) under the following conditions: 1-20 mg of cellulolytic enzyme protein/g of cellulose in PCS for 3-7 days at 50°C compared to a control hydrolysis without addition of cellulolytic enzyme protein. Typical conditions are 1 ml reactions, washed or unwashed PCS, 5% insoluble solids, 50 mM sodium acetate pH 5, 1 mM $MnSO_4$, 50°C, 72 hours, sugar analysis by AMINEX® HPX-87H column (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Endoglucanase: The term "endoglucanase" means an endo-1,4-(1,3;1,4)-beta-D-

glucan 4-glucanohydrolase (E.C. 3.2.1.4), which catalyses endohydrolysis of 1,4-beta-D-glycosidic linkages in cellulose, cellulose derivatives (such as carboxymethyl cellulose and hydroxyethyl cellulose), lichenin, beta-1,4 bonds in mixed beta-1,3 glucans such as cereal beta-D-glucans or xyloglucans, and other plant material containing cellulosic components. Endoglucanase activity can be determined by measuring reduction in substrate viscosity or increase in reducing ends determined by a reducing sugar assay (Zhang *et al.*, 2006, *Biotechnology Advances* 24: 452-481). For purposes of the present invention, endoglucanase activity is determined using carboxymethyl cellulose (CMC) as substrate according to the procedure of Ghose, 1987, *Pure and Appl. Chem.* 59: 257-268, at pH 5, 40°C.

Cellobiohydrolase: The term "cellobiohydrolase" means a 1,4-beta-D-glucan cellobiohydrolase (E.C. 3.2.1.91), which catalyzes the hydrolysis of 1,4-beta-D-glucosidic linkages in cellulose, cellooligosaccharides, or any beta-1,4-linked glucose containing polymer, releasing cellobiose from the reducing or non-reducing ends of the chain (Teeri, 1997, Crystalline cellulose degradation: New insight into the function of cellobiohydrolases, *Trends in Biotechnology* 15: 160-167; Teeri *et al.*, 1998, *Trichoderma reesei* cellobiohydrolases: why so efficient on crystalline cellulose?, *Biochem. Soc. Trans.* 26: 173-178). For purposes of the present invention, cellobiohydrolase activity is determined according to the procedures described by Lever *et al.*, 1972, *Anal. Biochem.* 47: 273-279; van Tilbeurgh *et al.*, 1982, *FEBS Letters,* 149: 152-156; van Tilbeurgh and Claeyssens, 1985, *FEBS Letters,* 187: 283-288; and Tomme *et al.*, 1988, *Eur. J. Biochem.* 170: 575-581. In the present invention, the Lever *et al.* method can be employed to assess hydrolysis of cellulose in corn stover, while the methods of van Tilbeurgh *et al.* and Tomme *et al.* can be used to determine the cellobiohydrolase activity on a fluorescent disaccharide derivative, 4-methylumbelliferyl-β-D-lactoside.

Beta-glucosidase: The term "beta-glucosidase" means a beta-D-glucoside glucohydrolase (E.C. 3.2.1.21), which catalyzes the hydrolysis of terminal non-reducing beta-D-glucose residues with the release of beta-D-glucose. For purposes of the present invention, beta-glucosidase activity is determined according to the basic procedure described by Venturi *et al.*, 2002, Extracellular beta-D-glucosidase from *Chaetomium thermophilum* var. *coprophilum*: production, purification and some biochemical properties, *J. Basic Microbiol.* 42: 55-66. One unit of beta-glucosidase is defined as 1.0 µmole of *p*-nitrophenolate anion produced per minute at 25°C, pH 4.8 from 1 mM *p*-nitrophenyl-beta-D-glucopyranoside as substrate in 50 mM sodium citrate containing 0.01% TWEEN® 20.

Polypeptide having cellulolytic enhancing activity: The term "polypeptide having cellulolytic enhancing activity" means a GH61 polypeptide that catalyzes the enhancement of the hydrolysis of a cellulosic material by enzyme having cellulolytic activity. For purposes of

the present invention, cellulolytic enhancing activity is determined by measuring the increase in reducing sugars or the increase of the total of cellobiose and glucose from the hydrolysis of a cellulosic material by cellulolytic enzyme under the following conditions: 1-50 mg of total protein/g of cellulose in PCS, wherein total protein is comprised of 50-99.5% w/w cellulolytic enzyme protein and 0.5-50% w/w protein of a GH61 polypeptide having cellulolytic enhancing activity for 1-7 days at 50°C compared to a control hydrolysis with equal total protein loading without cellulolytic enhancing activity (1-50 mg of cellulolytic protein/g of cellulose in PCS). In a preferred aspect, a mixture of CELLUCLAST® 1.5L (Novozymes A/S, Bagsværd, Denmark) in the presence of 2-3% of total protein weight *Aspergillus oryzae* beta-glucosidase (recombinantly produced in *Aspergillus oryzae* according to WO 02/095014) or 2-3% of total protein weight *Aspergillus fumigatus* beta-glucosidase (recombinantly produced in *Aspergillus oryzae* as described in WO 2002/095014) of cellulase protein loading is used as the source of the cellulolytic activity.

The GH61 polypeptides having cellulolytic enhancing activity enhance the hydrolysis of a cellulosic material catalyzed by enzyme having cellulolytic activity by reducing the amount of cellulolytic enzyme required to reach the same degree of hydrolysis preferably at least 1.01-fold, more preferably at least 1.05-fold, more preferably at least 1.10-fold, more preferably at least 1.25-fold, more preferably at least 1.5-fold, more preferably at least 2-fold, more preferably at least 3-fold, more preferably at least 4-fold, more preferably at least 5-fold, even more preferably at least 10-fold, and most preferably at least 20-fold.

Hemicellulolytic enzyme or hemicellulase: The term "hemicellulolytic enzyme" or "hemicellulase" means one or more (several) enzymes that hydrolyze a hemicellulosic material. See, for example, Shallom, D. and Shoham, Y. Microbial hemicellulases. *Current Opinion In Microbiology*, 2003, 6(3): 219-228). Hemicellulases are key components in the degradation of plant biomass. Examples of hemicellulases include, but are not limited to, an acetylmannan esterase, an acetyxylan esterase, an arabinanase, an arabinofuranosidase, a coumaric acid esterase, a feruloyl esterase, a galactosidase, a glucuronidase, a glucuronoyl esterase, a mannanase, a mannosidase, a xylanase, and a xylosidase. The substrates of these enzymes, the hemicelluloses, are a heterogeneous group of branched and linear polysaccharides that are bound via hydrogen bonds to the cellulose microfibrils in the plant cell wall, crosslinking them into a robust network. Hemicelluloses are also covalently attached to lignin, forming together with cellulose a highly complex structure. The variable structure and organization of hemicelluloses require the concerted action of many enzymes for its complete degradation. The catalytic modules of hemicellulases are either glycoside hydrolases (GHs) that hydrolyze glycosidic bonds, or carbohydrate esterases (CEs), which hydrolyze ester linkages of acetate or ferulic acid side groups. These catalytic modules, based on homology of their primary sequence, can be assigned into GH and CE families

marked by numbers. Some families, with overall similar fold, can be further grouped into clans, marked alphabetically (*e.g.*, GH-A). A most informative and updated classification of these and other carbohydrate active enzymes is available on the Carbohydrate-Active Enzymes (CAZy) database. Hemicellulolytic enzyme activities can be measured according to Ghose and Bisaria, 1987, *Pure & Appl. Chem.* 59: 1739-1752.

Xylan degrading activity or xylanolytic activity: The term "xylan degrading activity" or "xylanolytic activity" means a biological activity that hydrolyzes xylan-containing material. The two basic approaches for measuring xylanolytic activity include: (1) measuring the total xylanolytic activity, and (2) measuring the individual xylanolytic activities (*e.g.*, endoxylanases, beta-xylosidases, arabinofuranosidases, alpha-glucuronidases, acetylxylan esterases, feruloyl esterases, and alpha-glucuronyl esterases). Recent progress in assays of xylanolytic enzymes was summarized in several publications including Biely and Puchard, Recent progress in the assays of xylanolytic enzymes, 2006, *Journal of the Science of Food and Agriculture* 86(11): 1636-1647; Spanikova and Biely, 2006, Glucuronoyl esterase - Novel carbohydrate esterase produced by Schizophyllum commune, *FEBS Letters* 580(19): 4597-4601; Herrmann, Vrsanska, Jurickova, Hirsch, Biely, and Kubicek, 1997, The beta-D-xylosidase of *Trichoderma reesei* is a multifunctional beta-D-xylan xylohydrolase, *Biochemical Journal* 321: 375-381.

Total xylan degrading activity can be measured by determining the reducing sugars formed from various types of xylan, including, for example, oat spelt, beechwood, and larchwood xylans, or by photometric determination of dyed xylan fragments released from various covalently dyed xylans. The most common total xylanolytic activity assay is based on production of reducing sugars from polymeric 4-O-methyl glucuronoxylan as described in Bailey, Biely, Poutanen, 1992, Interlaboratory testing of methods for assay of xylanase activity, *Journal of Biotechnology* 23(3): 257-270. Xylanase activity can also be determined with 0.2% AZCL-arabinoxylan as substrate in 0.01% Triton X-100 and 200 mM sodium phosphate buffer pH 6 at 37°C. One unit of xylanase activity is defined as 1.0 μmole of azurine produced per minute at 37°C, pH 6 from 0.2% AZCL-arabinoxylan as substrate in 200 mM sodium phosphate pH 6 buffer.

For purposes of the present invention, xylan degrading activity is determined by measuring the increase in hydrolysis of birchwood xylan (Sigma Chemical Co., Inc., St. Louis, MO, USA) by xylan-degrading enzyme(s) under the following typical conditions: 1 ml reactions, 5 mg/ml substrate (total solids), 5 mg of xylanolytic protein/g of substrate, 50 mM sodium acetate pH 5, 50°C, 24 hours, sugar analysis using *p*-hydroxybenzoic acid hydrazide (PHBAH) assay as described by Lever, 1972, A new reaction for colorimetric determination of carbohydrates, *Anal. Biochem* 47: 273-279.

Xylanase: The term "xylanase" means a 1,4-beta-D-xylan-xylohydrolase (E.C.

3.2.1.8) that catalyzes the endohydrolysis of 1,4-beta-D-xylosidic linkages in xylans. For purposes of the present invention, xylanase activity is determined with 0.2% AZCL-arabinoxylan as substrate in 0.01% Triton X-100 and 200 mM sodium phosphate buffer pH 6 at 37°C. One unit of xylanase activity is defined as 1.0 µmole of azurine produced per minute at 37°C, pH 6 from 0.2% AZCL-arabinoxylan as substrate in 200 mM sodium phosphate pH 6 buffer.

Beta-xylosidase: The term "beta-xylosidase" means a beta-D-xyloside xylohydrolase (E.C. 3.2.1.37) that catalyzes the exo-hydrolysis of short beta (1→4)-xylooligosaccharides, to remove successive D-xylose residues from the non-reducing termini. For purposes of the present invention, one unit of beta-xylosidase is defined as 1.0 µmole of *p*-nitrophenolate anion produced per minute at 40°C, pH 5 from 1 mM *p*-nitrophenyl-beta-D-xyloside as substrate in 100 mM sodium citrate containing 0.01% TWEEN® 20.

Acetylxylan esterase: The term "acetylxylan esterase" means a carboxylesterase (EC 3.1.1.72) that catalyses the hydrolysis of acetyl groups from polymeric xylan, acetylated xylose, acetylated glucose, alpha-napthyl acetate, and *p*-nitrophenyl acetate. For purposes of the present invention, acetylxylan esterase activity is determined using 0.5 mM *p*-nitrophenylacetate as substrate in 50 mM sodium acetate pH 5.0 containing 0.01% TWEEN™ 20. One unit of acetylxylan esterase is defined as the amount of enzyme capable of releasing 1 µmole of *p*-nitrophenolate anion per minute at pH 5, 25°C.

Feruloyl esterase: The term "feruloyl esterase" means a 4-hydroxy-3-methoxycinnamoyl-sugar hydrolase (EC 3.1.1.73) that catalyzes the hydrolysis of the 4-hydroxy-3-methoxycinnamoyl (feruloyl) group from an esterified sugar, which is usually arabinose in "natural" substrates, to produce ferulate (4-hydroxy-3-methoxycinnamate). Feruloyl esterase is also known as ferulic acid esterase, hydroxycinnamoyl esterase, FAE-III, cinnamoyl ester hydrolase, FAEA, cinnAE, FAE-I, or FAE-II. For purposes of the present invention, feruloyl esterase activity is determined using 0.5 mM *p*-nitrophenylferulate as substrate in 50 mM sodium acetate pH 5.0. One unit of feruloyl esterase equals the amount of enzyme capable of releasing 1 µmole of *p*-nitrophenolate anion per minute at pH 5, 25°C.

Alpha-glucuronidase: The term "alpha-glucuronidase" means an alpha-D-glucosiduronate glucuronohydrolase (EC 3.2.1.139) that catalyzes the hydrolysis of an alpha-D-glucuronoside to D-glucuronate and an alcohol. For purposes of the present invention, alpha-glucuronidase activity is determined according to de Vries, 1998, *J. Bacteriol.* 180: 243-249. One unit of alpha-glucuronidase equals the amount of enzyme capable of releasing 1 µmole of glucuronic or 4-O-methylglucuronic acid per minute at pH 5, 40°C.

Alpha-L-arabinofuranosidase: The term "alpha-L-arabinofuranosidase" means an

alpha-L-arabinofuranoside arabinofuranohydrolase (EC 3.2.1.55) that catalyzes the hydrolysis of terminal non-reducing alpha-L-arabinofuranoside residues in alpha-L-arabinosides. The enzyme acts on alpha-L-arabinofuranosides, alpha-L-arabinans containing (1,3)- and/or (1,5)-linkages, arabinoxylans, and arabinogalactans. Alpha-L-arabinofuranosidase is also known as arabinosidase, alpha-arabinosidase, alpha-L-arabinosidase, alpha-arabinofuranosidase, polysaccharide alpha-L-arabinofuranosidase, alpha-L-arabinofuranoside hydrolase, L-arabinosidase, or alpha-L-arabinanase. For purposes of the present invention, alpha-L-arabinofuranosidase activity is determined using 5 mg of medium viscosity wheat arabinoxylan (Megazyme International Ireland, Ltd., Bray, Co. Wicklow, Ireland) per ml of 100 mM sodium acetate pH 5 in a total volume of 200 µl for 30 minutes at 40°C followed by arabinose analysis by AMINEX® HPX-87H column chromatography (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Family 3, 5, 6, 7, 10, 11, or 61, or GH3, GH5, GH6, GH7, GH10, GH11, or GH61, or Cel3, Cel5, Cel6 or Cel7: The terms "Family 3", "Family 5", "Family 6", "Family 7", "Family 10", "Family 11", "Family 61", "GH3", "GH5", "GH6", "GH7", "GH10", "GH11", "GH61", "Cel3", "Cel5", "Cel6", or "Cel7" are defined herein as a polypeptide falling into the glycoside hydrolase Families 3, 5, 6, 7, 10, 11, and 61 according to Henrissat B., 1991, A classification of glycosyl hydrolases based on amino-acid sequence similarities, *Biochem. J.* 280: 309-316, and Henrissat and Bairoch, 1996, Updating the sequence-based classification of glycosyl hydrolases, *Biochem. J.* 316: 695-696.

Cellulosic material: The cellulosic material can be any material containing cellulose. The predominant polysaccharide in the primary cell wall of biomass is cellulose, the second most abundant is hemicellulose, and the third is pectin. The secondary cell wall, produced after the cell has stopped growing, also contains polysaccharides and is strengthened by polymeric lignin covalently cross-linked to hemicellulose. Cellulose is a homopolymer of anhydrocellobiose and thus a linear beta-(1-4)-D-glucan, while hemicelluloses include a variety of compounds, such as xylans, xyloglucans, arabinoxylans, and mannans in complex branched structures with a spectrum of substituents. Although generally polymorphous, cellulose is found in plant tissue primarily as an insoluble crystalline matrix of parallel glucan chains. Hemicelluloses usually hydrogen bond to cellulose, as well as to other hemicelluloses, which help stabilize the cell wall matrix.

Cellulose is generally found, for example, in the stems, leaves, hulls, husks, and cobs of plants or leaves, branches, and wood of trees. The cellulosic material can be, but is not limited to, herbaceous material, agricultural residue, forestry residue, municipal solid waste, waste paper, and pulp and paper mill residue (see, for example, Wiselogel *et al.*, 1995, in Handbook on Bioethanol (Charles E. Wyman, editor), pp.105-118, Taylor & Francis, Washington D.C.; Wyman, 1994, *Bioresource Technology* 50: 3-16; Lynd, 1990, *Applied*

Biochemistry and Biotechnology 24/25: 695-719; Mosier *et al.*, 1999, Recent Progress in Bioconversion of Lignocellulosics, in *Advances in Biochemical Engineering/Biotechnology*, T. Scheper, managing editor, Volume 65, pp.23-40, Springer-Verlag, New York). It is understood herein that the cellulose may be in the form of lignocellulose, a plant cell wall material containing lignin, cellulose, and hemicellulose in a mixed matrix. In a preferred aspect, the cellulosic material is lignocellulose.

In one aspect, the cellulosic material is herbaceous material. In another aspect, the cellulosic material is agricultural residue. In another aspect, the cellulosic material is forestry residue. In another aspect, the cellulosic material is municipal solid waste. In another aspect, the cellulosic material is waste paper. In another aspect, the cellulosic material is pulp and paper mill residue.

In another aspect, the cellulosic material is corn stover. In another aspect, the cellulosic material is corn fiber. In another aspect, the cellulosic material is corn cob. In another aspect, the cellulosic material is orange peel. In another aspect, the cellulosic material is rice straw. In another aspect, the cellulosic material is wheat straw. In another aspect, the cellulosic material is switch grass. In another aspect, the cellulosic material is miscanthus. In another aspect, the cellulosic material is bagasse. In another aspect, the cellulosic material is softwood. In another aspect, the cellulosic material is hardwood.

In another aspect, the cellulosic material is microcrystalline cellulose. In another aspect, the cellulosic material is bacterial cellulose. In another aspect, the cellulosic material is algal cellulose. In another aspect, the cellulosic material is cotton linter. In another aspect, the cellulosic material is amorphous phosphoric-acid treated cellulose. In another aspect, the cellulosic material is filter paper.

The cellulosic material may be used as is or may be subjected to pretreatment, using conventional methods known in the art, as described herein. In a preferred aspect, the cellulosic material is pretreated.

Pretreated corn stover: The term "PCS" or "Pretreated Corn Stover" means a cellulosic material derived from corn stover by treatment with heat and dilute sulfuric acid.

Isolated or purified: The term "isolated" or "purified" means a polypeptide or polynucleotide that is removed from at least one component with which it is naturally associated. For example, a polypeptide may be at least 1% pure, *e.g.*, at least 5% pure, at least 10% pure, at least 20% pure, at least 40% pure, at least 60% pure, at least 80% pure, at least 90% pure, or at least 95% pure, as determined by SDS-PAGE, and a polynucleotide may be at least 1% pure, *e.g.*, at least 5% pure, at least 10% pure, at least 20% pure, at least 40% pure, at least 60% pure, at least 80% pure, at least 90% pure, or at least 95% pure, as determined by agarose electrophoresis.

Mature polypeptide: The term "mature polypeptide" means a polypeptide in its final

form following translation and any post-translational modifications, such as N-terminal processing, C-terminal truncation, glycosylation, phosphorylation, etc. It is known in the art that a host cell may produce a mixture of two of more different mature polypeptides (*i.e.*, with a different C-terminal and/or N-terminal amino acid) expressed by the same polynucleotide. The mature polypeptide can be predicted using the SignalP program (Nielsen *et al.*, 1997, *Protein Engineering* 10: 1-6).

Mature polypeptide coding sequence: The term "mature polypeptide coding sequence" is defined herein as a nucleotide sequence that encodes a mature polypeptide having biological activity. The mature polypeptide coding sequence can be predicted using the SignalP program (Nielsen *et al.*, 1997, *supra*).

Sequence Identity: The relatedness between two amino acid sequences or between two nucleotide sequences is described by the parameter "sequence identity".

For purposes of the present invention, the degree of sequence identity between two amino acid sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *J. Mol. Biol.* 48: 443-453) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *Trends Genet.* 16: 276-277), preferably version 3.0.0 or later. The optional parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of BLOSUM62) substitution matrix. The output of Needle labeled "longest identity" (obtained using the –nobrief option) is used as the percent identity and is calculated as follows:

(Identical Residues x 100)/(Length of Alignment – Total Number of Gaps in Alignment)

For purposes of the present invention, the degree of sequence identity between two deoxyribonucleotide sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *supra*) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *supra*), preferably version 3.0.0 or later. The optional parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EDNAFULL (EMBOSS version of NCBI NUC4.4) substitution matrix. The output of Needle labeled "longest identity" (obtained using the –nobrief option) is used as the percent identity and is calculated as follows:

(Identical Deoxyribonucleotides x 100)/(Length of Alignment – Total Number of Gaps in Alignment)

Polypeptide fragment: The term "fragment" means a polypeptide having one or more (several) amino acids deleted from the amino and/or carboxyl terminus of a mature polypeptide; wherein the fragment has biological activity.

Subsequence: The term "subsequence" means a polynucleotide having one or more (several) nucleotides deleted from the 5' and/or 3' end of a mature polypeptide coding

sequence; wherein the subsequence encodes a fragment having biological activity.

Allelic variant: The term “allelic variant” means any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and may result in polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequences. An allelic variant of a polypeptide is a polypeptide encoded by an allelic variant of a gene.

Coding sequence: The term “coding sequence” means a polynucleotide, which directly specifies the amino acid sequence of a polypeptide. The boundaries of the coding sequence are generally determined by an open reading frame, which usually begins with the ATG start codon or alternative start codons such as GTG and TTG and ends with a stop codon such as TAA, TAG, and TGA. The coding sequence may be a DNA, cDNA, synthetic, or recombinant polynucleotide.

cDNA: The term "cDNA" means a DNA molecule that can be prepared by reverse transcription from a mature, spliced, mRNA molecule obtained from a eukaryotic cell. cDNA lacks intron sequences that may be present in the corresponding genomic DNA. The initial, primary RNA transcript is a precursor to mRNA that is processed through a series of steps, including splicing, before appearing as mature spliced mRNA.

Nucleic acid construct: The term "nucleic acid construct" means a nucleic acid molecule, either single- or double-stranded, which is isolated from a naturally occurring gene or is modified to contain segments of nucleic acids in a manner that would not otherwise exist in nature or which is synthetic. The term nucleic acid construct is synonymous with the term “expression cassette” when the nucleic acid construct contains the control sequences required for expression of a coding sequence of the present invention.

Control sequences: The term “control sequences” means all components necessary for the expression of a polynucleotide encoding a polypeptide. Each control sequence may be native or foreign to the polynucleotide encoding the polypeptide or native or foreign to each other. Such control sequences include, but are not limited to, a leader, polyadenylation sequence, propeptide sequence, promoter, signal peptide sequence, and transcription terminator. At a minimum, the control sequences include a promoter, and transcriptional and translational stop signals. The control sequences may be provided with linkers for the purpose of introducing specific restriction sites facilitating ligation of the control sequences with the coding region of the polynucleotide encoding a polypeptide.

Operably linked: The term “operably linked” means a configuration in which a control sequence is placed at an appropriate position relative to the coding sequence of a polynucleotide such that the control sequence directs the expression of the coding sequence.

Expression: The term “expression” includes any step involved in the production of the polypeptide including, but not limited to, transcription, post-transcriptional modification, translation, post-translational modification, and secretion.

Expression vector: The term “expression vector” means a linear or circular DNA molecule that comprises a polynucleotide encoding a polypeptide and is operably linked to additional nucleotides that provide for its expression.

Host cell: The term "host cell" means any cell type that is susceptible to transformation, transfection, transduction, and the like with a nucleic acid construct or expression vector comprising a polynucleotide of the present invention. The term “host cell” encompasses any progeny of a parent cell that is not identical to the parent cell due to mutations that occur during replication.

Detailed Description of the Invention

Enzyme Compositions

The present invention relates to enzyme compositions, comprising two or more (several) components selected from the group consisting of:

(I) a polypeptide having cellobiohydrolase I activity selected from the group consisting of:

(A) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 2; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 1, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 1, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 1;

(B) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 4; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature

polypeptide coding sequence of SEQ ID NO: 3, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 3, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 3;

(C) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 6; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 5, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 5, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 5;

(D) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 8; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 7, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 7, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 7;

(E) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 158; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 157, (ii) the genomic DNA

sequence of the mature polypeptide coding sequence of SEQ ID NO: 157, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 157;

(F) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 160; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 159, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 159, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 159;

(G) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 162; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 161, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 161, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 161;

(H) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 164; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at

least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 163, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 163, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 163; and

(I) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 166; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 165, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 165, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 165;

(II) a polypeptide having cellobiohydrolase II activity selected from the group consisting of:

(A) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 10; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 9, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 9, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 9;

(B) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more

preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 12; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 11, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 11, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 11;

(C) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 14; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 13, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 13, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 13;

(D) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 16; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 15, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 15, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 15;

(E) (a) a polypeptide comprising an amino acid sequence having preferably at least

80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 18; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 17, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 17, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 17;

(F) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 168; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 167, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 167, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 167;

(G) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 170; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 169, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 169, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 169; and

(H) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 172; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 172, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 172, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 172;

(III) a polypeptide having endoglucanase I activity selected from the group consisting of: (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 20; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 19, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 19, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 19;

(IV) a polypeptide having endoglucanase II activity selected from the group consisting of:

(A) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 22; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 21, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 21, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a

nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 21;

(B) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 24; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 23, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 23, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 23;

(C) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 26; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 25, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 25, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 25;

(D) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 174; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 173, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 173, or (iii) a full-length

complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 173; and

(E) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 176; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 175, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 175, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 175; and

(V) a polypeptide having beta-glucosidase activity selected from the group consisting of:

(A) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 28; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 27, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 27, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 27;

(B) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 30; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high

stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 29, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 29, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 29;

(C) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 32; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 31, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 31, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 31;

(D) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 178; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 177, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 177, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 177;

(E) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 180; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at

least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 179, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 179, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 179;

(F) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 182; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 181, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 181, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 181;

(G) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 184; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 183, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 183, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 183;

(H) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature

polypeptide of SEQ ID NO: 186; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 185, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 185, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 185;

(l) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 188; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 187, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 187, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 187; and

(J) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 190; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 189, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 189, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 189.

In a preferred aspect, the polypeptide having cellobiohydrolase I activity is a

Chaetomium thermophilum Cel7A cellobiohydrolase I of the mature polypeptide of SEQ ID NO: 2. In another aspect, the *Chaetomium thermophilum* Cel7A cellobiohydrolase I is encoded by the mature polypeptide coding sequence of SEQ ID NO: 1.

In another aspect, the polypeptide having cellobiohydrolase I activity is a *Myceliophthora thermophila* Cel7A cellobiohydrolase I of the mature polypeptide of SEQ ID NO: 4. In another aspect, the *Myceliophthora thermophila* Cel7A cellobiohydrolase I is encoded by the mature polypeptide coding sequence of SEQ ID NO: 3.

In another aspect, the polypeptide having cellobiohydrolase I activity is a *Aspergillus fumigatus* Cel7A cellobiohydrolase I of the mature polypeptide of SEQ ID NO: 6. In another aspect, the *Aspergillus fumigatus* Cel7A cellobiohydrolase I is encoded by the mature polypeptide coding sequence of SEQ ID NO: 5.

In another aspect, the polypeptide having cellobiohydrolase I activity is a *Thermoascus aurantiacus* Cel7A cellobiohydrolase I of the mature polypeptide of SEQ ID NO: 8. In another aspect, the *Thermoascus aurantiacus* Cel7A cellobiohydrolase I is encoded by the mature polypeptide coding sequence of SEQ ID NO: 7.

In another aspect, the polypeptide having cellobiohydrolase I activity is a *Penicillium emersonii* Cel7 cellobiohydrolase I of the mature polypeptide of SEQ ID NO: 158. In another aspect, the *Penicillium emersonii* Cel7 cellobiohydrolase I is encoded by the mature polypeptide coding sequence of SEQ ID NO: 157.

In another aspect, the polypeptide having cellobiohydrolase I activity is a *Penicillium pinophilum* Cel7 cellobiohydrolase I of the mature polypeptide of SEQ ID NO: 160. In another aspect, the *Penicillium pinophilum* Cel7A cellobiohydrolase I is encoded by the mature polypeptide coding sequence of SEQ ID NO: 159.

In another aspect, the polypeptide having cellobiohydrolase I activity is an *Aspergillus terreus* Cel7 cellobiohydrolase I of the mature polypeptide of SEQ ID NO: 162. In another aspect, the *Aspergillus terreus* Cel7 cellobiohydrolase I is encoded by the mature polypeptide coding sequence of SEQ ID NO: 161.

In another aspect, the polypeptide having cellobiohydrolase I activity is a *Neosartorya fischeri* Cel7 cellobiohydrolase I of the mature polypeptide of SEQ ID NO: 164. In another aspect, the *Neosartorya fischeri* Cel7 cellobiohydrolase I is encoded by the mature polypeptide coding sequence of SEQ ID NO: 163.

In another aspect, the polypeptide having cellobiohydrolase I activity is an *Aspergillus nidulans* Cel7 cellobiohydrolase I of the mature polypeptide of SEQ ID NO: 166. In another aspect, the *Aspergillus nidulans* Cel7 cellobiohydrolase I is encoded by the mature polypeptide coding sequence of SEQ ID NO: 165.

In another aspect, the polypeptide having cellobiohydrolase II activity is a *Myceliophthora thermophila* Cel6A cellobiohydrolase II of the mature polypeptide of SEQ ID

NO: 10. In another aspect, the *Myceliophthora thermophila* Cel6A cellobiohydrolase II is encoded by the mature polypeptide coding sequence of SEQ ID NO: 9.

In another aspect, the polypeptide having cellobiohydrolase II activity is a *Myceliophthora thermophila* Cel6B cellobiohydrolase II of the mature polypeptide of SEQ ID NO: 12. In another aspect, the *Myceliophthora thermophila* Cel6B cellobiohydrolase II is encoded by the mature polypeptide coding sequence of SEQ ID NO: 11.

In another aspect, the polypeptide having cellobiohydrolase II activity is a *Thielavia terrestris* Cel6A cellobiohydrolase II of the mature polypeptide of SEQ ID NO: 14. In another aspect, the *Thielavia terrestris* Cel6A cellobiohydrolase II is encoded by the mature polypeptide coding sequence of SEQ ID NO: 13.

In another aspect, the polypeptide having cellobiohydrolase II activity is a *Trichophaea saccata* CBS 804.70 Cel6A cellobiohydrolase II of the mature polypeptide of SEQ ID NO: 16. In another aspect, the *Trichophaea saccata* Cel6A cellobiohydrolase II is encoded by the mature polypeptide coding sequence of SEQ ID NO: 15.

In another aspect, the polypeptide having cellobiohydrolase II activity is an *Aspergillus fumigatus* Cel6A cellobiohydrolase II of the mature polypeptide of SEQ ID NO: 18. In another aspect, the *Aspergillus fumigatus* Cel6A cellobiohydrolase II is encoded by the mature polypeptide coding sequence of SEQ ID NO: 17.

In another aspect, the polypeptide having cellobiohydrolase II activity is a *Fennellia nivea* Cel6 cellobiohydrolase II of the mature polypeptide of SEQ ID NO: 168. In another aspect, the *Fennellia nivea* Cel6 cellobiohydrolase II is encoded by the mature polypeptide coding sequence of SEQ ID NO: 167.

In another aspect, the polypeptide having cellobiohydrolase II activity is a *Penicillium emersonii* Cel6A cellobiohydrolase II of the mature polypeptide of SEQ ID NO: 170. In another aspect, the *Penicillium emersonii* Cel6A cellobiohydrolase II is encoded by the mature polypeptide coding sequence of SEQ ID NO: 169.

In another aspect, the polypeptide having cellobiohydrolase II activity is a *Penicillium pinophilum* Cel6A cellobiohydrolase II of the mature polypeptide of SEQ ID NO: 172. In another aspect, the *Penicillium pinophilum* Cel6A cellobiohydrolase II is encoded by the mature polypeptide coding sequence of SEQ ID NO: 171.

In another aspect, the polypeptide having endoglucanase I activity is a *Aspergillus terreus* Cel7A endoglucanase I of the mature polypeptide of SEQ ID NO: 20. In another aspect, the *Aspergillus terreus* Cel7A endoglucanase I is encoded by the mature polypeptide coding sequence of SEQ ID NO: 19.

In another aspect, the polypeptide having endoglucanase II activity is a *Trichoderma reesei* Cel5A endoglucanase II of the mature polypeptide of SEQ ID NO: 22. In another aspect, the *Trichoderma reesei* Cel5A endoglucanase II is encoded by the mature

polypeptide coding sequence of SEQ ID NO: 21.

In another aspect, the polypeptide having endoglucanase II activity is a *Myceliophthora thermophila* Cel5A endoglucanase II of the mature polypeptide of SEQ ID NO: 24. In another aspect, the *Myceliophthora thermophila* Cel5A endoglucanase II is encoded by the mature polypeptide coding sequence of SEQ ID NO: 23.

In another aspect, the polypeptide having endoglucanase II activity is a *Thermoascus aurantiacus* Cel5A endoglucanase II of the mature polypeptide of SEQ ID NO: 26. In another aspect, the *Thermoascus aurantiacus* Cel5A endoglucanase II is encoded by the mature polypeptide coding sequence of SEQ ID NO: 25.

In another aspect, the polypeptide having endoglucanase II activity is an *Aspergillus fumigatus* Cel5 endoglucanase II of the mature polypeptide of SEQ ID NO: 174. In another aspect, the *Aspergillus fumigatus* Cel5 endoglucanase II is encoded by the mature polypeptide coding sequence of SEQ ID NO: 173.

In another aspect, the polypeptide having endoglucanase II activity is a *Neosartorya fischeri* Cel5 endoglucanase II of the mature polypeptide of SEQ ID NO: 176. In another aspect, the *Neosartorya fischeri* Cel5 endoglucanase II is encoded by the mature polypeptide coding sequence of SEQ ID NO: 175.

In another aspect, the polypeptide having beta-glucosidase activity is a *Aspergillus fumigatus* beta-glucosidase of the mature polypeptide of SEQ ID NO: 28. In another aspect, the *Aspergillus fumigatus* beta-glucosidase is encoded by the mature polypeptide coding sequence of SEQ ID NO: 27.

In another aspect, the polypeptide having beta-glucosidase activity is a *Penicillium brasilianum* beta-glucosidase of the mature polypeptide of SEQ ID NO: 30. In another aspect, the *Penicillium brasilianum* beta-glucosidase is encoded by the mature polypeptide coding sequence of SEQ ID NO: 29.

In another aspect, the polypeptide having beta-glucosidase activity is a *Aspergillus niger* beta-glucosidase of the mature polypeptide of SEQ ID NO: 32. In another aspect, the *Aspergillus niger* beta-glucosidase is encoded by the mature polypeptide coding sequence of SEQ ID NO: 31.

In another aspect, the polypeptide having beta-glucosidase activity is an *Aspergillus aculeatus* Cel3 beta-glucosidase of the mature polypeptide of SEQ ID NO: 178. In another aspect, the *Aspergillus aculeatus* Cel3 beta-glucosidase is encoded by the mature polypeptide coding sequence of SEQ ID NO: 177.

In another aspect, the polypeptide having beta-glucosidase activity is an *Aspergillus kawashii* Cel3 beta-glucosidase of the mature polypeptide of SEQ ID NO: 180. In another aspect, the *Aspergillus kawashii* Cel3 beta-glucosidase is encoded by the mature polypeptide coding sequence of SEQ ID NO: 179.

In another aspect, the polypeptide having beta-glucosidase activity is an *Aspergillus clavatus* Cel3 beta-glucosidase of the mature polypeptide of SEQ ID NO: 182. In another aspect, the *Aspergillus clavatus* Cel3 beta-glucosidase is encoded by the mature polypeptide coding sequence of SEQ ID NO: 181.

In another aspect, the polypeptide having beta-glucosidase activity is a *Thielavia terrestris* NRRL 8126 Cel3 beta-glucosidase of the mature polypeptide of SEQ ID NO: 184. In another aspect, the *Thielavia terrestris* NRRL 8126 Cel3 beta-glucosidase is encoded by the mature polypeptide coding sequence of SEQ ID NO: 183.

In another aspect, the polypeptide having beta-glucosidase activity is a *Penicillium oxalicum* Cel3 beta-glucosidase of the mature polypeptide of SEQ ID NO: 186. In another aspect, the *Penicillium oxalicum* Cel3 beta-glucosidase is encoded by the mature polypeptide coding sequence of SEQ ID NO: 185.

In another aspect, the polypeptide having beta-glucosidase activity is a *Penicillium oxalicum* Cel3 beta-glucosidase of the mature polypeptide of SEQ ID NO: 188. In another aspect, the *Penicillium oxalicum* Cel3 beta-glucosidase is encoded by the mature polypeptide coding sequence of SEQ ID NO: 187.

In another aspect, the polypeptide having beta-glucosidase activity is a *Talaromyces emersonii* Cel3 beta-glucosidase of the mature polypeptide of SEQ ID NO: 190. In another aspect, the *Talaromyces emersonii* Cel3 beta-glucosidase is encoded by the mature polypeptide coding sequence of SEQ ID NO: 189.

In one aspect, the enzyme composition further comprises or even further comprises a polypeptide having cellulolytic enhancing activity selected from the group consisting of:

(I) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 34; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 33, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 33, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 33;

(II) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature

polypeptide of SEQ ID NO: 36; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 35, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 35, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 35;

(III) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 38; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 37, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 37, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 37;

(IV) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 40; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 39, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 39, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 39;

(V) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature

polypeptide of SEQ ID NO: 42; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 41, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 41, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 41;

(VI) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 44; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 43, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 43, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 43;

(VII) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 192; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 191, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 191, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 191; and

(VIII) a combination of any of I, II, III, IV, V, VI, and VII.

In a preferred aspect, the polypeptide having cellulolytic enhancing activity is a *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity of the

mature polypeptide of SEQ ID NO: 34. In another aspect, the *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity is encoded by the mature polypeptide coding sequence of SEQ ID NO: 33.

In another aspect, the polypeptide having cellulolytic enhancing activity is a *Thielavia terrestris* GH61E polypeptide having cellulolytic enhancing activity of the mature polypeptide of SEQ ID NO: 36. In another aspect, the *Thielavia terrestris* GH61E polypeptide having cellulolytic enhancing activity is encoded by the mature polypeptide coding sequence of SEQ ID NO: 35.

In another aspect, the polypeptide having cellulolytic enhancing activity is a *Aspergillus fumigatus* GH61B polypeptide having cellulolytic enhancing activity of the mature polypeptide of SEQ ID NO: 38. In another aspect, the *Aspergillus fumigatus* GH61B polypeptide having cellulolytic enhancing activity is encoded by the mature polypeptide coding sequence of SEQ ID NO: 37.

In another aspect, the polypeptide having cellulolytic enhancing activity is a *Penicillium pinophilum* GH61 polypeptide having cellulolytic enhancing activity of the mature polypeptide of SEQ ID NO: 40. In another aspect, the *Penicillium pinophilum* GH61A polypeptide having cellulolytic enhancing activity is encoded by the mature polypeptide coding sequence of SEQ ID NO: 39.

In another aspect, the polypeptide having cellulolytic enhancing activity is a *Penicillium* sp. GH61A polypeptide having cellulolytic enhancing activity of the mature polypeptide of SEQ ID NO: 42. In another aspect, the *Penicillium* sp. GH61 polypeptide having cellulolytic enhancing activity is encoded by the mature polypeptide coding sequence of SEQ ID NO: 41.

In another aspect, the polypeptide having cellulolytic enhancing activity is a *Thielavia terrestris* GH61N polypeptide having cellulolytic enhancing activity of the mature polypeptide of SEQ ID NO: 44. In another aspect, the *Thielavia terrestris* GH61N polypeptide having cellulolytic enhancing activity is encoded by the mature polypeptide coding sequence of SEQ ID NO: 43.

In another aspect, the polypeptide having cellulolytic enhancing activity is a *Thermoascus crustaceus* GH61A polypeptide having cellulolytic enhancing activity of the mature polypeptide of SEQ ID NO: 192. In another aspect, the *Thermoascus crustaceus* GH61A polypeptide having cellulolytic enhancing activity is encoded by the mature polypeptide coding sequence of SEQ ID NO: 191.

In another aspect, the enzyme composition further comprises or even further comprises a polypeptide having xylanase activity. In a preferred aspect, the polypeptide having xylanase activity is a Family 10 polypeptide having xylanase activity. In another aspect, the polypeptide having xylanase activity is a Family 11 polypeptide having xylanase

activity.

In a more preferred aspect, the Family 10 polypeptide having xylanase activity is selected from the group consisting of:

(I) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 46; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 45, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 45, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 45;

(II) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 48; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 47, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 47, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 47;

(III) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 50; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 49, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 49, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a

nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 49;

(IV) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 52; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 51, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 51, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 51;

(V) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 54; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 53, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 53, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 53;

(VI) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 194; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 193, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 193, or (iii) a full-length

complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 193;

(VII) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 196; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 195, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 195, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 195; and

(VIII) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 198; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 197, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 197, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 197.

In one aspect, the Family 10 polypeptide having xylanase activity is an *Aspergillus aculeatus* GH10 xylanase of the mature polypeptide of SEQ ID NO: 46. In another aspect, the *Aspergillus aculeatus* GH10 xylanase is encoded by the mature polypeptide coding sequence of SEQ ID NO: 45.

In another aspect, the Family 10 polypeptide having xylanase activity is an *Aspergillus fumigatus* GH10C xylanase of the mature polypeptide of SEQ ID NO: 48. In

another aspect, the *Aspergillus fumigatus* GH10C xylanase is encoded by the mature polypeptide coding sequence of SEQ ID NO: 47.

In another aspect, the Family 10 polypeptide having xylanase activity is a *Trichophaea saccata* GH10 xylanase of the mature polypeptide of SEQ ID NO: 50. In another aspect, the *Trichophaea saccata* GH10 xylanase is encoded by the mature polypeptide coding sequence of SEQ ID NO: 49.

In another aspect, the Family 10 polypeptide having xylanase activity is a *Penicillium pinophilum* GH10 xylanase of the mature polypeptide of SEQ ID NO: 52. In another aspect, the *Penicillium pinophilum* GH10 xylanase is encoded by the mature polypeptide coding sequence of SEQ ID NO: 51.

In another aspect, the Family 10 polypeptide having xylanase activity is a *Thielavia terrestris* GH10E xylanase of the mature polypeptide of SEQ ID NO: 54. In another aspect, the *Thielavia terrestris* GH10E xylanase is encoded by the mature polypeptide coding sequence of SEQ ID NO: 53.

In another aspect, the Family 10 polypeptide having xylanase activity is a *Talaromyces emersonii* GH10 xylanase of the mature polypeptide of SEQ ID NO: 194. In another aspect, the *Talaromyces emersonii* GH10 xylanase is encoded by the mature polypeptide coding sequence of SEQ ID NO: 193.

In another aspect, the Family 10 polypeptide having xylanase activity is a *Penicillium* sp. GH10 xylanase of the mature polypeptide of SEQ ID NO: 196. In another aspect, the *Penicillium* sp. GH10 xylanase is encoded by the mature polypeptide coding sequence of SEQ ID NO: 195.

In another aspect, the Family 10 polypeptide having xylanase activity is a *Meripilus giganteus* GH10 xylanase of the mature polypeptide of SEQ ID NO: 198. In another aspect, the *Meripilus giganteus* GH10 xylanase is encoded by the mature polypeptide coding sequence of SEQ ID NO: 197.

In another aspect, the Family 11 polypeptide having xylanase activity is selected from the group consisting of:

(I) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 56; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with the mature polypeptide coding sequence of SEQ ID NO: 55 or its full-length complementary strand; and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%,

even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 55; and

(II) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 200 or SEQ ID NO: 305; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with the mature polypeptide coding sequence of SEQ ID NO: 199 or SEQ ID NO: 304; or its full-length complementary strand; and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 199 or SEQ ID NO: 304.

In another aspect, the Family 11 polypeptide having xylanase activity is a *Thermobifida fusca* GH11 xylanase of the mature polypeptide of SEQ ID NO: 56. In another aspect, the *Thermobifida fusca* GH11 xylanase is encoded by the mature polypeptide coding sequence of SEQ ID NO: 55.

In another aspect, the Family 11 polypeptide having xylanase activity is a *Dictyoglomus thermophilum* GH11 xylanase of the mature polypeptide of SEQ ID NO: 200 or SEQ ID NO: 305. In another aspect, the *Dictyoglomus thermophilum* GH11 xylanase is encoded by the mature polypeptide coding sequence of SEQ ID NO: 199 or SEQ ID NO: 304.

In another aspect, the enzyme composition further comprises or even further comprises a polypeptide having beta-xylosidase activity. In a preferred aspect, the polypeptide having beta-xylosidase activity is a Family 3 polypeptide having beta-xylosidase activity.

In another aspect, the Family 3 polypeptide having beta-xylosidase activity is selected from the group consisting of:

(I) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 58; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with the mature polypeptide coding sequence of SEQ ID NO: 57 or its full-length complementary strand; and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having

preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 57;

(II) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 60; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with the mature polypeptide coding sequence of SEQ ID NO: 59 or its full-length complementary strand; and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 59;

(III) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 202; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with the mature polypeptide coding sequence of SEQ ID NO: 201 or its full-length complementary strand; and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 201;

(IV) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 204; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with the mature polypeptide coding sequence of SEQ ID NO: 203 or its full-length complementary strand; and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 203; and

(V) (a) a polypeptide comprising an amino acid sequence having preferably at least

80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 206; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with the mature polypeptide coding sequence of SEQ ID NO: 205 or its full-length complementary strand; and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 205.

In another aspect, the Family 3 polypeptide having beta-xylosidase activity is a *Trichoderma reesei* beta-xylosidase of the mature polypeptide of SEQ ID NO: 58. In another most preferred aspect, the *Trichoderma reesei* beta-xylosidase is encoded by the mature polypeptide coding sequence of SEQ ID NO: 57.

In another aspect, the Family 3 polypeptide having beta-xylosidase activity is a *Talaromyces emersonii* beta-xylosidase of the mature polypeptide of SEQ ID NO: 60. In another most preferred aspect, the *Talaromyces emersonii* beta-xylosidase is encoded by the mature polypeptide coding sequence of SEQ ID NO: 59.

In another aspect, the Family 3 polypeptide having beta-xylosidase activity is an *Aspergillus aculeatus* Cel3 beta-xylosidase of the mature polypeptide of SEQ ID NO: 202. In another most preferred aspect, the *Aspergillus aculeatus* Cel3 beta-xylosidase is encoded by the mature polypeptide coding sequence of SEQ ID NO: 201.

In another aspect, the Family 3 polypeptide having beta-xylosidase activity is an *Aspergillus aculeatus* Cel3 beta-xylosidase of the mature polypeptide of SEQ ID NO: 204. In another most preferred aspect, the *Aspergillus aculeatus* Cel3 beta-xylosidase is encoded by the mature polypeptide coding sequence of SEQ ID NO: 203.

In another aspect, the Family 3 polypeptide having beta-xylosidase activity is an *Aspergillus fumigatus* Cel3 beta-xylosidase of the mature polypeptide of SEQ ID NO: 206. In another most preferred aspect, the *Aspergillus fumigatus* Cel3 beta-xylosidase is encoded by the mature polypeptide coding sequence of SEQ ID NO: 205.

One or more components of the enzyme composition may be wild-type proteins, recombinant proteins, or a combination of wild-type proteins and recombinant proteins. For example, one or more components may be native proteins of a cell, which is used as a host cell to express recombinantly one or more (several) other components of the enzyme composition. One or more components of the enzyme composition may be produced as monocomponents, which are then combined to form the enzyme composition. The enzyme composition may be a combination of multicomponent and monocomponent protein

preparations.

An enzyme composition of the present invention may be used as a supplement to another enzyme composition, where the enzyme composition of the present invention is simply added to the other enzyme composition or is added as a replacement of a portion of the other enzyme composition. The other enzyme composition may be a commercial enzyme composition such as a cellulolytic enzyme preparation or a xylan degrading enzyme preparation.

Examples of commercial cellulolytic enzyme preparations include, but are not limited to, CELLIC™ CTec (Novozymes A/S), CELLIC™ CTec2 (Novozymes A/S), CELLUCLAST™ (Novozymes A/S), NOVOZYM™ 188 (Novozymes A/S), CELLUZYME™(Novozymes A/S), CEREFLO™(Novozymes A/S), ULTRAFLO™ (Novozymes A/S), ACCELERASE (Genencor Int.), LAMINEX™ (Genencor Int.), SPEZYME™ CP (Genencor Int.), ROHAMENT™ 7069 W (Röhm GmbH), and FIBREZYME® LDI (Dyadic International, Inc.), FIBREZYME® LBR (Dyadic International, Inc.), and VISCOSTAR® 150L (Dyadic International, Inc.).

Examples of commercial xylan degrading enzyme preparations include, but are not limited to, SHEARZYME™ (Novozymes A/S), CELLIC™ HTec (Novozymes A/S), CELLIC™ HTec2 (Novozymes A/S), VISCOZYME® (Novozymes A/S), ULTRAFLO® (Novozymes A/S), PULPZYME® HC (Novozymes A/S), MULTIFECT® Xylanase (Genencor Int.), ECOPULP® TX-200A (AB Enzymes), HSP 6000 Xylanase (DSM), DEPOL™ 333P (Biocatalysts Limit, Wales, UK), DEPOL™ 740L. (Biocatalysts Limit, Wales, UK), and DEPOL™ 762P (Biocatalysts Limit, Wales, UK).

In one aspect, an enzyme composition of the present invention is simply added to another enzyme composition. The enzyme composition of the present invention is added to the other enzyme composition at a level of preferably at least 1%, more preferably at least 5%, more preferably at least 10%, more preferably at least 25%, more preferably at least 50%, more preferably at least 75%, more preferably at least 100%, more preferably at least 150%, more preferably at least 200%, more preferably at least 250%, most preferably at least 300%, more preferably at least 400%, more preferably at least 500%, even more preferably at least 750%, most preferably at least 1000% of the other enzyme composition.

In another aspect, an enzyme composition of the present invention is added as a replacement of a portion of another enzyme composition. The enzyme composition of the present invention is added as a replacement of the other enzyme composition at a level of preferably at least 1%, more preferably at least 2%, more preferably at least 3%, more preferably at least 5%, more preferably at least 10%, more preferably at least 15%, more preferably at least 20%, more preferably at least 25%, more preferably at least 30%, more preferably at least 40%, most preferably at least 50%, more preferably at least 60%, more preferably at least 70%, even more preferably at least 80%, most preferably at least 90% of

the other enzyme composition.

In another aspect, the enzyme composition may further or even further comprise one or more (several) additional enzyme activities to improve the degradation of the cellulose-containing material. Preferred additional enzymes are hemicellulases (*e.g.*, alpha-D-glucuronidases, alpha-L-arabinofuranosidases, endo-mannanases, beta-mannosidases, alpha-galactosidases, endo-alpha-L-arabinanases, beta-galactosidases), carbohydrate-esterases (*e.g.*, acetyl-xylan esterases, acetyl-mannan esterases, ferulic acid esterases, coumaric acid esterases, glucuronoyl esterases), pectinases, proteases, ligninolytic enzymes (*e.g.*, catalases, laccases, manganese peroxidases, lignin peroxidases, H_2O_2-producing enzymes, oxidoreductases), expansins, swollenins, or mixtures thereof.

Such other endoglucanases include an *Acidothermus cellulolyticus* endoglucanase (WO 91/05039; WO 93/15186; U.S. Patent No. 5,275,944; WO 96/02551; U.S. Patent No. 5,536,655, WO 00/70031, WO 05/093050); *Thermobifida fusca* endoglucanase III (WO 05/093050); and *Thermobifida fusca* endoglucanase V (WO 05/093050); *Trichoderma reesei* endoglucanase I (Penttila *et al.*, 1986, *Gene* 45: 253-263; *Trichoderma reesei* Cel7B endoglucanase I; GENBANK™ accession no. M15665); *Trichoderma reesei* endoglucanase III (Okada *et al.*, 1988, *Appl. Environ. Microbiol.* 64: 555-563; GENBANK™ accession no. AB003694); *Trichoderma reesei* endoglucanase V (Saloheimo *et al.*, 1994, *Molecular Microbiology* 13: 219-228; GENBANK™ accession no. Z33381); *Aspergillus aculeatus* endoglucanase (Ooi *et al.*, 1990, *Nucleic Acids Research* 18: 5884); *Aspergillus kawachii* endoglucanase (Sakamoto *et al.*, 1995, *Current Genetics* 27: 435-439); *Erwinia carotovara* endoglucanase (Saarilahti *et al.*, 1990, *Gene* 90: 9-14); *Fusarium oxysporum* endoglucanase (GENBANK™ accession no. L29381); *Humicola grisea* var. *thermoidea* endoglucanase (GENBANK™ accession no. AB003107); *Melanocarpus albomyces* endoglucanase (GENBANK™ accession no. MAL515703); *Neurospora crassa* endoglucanase (GENBANK™ accession no. XM_324477); *Humicola insolens* endoglucanase V); basidiomycete CBS 495.95 endoglucanase; basidiomycete CBS 494.95 endoglucanase; *Thielavia terrestris* NRRL 8126 CEL6B endoglucanase; *Thielavia terrestris* NRRL 8126 CEL6C endoglucanase; *Thielavia terrestris* NRRL 8126 CEL7C endoglucanase; *Thielavia terrestris* NRRL 8126 CEL7E endoglucanase; *Thielavia terrestris* NRRL 8126 CEL7F endoglucanase; *Cladorrhinum foecundissimum* ATCC 62373 CEL7A endoglucanase; and *Trichoderma reesei* strain No. VTT-D-80133 endoglucanase (GENBANK™ accession no. M15665).

Such other cellobiohydrolases include *Trichoderma reesei* cellobiohydrolase I; *Trichoderma reesei* cellobiohydrolase II; *Humicola insolens* cellobiohydrolase I; and *Chaetomium thermophilum* cellobiohydrolase II.

Such other xylanases include *Aspergillus aculeatus* xylanase (GeneSeqP:AAR63790; WO 94/21785); *Aspergillus fumigatus* xylanases (WO 2006/078256); and *Thielavia terrestris* NRRL 8126 xylanases (WO 2009/079210).

Such other beta-xylosidases include *Trichoderma reesei* beta-xylosidase (UniProtKB/TrEMBL accession number Q92458) and *Neurospora crassa* (SwissProt accession number Q7SOW4).

Such other acetylxylan esterases include *Hypocrea jecorina* acetylxylan esterase (WO 2005/001036); *Neurospora crassa* acetylxylan esterase (UniProt accession number q7s259); *Thielavia terrestris* NRRL 8126 acetylxylan esterase (WO 2009/042846); *Chaetomium globosum* acetylxylan esterase (Uniprot accession number Q2GWX4); *Chaetomium gracile* acetylxylan esterase (GeneSeqP accession number AAB82124); *Phaeosphaeria nodorum* acetylxylan esterase (Uniprot accession number Q0UHJ1); and *Humicola insolens* DSM 1800 acetylxylan esterase (WO 2009/073709).

Such other ferulic acid esterases include *Humicola insolens* DSM 1800 feruloyl esterase (WO 2009/076122); *Neurospora crassa* feruloyl esterase (UniProt accession number Q9HGR3); and *Neosartorya fischeri* feruloyl esterase (UniProt Accession number A1D9T4).

Such other arabinofuranosidases include *Humicola insolens* DSM 1800 arabinofuranosidase (WO 2009/073383) and *Aspergillus niger* arabinofuranosidase (GeneSeqP accession number AAR94170).

Such other alpha-glucuronidases include *Aspergillus clavatus* alpha-glucuronidase (UniProt accession number alcc12); *Trichoderma reesei* alpha-glucuronidase (Uniprot accession number Q99024); *Talaromyces emersonii* alpha-glucuronidase (UniProt accession number Q8X211); *Aspergillus niger* alpha-glucuronidase (Uniprot accession number Q96WX9); *Aspergillus terreus* alpha-glucuronidase (SwissProt accession number Q0CJP9); and *Aspergillus fumigatus* alpha-glucuronidase (SwissProt accession number Q4WW45).

The enzymes used in the present invention may be in any form suitable for use in the processes described herein, such as, for example, a crude fermentation broth with or without cells removed, a dry powder or granulate, a non-dusting granulate, a liquid, a stabilized liquid, or a stabilized protected preparation. Liquid enzyme preparations may, for instance, be stabilized by adding stabilizers such as a sugar, a sugar alcohol or another polyol, and/or lactic acid or another organic acid according to established processes.

The enzyme compositions of the present invention are particularly useful for high-temperature enzymatic hydrolysis of cellulosic material at temperatures above 50°C. High-temperature enzymatic hydrolysis of cellulosic material, *e.g.*, lignocellulose, has a number of advantages over conventional saccharification at 40-50°C. The advantages include reduced

hydrolysis time due to improved activity of thermostable enzyme mixtures at elevated temperatures, decreased risk of contamination, and possibility of using higher biomass loadings due to reduced viscosity, leading to increased sugar and ethanol concentrations.

In one aspect, an enzyme composition of the present invention is employed at a temperature preferably in the range of about 40°C to about 70°C, more preferably about 50°C to about 65°C, even more preferably in the range of about 55°C to about 63°C, and most preferably in the range of about 55°C to about 60°C. In another aspect, an enzyme composition of the present invention is employed at a temperature of at least 50°C. In another aspect, an enzyme composition of the present invention is employed at a temperature of at least 55°C. In another aspect, an enzyme composition of the present invention is employed at a temperature of at least 60°C. In another aspect, an enzyme composition of the present invention is employed at a temperature of at least 65°C. In another aspect, an enzyme composition of the present invention is employed at a temperature of at least 70°C.

In another aspect, an enzyme composition of the present invention reduces the amount of protein required to reach the same degree of cellulose hydrolysis of PCS at 55°C, pH 3.5 to pH 6.0 preferably at least 1.1-fold, more preferably at least 1.25-fold, more preferably at least 1.5-fold, more preferably at least 2-fold, more preferably at least 2.5-fold, more preferably at least 3-fold, more preferably at least 4-fold, more preferably at least 5-fold, more preferably at least 7.5-fold, and most preferably at least 10-fold compared to *Trichoderma reesei*-based enzyme composition SaMe-MF268 at 50°C, pH 5.0.

In another aspect, an enzyme composition of the present invention reduces the amount of protein required to reach the same degree of cellulose hydrolysis of PCS at 60°C, pH 3.5 to pH 6.0 preferably at least 1.1-fold, more preferably at least 1.25-fold, more preferably at least 1.5-fold, more preferably at least 2-fold, more preferably at least 2.5-fold, more preferably at least 3-fold, more preferably at least 4-fold, more preferably at least 5-fold, more preferably at least 7.5-fold, and most preferably at least 10-fold compared to *Trichoderma reesei*-based enzyme composition SaMe-MF268 at 50°C, pH 5.0.

In another aspect, an enzyme composition of the present invention reduces the amount of protein required to reach the same degree of cellulose hydrolysis of PCS at 65°C, pH 3.5 to pH 6.0 preferably at least 1.1-fold, more preferably at least 1.25-fold, more preferably at least 1.5-fold, more preferably at least 2-fold, more preferably at least 2.5-fold, more preferably at least 3-fold, more preferably at least 4-fold, more preferably at least 5-fold, more preferably at least 7.5-fold, and most preferably at least 10-fold compared to *Trichoderma reesei*-based enzyme composition SaMe-MF268 at 50°C, pH 5.0.

In another aspect, an enzyme composition of the present invention reduces the amount of protein required to reach the same degree of cellulose hydrolysis of PCS at 70°C,

pH 3.5 to pH 6.0 preferably at least 1.1-fold, more preferably at least 1.25-fold, more preferably at least 1.5-fold, more preferably at least 2-fold, more preferably at least 2.5-fold, more preferably at least 3-fold, more preferably at least 4-fold, more preferably at least 5-fold, more preferably at least 7.5-fold, and most preferably at least 10-fold compared to *Trichoderma reesei*-based enzyme composition SaMe-MF268 at 50°C, pH 5.0.

In each of the aspects above, the range of pH from pH 3.5 to pH 6.0 is pH 3.5. In each of the aspects above, the range of pH from pH 3.5 to pH 6.0 is pH 4.0. In each of the aspects above, the range of pH from pH 4.0 to pH 6.0 is pH 4.5. In each of the aspects above, the range of pH from pH 4.0 to pH 6.0 is pH 5.0. In each of the aspects above, the range of pH from pH 4.0 to pH 6.0 is pH 5.5. In each of the aspects above, the range of pH from pH 4.0 to pH 6.0 is pH 6.0.

In each of the aspects above, PCS as a substrate can be substituted with any other cellulose-containing material, *e.g.*, corn fiber, corn cob, orange peel, rice straw, wheat straw, switch grass, miscanthus, bagasse, softwood, or hardwood . The other cellulose-containing material may be pretreated using any pretreatment such as those pretreatments described herein.

In another aspect, cellobiohydrolase I constitutes preferably 10% to 90%, more preferably 20% to 65%, and most preferably 35% to 45% of a composition of the present invention on a protein basis. In another aspect, cellobiohydrolase II constitutes preferably 5% to 60%, more preferably 10% to 40%, and most preferably 20% to 30% of a composition of the present invention on a protein basis. In another aspect, endoglucanase I constitutes preferably 1% to 30%, more preferably 2.5% to 20%, and most preferably 5% to 10% of a composition of the present invention on a protein basis. In another aspect, endoglucanase II constitutes preferably 1% to 30%, more preferably 2.5% to 20%, and most preferably 5% to 10% of a composition of the present invention on a protein basis. In another aspect, beta-glucosidase constitutes preferably 1% to 30%, more preferably 2.5% to 20%, and most preferably 5% to 10% of a composition of the present invention on a protein basis. In another aspect, GH61 protein constitutes preferably 1% to 50%, more preferably 10% to 40%, and most preferably 15% to 25% of a composition of the present invention on a protein basis. In another aspect, xylanase constitutes preferably 1% to 30%, more preferably 2.5% to 20%, and most preferably 5% to 10% of a composition of the present invention on a protein basis. In another aspect, beta-xylosidase constitutes preferably 1% to 20%, more preferably 1% to 15%, and most preferably 1% to 5% of a composition of the present invention on a protein basis.

In one aspect, an enzyme composition of the present invention comprises at least two components. In another aspect, an enzyme composition of the present invention comprises at least three components. In another aspect, an enzyme composition of the

present invention comprises at least four components. In another aspect, an enzyme composition of the present invention comprises at least five components. In another aspect, an enzyme composition of the present invention comprises at least six components. In another aspect, an enzyme composition of the present invention comprises at least seven components. In another aspect, an enzyme composition of the present invention comprises at least eight components. In another aspect, an enzyme composition of the present invention comprises at least nine components. In another aspect, an enzyme composition of the present invention comprises at least ten components. In another aspect, an enzyme composition of the present invention comprises at least eleven components. In another aspect, an enzyme composition of the present invention comprises at least twelve components.

In one aspect, the enzyme compositions comprise *Aspergillus fumigatus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Penicillium* sp GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Aspergillus fumigatus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Penicillium* sp GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Aspergillus fumigatus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Penicillium* sp GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Aspergillus fumigatus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Penicillium* sp GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Penicillium emersonii* Cel 7 CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Penicillium* sp GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Penicillium emersonii* Cel 7 CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Penicillium* sp GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Penicillium emersonii* Cel 7 CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Penicillium* sp GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Penicillium emersonii* Cel 7 CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Penicillium* sp GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Thermoascus aurantiacus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Penicillium* sp GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Thermoascus aurantiacus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Penicillium* sp GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Thermoascus aurantiacus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Penicillium* sp GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Thermoascus aurantiacus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Penicillium* sp GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Aspergillus fumigatus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Penicillium* sp

GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Aspergillus fumigatus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Penicillium* sp GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

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In another aspect, the enzyme compositions comprise *Penicillium emersonii* Cel 7 CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Penicillium* sp GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

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In another aspect, the enzyme compositions comprise *Penicillium emersonii* Cel 7 CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Penicillium* sp GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-

glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Thermoascus aurantiacus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Penicillium* sp GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Thermoascus aurantiacus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Penicillium* sp GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Thermoascus aurantiacus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Penicillium* sp GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Thermoascus aurantiacus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Penicillium* sp GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Aspergillus fumigatus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Aspergillus fumigatus* GH61B polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Aspergillus fumigatus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Aspergillus fumigatus* GH61B polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

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In another aspect, the enzyme compositions comprise *Penicillium emersonii* Cel 7 CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Aspergillus fumigatus* GH61B polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Thermoascus aurantiacus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Aspergillus fumigatus* GH61B polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Thermoascus aurantiacus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Aspergillus fumigatus* GH61B polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Thermoascus aurantiacus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII,

Aspergillus fumigatus GH61B GH61 polypeptides having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Thermoascus aurantiacus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Aspergillus fumigatus* GH61B polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise the enzyme compositions comprise *Aspergillus fumigatus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Aspergillus fumigatus* GH61B polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Aspergillus fumigatus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Aspergillus fumigatus* GH61B polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Aspergillus fumigatus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Aspergillus fumigatus* GH61B polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Aspergillus fumigatus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Aspergillus fumigatus* GH61B polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Penicillium emersonii* Cel 7 CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Aspergillus fumigatus* GH61B polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Penicillium emersonii* Cel 7 CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Aspergillus fumigatus* GH61B polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus*

GH3 beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Penicillium emersonii* Cel 7 CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Aspergillus fumigatus* GH61B polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Penicillium emersonii* Cel 7 CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Aspergillus fumigatus* GH61B polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Thermoascus aurantiacus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Aspergillus fumigatus* GH61B polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Thermoascus aurantiacus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Aspergillus fumigatus* GH61B polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Thermoascus aurantiacus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Aspergillus fumigatus* GH61B polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Thermoascus aurantiacus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Aspergillus fumigatus* GH61B polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Aspergillus fumigatus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Aspergillus fumigatus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Aspergillus fumigatus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Aspergillus fumigatus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Penicillium emersonii* Cel 7 CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Penicillium emersonii* Cel 7 CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Penicillium emersonii* Cel 7 CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Penicillium emersonii* Cel 7 CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Thermoascus aurantiacus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII,

Thermoascus aurantiacus GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Thermoascus aurantiacus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Thermoascus aurantiacus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Thermoascus aurantiacus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Thermoascus aurantiacus* Cel5A EGII, *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Aspergillus fumigatus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Aspergillus fumigatus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Aspergillus fumigatus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Aspergillus fumigatus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus*

GH3 beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Penicillium emersonii* Cel 7 CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Penicillium emersonii* Cel 7 CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Penicillium emersonii* Cel 7 CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Penicillium emersonii* Cel 7 CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Thermoascus aurantiacus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Thermoascus aurantiacus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Aspergillus fumigatus* GH10C xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Thermoascus aurantiacus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus fumigatus* GH3A beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

In another aspect, the enzyme compositions comprise *Thermoascus aurantiacus* Cel 7A CBHI, *Aspergillus fumigatus* Cel 6A CBHII, *Aspergillus fumigatus* Cel5A EGII, *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, *Aspergillus aculeatus* GH3 beta-glucosidase, *Trichophaea saccata* GH10 xylanase, and *Talaromyces emersonii* GH3 beta-xylosidase.

Enzyme Composition Components and Polynucleotides Thereof

In first aspect, the isolated polypeptides having cellobiohydrolase I activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 2 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have cellobiohydrolase I activity (hereinafter "homologous polypeptides"). In a preferred aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 2.

In one aspect, a polypeptide having cellobiohydrolase I activity comprises or consists of the amino acid sequence of SEQ ID NO: 2 or an allelic variant thereof; or a fragment thereof having cellobiohydrolase I activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 2. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 2. In another aspect, the polypeptide comprises or consists of amino acids 19 to 530 of SEQ ID NO: 2, or an allelic variant thereof; or a fragment thereof having cellobiohydrolase I activity. In another aspect, the polypeptide comprises or consists of amino acids 19 to 530 of SEQ ID NO: 2.

In another first aspect, the isolated polypeptides having cellobiohydrolase I activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 4 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have cellobiohydrolase I activity (hereinafter "homologous polypeptides"). In one aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 4.

In one aspect, a polypeptide having cellobiohydrolase I activity comprises or consists of the amino acid sequence of SEQ ID NO: 4 or an allelic variant thereof; or a fragment

thereof having cellobiohydrolase I activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 4. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 4. In another aspect, the polypeptide comprises or consists of amino acids 21 to 450 of SEQ ID NO: 4, or an allelic variant thereof; or a fragment thereof having cellobiohydrolase I activity. In another aspect, the polypeptide comprises or consists of amino acids 21 to 450 of SEQ ID NO: 4.

In another first aspect, the isolated polypeptides having cellobiohydrolase I activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 6 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have cellobiohydrolase I activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 6.

In one aspect, a polypeptide having cellobiohydrolase I activity comprises or consists of the amino acid sequence of SEQ ID NO: 6 or an allelic variant thereof; or a fragment thereof having cellobiohydrolase I activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 6. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 6. In another aspect, the polypeptide comprises or consists of amino acids 27 to 532 of SEQ ID NO: 6, or an allelic variant thereof; or a fragment thereof having cellobiohydrolase I activity. In another aspect, the polypeptide comprises or consists of amino acids 27 to 532 of SEQ ID NO: 6.

In another first aspect, the isolated polypeptides having cellobiohydrolase I activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 8 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have cellobiohydrolase I activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 8.

In one aspect, a polypeptide having cellobiohydrolase I activity comprises or consists of the amino acid sequence of SEQ ID NO: 8 or an allelic variant thereof; or a fragment thereof having cellobiohydrolase I activity. In another aspect, the polypeptide comprises or

consists of the amino acid sequence of SEQ ID NO: 8. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 8. In another aspect, the polypeptide comprises or consists of amino acids 18 to 457 of SEQ ID NO: 8, or an allelic variant thereof; or a fragment thereof having cellobiohydrolase I activity. In another aspect, the polypeptide comprises or consists of amino acids 18 to 457 of SEQ ID NO: 8.

In another first aspect, the isolated polypeptides having cellobiohydrolase I activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 158 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have cellobiohydrolase I activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 158.

In one aspect, a polypeptide having cellobiohydrolase I activity comprises or consists of or consists of the amino acid sequence of SEQ ID NO: 158 or an allelic variant thereof; or a fragment thereof having cellobiohydrolase I activity. In another aspect, the polypeptide comprises or consists of or consists of the amino acid sequence of SEQ ID NO: 158. In another aspect, the polypeptide comprises or consists of or consists of the mature polypeptide of SEQ ID NO: 158. In another aspect, the polypeptide comprises or consists of or consists of amino acids 19 to 455 of SEQ ID NO: 158, or an allelic variant thereof; or a fragment thereof having cellobiohydrolase I activity. In another aspect, the polypeptide comprises or consists of or consists of amino acids 19 to 455 of SEQ ID NO: 158.

In another first aspect, the isolated polypeptides having cellobiohydrolase I activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 160 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have cellobiohydrolase I activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 160.

In one aspect, a polypeptide having cellobiohydrolase I activity comprises or consists of the amino acid sequence of SEQ ID NO: 160 or an allelic variant thereof; or a fragment thereof having cellobiohydrolase I activity. In another aspect, the polypeptide comprises or

consists of the amino acid sequence of SEQ ID NO: 160. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 160. In another aspect, the polypeptide comprises or consists of amino acids 26 to 529 of SEQ ID NO: 160, or an allelic variant thereof; or a fragment thereof having cellobiohydrolase I activity. In another aspect, the polypeptide comprises or consists of amino acids 26 to 529 of SEQ ID NO: 160.

In another first aspect, the isolated polypeptides having cellobiohydrolase I activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 162 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have cellobiohydrolase I activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 162.

In one aspect, a polypeptide having cellobiohydrolase I activity comprises or consists of the amino acid sequence of SEQ ID NO: 162 or an allelic variant thereof; or a fragment thereof having cellobiohydrolase I activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 162. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 162. In another aspect, the polypeptide comprises or consists of amino acids 24 to 541 of SEQ ID NO: 162, or an allelic variant thereof; or a fragment thereof having cellobiohydrolase I activity. In another aspect, the polypeptide comprises or consists of amino acids 24 to 541 of SEQ ID NO: 162.

In another first aspect, the isolated polypeptides having cellobiohydrolase I activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 164 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have cellobiohydrolase I activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 164.

In one aspect, a polypeptide having cellobiohydrolase I activity comprises or consists of the amino acid sequence of SEQ ID NO: 164 or an allelic variant thereof; or a fragment thereof having cellobiohydrolase I activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 164. In another aspect, the polypeptide

comprises or consists of the mature polypeptide of SEQ ID NO: 164. In another aspect, the polypeptide comprises or consists of amino acids 27 to 535 of SEQ ID NO: 164, or an allelic variant thereof; or a fragment thereof having cellobiohydrolase I activity. In another aspect, the polypeptide comprises or consists of amino acids 27 to 535 of SEQ ID NO: 164.

In another first aspect, the isolated polypeptides having cellobiohydrolase I activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 166 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have cellobiohydrolase I activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 166.

In one aspect, a polypeptide having cellobiohydrolase I activity comprises or consists of the amino acid sequence of SEQ ID NO: 166 or an allelic variant thereof; or a fragment thereof having cellobiohydrolase I activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 166. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 166. In another aspect, the polypeptide comprises or consists of amino acids 24 to 526 of SEQ ID NO: 166, or an allelic variant thereof; or a fragment thereof having cellobiohydrolase I activity. In another aspect, the polypeptide comprises or consists of amino acids 24 to 526 of SEQ ID NO: 166.

In another first aspect, the isolated polypeptides having cellobiohydrolase II activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 10 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have cellobiohydrolase II activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 10.

In one aspect, a polypeptide having cellobiohydrolase II activity comprises or consists of the amino acid sequence of SEQ ID NO: 10 or an allelic variant thereof; or a fragment thereof having cellobiohydrolase II activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 10. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 10. In another

aspect, the polypeptide comprises or consists of amino acids 18 to 482 of SEQ ID NO: 10, or an allelic variant thereof; or a fragment thereof having cellobiohydrolase II activity. In another aspect, the polypeptide comprises or consists of amino acids 18 to 482 of SEQ ID NO: 10.

In another first aspect, the isolated polypeptides having cellobiohydrolase II activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 12 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have cellobiohydrolase II activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 12.

In one aspect, a polypeptide having cellobiohydrolase II activity comprises or consists of the amino acid sequence of SEQ ID NO: 12 or an allelic variant thereof; or a fragment thereof having cellobiohydrolase II activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 12. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 12. In another aspect, the polypeptide comprises or consists of amino acids 18 to 482 of SEQ ID NO: 12, or an allelic variant thereof; or a fragment thereof having cellobiohydrolase II activity. In another aspect, the polypeptide comprises or consists of amino acids 18 to 482 of SEQ ID NO: 12.

In another first aspect, the isolated polypeptides having cellobiohydrolase II activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 14 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have cellobiohydrolase II activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 14.

In one aspect, a polypeptide having cellobiohydrolase II activity comprises or consists of the amino acid sequence of SEQ ID NO: 14 or an allelic variant thereof; or a fragment thereof having cellobiohydrolase II activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 14. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 14. In another aspect, the polypeptide comprises or consists of amino acids 18 to 481 of SEQ ID NO: 14, or

an allelic variant thereof; or a fragment thereof having cellobiohydrolase II activity. In another aspect, the polypeptide comprises or consists of amino acids 18 to 481 of SEQ ID NO: 14.

In another first aspect, the isolated polypeptides having cellobiohydrolase II activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 16 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have cellobiohydrolase II activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 16.

In one aspect, a polypeptide having cellobiohydrolase II activity comprises or consists of the amino acid sequence of SEQ ID NO: 16 or an allelic variant thereof; or a fragment thereof having cellobiohydrolase II activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 16. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 16. In another aspect, the polypeptide comprises or consists of amino acids 17 to 447 of SEQ ID NO: 16, or an allelic variant thereof; or a fragment thereof having cellobiohydrolase II activity. In another aspect, the polypeptide comprises or consists of amino acids 17 to 447 of SEQ ID NO: 16.

In another first aspect, the isolated polypeptides having cellobiohydrolase II activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 18 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have cellobiohydrolase II activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 18.

In one aspect, a polypeptide having cellobiohydrolase II activity comprises or consists of the amino acid sequence of SEQ ID NO: 18 or an allelic variant thereof; or a fragment thereof having cellobiohydrolase II activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 18. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 18. In another aspect, the polypeptide comprises or consists of amino acids 20 to 454 of SEQ ID NO: 18, or an allelic variant thereof; or a fragment thereof having cellobiohydrolase II activity. In another

aspect, the polypeptide comprises or consists of amino acids 20 to 454 of SEQ ID NO: 18.

In another first aspect, the isolated polypeptides having cellobiohydrolase II activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 168 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have cellobiohydrolase II activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 168.

In one aspect, a polypeptide having cellobiohydrolase II activity comprises or consists of the amino acid sequence of SEQ ID NO: 168 or an allelic variant thereof; or a fragment thereof having cellobiohydrolase II activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 168. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 168. In another aspect, the polypeptide comprises or consists of amino acids 19 to 469 of SEQ ID NO: 168, or an allelic variant thereof; or a fragment thereof having cellobiohydrolase II activity. In another aspect, the polypeptide comprises or consists of amino acids 19 to 469 of SEQ ID NO: 168.

In another first aspect, the isolated polypeptides having cellobiohydrolase II activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 170 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have cellobiohydrolase II activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 170.

In one aspect, a polypeptide having cellobiohydrolase II activity comprises or consists of the amino acid sequence of SEQ ID NO: 170 or an allelic variant thereof; or a fragment thereof having cellobiohydrolase II activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 170. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 170. In another aspect, the polypeptide comprises or consists of amino acids 20 to 459 of SEQ ID NO: 170, or an allelic variant thereof; or a fragment thereof having cellobiohydrolase II

activity. In another aspect, the polypeptide comprises or consists of amino acids 20 to 459 of SEQ ID NO: 170.

In another first aspect, the isolated polypeptides having cellobiohydrolase II activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 172 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have cellobiohydrolase II activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 172.

In one aspect, a polypeptide having cellobiohydrolase II activity comprises or consists of the amino acid sequence of SEQ ID NO: 172 or an allelic variant thereof; or a fragment thereof having cellobiohydrolase II activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 172. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 172. In another aspect, the polypeptide comprises or consists of amino acids 20 to 457 of SEQ ID NO: 172, or an allelic variant thereof; or a fragment thereof having cellobiohydrolase II activity. In another aspect, the polypeptide comprises or consists of amino acids 20 to 457 of SEQ ID NO: 172.

In another first aspect, the isolated polypeptides having endoglucanase I activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 20 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have endoglucanase I activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 20.

In one aspect, a polypeptide having endoglucanase I activity comprises or consists of the amino acid sequence of SEQ ID NO: 20 or an allelic variant thereof; or a fragment thereof having endoglucanase I activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 20. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 20. In another aspect, the polypeptide comprises or consists of amino acids 22 to 471 of SEQ ID NO: 20, or an allelic

variant thereof; or a fragment thereof having endoglucanase I activity. In another aspect, the polypeptide comprises or consists of amino acids 22 to 471 of SEQ ID NO: 20.

In another first aspect, the isolated polypeptides having endoglucanase II activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 22 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have endoglucanase II activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 22.

In one aspect, a polypeptide having endoglucanase II activity comprises or consists of the amino acid sequence of SEQ ID NO: 22 or an allelic variant thereof; or a fragment thereof having endoglucanase II activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 22. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 22. In another aspect, the polypeptide comprises or consists of amino acids 22 to 418 of SEQ ID NO: 22, or an allelic variant thereof; or a fragment thereof having endoglucanase II activity. In another aspect, the polypeptide comprises or consists of amino acids 22 to 418 of SEQ ID NO: 22.

In another first aspect, the isolated polypeptides having endoglucanase II activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 24 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have endoglucanase II activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 24.

In one aspect, a polypeptide having endoglucanase II activity comprises or consists of the amino acid sequence of SEQ ID NO: 24 or an allelic variant thereof; or a fragment thereof having endoglucanase II activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 24. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 24. In another aspect, the polypeptide comprises or consists of amino acids 17 to 389 of SEQ ID NO: 24, or an allelic variant thereof; or a fragment thereof having endoglucanase II activity. In another aspect, the

polypeptide comprises or consists of amino acids 17 to 389 of SEQ ID NO: 24.

In another first aspect, the isolated polypeptides having endoglucanase II activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 26 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have endoglucanase II activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 26.

In another first aspect, the isolated polypeptides having endoglucanase II activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 174 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have endoglucanase II activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 174.

In one aspect, a polypeptide having endoglucanase II activity comprises or consists of the amino acid sequence of SEQ ID NO: 174 or an allelic variant thereof; or a fragment thereof having endoglucanase II activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 174. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 174. In another aspect, the polypeptide comprises or consists of amino acids 19 to 329 of SEQ ID NO: 174, or an allelic variant thereof; or a fragment thereof having endoglucanase II activity. In another aspect, the polypeptide comprises or consists of amino acids 19 to 329 of SEQ ID NO: 174.

In another first aspect, the isolated polypeptides having endoglucanase II activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 176 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have endoglucanase II activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids,

most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 176.

In one aspect, a polypeptide having endoglucanase II activity comprises or consists of the amino acid sequence of SEQ ID NO: 176 or an allelic variant thereof; or a fragment thereof having endoglucanase II activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 176. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 176. In another aspect, the polypeptide comprises or consists of amino acids 17 to 412 of SEQ ID NO: 176, or an allelic variant thereof; or a fragment thereof having endoglucanase II activity. In another aspect, the polypeptide comprises or consists of amino acids 17 to 412 of SEQ ID NO: 176.

In one aspect, a polypeptide having endoglucanase II activity comprises or consists of the amino acid sequence of SEQ ID NO: 26 or an allelic variant thereof; or a fragment thereof having endoglucanase II activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 26. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 26. In another aspect, the polypeptide comprises or consists of amino acids 31 to 335 of SEQ ID NO: 26, or an allelic variant thereof; or a fragment thereof having endoglucanase II activity. In another aspect, the polypeptide comprises or consists of amino acids 31 to 335 of SEQ ID NO: 26.

In another first aspect, the isolated polypeptides having beta-glucosidase activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 28 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have beta-glucosidase activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 28.

In one aspect, a polypeptide having beta-glucosidase activity comprises or consists of the amino acid sequence of SEQ ID NO: 28 or an allelic variant thereof; or a fragment thereof having beta-glucosidase activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 28. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 28. In another aspect, the polypeptide comprises or consists of amino acids 20 to 863 of SEQ ID NO: 28, or an allelic variant thereof; or a fragment thereof having beta-glucosidase activity. In another aspect, the polypeptide comprises or consists of amino acids 20 to 863 of SEQ ID NO: 28.

In another first aspect, the isolated polypeptides having beta-glucosidase activity

comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 30 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have beta-glucosidase activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 30.

In one aspect, a polypeptide having beta-glucosidase activity comprises or consists of the amino acid sequence of SEQ ID NO: 30 or an allelic variant thereof; or a fragment thereof having beta-glucosidase activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 30. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 30. In another aspect, the polypeptide comprises or consists of amino acids 37 to 878 of SEQ ID NO: 30, or an allelic variant thereof; or a fragment thereof having beta-glucosidase activity. In another aspect, the polypeptide comprises or consists of amino acids 37 to 878 of SEQ ID NO: 30.

In another first aspect, the isolated polypeptides having beta-glucosidase activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 32 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have beta-glucosidase activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 32.

In one aspect, a polypeptide having beta-glucosidase activity comprises or consists of the amino acid sequence of SEQ ID NO: 32 or an allelic variant thereof; or a fragment thereof having beta-glucosidase activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 32. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 32. In another aspect, the polypeptide comprises or consists of amino acids 20 to 860 of SEQ ID NO: 32, or an allelic variant thereof; or a fragment thereof having beta-glucosidase activity. In another aspect, the polypeptide comprises or consists of amino acids 20 to 860 of SEQ ID NO: 32.

In another first aspect, the isolated polypeptides having beta-glucosidase activity comprise amino acid sequences having a degree of identity to the mature polypeptide of

SEQ ID NO: 178 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have beta-glucosidase activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 178.

In one aspect, a polypeptide having beta-glucosidase activity comprises or consists of the amino acid sequence of SEQ ID NO: 178 or an allelic variant thereof; or a fragment thereof having beta-glucosidase activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 178. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 178. In another aspect, the polypeptide comprises or consists of amino acids 20 to 680 of SEQ ID NO: 178, or an allelic variant thereof; or a fragment thereof having beta-glucosidase activity. In another aspect, the polypeptide comprises or consists of amino acids 20 to 680 of SEQ ID NO: 178.

In another first aspect, the isolated polypeptides having beta-glucosidase activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 180 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have beta-glucosidase activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 180.

In one aspect, a polypeptide having beta-glucosidase activity comprises or consists of the amino acid sequence of SEQ ID NO: 180 or an allelic variant thereof; or a fragment thereof having beta-glucosidase activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 180. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 180. In another aspect, the polypeptide comprises or consists of amino acids 20 to 860 of SEQ ID NO: 180, or an allelic variant thereof; or a fragment thereof having beta-glucosidase activity. In another aspect, the polypeptide comprises or consists of amino acids 20 to 860 of SEQ ID NO: 180.

In another first aspect, the isolated polypeptides having beta-glucosidase activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 182 of preferably at least 80%, more preferably at least 85%, even more

preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have beta-glucosidase activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 182.

In one aspect, a polypeptide having beta-glucosidase activity comprises or consists of the amino acid sequence of SEQ ID NO: 182 or an allelic variant thereof; or a fragment thereof having beta-glucosidase activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 182. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 182. In another aspect, the polypeptide comprises or consists of amino acids 19 to 860 of SEQ ID NO: 182, or an allelic variant thereof; or a fragment thereof having beta-glucosidase activity. In another aspect, the polypeptide comprises or consists of amino acids 19 to 860 of SEQ ID NO: 182.

In another first aspect, the isolated polypeptides having beta-glucosidase activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 184 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have beta-glucosidase activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 184.

In one aspect, a polypeptide having beta-glucosidase activity comprises or consists of the amino acid sequence of SEQ ID NO: 184 or an allelic variant thereof; or a fragment thereof having beta-glucosidase activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 184. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 184. In another aspect, the polypeptide comprises or consists of amino acids 19 to 872 of SEQ ID NO: 184, or an allelic variant thereof; or a fragment thereof having beta-glucosidase activity. In another aspect, the polypeptide comprises or consists of amino acids 19 to 872 of SEQ ID NO: 184.

In another first aspect, the isolated polypeptides having beta-glucosidase activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 186 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least

96%, at least 97%, at least 98%, or at least 99%, which have beta-glucosidase activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 186.

In one aspect, a polypeptide having beta-glucosidase activity comprises or consists of the amino acid sequence of SEQ ID NO: 186 or an allelic variant thereof; or a fragment thereof having beta-glucosidase activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 186. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 186. In another aspect, the polypeptide comprises or consists of amino acids 22 to 883 of SEQ ID NO: 186, or an allelic variant thereof; or a fragment thereof having beta-glucosidase activity. In another aspect, the polypeptide comprises or consists of amino acids 22 to 883 of SEQ ID NO: 186.

In another first aspect, the isolated polypeptides having beta-glucosidase activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 188 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have beta-glucosidase activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 188.

In one aspect, a polypeptide having beta-glucosidase activity comprises or consists of the amino acid sequence of SEQ ID NO: 188 or an allelic variant thereof; or a fragment thereof having beta-glucosidase activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 188. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 188. In another aspect, the polypeptide comprises or consists of amino acids 22 to 861 of SEQ ID NO: 188, or an allelic variant thereof; or a fragment thereof having beta-glucosidase activity. In another aspect, the polypeptide comprises or consists of amino acids 22 to 861 of SEQ ID NO: 188.

In another first aspect, the isolated polypeptides having beta-glucosidase activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 190 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have beta-glucosidase activity

(hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 190.

In one aspect, a polypeptide having beta-glucosidase activity comprises or consists of the amino acid sequence of SEQ ID NO: 190 or an allelic variant thereof; or a fragment thereof having beta-glucosidase activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 190. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 190. In another aspect, the polypeptide comprises or consists of amino acids 22 to 861 of SEQ ID NO: 190, or an allelic variant thereof; or a fragment thereof having beta-glucosidase activity. In another aspect, the polypeptide comprises or consists of amino acids 22 to 861 of SEQ ID NO: 190.

In another first aspect, the isolated GH61 polypeptides having cellulolytic enhancing activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 34 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have cellulolytic enhancing activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 34.

In one aspect, a polypeptide having cellulolytic enhancing activity comprises or consists of the amino acid sequence of SEQ ID NO: 34 or an allelic variant thereof; or a fragment thereof having cellulolytic enhancing activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 34. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 34. In another aspect, the polypeptide comprises or consists of amino acids 23 to 250 of SEQ ID NO: 34, or an allelic variant thereof; or a fragment thereof having cellulolytic enhancing activity. In another aspect, the polypeptide comprises or consists of amino acids 23 to 250 of SEQ ID NO: 34.

In another first aspect, the isolated GH61 polypeptides having cellulolytic enhancing activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 36 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have cellulolytic enhancing

activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 36.

In one aspect, a polypeptide having cellulolytic enhancing activity comprises or consists of the amino acid sequence of SEQ ID NO: 36 or an allelic variant thereof; or a fragment thereof having cellulolytic enhancing activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 36. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 36. In another aspect, the polypeptide comprises or consists of amino acids 20 to 258 of SEQ ID NO: 36, or an allelic variant thereof; or a fragment thereof having cellulolytic enhancing activity. In another aspect, the polypeptide comprises or consists of amino acids 20 to 258 of SEQ ID NO: 36.

In another first aspect, the isolated GH61 polypeptides having cellulolytic enhancing activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 38 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have cellulolytic enhancing activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 38.

In one aspect, a polypeptide having cellulolytic enhancing activity comprises or consists of the amino acid sequence of SEQ ID NO: 38 or an allelic variant thereof; or a fragment thereof having cellulolytic enhancing activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 38. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 38. In another aspect, the polypeptide comprises or consists of amino acids 22 to 250 of SEQ ID NO: 38, or an allelic variant thereof; or a fragment thereof having cellulolytic enhancing activity. In another aspect, the polypeptide comprises or consists of amino acids 22 to 250 of SEQ ID NO: 38.

In another first aspect, the isolated GH61 polypeptides having cellulolytic enhancing activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 40 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at

least 96%, at least 97%, at least 98%, or at least 99%, which have cellulolytic enhancing activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 40.

In one aspect, a polypeptide having cellulolytic enhancing activity comprises or consists of the amino acid sequence of SEQ ID NO: 40 or an allelic variant thereof; or a fragment thereof having cellulolytic enhancing activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 40. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 40. In another aspect, the polypeptide comprises or consists of amino acids 22 to 322 of SEQ ID NO: 40, or an allelic variant thereof; or a fragment thereof having cellulolytic enhancing activity. In another aspect, the polypeptide comprises or consists of amino acids 22 to 322 of SEQ ID NO: 40.

In another first aspect, the isolated GH61 polypeptides having cellulolytic enhancing activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 42 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have cellulolytic enhancing activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 42.

In one aspect, a polypeptide having cellulolytic enhancing activity comprises or consists of the amino acid sequence of SEQ ID NO: 42 or an allelic variant thereof; or a fragment thereof having cellulolytic enhancing activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 42. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 42. In another aspect, the polypeptide comprises or consists of amino acids 26 to 253 of SEQ ID NO: 42, or an allelic variant thereof; or a fragment thereof having cellulolytic enhancing activity. In another aspect, the polypeptide comprises or consists of amino acids 26 to 253 of SEQ ID NO: 42.

In another first aspect, the isolated GH61 polypeptides having cellulolytic enhancing activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 44 of preferably at least 80%, more preferably at least 85%, even

more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have cellulolytic enhancing activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 44.

In one aspect, a polypeptide having cellulolytic enhancing activity comprises or consists of the amino acid sequence of SEQ ID NO: 44 or an allelic variant thereof; or a fragment thereof having cellulolytic enhancing activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 44. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 44. In another aspect, the polypeptide comprises or consists of amino acids 22 to 368 of SEQ ID NO: 44, or an allelic variant thereof; or a fragment thereof having cellulolytic enhancing activity. In another aspect, the polypeptide comprises or consists of amino acids 22 to 368 of SEQ ID NO: 44.

In another first aspect, the isolated GH61 polypeptides having cellulolytic enhancing activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 192 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have cellulolytic enhancing activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 192.

In one aspect, a polypeptide having cellulolytic enhancing activity comprises or consists of the amino acid sequence of SEQ ID NO: 192 or an allelic variant thereof; or a fragment thereof having cellulolytic enhancing activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 192. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 192. In another aspect, the polypeptide comprises or consists of amino acids 23 to 251 of SEQ ID NO: 186, or an allelic variant thereof; or a fragment thereof having cellulolytic enhancing activity. In another aspect, the polypeptide comprises or consists of amino acids 23 to 251 of SEQ ID NO: 186.

In another first aspect, the isolated GH10 polypeptides having xylanase activity comprise amino acid sequences having a degree of identity to the mature polypeptide of

SEQ ID NO: 46, of at preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have xylanase activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 46.

In one aspect, a GH10 polypeptide having xylanase activity comprises or consists of the amino acid sequence of SEQ ID NO: 46 or an allelic variant thereof; or a fragment thereof that has xylanase activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 46. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 46. In another aspect, the polypeptide comprises or consists of amino acids 23 to 406 of SEQ ID NO: 46, or an allelic variant thereof; or a fragment thereof that has xylanase activity. In another aspect, the polypeptide comprises or consists of amino acids 23 to 406 of SEQ ID NO: 46.

In another first aspect, the isolated GH10 polypeptides having xylanase activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 48, of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have xylanase activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 48.

In one aspect, a GH10 polypeptide having xylanase activity comprises or consists of the amino acid sequence of SEQ ID NO: 48 or an allelic variant thereof; or a fragment thereof that has xylanase activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 48. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 48. In another aspect, the polypeptide comprises or consists of amino acids 20 to 397 of SEQ ID NO: 48, or an allelic variant thereof; or a fragment thereof that has xylanase activity. In another aspect, the polypeptide comprises or consists of amino acids 20 to 397 of SEQ ID NO: 48.

In another first aspect, the isolated GH10 polypeptides having xylanase activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 50, of preferably at least 80%, more preferably at least 85%, even more

preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have xylanase activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 50.

In one aspect, a GH10 polypeptide having xylanase activity comprises or consists of the amino acid sequence of SEQ ID NO: 50 or an allelic variant thereof; or a fragment thereof that has xylanase activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 50. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 50. In another aspect, the polypeptide comprises or consists of amino acids 20 to 398 of SEQ ID NO: 50, or an allelic variant thereof; or a fragment thereof that has xylanase activity. In another aspect, the polypeptide comprises or consists of amino acids 20 to 398 of SEQ ID NO: 50.

In another first aspect, the isolated GH10 polypeptides having xylanase activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 52, of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have xylanase activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 52.

In one aspect, a GH10 polypeptide having xylanase activity comprises or consists of the amino acid sequence of SEQ ID NO: 50 or an allelic variant thereof; or a fragment thereof that has xylanase activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 52. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 52. In another aspect, the polypeptide comprises or consists of amino acids 20 to 407 of SEQ ID NO: 52, or an allelic variant thereof; or a fragment thereof that has xylanase activity. In another aspect, the polypeptide comprises or consists of amino acids 20 to 407 of SEQ ID NO: 52.

In another first aspect, the isolated GH10 polypeptides having xylanase activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 54, of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least

96%, at least 97%, at least 98%, or at least 99%, which have xylanase activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 54.

In one aspect, a GH10 polypeptide having xylanase activity comprises or consists of the amino acid sequence of SEQ ID NO: 50 or an allelic variant thereof; or a fragment thereof that has xylanase activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 54. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 54. In another aspect, the polypeptide comprises or consists of amino acids 20 to 395 of SEQ ID NO: 54, or an allelic variant thereof; or a fragment thereof that has xylanase activity. In another aspect, the polypeptide comprises or consists of amino acids 20 to 395 of SEQ ID NO: 54.

In another first aspect, the isolated GH10 polypeptides having xylanase activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 194, of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have xylanase activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 194.

A GH10 polypeptide having xylanase activity comprises or consists of the amino acid sequence of SEQ ID NO: 194 or an allelic variant thereof; or a fragment thereof that has xylanase activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 194. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 194. In another aspect, the polypeptide comprises or consists of amino acids 24 to 403 of SEQ ID NO: 194, or an allelic variant thereof; or a fragment thereof that has xylanase activity. In another aspect, the polypeptide comprises or consists of amino acids 24 to 403 of SEQ ID NO: 194.

In another first aspect, the isolated GH10 polypeptides having xylanase activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 196, of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have xylanase activity (hereinafter

"homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 196.

A GH10 polypeptide having xylanase activity comprises or consists of the amino acid sequence of SEQ ID NO: 194 or an allelic variant thereof; or a fragment thereof that has xylanase activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 196. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 196. In another aspect, the polypeptide comprises or consists of amino acids 24 to 403 of SEQ ID NO: 196, or an allelic variant thereof; or a fragment thereof that has xylanase activity. In another aspect, the polypeptide comprises or consists of amino acids 24 to 403 of SEQ ID NO: 196.

In another first aspect, the isolated GH10 polypeptides having xylanase activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 198, of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have xylanase activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 198.

A GH10 polypeptide having xylanase activity comprises or consists of the amino acid sequence of SEQ ID NO: 194 or an allelic variant thereof; or a fragment thereof that has xylanase activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 198. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 198. In another aspect, the polypeptide comprises or consists of amino acids 20 to 396 of SEQ ID NO: 198, or an allelic variant thereof; or a fragment thereof that has xylanase activity. In another aspect, the polypeptide comprises or consists of amino acids 20 to 396 of SEQ ID NO: 198.

In another first aspect, the isolated GH11 polypeptides having xylanase activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 56, of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have xylanase activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise

amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 56.

In one aspect, a GH11 polypeptide having xylanase activity comprises or consists of the amino acid sequence of SEQ ID NO: 56 or an allelic variant thereof; or a fragment thereof that has xylanase activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 56. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 56. In another aspect, the polypeptide comprises or consists of amino acids 43 to 338 of SEQ ID NO: 56, or an allelic variant thereof; or a fragment thereof that has xylanase activity. In another aspect, the polypeptide comprises or consists of amino acids 43 to 338 of SEQ ID NO: 56. In another aspect, the polypeptide comprises or consists of amino acids 43 to 338 of SEQ ID NO: 56. In another aspect, the polypeptide consists of the amino acid sequence of SEQ ID NO: 56 or an allelic variant thereof; or a fragment thereof that has xylanase activity.

In another first aspect, the isolated GH11 polypeptides having xylanase activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 200 or SEQ ID NO: 305, of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have xylanase activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 200 or SEQ ID NO: 305.

A GH11 polypeptide having xylanase activity comprises or consists of the amino acid sequence of SEQ ID NO: 200, or an allelic variant thereof; or a fragment thereof that has xylanase activity, or SEQ ID NO: 305 or a fragment thereof that has xylanase activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 200 or SEQ ID NO: 305. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 200 or SEQ ID NO: 305. In another aspect, the polypeptide comprises or consists of amino acids 25 to 360 of SEQ ID NO: 200, or an allelic variant thereof; or a fragment thereof that has xylanase activity, or amino acids 29 to 231 of SEQ ID NO: 305 or a fragment thereof that has xylanase activity. In another aspect, the polypeptide comprises or consists of amino acids 25 to 360 of SEQ ID NO: 200 or amino acids 29 to 231 of SEQ ID NO: 305. In another aspect, the polypeptide comprises or

consists of amino acids 25 to 360 of SEQ ID NO: 200 or amino acids 29 to 231 of SEQ ID NO: 305.

In another first aspect, the isolated polypeptides having beta-xylosidase activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 58, of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have beta-xylosidase activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 58.

In one aspect, a polypeptide having beta-xylosidase activity comprises or consists of the amino acid sequence of SEQ ID NO: 58 or an allelic variant thereof; or a fragment thereof that has beta-xylosidase activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 58. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 58. In another aspect, the polypeptide comprises or consists of amino acids 21 to 797 of SEQ ID NO: 58, or an allelic variant thereof; or a fragment thereof that has beta-xylosidase activity. In another aspect, the polypeptide comprises or consists of amino acids 21 to 797 of SEQ ID NO: 58.

In another first aspect, the isolated polypeptides having beta-xylosidase activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 60, of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have beta-xylosidase activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 60.

In one aspect, a polypeptide having beta-xylosidase activity comprises or consists of the amino acid sequence of SEQ ID NO: 60 or an allelic variant thereof; or a fragment thereof that has beta-xylosidase activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 60. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 60. In another aspect, the polypeptide comprises or consists of amino acids 22 to 795 of SEQ ID NO: 60, or an allelic variant thereof; or a fragment thereof that has beta-xylosidase activity. In another aspect, the

polypeptide comprises or consists of amino acids 22 to 795 of SEQ ID NO: 60.

In another first aspect, the isolated polypeptides having beta-xylosidase activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 202, of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have beta-xylosidase activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 202.

In one aspect, a polypeptide having beta-xylosidase activity comprises or consists of the amino acid sequence of SEQ ID NO: 202 or an allelic variant thereof; or a fragment thereof that has beta-xylosidase activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 202. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 202. In another aspect, the polypeptide comprises or consists of amino acids 18 to 803 of SEQ ID NO: 202, or an allelic variant thereof; or a fragment thereof that has beta-xylosidase activity. In another aspect, the polypeptide comprises or consists of amino acids 18 to 803 of SEQ ID NO: 202.

In one aspect, a polypeptide having beta-xylosidase activity comprises or consists of the amino acid sequence of SEQ ID NO: 204 or an allelic variant thereof; or a fragment thereof that has beta-xylosidase activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 204. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 204. In another aspect, the polypeptide comprises or consists of amino acids 18 to 817 of SEQ ID NO: 204, or an allelic variant thereof; or a fragment thereof that has beta-xylosidase activity. In another aspect, the polypeptide comprises or consists of amino acids 18 to 817 of SEQ ID NO: 204.

In another first aspect, the isolated polypeptides having beta-xylosidase activity comprise amino acid sequences having a degree of identity to the mature polypeptide of SEQ ID NO: 206, of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which have beta-xylosidase activity (hereinafter "homologous polypeptides"). In another aspect, the homologous polypeptides comprise amino acid sequences that differ by ten amino acids, preferably by five amino acids, more preferably by four amino acids, even more preferably by three amino acids, most preferably by two amino acids, and even most preferably by one amino acid from the mature polypeptide of SEQ ID NO: 206.

In one aspect, a polypeptide having beta-xylosidase activity comprises or consists of the amino acid sequence of SEQ ID NO: 206 or an allelic variant thereof; or a fragment thereof that has beta-xylosidase activity. In another aspect, the polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 206. In another aspect, the polypeptide comprises or consists of the mature polypeptide of SEQ ID NO: 206. In another aspect, the polypeptide comprises or consists of amino acids 21 to 792 of SEQ ID NO: 206, or an allelic variant thereof; or a fragment thereof that has beta-xylosidase activity. In another aspect, the polypeptide comprises or consists of amino acids 21 to 792 of SEQ ID NO: 206.

In a second aspect, the isolated polypeptides having cellobiohydrolase I activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 1, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 1, or (iii) a full-length complementary strand of (i) or (ii) (J. Sambrook, E.F. Fritsch, and T. Maniatis, 1989, *supra*).

In another second aspect, the isolated polypeptides having cellobiohydrolase I activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 3, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 3, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having cellobiohydrolase I activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 5, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 5, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having cellobiohydrolase I activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 7, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 7, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having cellobiohydrolase I activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO:

157, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 157, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having cellobiohydrolase I activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 159, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 159, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having cellobiohydrolase I activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 161, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 161, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having cellobiohydrolase I activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 163, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 163, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having cellobiohydrolase I activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 165, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 165, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having cellobiohydrolase II activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 9, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 9, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having cellobiohydrolase II activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 11, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 11, or

(iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having cellobiohydrolase II activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 13, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 13, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having cellobiohydrolase II activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 15, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 15, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having cellobiohydrolase II activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 17, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 17, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having cellobiohydrolase II activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 167, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 167, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having cellobiohydrolase II activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 169, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 169, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having cellobiohydrolase II activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 171, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 171, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having endoglucanase I activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 19, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 19, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having endoglucanase II activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 21, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 21, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having endoglucanase II activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 23, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 23, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having endoglucanase II activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 25, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 25, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having endoglucanase II activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 173, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 173, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having endoglucanase II activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 175, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 175, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having beta-glucosidase activity

are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 27, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 27, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having beta-glucosidase activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 29, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 29, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having beta-glucosidase activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 31, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 31, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having beta-glucosidase activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 177, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 177, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having beta-glucosidase activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 179, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 179, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having beta-glucosidase activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 181, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 181, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having beta-glucosidase activity are encoded by polynucleotides that hybridize under preferably medium-high stringency

conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 183, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 183, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having beta-glucosidase activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 185, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 185, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having beta-glucosidase activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 187, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 187, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having beta-glucosidase activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 189, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 189, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated GH61 polypeptides having cellulolytic enhancing activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 33, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 33, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated GH61 polypeptides having cellulolytic enhancing activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 35, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 35, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated GH61 polypeptides having cellulolytic enhancing activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably

very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 37, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 37, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated GH61 polypeptides having cellulolytic enhancing activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 39, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 39, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated GH61 polypeptides having cellulolytic enhancing activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 41, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 41, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated GH61 polypeptides having cellulolytic enhancing activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 43, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 43, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated GH10 polypeptides having xylanase activity is encoded by polynucleotides that hybridize under preferably medium stringency conditions, more preferably medium-high stringency conditions, even more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 191, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 191, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated GH10 polypeptides having xylanase activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 45, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 45, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated GH10 polypeptides having xylanase activity are encoded by polynucleotides that hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high

stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 47, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 47, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated GH10 polypeptides having xylanase activity is encoded by polynucleotides that hybridize under preferably medium stringency conditions, more preferably medium-high stringency conditions, even more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 49, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 49, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated GH10 polypeptides having xylanase activity is encoded by polynucleotides that hybridize under preferably medium stringency conditions, more preferably medium-high stringency conditions, even more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 51, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 51, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated GH10 polypeptides having xylanase activity is encoded by polynucleotides that hybridize under preferably medium stringency conditions, more preferably medium-high stringency conditions, even more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 53, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 53, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated GH10 polypeptides having xylanase activity is encoded by polynucleotides that hybridize under preferably medium stringency conditions, more preferably medium-high stringency conditions, even more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 193, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 193, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated GH10 polypeptides having xylanase activity is encoded by polynucleotides that hybridize under preferably medium stringency conditions, more preferably medium-high stringency conditions, even more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 195, (ii) the cDNA sequence of the mature

polypeptide coding sequence of SEQ ID NO: 195, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated GH10 polypeptides having xylanase activity is encoded by polynucleotides that hybridize under preferably medium stringency conditions, more preferably medium-high stringency conditions, even more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 197, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 197, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated GH11 polypeptides having xylanase activity is encoded by polynucleotides that hybridize under preferably medium stringency conditions, more preferably medium-high stringency conditions, even more preferably high stringency conditions, and most preferably very high stringency conditions with the mature polypeptide coding sequence of SEQ ID NO: 55 or its full-length complementary strand.

In another second aspect, the isolated GH11 polypeptides having xylanase activity is encoded by polynucleotides that hybridize under preferably medium stringency conditions, more preferably medium-high stringency conditions, even more preferably high stringency conditions, and most preferably very high stringency conditions with the mature polypeptide coding sequence of SEQ ID NO: 199 or SEQ ID NO: 304; or its full-length complementary strand.

In another second aspect, the isolated polypeptides having beta-xylosidase activity is encoded by polynucleotides that hybridize under preferably medium stringency conditions, more preferably medium-high stringency conditions, even more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 57, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 57, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having beta-xylosidase activity is encoded by polynucleotides that hybridize under preferably medium stringency conditions, more preferably medium-high stringency conditions, even more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 59, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 59, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having beta-xylosidase activity is encoded by polynucleotides that hybridize under preferably medium stringency conditions, more preferably medium-high stringency conditions, even more preferably high stringency

conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 201, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 201, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having beta-xylosidase activity is encoded by polynucleotides that hybridize under preferably medium stringency conditions, more preferably medium-high stringency conditions, even more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 203, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 203, or (iii) a full-length complementary strand of (i) or (ii).

In another second aspect, the isolated polypeptides having beta-xylosidase activity is encoded by polynucleotides that hybridize under preferably medium stringency conditions, more preferably medium-high stringency conditions, even more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 205, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 205, or (iii) a full-length complementary strand of (i) or (ii).

The nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 919, 193, 195, 197, 199, 201, 203, or 205; or a subsequence thereof; as well as the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, or 206; or a fragment thereof; may be used to design nucleic acid probes to identify and clone DNA encoding polypeptides having enzyme activity from strains of different genera or species according to methods well known in the art. In particular, such probes can be used for hybridization with the genomic or cDNA of the genus or species of interest, following standard Southern blotting procedures, in order to identify and isolate the corresponding gene therein. Such probes can be considerably shorter than the entire sequence, but should be at least 14, preferably at least 25, more preferably at least 35, and most preferably at least 70 nucleotides in length. It is, however, preferred that the nucleic acid probe is at least 100 nucleotides in length. For example, the nucleic acid probe may be at least 200 nucleotides, preferably at least 300 nucleotides, more preferably at least 400 nucleotides, or most preferably at least 500 nucleotides in length. Even longer probes may be used, *e.g.*, nucleic acid probes that are preferably at least 600 nucleotides, more preferably at least 700 nucleotides, even more preferably at least 800

nucleotides, or most preferably at least 900 nucleotides in length. Both DNA and RNA probes can be used. The probes are typically labeled for detecting the corresponding gene (for example, with ^{32}P, ^{3}H, ^{35}S, biotin, or avidin). Such probes are encompassed by the present invention.

A genomic DNA or cDNA library prepared from such other strains may, therefore, be screened for DNA that hybridizes with the probes described above and encodes a polypeptide having enzyme or biological activity. Genomic or other DNA from such other strains may be separated by agarose or polyacrylamide gel electrophoresis, or other separation techniques. DNA from the libraries or the separated DNA may be transferred to and immobilized on nitrocellulose or other suitable carrier material. In order to identify a clone or DNA that is homologous with SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 919, 193, 195, 197, 199, 201, 203, or 205 or a subsequence thereof, the carrier material is preferably used in a Southern blot.

For purposes of the present invention, hybridization indicates that the nucleotide sequence hybridizes to a labeled nucleic acid probe corresponding to the mature polypeptide coding sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 919, 193, 195, 197, 199, 201, 203, or 205; the cDNA sequence of or the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 919, 193, 195, 197, 199, 201, 203, or 205; its full-length complementary strand; or a subsequence thereof; under very low to very high stringency conditions. Molecules to which the nucleic acid probe hybridizes under these conditions can be detected using, for example, X-ray film.

In one aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 1. In another aspect, the nucleic acid probe is nucleotides 55 to 1590 of SEQ ID NO: 1. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 2, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 1. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Chaetomium thermophilum* CGMCC 0581, wherein the polynucleotide sequence thereof encodes a polypeptide having cellobiohydrolase I activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Chaetomium thermophilum* CGMCC 0581.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence

of SEQ ID NO: 3. In another aspect, the nucleic acid probe is nucleotides 61 to 1350 of SEQ ID NO: 3. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 4, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 3. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Myceliophthora thermophila* CBS 117.65, wherein the polynucleotide sequence thereof encodes a polypeptide having cellobiohydrolase I activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Myceliophthora thermophila* CBS 117.65.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 5. In another aspect, the nucleic acid probe is nucleotides 79 to 1596 of SEQ ID NO: 5. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 6, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 5. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Aspergillus fumigatus* NN055679, wherein the polynucleotide sequence thereof encodes a polypeptide having cellobiohydrolase I activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Aspergillus fumigatus* NN055679.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 7. In another aspect, the nucleic acid probe is nucleotides 52 to 1374 of SEQ ID NO: 7. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 8, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 7. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Thermoascus aurantiacus* CGMCC 0583, wherein the polynucleotide sequence thereof encodes a polypeptide having cellobiohydrolase I activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Thermoascus aurantiacus* CGMCC 0583.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 157. In another aspect, the nucleic acid probe is nucleotides 55 to 1428 of SEQ ID NO: 157. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 158, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 157. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Penicillium emersonii* NN051602, wherein the polynucleotide sequence thereof encodes a polypeptide having cellobiohydrolase I activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Penicillium emersonii* NN051602.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 159. In another aspect, the nucleic acid probe is nucleotides 76 to 1590 of

SEQ ID NO: 159. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 160, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 159. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Penicillium pinophilum* NN046877, wherein the polynucleotide sequence thereof encodes a polypeptide having cellobiohydrolase I activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Penicillium pinophilum* NN046877.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 161. In another aspect, the nucleic acid probe is nucleotides 52 to 1374 of SEQ ID NO: 161. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 162, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 161. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Aspergillus terreus* ATCC 28865, wherein the polynucleotide sequence thereof encodes a polypeptide having cellobiohydrolase I activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Aspergillus terreus* ATCC 28865.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 163. In another aspect, the nucleic acid probe is nucleotides 79 to 1605 of SEQ ID NO: 163. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 164, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 162. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Neosartorya fischeri* NRRL 181, wherein the polynucleotide sequence thereof encodes a polypeptide having cellobiohydrolase I activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Neosartorya fischeri* NRRL 181.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 165. In another aspect, the nucleic acid probe is nucleotides 52 to 1374 of SEQ ID NO: 165. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 166, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 165. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Aspergillus nidulans* FGSCA4, wherein the polynucleotide sequence thereof encodes a polypeptide having cellobiohydrolase I activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Aspergillus nidulans* FGSCA4.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 9. In another aspect, the nucleic acid probe is nucleotides 52 to 1799 of SEQ ID NO: 9. In another aspect, the nucleic acid probe is a polynucleotide sequence that

encodes the polypeptide of SEQ ID NO: 10, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 9. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Myceliophthora thermophila* CBS 117.65, wherein the polynucleotide sequence thereof encodes a polypeptide having cellobiohydrolase II activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Myceliophthora thermophila* CBS 117.65.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 11. In another aspect, the nucleic acid probe is nucleotides 52 to 1809 of SEQ ID NO: 11. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 12, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 11. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pSMai182 which is contained in *E. coli* NRRL B-50059 or contained in *Myceliophthora thermophila* CBS 202.73, wherein the polynucleotide sequence thereof encodes a polypeptide having cellobiohydrolase II activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in plasmid pSMai182 which is contained in *E. coli* NRRL B-50059 or contained in *Myceliophthora thermophila* CBS 202.73.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 13. In another aspect, the nucleic acid probe is nucleotides 52 to 1443 of SEQ ID NO: 13. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 14, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 13. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pTter6A which is contained in *E. coli* NRRL B-30802, wherein the polynucleotide sequence thereof encodes a polypeptide having cellobiohydrolase II activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in plasmid pTter6A which is contained in *E. coli* NRRL B-30802.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 15. In another aspect, the nucleic acid probe is nucleotides 109 to 1401 of SEQ ID NO: 15. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 16, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 15. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pMStr199 which is contained in *E. coli* DSM 23379, wherein the polynucleotide sequence thereof encodes a polypeptide having cellobiohydrolase II activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in plasmid pMStr199 which is contained in *E. coli* DSM 23379.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 17. In another aspect, the nucleic acid probe is nucleotides 58 to 1700 of SEQ ID NO: 17. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 18, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 17. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Aspergillus fumigatus* NN055679, wherein the polynucleotide sequence thereof encodes a polypeptide having cellobiohydrolase II activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Aspergillus fumigatus* NN055679.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 167. In another aspect, the nucleic acid probe is nucleotides 55 to 1749 of SEQ ID NO: 167. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 168, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 167. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Fennellia nivea* NN046949 or in pGEM-T-CBHII46949-2 which is contained in *E. coli* DSM 24143, wherein the polynucleotide sequence thereof encodes a polypeptide having cellobiohydrolase II activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Fennellia nivea* NN046949 or in pGEM-T-CBHII46949-2 which is contained in *E. coli* DSM 24143.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 169. In another aspect, the nucleic acid probe is nucleotides 58 to 1744 of SEQ ID NO: 169. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 170, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 169. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Penicillium emersonii* NN051602, wherein the polynucleotide sequence thereof encodes a polypeptide having cellobiohydrolase II activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Penicillium emersonii* NN051602.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 171. In another aspect, the nucleic acid probe is nucleotides 58 to 1701 of SEQ ID NO: 171. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 172, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 171. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Penicillium pinophilum* NN046877, wherein the polynucleotide sequence thereof encodes a polypeptide having cellobiohydrolase II activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Penicillium pinophilum* NN046877.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 19. In another aspect, the nucleic acid probe is nucleotides 64 to 1502 of SEQ ID NO: 19. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 20, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 19. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Aspergillus terreus* ATCC 28865, wherein the polynucleotide sequence thereof encodes a polypeptide having endoglucanase I activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Aspergillus terreus* ATCC 28865.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 21. In another aspect, the nucleic acid probe is nucleotides 64 to 1254 of SEQ ID NO: 21. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 22, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 21. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Trichoderma reesei* RutC30, wherein the polynucleotide sequence thereof encodes a polypeptide having endoglucanase II activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Trichoderma reesei* RutC30.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 23. In another aspect, the nucleic acid probe is nucleotides 67 to 1185 of SEQ ID NO: 23. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 24, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 23. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pCIC161 which is contained in *E. coli* NRRL B-30902, wherein the polynucleotide sequence thereof encodes a polypeptide having endoglucanase II activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in plasmid pCIC161 which is contained in *E. coli* NRRL B-30902.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 25. In another aspect, the nucleic acid probe is nucleotides 91 to 1005 of SEQ ID NO: 25. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 26, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 25. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Thermoascus aurantiacus* CGMCC 0670, wherein the polynucleotide sequence thereof encodes a polypeptide having endoglucanase II activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Thermoascus aurantiacus* CGMCC 0670.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence

of SEQ ID NO: 173. In another aspect, the nucleic acid probe is nucleotides 55 to 1260 of SEQ ID NO: 173. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 174, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 173. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Aspergillus fumigatus* NN051616, wherein the polynucleotide sequence thereof encodes a polypeptide having endoglucanase II activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Aspergillus fumigatus* NN051616.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 175. In another aspect, the nucleic acid probe is nucleotides 49 to 1378 of SEQ ID NO: 175. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 176, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 175. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Neosartorya fischeri* NRRL 181, wherein the polynucleotide sequence thereof encodes a polypeptide having endoglucanase II activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Neosartorya fischeri* NRRL 181.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 27. In another aspect, the nucleic acid probe is nucleotides 58 to 2580 of SEQ ID NO: 27. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 28, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 27. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pEJG113 which is contained in *E. coli* NRRL B-30695, wherein the polynucleotide sequence thereof encodes a polypeptide having beta-glucosidase activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in plasmid pEJG113 which is contained in *E. coli* NRRL B-30695.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 29. In another aspect, the nucleic acid probe is nucleotides 171 to 2753 of SEQ ID NO: 29. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 30, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 29. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pKKAB which is contained in *E. coli* NRRL B-30860, wherein the polynucleotide sequence thereof encodes a polypeptide having beta-glucosidase activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in plasmid pKKAB which is contained in *E. coli* NRRL B-30860.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 31. In another aspect, the nucleic acid probe is nucleotides 58 to 2934 of

SEQ ID NO: 31. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 32, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 31. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Aspergillus niger* IBT 10140, wherein the polynucleotide sequence thereof encodes a polypeptide having beta-glucosidase activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Aspergillus niger* IBT 10140.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 177. In another aspect, the nucleic acid probe is nucleotides 58 to 2937 of SEQ ID NO: 177. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 178, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 177. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Aspergillus aculeatus* WDCM190, which encodes a polypeptide having beta-glucosidase activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Aspergillus aculeatus* WDCM190.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 179. In another aspect, the nucleic acid probe is nucleotides 58 to 2932 of SEQ ID NO: 179. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 180, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 179. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Aspergillus kawashii* IFO4308, which encodes a polypeptide having beta-glucosidase activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Aspergillus kawashii* IFO4308.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 181. In another aspect, the nucleic acid probe is nucleotides 55 to 3059 of SEQ ID NO: 181. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 182, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 181. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Aspergillus clavatus* NRRL 1, which encodes a polypeptide having beta-glucosidase activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Aspergillus clavatus* NRRL 1.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 183. In another aspect, the nucleic acid probe is nucleotides 55 to 3029 of SEQ ID NO: 183. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 184, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 183. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Thielavia terrestris* NRRL 8126, which encodes a

polypeptide having beta-glucosidase activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Thielavia terrestris* NRRL 8126.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 185. In another aspect, the nucleic acid probe is nucleotides 64 to 2790 of SEQ ID NO: 185. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 186, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 185. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in pUC19 D55EX which is contained in *E. coli* NRRL B-50395, wherein the polynucleotide sequence thereof encodes a polypeptide having beta-glucosidase activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in in pUC19 D55EX which is contained in *E. coli* NRRL B-50395.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 187. In another aspect, the nucleic acid probe is nucleotides 64 to 2790 of SEQ ID NO: 187. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 188, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 187. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Penicillium oxalicum* IBT5387, wherein the polynucleotide sequence thereof encodes a polypeptide having beta-glucosidase activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Penicillium oxalicum* IBT5387.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 189. In another aspect, the nucleic acid probe is nucleotides 58 to 2961 of SEQ ID NO: 189. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 190, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 189. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Talaromyces emersonii* CBS 549.92, wherein the polynucleotide sequence thereof encodes a polypeptide having beta-glucosidase activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Talaromyces emersonii* CBS 549.92.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 33. In another aspect, the nucleic acid probe is nucleotides 67 to 796 of SEQ ID NO: 33. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 34, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 33. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pDZA2-7 which is contained in *E. coli* NRRL B-30704, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another aspect, the nucleic acid probe is the mature

polypeptide coding region contained in plasmid pDZA2-7 which is contained in *E. coli* NRRL B-30704.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 35. In another aspect, the nucleic acid probe is nucleotides 58 to 900 of SEQ ID NO: 35. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 36, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 35. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pTter61E which is contained in *E. coli* NRRL B-30814, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in plasmid pTter61E which is contained in *E. coli* NRRL B-30814.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 37. In another aspect, the nucleic acid probe is nucleotides 64 to 859 of SEQ ID NO: 37. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 38, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 37. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Aspergillus fumigatus* NN051616, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Aspergillus fumigatus* NN051616.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 39. In another aspect, the nucleic acid probe is nucleotides 64 to 1018 of SEQ ID NO: 39. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 40, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 39. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Penicillim pinophilum* NN046877, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in plasmid pGEM-T-Ppin7 which is contained in *E. coli* DSM 22711.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 41. In another aspect, the nucleic acid probe is nucleotides 76 to 832 of SEQ ID NO: 41. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 42, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 41. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pGEM-T-GH61D23Y4 which is contained in *E. coli* DSM 22882, wherein the polynucleotide sequence thereof encodes a polypeptide

having cellulolytic enhancing activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in plasmid pGEM-T-GH61D23Y4 which is contained in *E. coli* DSM 22882.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 43. In another aspect, the nucleic acid probe is nucleotides 64 to 1104 of SEQ ID NO: 43. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 44, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 43. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pAG68 which is contained in *E. coli* NRRL B-50320, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in plasmid pAG68 which is contained in *E. coli* NRRL B-50320.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 191. In another aspect, the nucleic acid probe is nucleotides 64 to 1104 of SEQ ID NO: 191. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 192, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 191. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pGEM-T-GH61a51486 which is contained in *E. coli* DSM 22656, wherein the polynucleotide sequence thereof encodes a polypeptide having cellulolytic enhancing activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in plasmid pGEM-T-GH61a51486 which is contained in *E. coli* DSM 22656.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 45. In another aspect, the nucleic acid probe is nucleotides 69 to 1314 of SEQ ID NO: 45. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 46, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 45. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Aspergillus aculeatus* CBS 101.43, wherein the polynucleotide sequence thereof encodes a polypeptide having xylanase activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Aspergillus aculeatus* CBS 101.43.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 47. In another aspect, the nucleic acid probe is nucleotides 107 to 1415 of SEQ ID NO: 47. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 48, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 47. In another aspect, the nucleic acid probe is the

polynucleotide sequence contained in plasmid pHyGe001 which is contained in *E. coli* NRRL B-30703, wherein the polynucleotide sequence thereof encodes a polypeptide having xylanase activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in plasmid pHyGe001 which is contained in *E. coli* NRRL B-30703.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 49. In another aspect, the nucleic acid probe is nucleotides 58 to 1194 of SEQ ID NO: 49. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 50, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 49. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pTF12Xyl170 which is contained in *E. coli* NRRL B-50309, wherein the polynucleotide sequence thereof encodes a polypeptide having xylanase activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in plasmid pTF12Xyl170 which is contained in *E. coli* NRRL B-50309.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 51. In another aspect, the nucleic acid probe is nucleotides 58 to 1439 of SEQ ID NO: 51. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 52, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 51. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in plasmid pGEM-T-Ppin3 which is contained in *E. coli* DSM 22922, wherein the polynucleotide sequence thereof encodes a polypeptide having xylanase activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in plasmid pGEM-T-Ppin3 which is contained in *E. coli* DSM 22922.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 53. In another aspect, the nucleic acid probe is nucleotides 58 to 1185 of SEQ ID NO: 53. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 54, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 53. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Thielavia terrestris* NRRL 8126, wherein the polynucleotide sequence thereof encodes a polypeptide having xylanase activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Thielavia terrestris* NRRL 8126.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 193. In another aspect, the nucleic acid probe is nucleotides 70 to 1383 of SEQ ID NO: 193. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 194, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 193. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Talaromyces emersonii* NN050022, wherein the

polynucleotide sequence thereof encodes a polypeptide having xylanase activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Talaromyces emersonii* NN050022.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 195. In another aspect, the nucleic acid probe is nucleotides 70 to 1384 of SEQ ID NO: 195. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 196, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 195. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in pMMar26 which is contained in *E. coli* NRRL B-50266, wherein the polynucleotide sequence thereof encodes a polypeptide having xylanase activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in pMMar26 which is contained in *E. coli* NRRL B-50266.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 197. In another aspect, the nucleic acid probe is nucleotides 58 to1188 of SEQ ID NO: 197. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 198, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 197. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *E. coli* DSM 10361, wherein the polynucleotide sequence thereof encodes a polypeptide having xylanase activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *E. coli* DSM 10361.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 55. In another aspect, the nucleic acid probe is nucleotides 127 to 1014 of SEQ ID NO: 55. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 56, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 55. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Thermobifida fusca* DSM 22883, wherein the polynucleotide sequence thereof encodes a polypeptide having xylanase activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Thermobifida fusca* DSM 22883.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 55. In another aspect, the nucleic acid probe is nucleotides 85 to 693 of SEQ ID NO: 55. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 56, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 55. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Dictyoglomus thermophilum* ATCC 35947, wherein the polynucleotide sequence thereof encodes a polypeptide having xylanase activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in

Dictyoglomus thermophilum ATCC 35947.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 57. In another aspect, the nucleic acid probe is nucleotides 61 to 2391 of SEQ ID NO: 57. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 58, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 57. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Trichoderma reesei* RutC30, wherein the polynucleotide sequence thereof encodes a polypeptide having beta-xylosidase activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Trichoderma reesei* RutC30.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 59. In another aspect, the nucleic acid probe is nucleotides 64 to 2388 of SEQ ID NO: 59. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 60, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 59. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Talaromyces emersonii* CBS 393.64, wherein the polynucleotide sequence thereof encodes a polypeptide having beta-xylosidase activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Talaromyces emersonii* CBS 393.64.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 201. In another aspect, the nucleic acid probe is nucleotides 52 to 2409 of SEQ ID NO: 201. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 202, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 201. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Aspergillus aculeatus* CBS 172.66, wherein the polynucleotide sequence thereof encodes a polypeptide having beta-xylosidase activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Aspergillus aculeatus* CBS 172.66.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 203. In another aspect, the nucleic acid probe is nucleotides 52 to 2451 of SEQ ID NO: 203. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 204, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 203. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Aspergillus aculeatus* CBS 186.67, wherein the polynucleotide sequence thereof encodes a polypeptide having beta-xylosidase activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Aspergillus aculeatus* CBS 186.67.

In another aspect, the nucleic acid probe is the mature polypeptide coding sequence of SEQ ID NO: 205. In another aspect, the nucleic acid probe is nucleotides 61 to 2376 of SEQ ID NO: 205. In another aspect, the nucleic acid probe is a polynucleotide sequence that encodes the polypeptide of SEQ ID NO: 206, or a subsequence thereof. In another aspect, the nucleic acid probe is SEQ ID NO: 205. In another aspect, the nucleic acid probe is the polynucleotide sequence contained in *Aspergillus fumigatus* NN051616, wherein the polynucleotide sequence thereof encodes a polypeptide having beta-xylosidase activity. In another aspect, the nucleic acid probe is the mature polypeptide coding region contained in *Aspergillus fumigatus* NN051616.

For long probes of at least 100 nucleotides in length, very low to very high stringency conditions are defined as prehybridization and hybridization at 42°C in 5X SSPE, 0.3% SDS, 200 micrograms/ml sheared and denatured salmon sperm DNA, and either 25% formamide for very low and low stringencies, 35% formamide for medium and medium-high stringencies, or 50% formamide for high and very high stringencies, following standard Southern blotting procedures for 12 to 24 hours optimally. The carrier material is finally washed three times each for 15 minutes using 2X SSC, 0.2% SDS at 45°C (very low stringency), at 50°C (low stringency), at 55°C (medium stringency), at 60°C (medium-high stringency), at 65°C (high stringency), and at 70°C (very high stringency).

For short probes of about 15 nucleotides to about 70 nucleotides in length, stringency conditions are defined as prehybridization and hybridization at about 5°C to about 10°C below the calculated T_m using the calculation according to Bolton and McCarthy (1962, *Proc. Natl. Acad. Sci. USA* 48:1390) in 0.9 M NaCl, 0.09 M Tris-HCl pH 7.6, 6 mM EDTA, 0.5% NP-40, 1X Denhardt's solution, 1 mM sodium pyrophosphate, 1 mM sodium monobasic phosphate, 0.1 mM ATP, and 0.2 mg of yeast RNA per ml following standard Southern blotting procedures for 12 to 24 hours optimally. The carrier material is finally washed once in 6X SCC plus 0.1% SDS for 15 minutes and twice each for 15 minutes using 6X SSC at 5°C to 10°C below the calculated T_m.

In a third aspect, the isolated polypeptides having cellobiohydrolase I activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 1 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase I activity.

In another third aspect, the isolated polypeptides having cellobiohydrolase I activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 3 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%,

most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase I activity.

In another third aspect, the isolated polypeptides having cellobiohydrolase I activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 5 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase I activity.

In another third aspect, the isolated polypeptides having cellobiohydrolase I activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 7 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase I activity.

In another third aspect, the isolated polypeptides having cellobiohydrolase I activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 157 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase I activity.

In another third aspect, the isolated polypeptides having cellobiohydrolase I activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 159 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase I activity.

In another third aspect, the isolated polypeptides having cellobiohydrolase I activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 161 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase I activity.

In another third aspect, the isolated polypeptides having cellobiohydrolase I activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 163 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least

98%, or at least 99%, which encode a polypeptide having cellobiohydrolase I activity.

In another third aspect, the isolated polypeptides having cellobiohydrolase I activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 165 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase I activity.

In another third aspect, the isolated polypeptides having cellobiohydrolase II activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 9 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase II activity.

In another third aspect, the isolated polypeptides having cellobiohydrolase II activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 11 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase II activity.

In another third aspect, the isolated polypeptides having cellobiohydrolase II activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 13 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase II activity.

In another third aspect, the isolated polypeptides having cellobiohydrolase II activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 15 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase II activity.

In another third aspect, the isolated polypeptides having cellobiohydrolase II activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 17 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase II activity.

In another third aspect, the isolated polypeptides having cellobiohydrolase II activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 167 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase II activity.

In another third aspect, the isolated polypeptides having cellobiohydrolase II activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 169 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase II activity.

In another third aspect, the isolated polypeptides having cellobiohydrolase II activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 171 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase II activity.

In another third aspect, the isolated polypeptides having endoglucanase I activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 19 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having endoglucanase I activity.

In another third aspect, the isolated polypeptides having endoglucanase II activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 21 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having endoglucanase II activity.

In another third aspect, the isolated polypeptides having endoglucanase II activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 23 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having endoglucanase II activity.

In another third aspect, the isolated polypeptides having endoglucanase II activity are

encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 25 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having endoglucanase II activity.

In another third aspect, the isolated polypeptides having endoglucanase II activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 173 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having endoglucanase II activity.

In another third aspect, the isolated polypeptides having endoglucanase II activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 175 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having endoglucanase II activity.

In another third aspect, the isolated polypeptides having beta-glucosidase activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 27 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having beta-glucosidase activity.

In another third aspect, the isolated polypeptides having beta-glucosidase activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 29 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having beta-glucosidase activity.

In another third aspect, the isolated polypeptides having beta-glucosidase activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 31 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having beta-glucosidase activity.

In another third aspect, the isolated polypeptides having beta-glucosidase activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a

degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 177 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having beta-glucosidase activity.

In another third aspect, the isolated polypeptides having beta-glucosidase activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 179 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having beta-glucosidase activity.

In another third aspect, the isolated polypeptides having beta-glucosidase activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 181 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having beta-glucosidase activity.

In another third aspect, the isolated polypeptides having beta-glucosidase activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 183 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having beta-glucosidase activity.

In another third aspect, the isolated polypeptides having beta-glucosidase activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 185 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having beta-glucosidase activity.

In another third aspect, the isolated polypeptides having beta-glucosidase activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 187 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having beta-glucosidase activity.

In another third aspect, the isolated polypeptides having beta-glucosidase activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 189 of

preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having beta-glucosidase activity.

In another third aspect, the isolated GH61 polypeptides having cellulolytic enhancing activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 33 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellulolytic enhancing activity.

In another third aspect, the isolated GH61 polypeptides having cellulolytic enhancing activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 35 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellulolytic enhancing activity.

In another third aspect, the isolated GH61 polypeptides having cellulolytic enhancing activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 37 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellulolytic enhancing activity.

In another third aspect, the isolated GH61 polypeptides having cellulolytic enhancing activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 39 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellulolytic enhancing activity.

In another third aspect, the isolated GH61 polypeptides having cellulolytic enhancing activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 41 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellulolytic enhancing activity.

In another third aspect, the isolated GH61 polypeptides having cellulolytic enhancing activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 43 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%,

most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellulolytic enhancing activity.

In another third aspect, the isolated GH61 polypeptides having cellulolytic enhancing activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 191 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellulolytic enhancing activity.

In another third aspect, the isolated polypeptides having xylanase activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 45 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having xylanase activity.

In another third aspect, the isolated polypeptides having xylanase activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 47 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having xylanase activity.

In another third aspect, the isolated polypeptides having xylanase activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 49 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having xylanase activity.

In another third aspect, the isolated polypeptides having xylanase activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 51 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having xylanase activity.

In another third aspect, the isolated polypeptides having xylanase activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 53 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%,

most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having xylanase activity.

In another third aspect, the isolated polypeptides having xylanase activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 193 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having xylanase activity.

In another third aspect, the isolated polypeptides having xylanase activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 195 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having xylanase activity.

In another third aspect, the isolated polypeptides having xylanase activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 197 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having xylanase activity.

In another third aspect, the isolated polypeptides having xylanase activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 55 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having xylanase activity.

In another third aspect, the isolated polypeptides having xylanase activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 199 or SEQ ID NO: 304 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having xylanase activity.

In another third aspect, the isolated polypeptides having beta-xylosidase activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 57 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least

98%, or at least 99%, which encode a polypeptide having beta-xylosidase activity.

In another third aspect, the isolated polypeptides having beta-xylosidase activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 59 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having beta-xylosidase activity.

In another third aspect, the isolated polypeptides having beta-xylosidase activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 201 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having beta-xylosidase activity.

In another third aspect, the isolated polypeptides having beta-xylosidase activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 203 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having beta-xylosidase activity.

In another third aspect, the isolated polypeptides having beta-xylosidase activity are encoded by polynucleotides comprising or consisting of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 205 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having beta-xylosidase activity.

Techniques used to isolate or clone a polynucleotide encoding a polypeptide are known in the art and include isolation from genomic DNA, preparation from cDNA, or a combination thereof. The cloning of a polynucleotide from such genomic DNA can be effected, *e.g.*, by using the well known polymerase chain reaction (PCR) or antibody screening of expression libraries to detect cloned DNA fragments with shared structural features. See, *e.g.*, Innis *et al.*, 1990, *PCR: A Guide to Methods and Application*, Academic Press, New York. Other nucleic acid amplification procedures such as ligase chain reaction (LCR), ligated activated transcription (LAT) and nucleotide sequence-based amplification (NASBA) may be used. The polynucleotides may be cloned from any strain and thus, for example, may be an allelic or species variant of the polypeptide encoding region of the nucleotide sequence.

In a fourth aspect, the isolated polynucleotides encoding polypeptides having

cellobiohydrolase I activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 1 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase I activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having cellobiohydrolase I activity comprises or consists of the nucleotide sequence of SEQ ID NO: 1. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Chaetomium thermophilum* CGMCC 0581. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 1. In another aspect, the nucleotide sequence comprises or consists of nucleotides 55 to 1590 of SEQ ID NO: 1. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Chaetomium thermophilum* CGMCC 0581.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase I activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 3 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase I activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having cellobiohydrolase I activity comprises or consists of the nucleotide sequence of SEQ ID NO: 3. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Myceliophthora thermophila* CBS 117.65. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 3. In another aspect, the nucleotide sequence comprises or consists of nucleotides 61 to 1350 of SEQ ID NO: 3. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Myceliophthora thermophila* CBS 117.65.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase I activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 5 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase I activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having cellobiohydrolase I activity comprises or consists of the nucleotide sequence of SEQ ID NO:

5. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Aspergillus fumigatus* NN055679. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 5. In another aspect, the nucleotide sequence comprises or consists of nucleotides 79 to 1596 of SEQ ID NO: 5. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Aspergillus fumigatus* NN055679.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase I activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 7 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase I activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having cellobiohydrolase I activity comprises or consists of the nucleotide sequence of SEQ ID NO: 7. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Thermoascus aurantiacus* CGMCC 0583. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 7. In another aspect, the nucleotide sequence comprises or consists of nucleotides 52 to 1374 of SEQ ID NO: 7. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Thermoascus aurantiacus* CGMCC 0583.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase I activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 157 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase I activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having cellobiohydrolase I activity comprises or consists of the nucleotide sequence of SEQ ID NO: 157. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Penicillium emersonii* NN051602. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 157. In another aspect, the nucleotide sequence comprises or consists of nucleotides 55 to 1428 of SEQ ID NO: 157. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Penicillium emersonii* NN051602

In another fourth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase I activity comprise or consist of nucleotide sequences that have a degree

of identity to the mature polypeptide coding sequence of SEQ ID NO: 159 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase I activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having cellobiohydrolase I activity comprises or consists of the nucleotide sequence of SEQ ID NO: 159. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Penicillium pinophilum* NN046877. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 159. In another aspect, the nucleotide sequence comprises or consists of nucleotides 76 to 1590 of SEQ ID NO: 159. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Penicillium pinophilum* NN046877.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase I activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 161 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase I activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having cellobiohydrolase I activity comprises or consists of the nucleotide sequence of SEQ ID NO: 161. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Aspergillus terreus* ATCC 28865. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 161. In another aspect, the nucleotide sequence comprises or consists of nucleotides 70 to 1675 of SEQ ID NO: 161. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Aspergillus terreus* ATCC 28865.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase I activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 163 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase I activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having cellobiohydrolase I activity comprises or consists of the nucleotide sequence of SEQ ID NO: 163. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Neosartorya fischeri* NRRL 181. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 163. In

another aspect, the nucleotide sequence comprises or consists of nucleotides 79 to 1605 of SEQ ID NO: 163. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Neosartorya fischeri* NRRL 181.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase I activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 165 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase I activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having cellobiohydrolase I activity comprises or consists of the nucleotide sequence of SEQ ID NO: 165. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Aspergillus nidulans* strain FGSCA4. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 165. In another aspect, the nucleotide sequence comprises or consists of nucleotides 70 to 1578 of SEQ ID NO: 165. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Aspergillus nidulans* strain FGSCA4.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase II activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 9 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase II activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having cellobiohydrolase II activity comprises or consists of the nucleotide sequence of SEQ ID NO: 9. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Myceliophthora thermophila* CBS 117.65. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 9. In another aspect, the nucleotide sequence comprises or consists of nucleotides 52 to 1799 of SEQ ID NO: 9. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Myceliophthora thermophila* CBS 117.65.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase II activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 11 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably

at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase II activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having cellobiohydrolase II activity comprises or consists of the nucleotide sequence of SEQ ID NO: 11. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in plasmid pSMai182 which is contained in *E. coli* NRRL B-50059 or contained in *Myceliophthora thermophila* CBS 202.73. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 11. In another aspect, the nucleotide sequence comprises or consists of nucleotides 52 to 1809 of SEQ ID NO: 11. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Myceliophthora thermophila* CBS 202.73 or contained in plasmid pSMai182 which is contained in *E. coli* NRRL B-50059.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase II activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 13 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase II activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having cellobiohydrolase II activity comprises or consists of the nucleotide sequence of SEQ ID NO: 13. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in plasmid pTter6A which is contained in *E. coli* NRRL B-30802. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 13. In another aspect, the nucleotide sequence comprises or consists of nucleotides 52 to 1443 of SEQ ID NO: 13. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in plasmid pTter6A which is contained in *E. coli* NRRL B-30802.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase II activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 15 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase II activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having cellobiohydrolase II activity comprises or consists of the nucleotide sequence of SEQ ID NO: 15. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in plasmid pMStr199 which is contained in *E. coli* DSM 23379. In another aspect,

the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 15. In another aspect, the nucleotide sequence comprises or consists of nucleotides 109 to 1401 of SEQ ID NO: 15. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in plasmid pMStr199 which is contained in *E. coli* DSM 23379.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase II activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 17 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase II activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having cellobiohydrolase II activity comprises or consists of the nucleotide sequence of SEQ ID NO: 17. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Aspergillus fumigatus* NN055679. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 17. In another aspect, the nucleotide sequence comprises or consists of nucleotides 58 to 1700 of SEQ ID NO: 17. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Aspergillus fumigatus* NN055679.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase II activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 167 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase II activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having cellobiohydrolase II activity comprises or consists of the nucleotide sequence of SEQ ID NO: 167. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in pGEM-T-CBHII46949-2 which is contained in *E. coli* DSM 24143. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 167. In another aspect, the nucleotide sequence comprises or consists of nucleotides 55 to 1749 of SEQ ID NO: 167. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in pGEM-T-CBHII46949-2 which is contained in *E. coli* DSM 24143.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase II activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 169 of preferably at

least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase II activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having cellobiohydrolase II activity comprises or consists of the nucleotide sequence of SEQ ID NO: 169. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Penicillium emersonii* NN051602. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 169. In another aspect, the nucleotide sequence comprises or consists of nucleotides 58 to 1744 of SEQ ID NO: 169. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Penicillium emersonii* NN051602.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase II activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 171 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellobiohydrolase I activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having cellobiohydrolase II activity comprises or consists of the nucleotide sequence of SEQ ID NO: 171. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Penicillium pinophilum* NN046877. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 171. In another aspect, the nucleotide sequence comprises or consists of nucleotides 58 to 1701 of SEQ ID NO: 171. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Penicillium pinophilum* NN046877.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having endoglucanase I activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 19 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having endoglucanase I activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having endoglucanase I activity comprises or consists of the nucleotide sequence of SEQ ID NO: 19. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Aspergillus terreus* ATCC 28865. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 19. In another aspect, the nucleotide sequence comprises or consists of nucleotides 64 to 1502 of

SEQ ID NO: 19. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Aspergillus terreus* ATCC 28865.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having endoglucanase II activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 21 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having endoglucanase II activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having endoglucanase II activity comprises or consists of the nucleotide sequence of SEQ ID NO: 21. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Trichoderma reesei* RutC30. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 21. In another aspect, the nucleotide sequence comprises or consists of nucleotides 64 to 1254 of SEQ ID NO: 21. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Trichoderma reesei* RutC30.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having endoglucanase II activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 23 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having endoglucanase II activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having endoglucanase II activity comprises or consists of the nucleotide sequence of SEQ ID NO: 23. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in plasmid pCIC161 which is contained in *E. coli* NRRL B-30902. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 23. In another aspect, the nucleotide sequence comprises or consists of nucleotides 67 to 1185 of SEQ ID NO: 23. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in plasmid pCIC161 which is contained in *E. coli* NRRL B-30902.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having endoglucanase II activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 25 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having endoglucanase II activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having endoglucanase II activity comprises or consists of the nucleotide sequence of SEQ ID NO: 25. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Thermoascus aurantiacus* CGMCC 0670. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 25. In another aspect, the nucleotide sequence comprises or consists of nucleotides 91 to 1005 of SEQ ID NO: 25. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Thermoascus aurantiacus* CGMCC 0670.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having endoglucanase II activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 173 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having endoglucanase II activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having endoglucanase II activity comprises or consists of the nucleotide sequence of SEQ ID NO: 173. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in p *Aspergillus fumigatus* NN051616. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 173. In another aspect, the nucleotide sequence comprises or consists of nucleotides 55 to 1230 of SEQ ID NO: 173. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Aspergillus fumigatus* NN051616.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having endoglucanase II activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 175 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having endoglucanase II activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having endoglucanase II activity comprises or consists of the nucleotide sequence of SEQ ID NO: 175. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Neosartorya fischeri* NRRL 181. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 175. In another aspect, the nucleotide sequence comprises or consists of nucleotides 49 to 1378of SEQ ID NO: 175. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Neosartorya fischeri* NRRL 181.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having beta-glucosidase activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 27 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having beta-glucosidase activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having beta-glucosidase activity comprises or consists of the nucleotide sequence of SEQ ID NO: 27. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in plasmid pEJG113 which is contained in *E. coli* NRRL B-30695. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 27. In another aspect, the nucleotide sequence comprises or consists of nucleotides 58 to 2580 of SEQ ID NO: 27. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in plasmid pEJG113 which is contained in *E. coli* NRRL B-30695.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having beta-glucosidase activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 29 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having beta-glucosidase activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having beta-glucosidase activity comprises or consists of the nucleotide sequence of SEQ ID NO: 29. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in plasmid pKKAB which is contained in *E. coli* NRRL B-30860. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 29. In another aspect, the nucleotide sequence comprises or consists of nucleotides 171 to 2753 of SEQ ID NO: 29. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in plasmid pKKAB which is contained in *E. coli* NRRL B-30860.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having beta-glucosidase activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 31 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having beta-glucosidase activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having beta-

glucosidase activity comprises or consists of the nucleotide sequence of SEQ ID NO: 31. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Aspergillus niger* IBT 10140. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 31. In another aspect, the nucleotide sequence comprises or consists of nucleotides 58 to 2934 of SEQ ID NO: 31. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Aspergillus niger* IBT 10140.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having beta-glucosidase activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 177 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having beta-glucosidase activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having beta-glucosidase activity comprises or consists of the nucleotide sequence of SEQ ID NO: 177. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Aspergillus aculeatus* WDCM190. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 177. In another aspect, the nucleotide sequence comprises or consists of nucleotides 58 to 2937 of SEQ ID NO: 177. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Aspergillus aculeatus* WDCM190.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having beta-glucosidase activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 179 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having beta-glucosidase activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having beta-glucosidase activity comprises or consists of the nucleotide sequence of SEQ ID NO: 179. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Aspergillus kawashii* IFO4308. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 179. In another aspect, the nucleotide sequence comprises or consists of nucleotides 58 to 2932 of SEQ ID NO: 179. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Aspergillus kawashii* IFO4308.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having beta-glucosidase activity comprise or consist of nucleotide sequences that have a degree of

identity to the mature polypeptide coding sequence of SEQ ID NO: 181 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having beta-glucosidase activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having beta-glucosidase activity comprises or consists of the nucleotide sequence of SEQ ID NO: 181. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in p *Aspergillus clavatus* NRRL 1. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 181. In another aspect, the nucleotide sequence comprises or consists of nucleotides 55 to 3059 of SEQ ID NO: 181. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Aspergillus clavatus* NRRL 1.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having beta-glucosidase activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 183 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having beta-glucosidase activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having beta-glucosidase activity comprises or consists of the nucleotide sequence of SEQ ID NO: 183. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Thielavia terrestris* NRRL 8126. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 183. In another aspect, the nucleotide sequence comprises or consists of nucleotides 55 to 3029 of SEQ ID NO: 183. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Thielavia terrestris* NRRL 8126.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having beta-glucosidase activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 185 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having beta-glucosidase activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having beta-glucosidase activity comprises or consists of the nucleotide sequence of SEQ ID NO: 185. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in pUC19 D55EX which is contained in *E. coli* NRRL B-50395. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of

SEQ ID NO: 185. In another aspect, the nucleotide sequence comprises or consists of nucleotides 64 to 2790 of SEQ ID NO: 185. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in pUC19 D55EX which is contained in *E. coli* NRRL B-50395.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having beta-glucosidase activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 187 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having beta-glucosidase activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having beta-glucosidase activity comprises or consists of the nucleotide sequence of SEQ ID NO: 187. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Penicillium oxalicum*. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 187. In another aspect, the nucleotide sequence comprises or consists of nucleotides 64 to 2790 of SEQ ID NO: 187. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Penicillium oxalicum*.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having beta-glucosidase activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 189 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having beta-glucosidase activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having beta-glucosidase activity comprises or consists of the nucleotide sequence of SEQ ID NO: 189. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Talaromyces emersonii* CBS 549.92. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 189. In another aspect, the nucleotide sequence comprises or consists of nucleotides 58 to 2961 of SEQ ID NO: 189. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Talaromyces emersonii* CBS 549.92.

In another fourth aspect, the isolated polynucleotides encoding GH61 polypeptides having cellulolytic enhancing activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 33 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least

98%, or at least 99%, which encode a polypeptide having cellulolytic enhancing activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having cellulolytic enhancing activity comprises or consists of the nucleotide sequence of SEQ ID NO: 33. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in plasmid pDZA2-7 which is contained in *E. coli* NRRL B-30704. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 33. In another aspect, the nucleotide sequence comprises or consists of nucleotides 67 to 796 of SEQ ID NO: 33. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in plasmid pDZA2-7 which is contained in *E. coli* NRRL B-30704.

In another fourth aspect, the isolated polynucleotides encoding GH61 polypeptides having cellulolytic enhancing activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 35 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellulolytic enhancing activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having cellulolytic enhancing activity comprises or consists of the nucleotide sequence of SEQ ID NO: 35. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in plasmid pTter61E which is contained in *E. coli* NRRL B-30814. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 35. In another aspect, the nucleotide sequence comprises or consists of nucleotides 58 to 900 of SEQ ID NO: 35. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in plasmid pTter61E which is contained in *E. coli* NRRL B-30814.

In another fourth aspect, the isolated polynucleotides encoding GH61 polypeptides having cellulolytic enhancing activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 37 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellulolytic enhancing activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having cellulolytic enhancing activity comprises or consists of the nucleotide sequence of SEQ ID NO: 37. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Aspergillus fumigatus* NN051616. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 37. In another aspect, the nucleotide sequence comprises or consists of nucleotides 64 to 859 of SEQ ID NO: 37.

In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Aspergillus fumigatus* NN051616.

In another fourth aspect, the isolated polynucleotides encoding GH61 polypeptides having cellulolytic enhancing activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 39 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellulolytic enhancing activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having cellulolytic enhancing activity comprises or consists of the nucleotide sequence of SEQ ID NO: 39. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in plasmid pGEM-T-Ppin7 which is contained in *E. coli* DSM 22711. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 39. In another aspect, the nucleotide sequence comprises or consists of nucleotides 64 to 1018 of SEQ ID NO: 39. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in plasmid pGEM-T-Ppin7 which is contained in *E. coli* DSM 22711.

In another fourth aspect, the isolated polynucleotides encoding GH61 polypeptides having cellulolytic enhancing activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 41 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having cellulolytic enhancing activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having cellulolytic enhancing activity comprises or consists of the nucleotide sequence of SEQ ID NO: 41. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in plasmid pGEM-T-GH61D23Y4 which is contained in *E. coli* DSM 22882. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 41. In another aspect, the nucleotide sequence comprises or consists of nucleotides 76 to 832 of SEQ ID NO: 41. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in plasmid pGEM-T-GH61D23Y4 which is contained in *E. coli* DSM 22882.

In another fourth aspect, the isolated polynucleotides encoding GH61 polypeptides having cellulolytic enhancing activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 43 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least

98%, or at least 99%, which encode a polypeptide having cellulolytic enhancing activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having cellulolytic enhancing activity comprises or consists of the nucleotide sequence of SEQ ID NO: 43. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in plasmid pAG68 which is contained in *E. coli* NRRL B-50320. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 43. In another aspect, the nucleotide sequence comprises or consists of nucleotides 64 to 1104 of SEQ ID NO: 43. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in plasmid pAG68 which is contained in *E. coli* NRRL B-50320.

In another fourth aspect, the isolated polynucleotides encoding GH61 polypeptides having cellulolytic enhancing activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 191 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a GH61 polypeptide having cellulolytic enhancing activity.

In one aspect, the isolated polynucleotide encoding a GH61 polypeptide having cellulolytic enhancing activity comprises or consists of the nucleotide sequence of SEQ ID NO: 191. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in pGEM-T-GH61a51486 which is contained in *E. coli* DSM 22656. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 191. In another aspect, the nucleotide sequence comprises or consists of nucleotides 67 to 868 of SEQ ID NO: 191. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in pGEM-T-GH61a51486 which is contained in *E. coli* NRRL DSM 22656.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having xylanase activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 45 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode an active polypeptide.

In one aspect, the isolated polynucleotide encoding a polypeptide having xylanase activity comprises or consists of the nucleotide sequence of SEQ ID NO: 45. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Aspergillus aculeatus* CBS 101.43. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 45. In another aspect,

the nucleotide sequence comprises or consists of nucleotides 69 to 1314 of SEQ ID NO: 45. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Aspergillus aculeatus* CBS 101.43.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having xylanase activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 47 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode an active polypeptide.

In one aspect, the isolated polynucleotide encoding a polypeptide having xylanase activity comprises or consists of the nucleotide sequence of SEQ ID NO: 47. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in plasmid pHyGe009 which is contained in *E. coli* NRRL B-30703. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 47. In another aspect, the nucleotide sequence comprises or consists of nucleotides 107 to 1415 of SEQ ID NO: 47. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in plasmid pHyGe009 which is contained in *E. coli* NRRL B-30703.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having xylanase activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 49 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode an active polypeptide.

In one aspect, the isolated polynucleotide encoding a polypeptide having xylanase activity comprises or consists of the nucleotide sequence of SEQ ID NO: 49. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in plasmid pTF12Xyl170 which is contained in *E. coli* NRRL B-50309. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 49. In another aspect, the nucleotide sequence comprises or consists of nucleotides 58 to 1194 of SEQ ID NO: 49. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in plasmid pTF12Xyl170 which is contained in *E. coli* NRRL B-50309.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having xylanase activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 51 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least

95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode an active polypeptide.

In one aspect, the isolated polynucleotide encoding a polypeptide having xylanase activity comprises or consists of the nucleotide sequence of SEQ ID NO: 51. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in plasmid pGEM-T-Ppin3 which is contained in *E. coli* DSM 22922. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 51. In another aspect, the nucleotide sequence comprises or consists of nucleotides 58 to 1439 of SEQ ID NO: 51. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in plasmid pGEM-T-Ppin3 which is contained in *E. coli* DSM 22922.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having xylanase activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 53 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode an active polypeptide.

In one aspect, the isolated polynucleotide encoding a polypeptide having xylanase activity comprises or consists of the nucleotide sequence of SEQ ID NO: 53. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Thielavia terrestris* NRRL 8126. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 53. In another aspect, the nucleotide sequence comprises or consists of nucleotides 58 to 1185 of SEQ ID NO: 53. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Thielavia terrestris* NRRL 8126.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having xylanase activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 193 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having xylanase activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having xylanase activity comprises or consists of the nucleotide sequence of SEQ ID NO: 193. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Talaromyces emersonii* NN05002. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 193. In another aspect, the nucleotide sequence comprises or consists of nucleotides 70 to 1383 of SEQ ID NO:

193. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Talaromyces emersonii* NN05002.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having xylanase activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 195 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having xylanase activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having xylanase activity comprises or consists of the nucleotide sequence of SEQ ID NO: 195. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in pMMar26 which is contained in *E. coli* NRRL B-50266. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 195. In another aspect, the nucleotide sequence comprises or consists of nucleotides 70 to 1384 of SEQ ID NO: 195. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in pMMar26 which is contained in *E. coli* NRRL B-50266.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having xylanase activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 197 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having xylanase activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having xylanase activity comprises or consists of the nucleotide sequence of SEQ ID NO: 197. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *E. coli* DSM 10361. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 197. In another aspect, the nucleotide sequence comprises or consists of nucleotides 58 to1188 of SEQ ID NO: 197. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *E. coli* DSM 10361.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having xylanase activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 55 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode an active polypeptide.

In one aspect, the isolated polynucleotide encoding a polypeptide having xylanase activity comprises or consists of the nucleotide sequence of SEQ ID NO: 55. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Thermobifida fusca* DSM 22883. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 55. In another aspect, the nucleotide sequence comprises or consists of nucleotides 127 to 1014 of SEQ ID NO: 55. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Thermobifida fusca* DSM 22883.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having xylanase activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 199 or SEQ ID NO: 304 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having xylanase activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having xylanase activity comprises or consists of the nucleotide sequence of SEQ ID NO: 199 or SEQ ID NO: 304. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Dictyoglomus thermophilum* ATCC 35947. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 199 or SEQ ID NO: 304. In another aspect, the nucleotide sequence comprises or consists of nucleotides 76 to 1137 of SEQ ID NO: 199 or nucleotides 85 to 693 of SEQ ID NO: 304. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Dictyoglomus thermophilum* ATCC 35947.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having beta-xylosidase activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 57 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode an active polypeptide.

In one aspect, the isolated polynucleotide encoding a polypeptide having beta-xylosidase activity comprises or consists of the nucleotide sequence of SEQ ID NO: 57. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Trichoderma reesei* RutC30. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 57. In another aspect, the nucleotide sequence comprises or consists of nucleotides 61 to 2391 of SEQ ID NO: 57. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Trichoderma reesei* RutC30.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having beta-xylosidase activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 59 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode an active polypeptide.

In one aspect, the isolated polynucleotide encoding a polypeptide having beta-xylosidase activity comprises or consists of the nucleotide sequence of SEQ ID NO: 59. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Talaromyces emersonii* CBS 393.64. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 59. In another aspect, the nucleotide sequence comprises or consists of nucleotides 64 to 2388 of SEQ ID NO: 59. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Talaromyces emersonii* CBS 393.64.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having beta-xylosidase activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 201 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having beta-xylosidase activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having beta-xylosidase activity comprises or consists of the nucleotide sequence of SEQ ID NO: 201. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Aspergillus aculeatus* CBS 172.66. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 201. In another aspect, the nucleotide sequence comprises or consists of nucleotides 52 to 2409 of SEQ ID NO: 201. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Aspergillus aculeatus* CBS 172.66.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having beta-xylosidase activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 203 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having beta-xylosidase activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having beta-xylosidase activity comprises or consists of the nucleotide sequence of SEQ ID NO: 203. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in

Aspergillus aculeatus CBS 186.67. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 203. In another aspect, the nucleotide sequence comprises or consists of nucleotides 52 to 2451 of SEQ ID NO: 203. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Aspergillus aculeatus* CBS 186.67.

In another fourth aspect, the isolated polynucleotides encoding polypeptides having beta-xylosidase activity comprise or consist of nucleotide sequences that have a degree of identity to the mature polypeptide coding sequence of SEQ ID NO: 205 of preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, most preferably at least 95%, and even most preferably at least 96%, at least 97%, at least 98%, or at least 99%, which encode a polypeptide having beta-xylosidase activity.

In one aspect, the isolated polynucleotide encoding a polypeptide having beta-xylosidase activity comprises or consists of the nucleotide sequence of SEQ ID NO: 205. In another aspect, the nucleotide sequence comprises or consists of the sequence contained in *Aspergillus fumigatus* NN051616. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence of SEQ ID NO: 205. In another aspect, the nucleotide sequence comprises or consists of nucleotides 61 to 2376 of SEQ ID NO: 205. In another aspect, the nucleotide sequence comprises or consists of the mature polypeptide coding sequence contained in *Aspergillus fumigatus* NN051616.

In a fifth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase I activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 1, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 1, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase I activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 3, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 3, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase I activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 5, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 5, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase I activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 7, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 7, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase I activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 157, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 157, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase I activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 159, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 159, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase I activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 161, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 161, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase I activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 163, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 163, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase I activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 165, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 165, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having

cellobiohydrolase II activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 9, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 9, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase II activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 11, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 11, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase II activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 13, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 13, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase II activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 15, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 15, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase II activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 17, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 17, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase II activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 167, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 167, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase II activity hybridize under preferably medium-high stringency conditions,

more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 169, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 169, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having cellobiohydrolase II activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 171, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 171, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having endoglucanase I activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 19, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 19, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having endoglucanase II activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 21, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 21, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having endoglucanase II activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 23, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 23, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having endoglucanase II activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 25, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 25, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having endoglucanase II activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency

conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 173, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 173, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having endoglucanase II activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 175, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 175, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having beta-glucosidase activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 27, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 27, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having beta-glucosidase activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 29, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 29, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having beta-glucosidase activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 31, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 31, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having beta-glucosidase activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 177, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 177, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having beta-glucosidase activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 179, (ii) the cDNA

sequence of the mature polypeptide coding sequence of SEQ ID NO: 179, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having beta-glucosidase activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 181, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 181, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having beta-glucosidase activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 183, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 183, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having beta-glucosidase activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 185, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 185, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having beta-glucosidase activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 187, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 187, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having beta-glucosidase activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 189, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 189, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding GH61 polypeptides having cellulolytic enhancing activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 33, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 33, or (iii) a

full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding GH61 polypeptides having cellulolytic enhancing activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 35, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 35, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding GH61 polypeptides having cellulolytic enhancing activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 37, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 37, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding GH61 polypeptides having cellulolytic enhancing activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 39, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 39, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding GH61 polypeptides having cellulolytic enhancing activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 41, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 41, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding GH61 polypeptides having cellulolytic enhancing activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 43, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 43, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding GH61 polypeptides having cellulolytic enhancing activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 191, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 191, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having xylanase activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 45, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 45, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having xylanase activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 47, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 47, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having xylanase activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 49, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 49, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having xylanase activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 51, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 51, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having xylanase activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 53, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 53, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having xylanase activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 193, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 193, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having

xylanase activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 195, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 195, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having xylanase activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 197, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 197, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having xylanase activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with the mature polypeptide coding sequence of SEQ ID NO: 55 or its full-length complementary strand.

In another fifth aspect, the isolated polynucleotides encoding polypeptides having xylanase activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 199 or SEQ ID NO: 304, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 199 or SEQ ID NO: 304, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having beta-xylosidase activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 57, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 57, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having beta-xylosidase activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 59, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 59, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having beta-xylosidase activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions

with (i) the mature polypeptide coding sequence of SEQ ID NO: 201, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 201, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having beta-xylosidase activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 203, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 203, or (iii) a full-length complementary strand of (i) or (ii).

In another fifth aspect, the isolated polynucleotides encoding polypeptides having beta-xylosidase activity hybridize under preferably medium-high stringency conditions, more preferably high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 205, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 205, or (iii) a full-length complementary strand of (i) or (ii).

Sources of Polypeptides Having Cellobiohydrolase I, Cellobiohydrolase II, Endoglucanase I, Endoglucanase II, Beta-Glucosidase, Cellulolytic Enhancing, Xylanase, or Beta-Xylosidase Activity

A polypeptide having cellobiohydrolase I, cellobiohydrolase II, endoglucanase I, endoglucanase II, beta-glucosidase, cellulolytic enhancing, xylanase, or beta-xylosidase activity may be obtained from microorganisms of any genus. For purposes of the present invention, the term “obtained from” as used herein in connection with a given source shall mean that the polypeptide encoded by a nucleotide sequence is produced by the source or by a strain in which the nucleotide sequence from the source has been inserted. In a preferred aspect, the polypeptide obtained from a given source is secreted extracellularly.

A polypeptide having cellobiohydrolase I, cellobiohydrolase II, endoglucanase I, endoglucanase II, beta-glucosidase, cellulolytic enhancing, xylanase, or beta-xylosidase activity may be a bacterial polypeptide. For example, the polypeptide may be a gram positive bacterial polypeptide such as a *Bacillus*, *Streptococcus, Streptomyces*, *Staphylococcus*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Clostridium*, *Geobacillus*, or *Oceanobacillus* polypeptide, or a Gram negative bacterial polypeptide such as an *E. coli*, *Pseudomonas*, *Salmonella*, *Campylobacter*, *Helicobacter*, *Flavobacterium*, *Fusobacterium*, *Ilyobacter*, *Neisseria*, or *Ureaplasma* polypeptide.

In a preferred aspect, the polypeptide is a *Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus brevis, Bacillus circulans, Bacillus clausii, Bacillus coagulans, Bacillus firmus, Bacillus lautus, Bacillus lentus, Bacillus licheniformis, Bacillus megaterium,*

Bacillus pumilus, Bacillus stearothermophilus, Bacillus subtilis, or *Bacillus thuringiensis* polypeptide.

In another preferred aspect, the polypeptide is a *Streptococcus equisimilis, Streptococcus pyogenes, Streptococcus uberis,* or *Streptococcus equi* subsp. *Zooepidemicus* polypeptide.

In another preferred aspect, the polypeptide is a *Streptomyces achromogenes*, *Streptomyces avermitilis*, *Streptomyces coelicolor*, *Streptomyces griseus*, or *Streptomyces lividans* polypeptide.

A polypeptide having cellobiohydrolase I, cellobiohydrolase II, endoglucanase I, endoglucanase II, beta-glucosidase, cellulolytic enhancing activity, xylanase, or beta-xylosidase may also be a fungal polypeptide, and more preferably a yeast polypeptide such as a *Candida*, *Kluyveromyces*, *Pichia*, *Saccharomyces*, *Schizosaccharomyces*, or *Yarrowia* polypeptide; or more preferably a filamentous fungal polypeptide such as an *Acremonium*, *Agaricus*, *Alternaria*, *Aspergillus*, *Aureobasidium*, *Botryospaeria*, *Ceriporiopsis*, *Chaetomidium*, *Chrysosporium*, *Claviceps*, *Cochliobolus*, *Coprinopsis*, *Coptotermes*, *Corynascus*, *Cryphonectria*, *Cryptococcus*, *Diplodia*, *Exidia*, *Filibasidium*, *Fusarium*, *Gibberella*, *Holomastigotoides*, *Humicola*, *Irpex*, *Lentinula*, *Leptospaeria*, *Magnaporthe*, *Melanocarpus*, *Meripilus*, *Mucor*, *Myceliophthora*, *Neocallimastix*, *Neurospora*, *Paecilomyces*, *Penicillium*, *Phanerochaete*, *Piromyces*, *Poitrasia*, *Pseudoplectania*, *Pseudotrichonympha*, *Rhizomucor*, *Schizophyllum*, *Scytalidium*, *Talaromyces*, *Thermoascus*, *Thielavia*, *Tolypocladium*, *Trichoderma*, *Trichophaea*, *Verticillium*, *Volvariella*, or *Xylaria* polypeptide.

In a preferred aspect, the polypeptide is a *Saccharomyces carlsbergensis*, *Saccharomyces cerevisiae*, *Saccharomyces diastaticus*, *Saccharomyces douglasii*, *Saccharomyces kluyveri*, *Saccharomyces norbensis*, or *Saccharomyces oviformis* polypeptide.

In another preferred aspect, the polypeptide is an *Acremonium cellulolyticus*, *Aspergillus aculeatus*, *Aspergillus awamori*, *Aspergillus fumigatus*, *Aspergillus foetidus*, *Aspergillus japonicus*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*, *Chrysosporium keratinophilum*, *Chrysosporium lucknowense, Chrysosporium tropicum*, *Chrysosporium merdarium*, *Chrysosporium inops*, *Chrysosporium pannicola*, *Chrysosporium queenslandicum*, *Chrysosporium zonatum*, *Fusarium bactridioides*, *Fusarium cerealis*, *Fusarium crookwellense*, *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium graminum*, *Fusarium heterosporum*, *Fusarium negundi*, *Fusarium oxysporum*, *Fusarium reticulatum*, *Fusarium roseum*, *Fusarium sambucinum*, *Fusarium sarcochroum*, *Fusarium sporotrichioides*, *Fusarium sulphureum*, *Fusarium torulosum*, *Fusarium trichothecioides*, *Fusarium venenatum*, *Humicola grisea*, *Humicola insolens*, *Humicola lanuginosa*, *Irpex*

lacteus, *Mucor miehei*, *Myceliophthora thermophila*, *Neurospora crassa*, *Penicillium funiculosum*, *Penicillium purpurogenum*, *Phanerochaete chrysosporium*, *Thielavia achromatica*, *Thielavia albomyces*, *Thielavia albopilosa*, *Thielavia australeinsis*, *Thielavia fimeti*, *Thielavia microspora*, *Thielavia ovispora*, *Thielavia peruviana*, *Thielavia spededonium*, *Thielavia setosa*, *Thielavia subthermophila*, *Thielavia terrestris*, *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma longibrachiatum*, *Trichoderma reesei*, or *Trichoderma viride* polypeptide.

In another aspect, the polypeptide having cellobiohydrolase I activity is a *Chaetomium thermophilum* CGMCC 0581 Cel7A polypeptide having cellobiohydrolase I activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 2.

In another aspect, the polypeptide having cellobiohydrolase I activity is a *Myceliophthora thermophila* CBS 117.65 Cel7A polypeptide having cellobiohydrolase I activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 4.

In another aspect, the polypeptide having cellobiohydrolase I activity is an *Aspergillus fumigatus* NN055679 Cel7A polypeptide having cellobiohydrolase I activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 6.

In another aspect, the polypeptide having cellobiohydrolase I activity is a *Thermoascus aurantiacus* CGMCC 0583 Cel7A polypeptide having cellobiohydrolase I activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 8.

In another aspect, the polypeptide having cellobiohydrolase I activity is a *Penicillium emersonii* NN051602 Cel7 polypeptide having cellobiohydrolase I activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 158.

In another aspect, the polypeptide having cellobiohydrolase I activity is a *Penicillium pinophilum* NN046877 Cel7 polypeptide having cellobiohydrolase I activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 160.

In another aspect, the polypeptide having cellobiohydrolase I activity is an *Aspergillus terreus* ATCC 28865 Cel7 polypeptide having cellobiohydrolase I activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 162.

In another aspect, the polypeptide having cellobiohydrolase I activity is a *Neosartorya fischeri* NRRL 181 Cel7 polypeptide having cellobiohydrolase I activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 164.

In another aspect, the polypeptide having cellobiohydrolase I activity is an *Aspergillus nidulans* FGSCA4 Cel7 polypeptide having cellobiohydrolase I activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 166.

In another aspect, the polypeptide having cellobiohydrolase II activity is a *Myceliophthora thermophila* CBS 117.65 Cel6A polypeptide having cellobiohydrolase II activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 10.

In another aspect, the polypeptide having cellobiohydrolase II activity is a *Myceliophthora thermophila* CBS 202.75 Cel6B polypeptide having cellobiohydrolase II activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 12.

In another aspect, the polypeptide having cellobiohydrolase II activity is a *Thielavia terrestris* NRRL 8126 Cel6A polypeptide having cellobiohydrolase II activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 14.

In another aspect, the polypeptide having cellobiohydrolase II activity is a *Trichophaea saccata* CBS 804.70 Cel6 polypeptide having cellobiohydrolase II activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 16.

In another aspect, the polypeptide having cellobiohydrolase II activity is a *Aspergillus fumigatus* NN055679 Cel6A polypeptide having cellobiohydrolase II activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 18.

In another aspect, the polypeptide having cellobiohydrolase II activity is a *Fennellia nivea* NN046949 Cel6 polypeptide having cellobiohydrolase II activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 168.

In another aspect, the polypeptide having cellobiohydrolase II activity is a *Penicillium emersonii* NN051602 Cel6A polypeptide having cellobiohydrolase II activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 170.

In another aspect, the polypeptide having cellobiohydrolase II activity is a *Penicillium pinophilum* NN046877 Cel6A polypeptide having cellobiohydrolase II activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 172.

In another aspect, the polypeptide having endoglucanase I activity is an *Aspergillus terreus* ATCC 28865 Cel6A polypeptide having endoglucanase I activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 20.

In another aspect, the polypeptide having endoglucanase II activity is a *Trichoderma reesei* RutC30 Cel5A polypeptide having endoglucanase II activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 22.

In another aspect, the polypeptide having endoglucanase II activity is a *Myceliophthora thermophila* CBS 202.75 Cel5A polypeptide having endoglucanase II activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 24.

In another aspect, the polypeptide having endoglucanase II activity is a *Thermoascus aurantiacus* CGMCC 0670 Cel5A polypeptide having endoglucanase II activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 26.

In another aspect, the polypeptide having endoglucanase II activity is an *Aspergillus fumigatus* NN051616 Cel5 polypeptide having endoglucanase II activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 174.

In another aspect, the polypeptide having endoglucanase II activity is a *Neosartorya*

fischeri NRRL 181 polypeptide having endoglucanase II activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 176.

In another aspect, the polypeptide having beta-glucosidase activity is an *Aspergillus fumigatus* NN055679 Cel5A polypeptide having beta-glucosidase activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 28.

In another aspect, the polypeptide having beta-glucosidase activity is a *Penicillium brasilianum* IBT 20888 Cel5A polypeptide having beta-glucosidase activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 30.

In another aspect, the polypeptide having beta-glucosidase activity is an *Aspergillus niger* IBT 10140 GH3 polypeptide having beta-glucosidase activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 32.

In another aspect, the polypeptide having beta-glucosidase activity is an *Aspergillus aculeatus* WDCM190 Cel3 polypeptide having beta-glucosidase activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 178.

In another aspect, the polypeptide having beta-glucosidase activity is an *Aspergillus kawashii* IFO4308 Cel3 polypeptide having beta-glucosidase activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 180.

In another aspect, the polypeptide having beta-glucosidase activity is an *Aspergillus clavatus* NRRL 1 Cel3 polypeptide having beta-glucosidase activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 182.

In another aspect, the polypeptide having beta-glucosidase activity is a *Thielavia terrestris* NRRL 8126 Cel3 polypeptide having beta-glucosidase activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 184.

In another aspect, the polypeptide having beta-glucosidase activity is a *Penicillium oxalicum* IBT5387 Cel3 polypeptide having beta-glucosidase activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 186.

In another aspect, the polypeptide having beta-glucosidase activity is a *Penicillium oxalicum* IBT5387 Cel3 polypeptide having beta-glucosidase activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 188.

In another aspect, the polypeptide having beta-glucosidase activity is a *Talaromyces emersonii* CBS 549.92 Cel3 polypeptide having beta-glucosidase activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 190.

In another aspect, the polypeptide having cellulolytic enhancing activity is a *Thermoascus aurantiacus* CGMCC 0583 GH61A polypeptide having cellulolytic enhancing activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 34.

In another aspect, the polypeptide having cellulolytic enhancing activity is a *Thielavia terrestris* NRRL 8126 GH61E polypeptide having cellulolytic enhancing activity, *i.e.*, the

polypeptide comprising the mature polypeptide of SEQ ID NO: 36.

In another aspect, the polypeptide having cellulolytic enhancing activity is an *Aspergillus fumigatus* NN051616 GH61B polypeptide having cellulolytic enhancing activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 38.

In another aspect, the polypeptide having cellulolytic enhancing activity is a *Penicillium pinophilum* NN046877 GH61A polypeptide having cellulolytic enhancing activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 40.

In another aspect, the polypeptide having cellulolytic enhancing activity is a *Penicillium* sp. NN051602 GH61A polypeptide having cellulolytic enhancing activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 42.

In another aspect, the polypeptide having cellulolytic enhancing activity is a *Thielavia terrestris* NRRL 8126 GH61N polypeptide having cellulolytic enhancing activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 44.

In another aspect, the polypeptide having cellulolytic enhancing activity is a *Thermoascus crustaceus* CBS 181.67 GH61A polypeptide having cellulolytic enhancing activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 192.

In another aspect, the polypeptide having xylanase activity is an *Aspergillus aculeatus* CBS 101.43 polypeptide having xylanase activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 46.

In another aspect, the polypeptide having xylanase activity is an *Aspergillus fumigatus* NN055679 polypeptide having xylanase activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 48.

In another aspect, the polypeptide having xylanase activity is a *Trichophaea saccata* CBS 804.70 polypeptide having xylanase activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 50.

In another aspect, the polypeptide having xylanase activity is a *Penicillium pinophilum* NN046877 polypeptide having xylanase activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 52.

In another aspect, the polypeptide having xylanase activity is a *Thielavia terrestris* NRRL 8126 polypeptide having xylanase activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 54.

In another aspect, the polypeptide having xylanase activity is a *Talaromyces emersonii* NN050022 polypeptide having xylanase activity, *i.e.*, the polypeptide comprising of the mature polypeptide of SEQ ID NO: 194.

In another aspect, the polypeptide having xylanase activity is a *Penicillium* sp. NN51602 polypeptide having xylanase activity, *i.e.*, the polypeptide comprising of the mature polypeptide of SEQ ID NO: 196.

In another aspect, the polypeptide having xylanase activity is a *Meripilus giganteus* CBS 521.95 polypeptide having xylanase activity, *i.e.*, the polypeptide comprising of the mature polypeptide of SEQ ID NO: 198.

In another aspect, the polypeptide having xylanase activity is a *Thermobifida fusca* DSM 22883 polypeptide having xylanase activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 56.

In another aspect, the polypeptide having xylanase activity is a *Dictyoglomus thermophilum* ATCC 35947 polypeptide having xylanase activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 200 or SEQ ID NO: 305.

In another aspect, the polypeptide having beta-xylosidase activity is a *Trichoderma reesei* RutC30 polypeptide having beta-xylosidase activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 58.

In another aspect, the polypeptide having beta-xylosidase activity is a *Talaromyces emersonii* CBS 393.64 polypeptide having beta-xylosidase activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 60.

In another aspect, the Family 3 polypeptide having beta-xylosidase activity is an *Aspergillus aculeatus* CBS 172.66 polypeptide having beta-xylosidase activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 202.

In another aspect, the Family 3 polypeptide having beta-xylosidase activity is an *Aspergillus aculeatus* CBS 186.67 polypeptide having beta-xylosidase activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 204.

In another aspect, the Family 3 polypeptide having beta-xylosidase activity is an *Aspergillus fumigatus* NN051616 polypeptide having beta-xylosidase activity, *i.e.*, the polypeptide comprising the mature polypeptide of SEQ ID NO: 206.

It will be understood that for the aforementioned species the invention encompasses both the perfect and imperfect states, and other taxonomic equivalents, *e.g.*, anamorphs, regardless of the species name by which they are known. Those skilled in the art will readily recognize the identity of appropriate equivalents.

Strains of these species are readily accessible to the public in a number of culture collections, such as the American Type Culture Collection (ATCC), Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM), Centraalbureau Voor Schimmelcultures (CBS), and Agricultural Research Service Patent Culture Collection, Northern Regional Research Center (NRRL).

Furthermore, such polypeptides may be identified and obtained from other sources including microorganisms isolated from nature (*e.g.*, soil, composts, water, etc.) using the above-mentioned probes. Techniques for isolating microorganisms from natural habitats are well known in the art. The polynucleotide may then be obtained by similarly screening a

genomic or cDNA library of such a microorganism. Once a polynucleotide encoding a polypeptide has been detected with the probe(s), the polynucleotide can be isolated or cloned by utilizing techniques that are well known to those of ordinary skill in the art (see, *e.g.*, Sambrook *et al.*, 1989, *supra*).

Such polypeptides also include fused polypeptides or cleavable fusion polypeptides in which another polypeptide is fused at the N-terminus or the C-terminus of the polypeptide or fragment thereof. A fused polypeptide is produced by fusing a nucleotide sequence (or a portion thereof) encoding another polypeptide to a nucleotide sequence (or a portion thereof) of the present invention. Techniques for producing fusion polypeptides are known in the art, and include ligating the coding sequences encoding the polypeptides so that they are in frame and that expression of the fused polypeptide is under control of the same promoter(s) and terminator.

A fusion polypeptide can further comprise a cleavage site between the two polypeptides. Upon secretion of the fusion protein, the site is cleaved releasing the two polypeptides. Examples of cleavage sites include, but are not limited to, the sites disclosed in Martin *et al.*, 2003, *J. Ind. Microbiol. Biotechnol.* 3: 568-576; Svetina *et al.*, 2000, *J. Biotechnol.* 76: 245-251; Rasmussen-Wilson *et al.*, 1997, *Appl. Environ. Microbiol.* 63: 3488-3493; Ward *et al.*, 1995, *Biotechnology* 13: 498-503; and Contreras *et al.*, 1991, *Biotechnology* 9: 378-381; Eaton *et al.*, 1986, *Biochemistry* 25: 505-512; Collins-Racie *et al.*, 1995, *Biotechnology* 13: 982-987; Carter *et al.*, 1989, *Proteins: Structure, Function, and Genetics* 6: 240-248; and *Stevens, 2003, Drug Discovery World 4: 35-48.*

Nucleic Acid Constructs

A nucleic acid construct comprising an isolated polynucleotide encoding a polypeptide component of an enzyme composition of the present invention may be constructed by operably linking the polynucleotide to one or more (several) control sequences that direct the expression of the coding sequence in a suitable host cell under conditions compatible with the control sequences. Manipulation of the polynucleotide's sequence prior to its insertion into a vector may be desirable or necessary depending on the expression vector. The techniques for modifying polynucleotide sequences utilizing recombinant DNA methods are well known in the art.

The control sequence may be an appropriate promoter sequence, a nucleotide sequence that is recognized by a host cell for expression of a polynucleotide encoding a polypeptide. The promoter sequence contains transcriptional control sequences that mediate the expression of the polypeptide. The promoter may be any nucleotide sequence that shows transcriptional activity in the host cell of choice including mutant, truncated, and hybrid promoters, and may be obtained from genes encoding extracellular or intracellular

polypeptides either homologous or heterologous to the host cell.

Examples of suitable promoters for directing the transcription of the nucleic acid constructs of the present invention, especially in a bacterial host cell, are the promoters obtained from the *E. coli lac* operon, *Streptomyces coelicolor* agarase gene (*dagA*), *Bacillus subtilis* levansucrase gene (*sacB*), *Bacillus licheniformis* alpha-amylase gene (*amyL*), *Bacillus stearothermophilus* maltogenic amylase gene (*amyM*), *Bacillus amyloliquefaciens* alpha-amylase gene (*amyQ*), *Bacillus licheniformis* penicillinase gene (*penP*), *Bacillus subtilis xylA* and *xylB* genes, and prokaryotic beta-lactamase gene (Villa-Kamaroff *et al.*, 1978, *Proceedings of the National Academy of Sciences USA* 75: 3727-3731), as well as the *tac* promoter (DeBoer *et al.*, 1983, *Proceedings of the National Academy of Sciences USA* 80: 21-25). Further promoters are described in "Useful proteins from recombinant bacteria" in *Scientific American*, 1980, 242: 74-94; and in Sambrook *et al.*, 1989, *supra*.

Examples of suitable promoters for directing the transcription of the nucleic acid constructs in a filamentous fungal host cell are promoters obtained from the genes for *Aspergillus nidulans* acetamidase, *Aspergillus niger* neutral alpha-amylase, *Aspergillus niger* acid stable alpha-amylase, *Aspergillus niger* or *Aspergillus awamori* glucoamylase (*glaA*), *Aspergillus oryzae* TAKA amylase, *Aspergillus oryzae* alkaline protease, *Aspergillus oryzae* triose phosphate isomerase, *Fusarium oxysporum* trypsin-like protease (WO 96/00787), *Fusarium venenatum* amyloglucosidase (WO 00/56900), *Fusarium venenatum* Daria (WO 00/56900), *Fusarium venenatum* Quinn (WO 00/56900), *Rhizomucor miehei* lipase, *Rhizomucor miehei* aspartic proteinase, *Trichoderma reesei* beta-glucosidase, *Trichoderma reesei* cellobiohydrolase I, *Trichoderma reesei* cellobiohydrolase II, *Trichoderma reesei* endoglucanase I, *Trichoderma reesei* endoglucanase II, *Trichoderma reesei* endoglucanase III, *Trichoderma reesei* endoglucanase IV, *Trichoderma reesei* endoglucanase V, *Trichoderma reesei* xylanase I, *Trichoderma reesei* xylanase II, *Trichoderma reesei* beta-xylosidase, as well as the NA2-tpi promoter (a modified promoter from a gene encoding a neutral alpha-amylase in *Aspergilli* in which the untranslated leader has been replaced by an untranslated leader from a gene encoding triose phosphate isomerase in *Aspergilli*; non-limiting examples include modified promoters from the gene encoding neutral alpha-amylase in *Aspergillus niger* in which the untranslated leader has been replaced by an untranslated leader from the gene encoding triose phosphate isomerase in *Aspergillus nidulans* or *Aspergillus oryzae*); and mutant, truncated, and hybrid promoters thereof.

In a yeast host, useful promoters are obtained from the genes for *Saccharomyces cerevisiae* enolase (ENO-1), *Saccharomyces cerevisiae* galactokinase (GAL1), *Saccharomyces cerevisiae* alcohol dehydrogenase/glyceraldehyde-3-phosphate dehydrogenase (ADH1, ADH2/GAP), *Saccharomyces cerevisiae* triose phosphate isomerase (TPI), *Saccharomyces cerevisiae* metallothionein (CUP1), and *Saccharomyces*

cerevisiae 3-phosphoglycerate kinase. Other useful promoters for yeast host cells are described by Romanos *et al.*, 1992, *Yeast* 8: 423-488.

The control sequence may also be a suitable transcription terminator sequence, a sequence recognized by a host cell to terminate transcription. The terminator sequence is operably linked to the 3' terminus of the nucleotide sequence encoding the polypeptide. Any terminator that is functional in the host cell of choice may be used in the present invention.

Preferred terminators for filamentous fungal host cells are obtained from the genes for *Aspergillus nidulans* anthranilate synthase, *Aspergillus niger* glucoamylase, *Aspergillus niger* alpha-glucosidase, *Aspergillus oryzae* TAKA amylase, and *Fusarium oxysporum* trypsin-like protease.

Preferred terminators for yeast host cells are obtained from the genes for *Saccharomyces cerevisiae* enolase, *Saccharomyces cerevisiae* cytochrome C (CYC1), and *Saccharomyces cerevisiae* glyceraldehyde-3-phosphate dehydrogenase. Other useful terminators for yeast host cells are described by Romanos *et al.*, 1992, *supra*.

The control sequence may also be a suitable leader sequence, a nontranslated region of an mRNA that is important for translation by the host cell. The leader sequence is operably linked to the 5' terminus of the nucleotide sequence encoding the polypeptide. Any leader sequence that is functional in the host cell of choice may be used in the present invention.

Preferred leaders for filamentous fungal host cells are obtained from the genes for *Aspergillus oryzae* TAKA amylase and *Aspergillus nidulans* triose phosphate isomerase.

Suitable leaders for yeast host cells are obtained from the genes for *Saccharomyces cerevisiae* enolase (ENO-1), *Saccharomyces cerevisiae* 3-phosphoglycerate kinase, *Saccharomyces cerevisiae* alpha-factor, and *Saccharomyces cerevisiae* alcohol dehydrogenase/glyceraldehyde-3-phosphate dehydrogenase (ADH2/GAP).

The control sequence may also be a polyadenylation sequence, a sequence operably linked to the 3' terminus of the nucleotide sequence and, when transcribed, is recognized by the host cell as a signal to add polyadenosine residues to transcribed mRNA. Any polyadenylation sequence that is functional in the host cell of choice may be used in the present invention.

Preferred polyadenylation sequences for filamentous fungal host cells are obtained from the genes for *Aspergillus oryzae* TAKA amylase, *Aspergillus niger* glucoamylase, *Aspergillus nidulans* anthranilate synthase, *Fusarium oxysporum* trypsin-like protease, and *Aspergillus niger* alpha-glucosidase.

Useful polyadenylation sequences for yeast host cells are described by Guo and Sherman, 1995, *Molecular Cellular Biology* 15: 5983-5990.

The control sequence may also be a signal peptide coding region that encodes a signal peptide linked to the N-terminus of a polypeptide and directs the polypeptide into the cell's secretory pathway. The 5'-end of the coding sequence of the polynucleotide may inherently contain a signal peptide coding sequence naturally linked in translation reading frame with the segment of the coding sequence that encodes the polypeptide. Alternatively, the 5'-end of the coding sequence may contain a signal peptide coding sequence that is foreign to the coding sequence. The foreign signal peptide coding sequence may be required where the coding sequence does not naturally contain a signal peptide coding sequence. Alternatively, the foreign signal peptide coding sequence may simply replace the natural signal peptide coding sequence in order to enhance secretion of the polypeptide. However, any signal peptide coding sequence that directs the expressed polypeptide into the secretory pathway of a host cell of choice may be used.

Effective signal peptide coding sequences for bacterial host cells are the signal peptide coding sequences obtained from the genes for *Bacillus* NCIB 11837 maltogenic amylase, *Bacillus licheniformis* subtilisin, *Bacillus licheniformis* beta-lactamase, *Bacillus stearothermophilus* alpha-amylase, *Bacillus stearothermophilus* neutral proteases (*nprT*, *nprS*, *nprM*), and *Bacillus subtilis prsA*. Further signal peptides are described by Simonen and Palva, 1993, *Microbiological Reviews* 57: 109-137.

Effective signal peptide coding sequences for filamentous fungal host cells are the signal peptide coding sequences obtained from the genes for *Aspergillus niger* neutral amylase, *Aspergillus niger* glucoamylase, *Aspergillus oryzae* TAKA amylase, *Humicola insolens* cellulase, *Humicola insolens* endoglucanase V, *Humicola lanuginosa* lipase, and *Rhizomucor miehei* aspartic proteinase.

Useful signal peptides for yeast host cells are obtained from the genes for *Saccharomyces cerevisiae* alpha-factor and *Saccharomyces cerevisiae* invertase. Other useful signal peptide coding sequences are described by Romanos *et al.*, 1992, *supra*.

The control sequence may also be a propeptide coding sequence that encodes a propeptide positioned at the N-terminus of a polypeptide. The resultant polypeptide is known as a proenzyme or propolypeptide (or a zymogen in some cases). A propolypeptide is generally inactive and can be converted to an active polypeptide by catalytic or autocatalytic cleavage of the propeptide from the propolypeptide. The propeptide coding sequence may be obtained from the genes for *Bacillus subtilis* alkaline protease (*aprE*), *Bacillus subtilis* neutral protease (*nprT*), *Myceliophthora thermophila* laccase (WO 95/33836), *Rhizomucor miehei* aspartic proteinase, and *Saccharomyces cerevisiae* alpha-factor.

Where both signal peptide and propeptide sequences are present at the N-terminus of a polypeptide, the propeptide sequence is positioned next to the N-terminus of a polypeptide and the signal peptide sequence is positioned next to the N-terminus of the

propeptide sequence.

It may also be desirable to add regulatory sequences that allow the regulation of the expression of the polypeptide relative to the growth of the host cell. Examples of regulatory systems are those that cause the expression of the gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Regulatory systems in prokaryotic systems include the *lac*, *tac*, and *trp* operator systems. In yeast, the ADH2 system or GAL1 system may be used. In filamentous fungi, the *Aspergillus niger* glucoamylase promoter, *Aspergillus* oryzae TAKA alpha-amylase promoter, and *Aspergillus oryzae* glucoamylase promoter may be used. Other examples of regulatory sequences are those that allow for gene amplification. In eukaryotic systems, these regulatory sequences include the dihydrofolate reductase gene that is amplified in the presence of methotrexate, and the metallothionein genes that are amplified with heavy metals. In these cases, the polynucleotide encoding the polypeptide would be operably linked with the regulatory sequence.

Expression Vectors

The various nucleic acids and control sequences described herein may be joined together to construct a recombinant expression vector that may include one or more (several) convenient restriction sites to allow for insertion or substitution of an isolated polynucleotide encoding a polypeptide component of the enzyme composition at such sites. Alternatively, a polynucleotide sequence may be expressed by inserting the nucleotide sequence or a nucleic acid construct comprising the sequence into an appropriate vector for expression. In creating the expression vector, the coding sequence is located in the vector so that the coding sequence is operably linked with the appropriate control sequences for expression.

The recombinant expression vector may be any vector (*e.g.*, a plasmid or virus) that can be conveniently subjected to recombinant DNA procedures and can bring about expression of the nucleotide sequence. The choice of the vector will typically depend on the compatibility of the vector with the host cell into which the vector is to be introduced. The vectors may be linear or closed circular plasmids.

The vector may be an autonomously replicating vector, *i.e.*, a vector that exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, *e.g.*, a plasmid, an extrachromosomal element, a minichromosome, or an artificial chromosome. The vector may contain any means for assuring self-replication. Alternatively, the vector may be one that, when introduced into the host cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. Furthermore, a single vector or plasmid or two or more vectors or plasmids that together

contain the total DNA to be introduced into the genome of the host cell, or a transposon, may be used.

The vectors preferably contain one or more (several) selectable markers that permit easy selection of transformed, transfected, transduced, or the like cells. A selectable marker is a gene the product of which provides for biocide or viral resistance, resistance to heavy metals, prototrophy to auxotrophs, and the like.

Examples of bacterial selectable markers are the *dal* genes from *Bacillus subtilis* or *Bacillus licheniformis*, or markers that confer antibiotic resistance such as ampicillin, kanamycin, chloramphenicol, or tetracycline resistance. Suitable markers for yeast host cells are ADE2, HIS3, LEU2, LYS2, MET3, TRP1, and URA3. Selectable markers for use in a filamentous fungal host cell include, but are not limited to, *amdS* (acetamidase), *argB* (ornithine carbamoyltransferase), *bar* (phosphinothricin acetyltransferase), *hph* (hygromycin phosphotransferase), *niaD* (nitrate reductase), *pyrG* (orotidine-5'-phosphate decarboxylase), *sC* (sulfate adenyltransferase), and *trpC* (anthranilate synthase), as well as equivalents thereof. Preferred for use in an *Aspergillus* cell are the *amdS* and *pyrG* genes of *Aspergillus nidulans* or *Aspergillus oryzae* and the *bar* gene of *Streptomyces hygroscopicus*.

The vectors preferably contain an element(s) that permits integration of the vector into the host cell's genome or autonomous replication of the vector in the cell independent of the genome.

For integration into the host cell genome, the vector may rely on the polynucleotide's sequence encoding the polypeptide or any other element of the vector for integration into the genome by homologous or nonhomologous recombination. Alternatively, the vector may contain additional nucleotide sequences for directing integration by homologous recombination into the genome of the host cell at a precise location(s) in the chromosome(s). To increase the likelihood of integration at a precise location, the integrational elements should preferably contain a sufficient number of nucleic acids, such as 100 to 10,000 base pairs, preferably 400 to 10,000 base pairs, and most preferably 800 to 10,000 base pairs, which have a high degree of identity to the corresponding target sequence to enhance the probability of homologous recombination. The integrational elements may be any sequence that is homologous with the target sequence in the genome of the host cell. Furthermore, the integrational elements may be non-encoding or encoding nucleotide sequences. On the other hand, the vector may be integrated into the genome of the host cell by non-homologous recombination.

For autonomous replication, the vector may further comprise an origin of replication enabling the vector to replicate autonomously in the host cell in question. The origin of replication may be any plasmid replicator mediating autonomous replication that functions in a cell. The term "origin of replication" or "plasmid replicator" is defined herein as a nucleotide

sequence that enables a plasmid or vector to replicate *in vivo*.

Examples of bacterial origins of replication are the origins of replication of plasmids pBR322, pUC19, pACYC177, and pACYC184 permitting replication in *E. coli*, and pUB110, pE194, pTA1060, and pAMß1 permitting replication in *Bacillus*.

Examples of origins of replication for use in a yeast host cell are the 2 micron origin of replication, ARS1, ARS4, the combination of ARS1 and CEN3, and the combination of ARS4 and CEN6.

Examples of origins of replication useful in a filamentous fungal cell are AMA1 and ANS1 (Gems *et al.*, 1991, *Gene* 98: 61-67; Cullen *et al.*, 1987, *Nucleic Acids Research* 15: 9163-9175; WO 00/24883). Isolation of the AMA1 gene and construction of plasmids or vectors comprising the gene can be accomplished according to the methods disclosed in WO 00/24883.

More than one copy of a polynucleotide may be inserted into a host cell to increase production of the gene product. An increase in the copy number of the polynucleotide can be obtained by integrating at least one additional copy of the sequence into the host cell genome or by including an amplifiable selectable marker gene with the polynucleotide where cells containing amplified copies of the selectable marker gene, and thereby additional copies of the polynucleotide, can be selected for by cultivating the cells in the presence of the appropriate selectable agent.

The procedures used to ligate the elements described above to construct the recombinant expression vectors are well known to one skilled in the art (see, *e.g.*, Sambrook *et al.*, 1989, *supra*).

Host Cells

The present invention also relates to recombinant host cells, comprising one or more isolated polynucleotides encoding polypeptide components of the enzyme composition, which are advantageously used in the recombinant production of the polypeptides. A vector is introduced into a host cell so that the vector is maintained as a chromosomal integrant or as a self-replicating extra-chromosomal vector as described earlier. The term "host cell" encompasses any progeny of a parent cell that is not identical to the parent cell due to mutations that occur during replication. The choice of a host cell will to a large extent depend upon the gene encoding the polypeptide and its source.

The host cell may be any cell useful in the recombinant production of a polypeptide of the present invention, *e.g.*, a prokaryote or a eukaryote.

The prokaryotic host cell may be any Gram positive bacterium or a Gram negative bacterium. Gram positive bacteria include, but not limited to, *Bacillus*, *Streptococcus,* *Streptomyces*, *Staphylococcus*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Clostridium*,

Geobacillus, and *Oceanobacillus*. Gram negative bacteria include, but not limited to, *E. coli*, *Pseudomonas*, *Salmonella*, *Campylobacter*, *Helicobacter*, *Flavobacterium*, *Fusobacterium*, *Ilyobacter*, *Neisseria*, and *Ureaplasma*.

The bacterial host cell may be any *Bacillus* cell. *Bacillus* cells useful in the practice of the present invention include, but are not limited to, *Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus brevis, Bacillus circulans, Bacillus clausii*, *Bacillus coagulans, Bacillus firmus, Bacillus lautus, Bacillus lentus, Bacillus licheniformis, Bacillus megaterium, Bacillus pumilus, Bacillus stearothermophilus, Bacillus subtilis*, and *Bacillus thuringiensis* cells.

In a preferred aspect, the bacterial host cell is a *Bacillus amyloliquefaciens, Bacillus lentus, Bacillus licheniformis, Bacillus stearothermophilus* or *Bacillus subtilis* cell. In a more preferred aspect, the bacterial host cell is a *Bacillus amyloliquefaciens* cell. In another more preferred aspect, the bacterial host cell is a *Bacillus clausii* cell. In another more preferred aspect, the bacterial host cell is a *Bacillus licheniformis* cell. In another more preferred aspect, the bacterial host cell is a *Bacillus subtilis* cell.

The bacterial host cell may also be any *Streptococcus* cell. *Streptococcus* cells useful in the practice of the present invention include, but are not limited to, *Streptococcus equisimilis, Streptococcus pyogenes, Streptococcus uberis,* and *Streptococcus equi* subsp. *Zooepidemicus* cells.

In a preferred aspect, the bacterial host cell is a *Streptococcus equisimilis* cell. In another preferred aspect, the bacterial host cell is a *Streptococcus pyogenes* cell. In another preferred aspect, the bacterial host cell is a *Streptococcus uberis* cell. In another preferred aspect, the bacterial host cell is a *Streptococcus equi* subsp. *Zooepidemicus* cell.

The bacterial host cell may also be any *Streptomyces* cell. *Streptomyces* cells useful in the practice of the present invention include, but are not limited to, *Streptomyces achromogenes*, *Streptomyces avermitilis*, *Streptomyces coelicolor*, *Streptomyces griseus*, and *Streptomyces lividans* cells.

In a preferred aspect, the bacterial host cell is a *Streptomyces achromogenes* cell. In another preferred aspect, the bacterial host cell is a *Streptomyces avermitilis* cell. In another preferred aspect, the bacterial host cell is a *Streptomyces coelicolor* cell. In another preferred aspect, the bacterial host cell is a *Streptomyces griseus* cell. In another preferred aspect, the bacterial host cell is a *Streptomyces lividans* cell.

The introduction of DNA into a *Bacillus* cell may, for instance, be effected by protoplast transformation (see, *e.g.*, Chang and Cohen, 1979, *Molecular General Genetics* 168: 111-115), by using competent cells (see, *e.g.*, Young and Spizizen, 1961, *Journal of Bacteriology* 81: 823-829, or Dubnau and Davidoff-Abelson, 1971, *Journal of Molecular Biology* 56: 209-221), by electroporation (see, *e.g.*, Shigekawa and Dower, 1988,

Biotechniques 6: 742-751), or by conjugation (see, *e.g.*, Koehler and Thorne, 1987, *Journal of Bacteriology* 169: 5271-5278). The introduction of DNA into an *E coli* cell may, for instance, be effected by protoplast transformation (see, *e.g.*, Hanahan, 1983, *J. Mol. Biol.* 166: 557-580) or electroporation (see, *e.g.*, Dower *et al.*, 1988, *Nucleic Acids Res.* 16: 6127-6145). The introduction of DNA into a *Streptomyces* cell may, for instance, be effected by protoplast transformation and electroporation (see, *e.g.*, Gong *et al.*, 2004, *Folia Microbiol. (Praha)* 49: 399-405), by conjugation (see, *e.g.*, Mazodier *et al.*, 1989, *J. Bacteriol.* 171: 3583-3585), or by transduction (see, *e.g.*, Burke *et al.*, 2001, *Proc. Natl. Acad. Sci. USA* 98: 6289-6294). The introduction of DNA into a *Pseudomonas* cell may, for instance, be effected by electroporation (see, *e.g.*, Choi *et al.*, 2006, *J. Microbiol. Methods* 64: 391-397) or by conjugation (see, *e.g.*, Pinedo and Smets, 2005, *Appl. Environ. Microbiol.* 71: 51-57). The introduction of DNA into a *Streptococcus* cell may, for instance, be effected by natural competence (see, *e.g.*, Perry and Kuramitsu, 1981, *Infect. Immun.* 32: 1295-1297), by protoplast transformation (see, *e.g.*, Catt and Jollick, 1991, *Microbios.* 68: 189-207, by electroporation (see, *e.g.*, Buckley *et al.*, 1999, *Appl. Environ. Microbiol.* 65: 3800-3804) or by conjugation (see, *e.g.*, Clewell, 1981, *Microbiol. Rev.* 45: 409-436). However, any method known in the art for introducing DNA into a host cell can be used.

The host cell may also be a eukaryote, such as a mammalian, insect, plant, or fungal cell.

In a preferred aspect, the host cell is a fungal cell. "Fungi" as used herein includes the phyla Ascomycota, Basidiomycota, Chytridiomycota, and Zygomycota (as defined by Hawksworth *et al.*, *In*, *Ainsworth and Bisby's Dictionary of The Fungi*, 8th edition, 1995, CAB International, University Press, Cambridge, UK) as well as the Oomycota (as cited in Hawksworth *et al.*, 1995, *supra*, page 171) and all mitosporic fungi (Hawksworth *et al.*, 1995, *supra*).

In a more preferred aspect, the fungal host cell is a yeast cell. "Yeast" as used herein includes ascosporogenous yeast (Endomycetales), basidiosporogenous yeast, and yeast belonging to the Fungi Imperfecti (Blastomycetes). Since the classification of yeast may change in the future, for the purposes of this invention, yeast shall be defined as described in *Biology and Activities of Yeast* (Skinner, F.A., Passmore, S.M., and Davenport, R.R., eds, *Soc. App. Bacteriol. Symposium Series* No. 9, 1980).

In an even more preferred aspect, the yeast host cell is a *Candida*, *Hansenula*, *Kluyveromyces*, *Pichia*, *Saccharomyces*, *Schizosaccharomyces*, or *Yarrowia* cell.

In a most preferred aspect, the yeast host cell is a *Saccharomyces carlsbergensis*, *Saccharomyces cerevisiae*, *Saccharomyces diastaticus*, *Saccharomyces douglasii*, *Saccharomyces kluyveri*, *Saccharomyces norbensis*, or *Saccharomyces oviformis* cell. In another most preferred aspect, the yeast host cell is a *Kluyveromyces lactis* cell. In another

most preferred aspect, the yeast host cell is a *Yarrowia lipolytica* cell.

In another more preferred aspect, the fungal host cell is a filamentous fungal cell. "Filamentous fungi" include all filamentous forms of the subdivision Eumycota and Oomycota (as defined by Hawksworth *et al.*, 1995, *supra*). The filamentous fungi are generally characterized by a mycelial wall composed of chitin, cellulose, glucan, chitosan, mannan, and other complex polysaccharides. Vegetative growth is by hyphal elongation and carbon catabolism is obligately aerobic. In contrast, vegetative growth by yeasts such as *Saccharomyces cerevisiae* is by budding of a unicellular thallus and carbon catabolism may be fermentative.

In an even more preferred aspect, the filamentous fungal host cell is an *Acremonium*, *Aspergillus*, *Aureobasidium*, *Bjerkandera*, *Ceriporiopsis*, *Chrysosporium*, *Coprinus*, *Coriolus*, *Cryptococcus*, *Filibasidium*, *Fusarium*, *Humicola*, *Magnaporthe*, *Mucor*, *Myceliophthora*, *Neocallimastix*, *Neurospora*, *Paecilomyces*, *Penicillium*, *Phanerochaete*, *Phlebia*, *Piromyces*, *Pleurotus*, *Schizophyllum*, *Talaromyces*, *Thermoascus*, *Thielavia*, *Tolypocladium*, *Trametes*, or *Trichoderma* cell.

In a most preferred aspect, the filamentous fungal host cell is an *Aspergillus awamori*, *Aspergillus fumigatus*, *Aspergillus foetidus*, *Aspergillus japonicus*, *Aspergillus nidulans*, *Aspergillus niger* or *Aspergillus oryzae* cell. In another most preferred aspect, the filamentous fungal host cell is a *Fusarium bactridioides*, *Fusarium cerealis*, *Fusarium crookwellense*, *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium graminum*, *Fusarium heterosporum*, *Fusarium negundi*, *Fusarium oxysporum*, *Fusarium reticulatum*, *Fusarium roseum*, *Fusarium sambucinum*, *Fusarium sarcochroum*, *Fusarium sporotrichioides*, *Fusarium sulphureum*, *Fusarium torulosum*, *Fusarium trichothecioides*, or *Fusarium venenatum* cell. In another most preferred aspect, the filamentous fungal host cell is a *Bjerkandera adusta*, *Ceriporiopsis aneirina*, *Ceriporiopsis aneirina*, *Ceriporiopsis caregiea*, *Ceriporiopsis gilvescens*, *Ceriporiopsis pannocinta*, *Ceriporiopsis rivulosa*, *Ceriporiopsis subrufa*, *Ceriporiopsis subvermispora*, *Chrysosporium keratinophilum*, *Chrysosporium lucknowense, Chrysosporium tropicum*, *Chrysosporium merdarium*, *Chrysosporium inops*, *Chrysosporium pannicola*, *Chrysosporium queenslandicum*, *Chrysosporium zonatum*, *Coprinus cinereus*, *Coriolus hirsutus*, *Humicola insolens*, *Humicola lanuginosa*, *Mucor miehei*, *Myceliophthora thermophila*, *Neurospora crassa*, *Penicillium purpurogenum*, *Phanerochaete chrysosporium*, *Phlebia radiata*, *Pleurotus eryngii*, *Thielavia terrestris*, *Trametes villosa*, *Trametes versicolor*, *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma longibrachiatum*, *Trichoderma reesei*, or *Trichoderma viride* cell.

Fungal cells may be transformed by a process involving protoplast formation, transformation of the protoplasts, and regeneration of the cell wall in a manner known *per se*. Suitable procedures for transformation of *Aspergillus* and *Trichoderma* host cells are

described in EP 238 023 and Yelton *et al.*, 1984, *Proceedings of the National Academy of Sciences USA* 81: 1470-1474. Suitable methods for transforming *Fusarium* species are described by Malardier *et al.*, 1989, *Gene* 78: 147-156, and WO 96/00787. Yeast may be transformed using the procedures described by Becker and Guarente, *In* Abelson, J.N. and Simon, M.I., editors, *Guide to Yeast Genetics and Molecular Biology*, *Methods in Enzymology*, Volume 194, pp 182-187, Academic Press, Inc., New York; Ito *et al.*, 1983, *Journal of Bacteriology* 153: 163; and Hinnen *et al.*, 1978, *Proceedings of the National Academy of Sciences USA* 75: 1920.

Methods of Production

The present invention also relates to methods of producing an enzyme composition of the present invention, comprising: (a) cultivating a recombinant host cell, as described herein, under conditions conducive for production of the enzyme composition; and (b) recovering the enzyme composition.

In the production methods of the present invention, the cells are cultivated in a nutrient medium suitable for production of the enzyme composition using methods well known in the art. For example, the cell may be cultivated by shake flask cultivation, and small-scale or large-scale fermentation (including continuous, batch, fed-batch, or solid state fermentations) in laboratory or industrial fermentors performed in a suitable medium and under conditions allowing the enzyme composition to be expressed and/or isolated. The cultivation takes place in a suitable nutrient medium comprising carbon and nitrogen sources and inorganic salts, using procedures known in the art. Suitable media are available from commercial suppliers or may be prepared according to published compositions (*e.g.*, in catalogues of the American Type Culture Collection). If the components of the enzyme composition are secreted into the nutrient medium, the enzyme composition can be recovered directly from the medium. If the components of the enzyme composition are not secreted into the medium, the enzyme composition can be recovered from cell lysates.

The polypeptide components may be detected using methods known in the art that are specific for the polypeptides. These detection methods may include use of specific antibodies, formation of an enzyme product, or disappearance of an enzyme substrate.

The resulting enzyme composition may be recovered using methods known in the art. For example, the enzyme composition may be recovered from the nutrient medium by conventional procedures including, but not limited to, centrifugation, filtration, extraction, spray-drying, evaporation, or precipitation.

An enzyme composition of the present invention may be purified by a variety of procedures known in the art including, but not limited to, chromatography (*e.g.*, ion exchange, affinity, hydrophobic, chromatofocusing, and size exclusion), electrophoretic

procedures (*e.g.*, preparative isoelectric focusing), differential solubility (*e.g.*, ammonium sulfate precipitation), SDS-PAGE, or extraction (see, *e.g.*, *Protein Purification*, J.-C. Janson and Lars Ryden, editors, VCH Publishers, New York, 1989) to obtain substantially pure polypeptides.

Methods of Processing Cellulosic Material

The present invention also relates to methods for degrading or converting a cellulosic material, comprising: treating the cellulosic material with an enzyme composition of the present invention. In a preferred aspect, the method further comprises recovering the degraded or converted cellulosic material.

The present invention also relates to methods of producing a fermentation product, comprising: (a) saccharifying a cellulosic material with an enzyme composition of the present invention; (b) fermenting the saccharified cellulosic material with one or more fermenting microorganisms to produce the fermentation product; and (c) recovering the fermentation product from the fermentation.

The present invention also relates to methods of fermenting a cellulosic material, comprising: fermenting the cellulosic material with one or more fermenting microorganisms, wherein the cellulosic material is saccharified with an enzyme composition of the present invention. In a preferred aspect, the fermenting of the cellulosic material produces a fermentation product. In another preferred aspect, the method further comprises recovering the fermentation product from the fermentation.

The methods of the present invention can be used to saccharify a cellulosic material to fermentable sugars and convert the fermentable sugars to many useful substances, *e.g.*, chemicals and fuels. The production of a desired fermentation product from cellulosic material typically involves pretreatment, enzymatic hydrolysis (saccharification), and fermentation.

The processing of cellulosic material according to the present invention can be accomplished using processes conventional in the art. Moreover, the methods of the present invention can be implemented using any conventional biomass processing apparatus configured to operate in accordance with the invention.

Hydrolysis (saccharification) and fermentation, separate or simultaneous, include, but are not limited to, separate hydrolysis and fermentation (SHF); simultaneous saccharification and fermentation (SSF); simultaneous saccharification and cofermentation (SSCF); hybrid hydrolysis and fermentation (HHF); separate hydrolysis and co-fermentation (SHCF), (hybrid hydrolysis and fermentation (HHCF), and direct microbial conversion (DMC). SHF uses separate process steps to first enzymatically hydrolyze lignocellulose to fermentable sugars, *e.g.,* glucose, cellobiose, cellotriose, and pentose sugars, and then ferment the fermentable sugars to ethanol. In SSF, the enzymatic hydrolysis of lignocellulose and the fermentation of

sugars to ethanol are combined in one step (Philippidis, G. P., 1996, Cellulose bioconversion technology, in *Handbook on Bioethanol: Production and Utilization*, Wyman, C. E., ed., Taylor & Francis, Washington, DC, 179-212). SSCF involves the cofermentation of multiple sugars (Sheehan, J., and Himmel, M., 1999, Enzymes, energy and the environment: A strategic perspective on the U.S. Department of Energy's research and development activities for bioethanol, *Biotechnol. Prog.* 15: 817-827). HHF involves a separate hydrolysis step, and in addition a simultaneous saccharification and hydrolysis step, which can be carried out in the same reactor. The steps in an HHF process can be carried out at different temperatures, *i.e.*, high temperature enzymatic saccharification followed by SSF at a lower temperature that the fermentation strain can tolerate. DMC combines all three processes (enzyme production, lignocellulose hydrolysis, and fermentation) in one or more steps where the same organism is used to produce the enzymes for conversion of the lignocellulose to fermentable sugars and to convert the fermentable sugars into a final product (Lynd, L. R., Weimer, P. J., van Zyl, W. H., and Pretorius, I. S., 2002, Microbial cellulose utilization: Fundamentals and biotechnology, *Microbiol. Mol. Biol. Reviews* 66: 506-577). It is understood herein that any method known in the art comprising pretreatment, enzymatic hydrolysis (saccharification), fermentation, or a combination thereof can be used in the practicing the methods of the present invention.

A conventional apparatus can include a fed-batch stirred reactor, a batch stirred reactor, a continuous flow stirred reactor with ultrafiltration, and/or a continuous plug-flow column reactor (Fernanda de Castilhos Corazza, Flávio Faria de Moraes, Gisella Maria Zanin and Ivo Neitzel, 2003, Optimal control in fed-batch reactor for the cellobiose hydrolysis, *Acta Scientiarum. Technology* 25: 33-38; Gusakov, A. V., and Sinitsyn, A. P., 1985, Kinetics of the enzymatic hydrolysis of cellulose: 1. A mathematical model for a batch reactor process, *Enz. Microb. Technol.* 7: 346-352), an attrition reactor (Ryu, S. K., and Lee, J. M., 1983, Bioconversion of waste cellulose by using an attrition bioreactor, *Biotechnol. Bioeng.* 25: 53-65), or a reactor with intensive stirring induced by an electromagnetic field (Gusakov, A. V., Sinitsyn, A. P., Davydkin, I. Y., Davydkin, V. Y., Protas, O. V., 1996, Enhancement of enzymatic cellulose hydrolysis using a novel type of bioreactor with intensive stirring induced by electromagnetic field, *Appl. Biochem. Biotechnol.* 56: 141-153). Additional reactor types include fluidized bed, upflow blanket, immobilized, and extruder type reactors for hydrolysis and/or fermentation.

Pretreatment. In practicing the methods of the present invention, any pretreatment process known in the art can be used to disrupt the plant cell wall components (Chandra *et al.*, 2007, Substrate pretreatment: The key to effective enzymatic hydrolysis of lignocellulosics? *Adv. Biochem. Engin./Biotechnol.* 108: 67-93; Galbe and Zacchi, 2007, Pretreatment of lignocellulosic materials for efficient bioethanol production, *Adv. Biochem.*

Engin. / Biotechnol. 108: 41-65; Hendriks and Zeeman, 2009, Pretreatments to enhance the digestibility of lignocellulosic biomass, *Bioresource Technol.* 100: 10-18; Mosier *et al.*, 2005, Features of promising technologies for pretreatment of lignocellulosic biomass, *Bioresource Technol.* 96: 673-686; Taherzadeh and Karimi, 2008, Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review, *Int. J. of Mol. Sci.* 9: 1621-1651; Yang and Wyman, 2008, Pretreatment: the key to unlocking low-cost cellulosic ethanol, *Biofuels Bioproducts and Biorefining-Biofpr* 2: 26-40).

Mechanical Pretreatment. The term "mechanical pretreatment" refers to various types of grinding or milling (*e.g.*, dry milling, wet milling, or vibratory ball milling) to disrupt and/or reduce particle sizeplant cell wall components of the cellulosic material.

Chemical Pretreatment. In practicing the methods of the present invention, any chemical pretreatment process known in the art can be used to disrupt plant cell wall components of the cellulosic material (Chandra *et al.*, 2007, *supra*; Galbe and Zacchi, 2007, Pretreatment of lignocellulosic materials for efficient bioethanol production, *Adv. Biochem. Engin. / Biotechnol.* 108: 41-65; Hendriks and Zeeman, 2009, Pretreatments to enhance the digestibility of lignocellulosic biomass, *Bioresource Technol.* 100: 10-18; Mosier *et al.*, 2005, Features of promising technologies for pretreatment of lignocellulosic biomass, *Bioresource Technol.* 96: 673-686; Taherzadeh and Karimi, 2008, Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review, *Int. J. of Mol. Sci.* 9: 1621-1651; Yang and Wyman, 2008, Pretreatment: the key to unlocking low-cost cellulosic ethanol, *Biofuels Bioproducts and Biorefining-Biofpr.* 2: 26-40).

Conventional chemical pretreatments include, but are not limited to, steam pretreatment (with or without explosion), dilute acid pretreatment, hot water pretreatment, alkaline pretreatment, lime pretreatment, wet oxidation, wet explosion, ammonia fiber explosion, organosolv pretreatment, and biological pretreatment. Additional pretreatments include ammonia percolation, ultrasound, electroporation, microwave, supercritical CO_2, supercritical H_2O, ozone, and gamma irradiation pretreatments.

The cellulosic material can be chemically pretreated before hydrolysis and/or fermentation. Pretreatment is preferably performed prior to the hydrolysis. Alternatively, the pretreatment can be carried out simultaneously with enzyme hydrolysis to release fermentable sugars, such as glucose, cellobiose, and/or xylose. In most cases the pretreatment step itself can result in some conversion of the cellulosic material to fermentable sugars (even in absence of enzymes).

Steam Pretreatment: In steam pretreatment, the cellulosic material is heated to disrupt the plant cell wall components, including lignin, hemicellulose, and cellulose to make the cellulose and other fractions, *e.g.*, hemicellulose, accessible to enzymes. The cellulosic material is passed to or through a reaction vessel where steam is injected to increase the

temperature to the required temperature and pressure and is retained therein for the desired reaction time. Steam pretreatment is preferably done at 140-230°C, more preferably 160-200°C, and most preferably 170-190°C, where the optimal temperature range depends on any addition of a chemical catalyst. Residence time for the steam pretreatment is preferably 1-15 minutes, more preferably 3-12 minutes, and most preferably 4-10 minutes, where the optimal residence time depends on temperature range and any addition of a chemical catalyst. Steam pretreatment allows for relatively high solids loadings, so that cellulosic material is generally only moist during the pretreatment. The steam pretreatment is often combined with an explosive discharge of the material after the pretreatment, which is known as steam explosion, that is, rapid flashing to atmospheric pressure and turbulent flow of the material to increase the accessible surface area by fragmentation (Duff and Murray, 1996, *Bioresource Technology* 855: 1-33; Galbe and Zacchi, 2002, *Appl. Microbiol. Biotechnol.* 59: 618-628; U.S. Patent Application No. 20020164730). During steam pretreatment, hemicellulose acetyl groups are cleaved and the resulting acid autocatalyzes partial hydrolysis of the hemicellulose to monosaccharides and oligosaccharides. Lignin is removed to only a limited extent.

A catalyst such as H_2SO_4 or SO_2 (typically 0.3 to 3% w/w) is often added prior to steam pretreatment, which decreases the time and temperature, increases the recovery, and improves enzymatic hydrolysis (Ballesteros *et al.*, 2006, *Appl. Biochem. Biotechnol.* 129-132: 496-508; Varga *et al.*, 2004, *Appl. Biochem. Biotechnol.* 113-116: 509-523; Sassner *et al.*, 2006, *Enzyme Microb. Technol.* 39: 756-762).

Chemical Pretreatment: The term "chemical treatment" refers to any chemical pretreatment that promotes the separation and/or release of cellulose, hemicellulose, and/or lignin. Examples of suitable chemical pretreatment processes include, for example, dilute acid pretreatment, lime pretreatment, wet oxidation, ammonia fiber/freeze explosion (AFEX), ammonia percolation (APR), and organosolv pretreatments.

In dilute acid pretreatment, the cellulosic material is mixed with dilute acid, typically H_2SO_4, and water to form a slurry, heated by steam to the desired temperature, and after a residence time flashed to atmospheric pressure. The dilute acid pretreatment can be performed with a number of reactor designs, *e.g.*, plug-flow reactors, counter-current reactors, or continuous counter-current shrinking bed reactors (Duff and Murray, 1996, *supra*; Schell *et al.*, 2004, *Bioresource Technol.* 91: 179-188; Lee *et al.*, 1999, *Adv. Biochem. Eng. Biotechnol.* 65: 93-115).

Several methods of pretreatment under alkaline conditions can also be used. These alkaline pretreatments include, but are not limited to, lime pretreatment, wet oxidation, ammonia percolation (APR), and ammonia fiber/freeze explosion (AFEX).

Lime pretreatment is performed with calcium carbonate, sodium hydroxide, or ammonia at low temperatures of 85-150°C and residence times from 1 hour to several days (Wyman *et al.*, 2005, *Bioresource Technol.* 96: 1959-1966; Mosier *et al.*, 2005, *Bioresource Technol.* 96: 673-686). WO 2006/110891, WO 2006/11899, WO 2006/11900, and WO 2006/110901 disclose pretreatment methods using ammonia.

Wet oxidation is a thermal pretreatment performed typically at 180-200°C for 5-15 minutes with addition of an oxidative agent such as hydrogen peroxide or over-pressure of oxygen (Schmidt and Thomsen, 1998, *Bioresource Technol.* 64: 139-151; Palonen *et al.*, 2004, *Appl. Biochem. Biotechnol.* 117: 1-17; Varga *et al.*, 2004, *Biotechnol. Bioeng.* 88: 567-574; Martin *et al.*, 2006, *J. Chem. Technol. Biotechnol.* 81: 1669-1677). The pretreatment is performed at preferably 1-40% dry matter, more preferably 2-30% dry matter, and most preferably 5-20% dry matter, and often the initial pH is increased by the addition of alkali such as sodium carbonate.

A modification of the wet oxidation pretreatment method, known as wet explosion (combination of wet oxidation and steam explosion), can handle dry matter up to 30%. In wet explosion, the oxidizing agent is introduced during pretreatment after a certain residence time. The pretreatment is then ended by flashing to atmospheric pressure (WO 2006/032282).

Ammonia fiber explosion (AFEX) involves treating the cellulosic material with liquid or gaseous ammonia at moderate temperatures such as 90-100°C and high pressure such as 17-20 bar for 5-10 minutes, where the dry matter content can be as high as 60% (Gollapalli *et al.*, 2002, *Appl. Biochem. Biotechnol.* 98: 23-35; Chundawat *et al.*, 2007, *Biotechnol. Bioeng.* 96: 219-231; Alizadeh *et al.*, 2005, *Appl. Biochem. Biotechnol.* 121: 1133-1141; Teymouri *et al.*, 2005, *Bioresource Technol.* 96: 2014-2018). AFEX pretreatment results in the depolymerization of cellulose and partial hydrolysis of hemicellulose. Lignin-carbohydrate complexes are cleaved.

Organosolv pretreatment delignifies the cellulosic material by extraction using aqueous ethanol (40-60% ethanol) at 160-200°C for 30-60 minutes (Pan *et al.*, 2005, *Biotechnol. Bioeng.* 90: 473-481; Pan *et al.*, 2006, *Biotechnol. Bioeng.* 94: 851-861; Kurabi *et al.*, 2005, *Appl. Biochem. Biotechnol.* 121: 219-230). Sulphuric acid is usually added as a catalyst. In organosolv pretreatment, the majority of hemicellulose is removed.

Other examples of suitable pretreatment methods are described by Schell *et al.*, 2003, *Appl. Biochem. and Biotechnol.* Vol. 105-108, p. 69-85, and Mosier *et al.*, 2005, *Bioresource Technology* 96: 673-686, and U.S. Published Application 2002/0164730.

In one aspect, the chemical pretreatment is preferably carried out as an acid treatment, and more preferably as a continuous dilute and/or mild acid treatment. The acid is typically sulfuric acid, but other acids can also be used, such as acetic acid, citric acid, nitric acid, phosphoric acid, tartaric acid, succinic acid, hydrogen chloride, or mixtures thereof. Mild acid treatment is conducted in the pH range of preferably 1-5, more preferably 1-4, and most

preferably 1-3. In one aspect, the acid concentration is in the range from preferably 0.01 to 20 wt% acid, more preferably 0.05 to 10 wt% acid, even more preferably 0.1 to 5 wt% acid, and most preferably 0.2 to 2.0 wt% acid. The acid is contacted with the cellulosic material and held at a temperature in the range of preferably 160-220°C, and more preferably 165-195°C, for periods ranging from seconds to minutes to, *e.g.*, 1 second to 60 minutes.

In another aspect, pretreatment is carried out as an ammonia fiber explosion step (AFEX pretreatment step).

In another aspect, pretreatment takes place in an aqueous slurry. In preferred aspects, the cellulosic material is present during pretreatment in amounts preferably between 10-80 wt%, more preferably between 20-70 wt%, and most preferably between 30-60 wt%, such as around 50 wt%. The pretreated cellulosic material can be unwashed or washed using any method known in the art, *e.g.*, washed with water.

Biological Pretreatment: The term "biological pretreatment" refers to any biological pretreatment that promotes the separation and/or release of cellulose, hemicellulose, and/or lignin from the cellulosic material. Biological pretreatment techniques can involve applying lignin-solubilizing microorganisms (see, for example, Hsu, T.-A., 1996, Pretreatment of biomass, in *Handbook on Bioethanol: Production and Utilization*, Wyman, C. E., ed., Taylor & Francis, Washington, DC, 179-212; Ghosh and Singh, 1993, Physicochemical and biological treatments for enzymatic/microbial conversion of cellulosic biomass, *Adv. Appl. Microbiol*. 39: 295-333; McMillan, J. D., 1994, Pretreating lignocellulosic biomass: a review, in *Enzymatic Conversion of Biomass for Fuels Production*, Himmel, M. E., Baker, J. O., and Overend, R. P., eds., ACS Symposium Series 566, American Chemical Society, Washington, DC, chapter 15; Gong, C. S., Cao, N. J., Du, J., and Tsao, G. T., 1999, Ethanol production from renewable resources, in *Advances in Biochemical Engineering/Biotechnology*, Scheper, T., ed., Springer-Verlag Berlin Heidelberg, Germany, 65: 207-241; Olsson and Hahn-Hagerdal, 1996, Fermentation of lignocellulosic hydrolysates for ethanol production, *Enz. Microb. Tech*. 18: 312-331; and Vallander and Eriksson, 1990, Production of ethanol from lignocellulosic materials: State of the art, *Adv. Biochem. Eng./Biotechnol*. 42: 63-95).

Physical Pretreatment. The term "physical pretreatment" refers to any pretreatment that promotes the separation and/or release of cellulose, hemicellulose, and/or lignin from the cellulosic material. For example, physical pretreatment can involve irradiation (*e.g.*, microwave irradiation), steaming/steam explosion, hydrothermolysis, and combinations thereof.

Physical pretreatment can involve high pressure and/or high temperature (steam explosion). In one aspect, high pressure means pressure in the range of preferably about 300 to about 600 psi, more preferably about 350 to about 550 psi, and most preferably about 400 to about 500 psi, such as around 450 psi. In another aspect, high temperature means

temperatures in the range of about 100 to about 300°C, preferably about 140 to about 235°C. In a preferred aspect, physical pretreatment is performed in a batch-process, steam gun hydrolyzer system that uses high pressure and high temperature as defined above, *e.g.*, a Sunds Hydrolyzer available from Sunds Defibrator AB, Sweden.

The cellulosic material can be subjected to pre-soaking, wetting, washing, or conditioning prior to pretreatment using methods known in the art.

Combined Physical and Chemical Pretreatment: The cellulosic material can be pretreated both physically and chemically. For instance, the pretreatment step can involve dilute or mild acid treatment and high temperature and/or pressure treatment. The physical and chemical pretreatments can be carried out sequentially or simultaneously, as desired. A mechanical pretreatment can also be included.

Accordingly, in a preferred aspect, the cellulosic material is subjected to mechanical, chemical, or physical pretreatment, or any combination thereof, to promote the separation and/or release of cellulose, hemicellulose, and/or lignin.

Saccharification. In the hydrolysis step, also known as saccharification, the cellulosic material, *e.g.*, pretreated, is hydrolyzed to break down cellulose and alternatively also hemicellulose to fermentable sugars, such as glucose, cellobiose, xylose, xylulose, arabinose, mannose, galactose, and/or soluble oligosaccharides. The hydrolysis is performed enzymatically by an enzyme composition of the present invention.

Enzymatic hydrolysis is preferably carried out in a suitable aqueous environment under conditions that can be readily determined by one skilled in the art. In a preferred aspect, hydrolysis is performed under conditions suitable for the activity of the enzyme(s), *i.e.*, optimal for the enzyme(s). The hydrolysis can be carried out as a fed batch or continuous process where the pretreated cellulosic material (substrate) is fed gradually to, for example, an enzyme containing hydrolysis solution.

The saccharification is generally performed in stirred-tank reactors or fermentors under controlled pH, temperature, and mixing conditions. Suitable process time, temperature and pH conditions can readily be determined by one skilled in the art. For example, the saccharification can last up to 200 hours, but is typically performed for preferably about 12 to about 96 hours, more preferably about 16 to about 72 hours, and most preferably about 24 to about 48 hours. The temperature is in the range of preferably about 25°C to about 70°C, more preferably about 30°C to about 65°C, and more preferably about 40°C to 60°C, in particular about 50°C. The pH is in the range of preferably about 3 to about 8, more preferably about 3.5 to about 7, and most preferably about 4 to about 6, in particular about pH 5. The dry solids content is in the range of preferably about 5 to about 50 wt%, more preferably about 10 to about 40 wt%, and most preferably about 20 to about 30 wt%.

In a preferred aspect, an effective amount of an enzyme composition of the present

invention is about 0.5 to about 50 mg, preferably at about 0.5 to about 40 mg, more preferably at about 0.5 to about 30 mg, more preferably at about 0.75 to about 20 mg, more preferably at about 0.75 to about 15 mg, even more preferably at about 1.0 to about 10 mg, and most preferably at about 2.0 to about 5 mg per g of cellulose in a cellulosic material.

Fermentation. The fermentable sugars obtained from the hydrolyzed cellulosic material can be fermented by one or more (several) fermenting microorganisms capable of fermenting the sugars directly or indirectly into a desired fermentation product. "Fermentation" or "fermentation process" refers to any fermentation process or any process comprising a fermentation step. Fermentation processes also include fermentation processes used in the consumable alcohol industry (*e.g.*, beer and wine), dairy industry (*e.g.*, fermented dairy products), leather industry, and tobacco industry. The fermentation conditions depend on the desired fermentation product and fermenting organism and can easily be determined by one skilled in the art.

In the fermentation step, sugars, released from cellulosic material as a result of the pretreatment and enzymatic hydrolysis steps, are fermented to a product, *e.g.*, ethanol, by a fermenting organism, such as yeast. Hydrolysis (saccharification) and fermentation can be separate or simultaneous, as described herein.

Any suitable hydrolyzed cellulosic material can be used in the fermentation step in practicing the present invention. The material is generally selected based on the desired fermentation product, *i.e.*, the substance to be obtained from the fermentation, and the process employed, as is well known in the art.

The term "fermentation medium" is understood herein to refer to a medium before the fermenting microorganism(s) is(are) added, such as, a medium resulting from a saccharification process, as well as a medium used in a simultaneous saccharification and fermentation process (SSF).

"Fermenting microorganism" refers to any microorganism, including bacterial and fungal organisms, suitable for use in a desired fermentation process to produce a fermentation product. The fermenting organism can be C_6 and/or C_5 fermenting organisms, or a combination thereof. Both C_6 and C_5 fermenting organisms are well known in the art. Suitable fermenting microorganisms are able to ferment, *i.e.*, convert, sugars, such as glucose, xylose, xylulose, arabinose, maltose, mannose, galactose, or oligosaccharides, directly or indirectly into the desired fermentation product.

Examples of bacterial and fungal fermenting organisms producing ethanol are described by Lin *et al.*, 2006, *Appl. Microbiol. Biotechnol.* 69: 627-642.

Examples of fermenting microorganisms that can ferment C_6 sugars include bacterial and fungal organisms, such as yeast. Preferred yeast includes strains of the *Saccharomyces* spp., preferably *Saccharomyces cerevisiae*.

Examples of fermenting organisms that can ferment C_5 sugars include bacterial and fungal organisms, such as some yeast. Preferred C_5 fermenting yeast include strains of *Pichia*, preferably *Pichia stipitis*, such as *Pichia stipitis* CBS 5773; strains of *Candida*, preferably *Candida boidinii*, *Candida brassicae*, *Candida sheatae*, *Candida diddensii*, *Candida pseudotropicalis*, or *Candida utilis.*

Other fermenting organisms include strains of *Zymomonas*, such as *Zymomonas mobilis*; *Hansenula*, such as *Hansenula anomala*; *Kluyveromyces*, such as *K. fragilis*; *Schizosaccharomyces*, such as *S. pombe*; *E. coli*, especially *E. coli* strains that have been genetically modified to improve the yield of ethanol; *Clostridium*, such as *Clostridium acetobutylicum*, *Chlostridium thermocellum*, and *Chlostridium phytofermentans*; *Geobacillus sp.*; *Thermoanaerobacter*, such as *Thermoanaerobacter saccharolyticum*; and *Bacillus*, such as *Bacillus coagulans.*

In a preferred aspect, the yeast is a *Saccharomyces* spp. In a more preferred aspect, the yeast is *Saccharomyces cerevisiae*. In another more preferred aspect, the yeast is *Saccharomyces distaticus*. In another more preferred aspect, the yeast is *Saccharomyces uvarum*. In another preferred aspect, the yeast is a *Kluyveromyces*. In another more preferred aspect, the yeast is *Kluyveromyces marxianus*. In another more preferred aspect, the yeast is *Kluyveromyces fragilis*. In another preferred aspect, the yeast is a *Candida*. In another more preferred aspect, the yeast is *Candida boidinii*. In another more preferred aspect, the yeast is *Candida brassicae*. In another more preferred aspect, the yeast is *Candida diddensii*. In another more preferred aspect, the yeast is *Candida pseudotropicalis*. In another more preferred aspect, the yeast is *Candida utilis*. In another preferred aspect, the yeast is a *Clavispora*. In another more preferred aspect, the yeast is *Clavispora lusitaniae*. In another more preferred aspect, the yeast is *Clavispora opuntiae*. In another preferred aspect, the yeast is a *Pachysolen*. In another more preferred aspect, the yeast is *Pachysolen tannophilus*. In another preferred aspect, the yeast is a *Pichia*. In another more preferred aspect, the yeast is a *Pichia stipitis*. In another preferred aspect, the yeast is a *Bretannomyces*. In another more preferred aspect, the yeast is *Bretannomyces clausenii* (Philippidis, G. P., 1996, Cellulose bioconversion technology, in *Handbook on Bioethanol: Production and Utilization*, Wyman, C. E., ed., Taylor & Francis, Washington, DC, 179-212).

Bacteria that can efficiently ferment hexose and pentose to ethanol include, for example, *Zymomonas mobilis*, *Clostridium acetobutylicum*, *Clostridium thermocellum*, *Chlostridium phytofermentans*, *Geobacillus* sp., *Thermoanaerobacter saccharolyticum*, and *Bacillus coagulans* (Philippidis, 1996, *supra*).

In a preferred aspect, the bacterium is a *Zymomonas*. In a more preferred aspect, the bacterium is *Zymomonas mobilis*. In another preferred aspect, the bacterium is a *Clostridium*. In another more preferred aspect, the bacterium is *Clostridium thermocellum*.

Commercially available yeast suitable for ethanol production includes, *e.g.*, ETHANOL RED™ yeast (available from Fermentis/Lesaffre, USA), FALI™ (available from Fleischmann's Yeast, USA), SUPERSTART™ and THERMOSACC™ fresh yeast (available from Ethanol Technology, WI, USA), BIOFERM™ AFT and XR (available from NABC - North American Bioproducts Corporation, GA, USA), GERT STRAND™ (available from Gert Strand AB, Sweden), and FERMIOL™ (available from DSM Specialties).

In a preferred aspect, the fermenting microorganism has been genetically modified to provide the ability to ferment pentose sugars, such as xylose utilizing, arabinose utilizing, and xylose and arabinose co-utilizing microorganisms.

The cloning of heterologous genes into various fermenting microorganisms has led to the construction of organisms capable of converting hexoses and pentoses to ethanol (cofermentation) (Chen and Ho, 1993, Cloning and improving the expression of *Pichia stipitis* xylose reductase gene in *Saccharomyces cerevisiae*, *Appl. Biochem. Biotechnol.* 39-40: 135-147; Ho *et al.*, 1998, Genetically engineered *Saccharomyces* yeast capable of effectively cofermenting glucose and xylose, *Appl. Environ. Microbiol.* 64: 1852-1859; Kotter and Ciriacy, 1993, Xylose fermentation by *Saccharomyces cerevisiae*, *Appl. Microbiol. Biotechnol.* 38: 776-783; Walfridsson *et al.*, 1995, Xylose-metabolizing *Saccharomyces cerevisiae* strains overexpressing the TKL1 and TAL1 genes encoding the pentose phosphate pathway enzymes transketolase and transaldolase, *Appl. Environ. Microbiol.* 61: 4184-4190; Kuyper *et al.*, 2004, Minimal metabolic engineering of *Saccharomyces cerevisiae* for efficient anaerobic xylose fermentation: a proof of principle, *FEMS Yeast Research* 4: 655-664; Beall *et al.*, 1991, Parametric studies of ethanol production from xylose and other sugars by recombinant *Escherichia coli*, *Biotech. Bioeng.* 38: 296-303; Ingram *et al.*, 1998, Metabolic engineering of bacteria for ethanol production, *Biotechnol. Bioeng.* 58: 204-214; Zhang *et al.*, 1995, Metabolic engineering of a pentose metabolism pathway in ethanologenic *Zymomonas mobilis*, *Science* 267: 240-243; Deanda *et al.*, 1996, Development of an arabinose-fermenting *Zymomonas mobilis* strain by metabolic pathway engineering, *Appl. Environ. Microbiol.* 62: 4465-4470; WO 2003/062430, xylose isomerase).

In a preferred aspect, the genetically modified fermenting microorganism is *Saccharomyces cerevisiae.* In another preferred aspect, the genetically modified fermenting microorganism is *Zymomonas mobilis*. In another preferred aspect, the genetically modified fermenting microorganism is *Escherichia coli*. In another preferred aspect, the genetically modified fermenting microorganism is *Klebsiella oxytoca*. In another preferred aspect, the genetically modified fermenting microorganism is *Kluyveromyces* sp.

It is well known in the art that the organisms described above can also be used to produce other substances, as described herein.

The fermenting microorganism is typically added to the degraded lignocellulose or

hydrolysate and the fermentation is performed for about 8 to about 96 hours, such as about 24 to about 60 hours. The temperature is typically between about 26°C to about 60°C, in particular about 32°C or 50°C, and at about pH 3 to about pH 8, such as around pH 4-5, 6, or 7.

In a preferred aspect, the yeast and/or another microorganism is applied to the degraded cellulosic material and the fermentation is performed for about 12 to about 96 hours, such as typically 24-60 hours. In a preferred aspect, the temperature is preferably between about 20°C to about 60°C, more preferably about 25°C to about 50°C, and most preferably about 32°C to about 50°C, in particular about 32°C or 50°C, and the pH is generally from about pH 3 to about pH 7, preferably around pH 4-7. However, some fermenting organisms, *e.g.*, bacteria, have higher fermentation temperature optima. Yeast or another microorganism is preferably applied in amounts of approximately 10^5 to 10^{12}, preferably from approximately 10^7 to 10^{10}, especially approximately 2 x 10^8 viable cell count per ml of fermentation broth. Further guidance in respect of using yeast for fermentation can be found in, *e.g.*, "The Alcohol Textbook" (Editors K. Jacques, T.P. Lyons and D.R. Kelsall, Nottingham University Press, United Kingdom 1999), which is hereby incorporated by reference.

For ethanol production, following the fermentation the fermented slurry is distilled to extract the ethanol. The ethanol obtained according to the methods of the invention can be used as, *e.g.*, fuel ethanol, drinking ethanol, *i.e.*, potable neutral spirits, or industrial ethanol.

A fermentation stimulator can be used in combination with any of the processes described herein to further improve the fermentation process, and in particular, the performance of the fermenting microorganism, such as, rate enhancement and ethanol yield. A "fermentation stimulator" refers to stimulators for growth of the fermenting microorganisms, in particular, yeast. Preferred fermentation stimulators for growth include vitamins and minerals. Examples of vitamins include multivitamins, biotin, pantothenate, nicotinic acid, meso-inositol, thiamine, pyridoxine, para-aminobenzoic acid, folic acid, riboflavin, and Vitamins A, B, C, D, and E. See, for example, Alfenore *et al.*, Improving ethanol production and viability of *Saccharomyces cerevisiae* by a vitamin feeding strategy during fed-batch process, Springer-Verlag (2002), which is hereby incorporated by reference. Examples of minerals include minerals and mineral salts that can supply nutrients comprising P, K, Mg, S, Ca, Fe, Zn, Mn, and Cu.

Fermentation products: A fermentation product can be any substance derived from the fermentation. The fermentation product can be, without limitation, an alcohol (*e.g.*, arabinitol, butanol, ethanol, glycerol, methanol, 1,3-propanediol, sorbitol, and xylitol); an organic acid (*e.g.*, acetic acid, acetonic acid, adipic acid, ascorbic acid, citric acid, 2,5-diketo-D-gluconic acid, formic acid, fumaric acid, glucaric acid, gluconic acid, glucuronic acid,

glutaric acid, 3-hydroxypropionic acid, itaconic acid, lactic acid, malic acid, malonic acid, oxalic acid, oxaloacetic acid, propionic acid, succinic acid, and xylonic acid); a ketone (*e.g.*, acetone); an amino acid (*e.g.*, aspartic acid, glutamic acid, glycine, lysine, serine, and threonine); and a gas (*e.g.*, methane, hydrogen (H_2), carbon dioxide (CO_2), and carbon monoxide (CO)). The fermentation product can also be protein as a high value product.

In a preferred aspect, the fermentation product is an alcohol. It will be understood that the term "alcohol" encompasses a substance that contains one or more hydroxyl moieties. In a more preferred aspect, the alcohol is arabinitol. In another more preferred aspect, the alcohol is butanol. In another more preferred aspect, the alcohol is ethanol. In another more preferred aspect, the alcohol is glycerol. In another more preferred aspect, the alcohol is methanol. In another more preferred aspect, the alcohol is 1,3-propanediol. In another more preferred aspect, the alcohol is sorbitol. In another more preferred aspect, the alcohol is xylitol. See, for example, Gong, C. S., Cao, N. J., Du, J., and Tsao, G. T., 1999, Ethanol production from renewable resources, in *Advances in Biochemical Engineering/Biotechnology*, Scheper, T., ed., Springer-Verlag Berlin Heidelberg, Germany, 65: 207-241; Silveira, M. M., and Jonas, R., 2002, The biotechnological production of sorbitol, *Appl. Microbiol. Biotechnol.* 59: 400-408; Nigam, P., and Singh, D., 1995, Processes for fermentative production of xylitol – a sugar substitute, *Process Biochemistry* 30 (2): 117-124; Ezeji, T. C., Qureshi, N. and Blaschek, H. P., 2003, Production of acetone, butanol and ethanol by *Clostridium beijerinckii* BA101 and *in situ* recovery by gas stripping, *World Journal of Microbiology and Biotechnology* 19 (6): 595-603.

In another preferred aspect, the fermentation product is an organic acid. In another more preferred aspect, the organic acid is acetic acid. In another more preferred aspect, the organic acid is acetonic acid. In another more preferred aspect, the organic acid is adipic acid. In another more preferred aspect, the organic acid is ascorbic acid. In another more preferred aspect, the organic acid is citric acid. In another more preferred aspect, the organic acid is 2,5-diketo-D-gluconic acid. In another more preferred aspect, the organic acid is formic acid. In another more preferred aspect, the organic acid is fumaric acid. In another more preferred aspect, the organic acid is glucaric acid. In another more preferred aspect, the organic acid is gluconic acid. In another more preferred aspect, the organic acid is glucuronic acid. In another more preferred aspect, the organic acid is glutaric acid. In another preferred aspect, the organic acid is 3-hydroxypropionic acid. In another more preferred aspect, the organic acid is itaconic acid. In another more preferred aspect, the organic acid is lactic acid. In another more preferred aspect, the organic acid is malic acid. In another more preferred aspect, the organic acid is malonic acid. In another more preferred aspect, the organic acid is oxalic acid. In another more preferred aspect, the organic acid is propionic acid. In another more preferred aspect, the organic acid is succinic acid. In another

more preferred aspect, the organic acid is xylonic acid. See, for example, Chen, R., and Lee, Y. Y., 1997, Membrane-mediated extractive fermentation for lactic acid production from cellulosic biomass, *Appl. Biochem. Biotechnol.* 63-65: 435-448.

In another preferred aspect, the fermentation product is a ketone. It will be understood that the term "ketone" encompasses a substance that contains one or more ketone moieties. In another more preferred aspect, the ketone is acetone. See, for example, Qureshi and Blaschek, 2003, *supra*.

In another preferred aspect, the fermentation product is an amino acid. In another more preferred aspect, the organic acid is aspartic acid. In another more preferred aspect, the amino acid is glutamic acid. In another more preferred aspect, the amino acid is glycine. In another more preferred aspect, the amino acid is lysine. In another more preferred aspect, the amino acid is serine. In another more preferred aspect, the amino acid is threonine. See, for example, Richard, A., and Margaritis, A., 2004, Empirical modeling of batch fermentation kinetics for poly(glutamic acid) production and other microbial biopolymers, *Biotechnology and Bioengineering* 87 (4): 501-515.

In another preferred aspect, the fermentation product is a gas. In another more preferred aspect, the gas is methane. In another more preferred aspect, the gas is H_2. In another more preferred aspect, the gas is CO_2. In another more preferred aspect, the gas is CO. See, for example, Kataoka, N., A. Miya, and K. Kiriyama, 1997, Studies on hydrogen production by continuous culture system of hydrogen-producing anaerobic bacteria, *Water Science and Technology* 36 (6-7): 41-47; and Gunaseelan V.N. in *Biomass and Bioenergy*, Vol. 13 (1-2), pp. 83-114, 1997, Anaerobic digestion of biomass for methane production: A review.

Recovery. The fermentation product(s) can be optionally recovered from the fermentation medium using any method known in the art including, but not limited to, chromatography, electrophoretic procedures, differential solubility, distillation, or extraction. For example, alcohol is separated from the fermented cellulosic material and purified by conventional methods of distillation. Ethanol with a purity of up to about 96 vol.% can be obtained, which can be used as, for example, fuel ethanol, drinking ethanol, *i.e.*, potable neutral spirits, or industrial ethanol.

The present invention is further described by the following examples that should not be construed as limiting the scope of the invention.

Examples

Materials

Chemicals used as buffers and substrates were commercial products of at least reagent grade.

Media

PDA plates were composed of 39 grams of potato dextrose agar and deionized water to 1 liter.

Minimal medium plates were composed of 6 g of $NaNO_3$, 0.52 g of KCl, 1.52 g of KH_2PO_4, 1 ml of COVE trace elements solution, 20 g of Noble agar, 20 ml of 50% glucose, 2.5 ml of $MgSO_4 \cdot 7H_2O$, 20 ml of a 0.02% biotin solution, and deionized water to 1 liter.

COVE trace elements solution was composed of 0.04 g of $Na_2B_4O_7 \cdot 10H_2O$, 0.4 g of $CuSO_4 \cdot 5H_2O$, 1.2 g of FeSO4·$7H_2O$, 0.7 g of $MnSO_4 \cdot H_2O$, 0.8 g of $Na_2MoO_2 \cdot 2H_2O$, 10 g of $ZnSO_4 \cdot 7H_2O$, and deionized water to 1 liter.

MDU2BP medium was composed per liter of 45 g of maltose, 1 g of $MgSO_4 \cdot 7H_2O$, 1 g of NaCl, 2 g of K_2SO_4, 12 g of KH_2PO_4, 7 g of yeast extract, 2 g of urea, and 0.5 ml of AMG trace metals solution; pH 5.0.

AMG trace metals solution was composed per liter of 14.3 g of $ZnSO_4 \cdot 7H_2O$, 2.5 g of $CuSO_4 \cdot 5H_2O$, 0.5 g of $NiCl_2 \cdot 6H_2O$, 13.8 g of $FeSO_4 \cdot 7H_2O$, 8.5 g of $MnSO_4 \cdot 7H_2O$, and 3 g of citric acid.

NNCYP-PCS medium was composed of 5.0 g of $NaNO_3$, 3.0 g of NH_4Cl, 2.0 g of MES, 2.5 g of citric acid, 0.2 g of $CaCl_2$ $2H_2O$, 1.0 g of Bacto Peptone, 5.0 g of yeast extract, 0.2 g of $MgSO_4$ $7H_2O$, 4.0 g of K_2HPO_4, 1.0 ml of COVE trace elements solution, 2.5 g of glucose, 25.0 g of pretreated corn stover (PCS), and deionized water to 1 liter.

2X YT medium was composed per liter of 16 g of tryptone, 10 g of yeast extract, and 5 g of NaCl.

2X YT plates were composed per liter of 16 g of tryptone, 10 g of yeast extract, 5 g of NaCl and 15 g of Noble agar.

YG agar plates were composed per liter of 5.0 g of yeast extract, 10.0 g of glucose, and 20.0 g of agar.

YEG medium was composed per liter of 20 g of dextrose and 5 g of yeast extract.

LB medium was composed per liter of 10 g of tryptone, 5 g of yeast extract, and 5 g of sodium chloride.

LB agar plates were composed of 10 g of tryptone, 5 g of yeast extract, 10 g of sodium chloride, 15 g of agar, and 1 liter of distilled water.

YP medium was composed per liter of 10 g of yeast extract and 20 g of Bacto peptone.

MEX-1 medium was composed per liter of 20 g of soya bean meal, 15 g of wheat bran, 10 g of microcrystalline cellulose (AVICEL®; FMC, Philadelphia, PA, USA), 5 g of

maltodextrin, 3 g of Bactopeptone, 0.2 g of pluronic, and 1 g of olive oil.

LB ampicillin medium was composed per liter of 10 g of tryptone, 5 g of yeast extract, 5 g of sodium chloride, and 50 mg of ampicillin (filter sterilized, added after autoclaving).

LB ampicillin plates were composed of 15 g of bacto agar per liter of LB ampicillin medium.

MY25 medium was composed per liter of 25 g of maltodextrin, 2 g of $MgSO_4 \cdot 7H_2O$, 10 g of KH_2PO_4, 2 g of citric acid, 2 g of K_2SO_4, 2 g of urea, 10 g of yeast extract, and 1.5 ml of AMG trace metals solution, adjusted to pH 6.

YPD medium was composed of 1% yeast extract, 2% peptone, and filter-sterilized 2% glucose added after autoclaving.

YPM medium was composed of 1% yeast extract, 2% peptone, and filter-sterilized 2% maltodextrin added after autoclaving.

SC-URA medium with glucose or galactose was composed of 100 ml of 10X Basal salts, 25 ml of 20% casamino acids without vitamins, 10 ml of 1% tryptophan, 4 ml of 5% threonine (filter sterilized, added after autoclaving), and 100 ml of 20% glucose or 100 ml of 20% galactose (filter sterilized, added after autoclaving), and deionized water to 1 liter.

10X Basal salts solution was composed of 75 g of yeast nitrogen base, 113 g of succinic acid, 68 g of NaOH, and deionized water to 1 liter.

SC-agar plates were composed of 20 g of agar per liter of SC-URA medium (with glucose or galactose as indicated).

0.1% AZCL xylan SC-URA agar plates with galactose were composed of 20 g of agar per liter of SC-URA medium with galactose and 0.1% AZCL oat xylan (Megazyme, Wicklow, Ireland).

SC-URA medium with galactose was composed of 900 ml of SC-Grund Agar (autoclaved), 4 ml of 5% threonine (filter sterilized), and 100 ml of 20% galactose (filter sterilized).

SC-Grund Agar was composed of 7.5 g Yeast Nitrogen Base (without amino acids), 11.3 g of succinic acid, 6.8 g of sodium hydroxide, 5.6 g of casamino acids, 0.1 g of L-tryptophan, 20 g of agar, and deionized water to 1 liter.

COVE plates were composed per liter of 342.3 g of sucrose, 25 g of Noble agar, 20 ml of COVE salts solution, 10 mM acetamide, and 15 or 20 mM CsCl. The solution was adjusted to pH 7.0 before autoclaving.

COVE2 plates were composed per liter of 30 g of sucrose, 20 ml of COVE salts solution, 20 ml of 1 M acetamide, and 25 g of Agar Noble.

COVE salts solution was composed per liter of 26 g of KCl, 26 g of $MgSO_4 \cdot 7H_2O$, 76 g of KH_2PO_4, and 50 ml of COVE trace metals.

YPG medium was composed per liter of 10 g of yeast extract, 10 g of Bacto peptone,

and 20 g of glucose.

M410 medium was composed per liter of 50 g of maltose, 50 g of glucose, 2 g of $MgSO_4$-$7H_2O$, 2 g of KH_2PO_4, 4 g of citric acid anhydrous powder, 8 g of yeast extract, 2 g of urea, 0.5 g of AMG trace metals solution, and 0.5 g of $CaCl_2$ at pH 6.0.

SOC medium was composed of 2% tryptone, 0.5% yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, and 10 mM $MgSO_4$; sterilized by autoclaving and then filter-sterilized glucose was added to 20 mM.

SY50 medium was composed per liter of 50 g of sucrose, 2 g of $MgSO_4$·7H2O, 10 g of KH_2PO_4, anhydrous, 2 g of K_2SO_4, 2 g of citric acid, 10 g of yeast extract, 2 g of urea, 0.5 g of $CaCl_2$·$2H_2O$, and 0.5 g of 200X AMG trace metals solution, pH 6.0.

200X AMG trace metals solution was composed per liter of 3 g of citric acid, 14.3 g of $ZnSO_4$·$7H_2O$, 2.5 g of $CuSO_4$·$5H_2O$, 13.8 g of $FeSO_4$·$7H_2O$, and 8.5 g of $MnSO_4$·H_2O.

Cal-18 medium was composed per liter of 40 g of yeast extract, 1.3 g of magnesium sulfate, 50 g of maltodextrin, 20 g of NaH_2PO_4, and 0.1 g of antifoam.

Cellulase-inducing medium was composed of 20 g of cellulose, 10 g of corn steep solids, 1.45 g of $(NH_4)_2SO_4$, 2.08 g of KH_2PO_4, 0.28 g of $CaCl_2$, 0.42 g of $MgSO_4$·$7H_2O$, 0.42 ml of *Trichoderma* trace metals solution, and 1-2 drops of antifoam.

Trichoderma trace metals solution was composed per liter of 216 g of $FeCl_3$·$6H_2O$, 58 g of $ZnSO_4$·$7H_2O$, 27 g of $MnSO_4$·H_2O, 10 g of $CuSO_4$·$5H_2O$, 2.4 g of H_3BO_3, and 336 g of citric acid.

TE was composed of 10 mM Tris pH 7.4 and 0.1 mM EDTA.

YPM medium contained 1% yeast extract, 2% of peptone, and 2% of maltose in deionized water.

MY50 medium was composed of 50 g of Maltodextrin, 2 g of MgSO47H20, 10 g of KH2PO4, 2 g of K2SO4, 2 g of citric acid, 10 g of yeast extract, 2 g of urea, 0.5 ml of AMG trace metals solution, and distilled water to 1 liter.

AMG trace metals solution was composed per liter of 14.3 g of $ZnSO_4$·$7H_2O$, 2.5 g of $CuSO_4$·$5H_2O$, 0.5 g of $NiCl_2$·$6H_2O$, 13.8 g of $FeSO_4$·$7H_2O$, 8.5 g of $MnSO_4$·$7H_2O$, 3 g of citric acid, and distilled water to 1 liter.

50X Vogels medium was composed per liter of 150 g of sodium citrate, 250 g of KH_2PO_4, 10 g of $MgSO_4$·$7H_2O$, 10 g of $CaCl_2$·$2H_2O$, 2.5 ml of biotin stock solution, 5.0 ml of AMG trace metals solution, and distilled water to 1 liter.

COVE agar selective plates were composed of 218 g sorbitol, 20 g agar, 20 ml COVE salts solution, 10 mM acetamide, 15 mM CsCl, and deionized water to 1 liter. The solution was adjusted to pH 7.0 before autoclaving.

COVE salts solution was composed of 26 g KCl, 26 g $MgSO_4$·$7H_2O$, 76 g KH_2PO_4, 50 ml COVE trace metals solution, and deionized water to 1 liter.

COVE trace metals solution was composed of 0.04 g $Na_2B_4O_7 \cdot 10H_2O$, 0.4 g $CuSO_4 \cdot 5H_2O$, 1.2 g $FeSO_4 \cdot 7H_2O$, 0.7 g $MnSO_4 \cdot H_2O$, 0.8 g $Na_2MoO_2 \cdot 2H_2O$, 10 g $ZnSO_4 \cdot 7H_2O$, and deionized water to 1 liter.

YP+2% glucose medium was composed of 1% yeast extract, 2% peptone and 2% glucose in deionized water.

YP+2% maltodextrin medium is composed of 2% peptone, 2% maltodextrin, and 1% yeast extract in deionized water.

DAP-2C-1 medium is composed of 3% maltodextrin, 1.1% magnesium sulfate, 0.52% tri-potassium phosphate, 0.2% citric acid, 0.1% potassium dihydrogen phosphate, 0.1% Dowfax 63N10, 0.05% yeast extract, and 0.05% of a trace element solution (1.39% ferrous sulfate, 0.845% maganese sulfate, 0.68% zinc chloride, 0.3% citric acid, 0.25% copper sulfate, and 0.013% nickel chloride) in deionized water.

DAP-2C-1 medium is composed of 2% glucose, 1.1% magnesium sulfate, 1.0% maltose, 0.52% tri-potassium phosphate, 0.2% citric acid, 0.1% potassium dihydrogen phosphate, 0.1% Dowfax 63N10, 0.05% yeast extract, and 0.05% of a trace element solution (1.39% ferrous sulfate, 0.845% maganese sulfate, 0.68% zinc chloride, 0.3% citric acid, 0.25% copper sulfate, and 0.013% nickel chloride) in deionized water.

Example 1: Preparation of *Chaetomium thermophilum* CGMCC 0581 Cel7A cellobiohydrolase I

The *Chaetomium thermophilum* CGMCC 0581 Cel7A cellobiohydrolase I (CBHI) gene (SEQ ID NO: 1 [DNA sequence] and SEQ ID NO: 2 [deduced amino acid sequence]) was isolated according to WO 2003/000941 and expressed in *Aspergillus oryzae* JaL250 (WO 99/61651).

The fungal strain *Chaetomium thermophilum* CGMCC 0581 was grown on agar plate composed of 0.5% yeast extract, 1% glucose and 2% agar for 3 days at 45°C. The fully grown culture was used to inoculate shake flasks containing liquid medium composed of 3% soymeal, 1.5% maltose, and 0.5% peptone. The flasks were incubated at 45°C for 48 hours with shaking. The mycelia were harvested by centrifugation of the culture broth at 8000 rpm, 4°C for 30 minutes, transferred into a clean plastic bag followed by immediate freezing in liquid nitrogen, and stored at -80°C before total RNA was isolated.

The frozen mycelia were grounded into a very fine powder with a sterilized mortar and pestle baked at 200°C for 24 hours. An RNEASY® Plant Mini Kit (QIAGEN Inc., Valencia, CA, USA) was used to isolate total RNA according to the manufacturer's instructions.

First strand cDNA synthesis from the total RNA was performed using a 3' RACE

System for Rapid Amplification of cDNA Ends (Invitrogen Corporation, Carlsbad, CA, USA) according to the manufacturer's instructions. The first strand cDNA of 3' RACE was used as PCR template for PCR screening.

Two oligonucleotides shown below were used for PCR screening of cDNA of *Chaetomium thermophilum* CGMCC 0581. The forward primer was derived from an alignment of conserved regions of cellobiohydrolase I genes and the reverse primer was provided by the 3' RACE System.

Forward primer:

5'-GGnACnGGnTA(t/c)TG(t/c)GA-3' (SEQ ID NO: 67)

Reverse primer:

5'-GGCCACGCGTCGACTAGTAC-3' (SEQ ID NO: 68)

One hundred picomoles of the forward primer and 10 picomoles of the reverse primer were used in a PCR reaction composed of 2 µl of the first strand cDNA of 3' RACE, 5 µl of 10X *Taq* DNA polymerase buffer (Promega Corporation, Madison, WI, USA), 3 µl of 25 mM $MgCl_2$, 1 µl of 10 mM dNTP, and 2.5 units of *Taq* DNA polymerase (Promega Corporation, Madison, WI, USA) in a final volume of 50 µl. The amplification was performed in a thermocycler programmed for 1 cycle at 95°C for 3 minutes; 30 cycles each at 95°C for 30 seconds, 50°C for 30 seconds, and 72°C for 50 seconds; and 1 cycle at 72C for 10 minutes. The heat block then went to a 4°C soak cycle.

The reaction products were isolated by 1.0% agarose gel electrophoresis using TBE buffer where an approximately 1.3 kb product band was excised from the gel, and purified using a WIZARD® PCR Preps DNA Purification System (Promega Corporation, Madison, WI, USA) according to the manufacturer's instructions. The PCR product was sequenced using a 377 DNA Analyzer (Applied Biosystems Inc, Foster City, CA, USA). Sequencing showed that the 1.3 kb fragment was homologous to cellobiohydrolase I.

Two oligos shown below were designed for the 5' end cloning of the *Chaetomium thermophilum* CGMCC 0581 Cel7A cellobiohydrolase I by using a 5' RACE System for Rapid Amplification of cDNA Ends (Invitrogen Corporation, Carlsbad, CA, USA).

Primer 4310AS1:

5'-AGATATCCATCTCAGAGCA-3' (SEQ ID NO: 69)

Primer 4310AS2:

5'-GTTGGCATCATTGGTCG-3' (SEQ ID NO: 70)

The gene specific primer 4310AS1 was used for the first strand cDNA synthesis using a 5' RACE System according to the manufacturer's instructions. The first strand cDNA of 5' RACE (5 µl) was used as template for PCR amplification composed of 5 µl of 10X *Taq* DNA polymerase buffer, 3 µl of 25 mM $MgCl_2$, 1 µl of 10 mM dNTP, 1 µl of 10 µM 4310AS2 primer, 1 µl of 10 µM primer AAP (Abridged Anchor Primer, provided by the kit), and 2.5

units of *Taq* DNA polymerase in a final volume of 50 µl. The amplification was performed in a thermocycler programmed for 1 cycle at 94°C for 3 minutes; 30 cycles each at 95°C for 30 seconds, 50°C for 30 seconds, and 72°C for 50 seconds; and 1 cycle at 72°C for 10 minutes. The heat block then went to a 4°C soak cycle.

The PCR products were isolated by 1.0% agarose gel electrophoresis using TBE buffer and purified using a WIZARD® PCR Preps DNA Purification System. A dominant DNA fragment of 0.8 kb was confirmed to be the 5' end of *Chaetomium thermophilum* CGMCC 0581 Cel7A cellobiohydrolase I gene by sequencing using a 377 DNA Analyzer.

Two primers shown below were designed based on the sequence information from both 5' and 3' end cloning. They were used for full-length cloning of the *Chaetomium thermophilum* CGMCC 0581 Cel7A cellobiohydrolase I gene.

Primer 4310S:

5'-ATCCTCTCCTTCCAGTTTTC-3' (SEQ ID NO: 71)

Primer 4310AS:

5'-TATCCAAGTAGTCCACAACC-3' (SEQ ID NO: 72)

Ten picomoles of the above two primers were used in a PCR reaction composed of 5 µl of first strand cDNA of 3' RACE, 5 µl of 10X *Taq* DNA polymerase buffer, 3 µl of 25 mM $MgCl_2$, 1 µl of 10 mM dNTP, and 2.5 units of *Taq* DNA polymerase in a final volume of 50 µl. The amplification was performed in a thermocycler programmed for 1 cycle at 95°C for 3 minutes; 30 cycles each at 95°C for 50 seconds, 55°C for 50 seconds, and 72°C for 90 seconds; and 1 cycle at 72°C for 10 minutes. The heat block then went to a 4°C soak cycle.

The reaction products were isolated by 1.0% agarose gel electrophoresis using TBE buffer where an approximately 1.5 kb product band was excised from the gel, and purified using a WIZARD® PCR Preps DNA Purification System. The PCR fragment was then ligated to pGEM-T using a pGEM-T Vector System (Promega Corporation, Madison, WI, USA). The plasmid DNA was confirmed by sequencing using a 377 DNA Analyzer. The correct clone was designated pT43-10.

Two synthetic oligonucleotide primers containing *Bsp* HI sites at their ends, shown below, were designed to PCR amplify the full-length open reading frame of the *Chaetomium thermophilum* CGMCC 0581 Family GH7A cellobiohydrolase I gene. A Rapid Ligation Kit (Roche Applied Science, Indianapolis, IN, USA) was used to clone the fragment into pAlLo2 (WO 2004/099228).

PCR Forward primer:

5'-TC**ATGA**TGTACAAGAAGTTCGCCG-3' (SEQ ID NO: 73)

PCR Reverse primer:

5'-TCATGA**TTACAGGCACTGGCTGTAC**-3' (SEQ ID NO: 74)

Bold letters represent coding sequence. The underlined sequence contains sequence

identity to the *BspHI* restriction site.

Fifty picomoles of each of the primers above were used in a PCR reaction containing 50 ng of plasmid pT43-10 containing the *Chaetomium thermophilum* CGMCC 0581 cellobiohydrolase I gene, 1X *Pwo* Amplification Buffer with $MgSO_4$ (Boehringer Mannheim, Indianapolis, IN, USA), 4 µl of 10 mM blend of dATP, dTTP, dGTP, and dCTP, and 2.5 units of *Pwo* DNA Polymerase (Boehringer Mannheim, Indianapolis, IN, USA), in a final volume of 50 µl. A DNA ENGINE™ Thermal Cycler (MJ Research, Waltham, MA, USA) was used to amplify the fragment programmed for one cycle at 94°C for 2 minutes; 35 cycles each at 94°C for 30 seconds, 62°C for 30 seconds, and 72°C for 1.5 minutes. After the 35 cycles, the reaction was incubated at 72°C for 10 minutes and then cooled at 10°C until further processed.

A 1.6 kb PCR reaction product was isolated on a 0.8% GTG® agarose gel (Cambrex Bioproducts, East Rutherford, NJ, USA) using 40 mM Tris base-20 mM sodium acetate-1 mM disodium EDTA (TAE) buffer and 0.1 µg of ethidium bromide per ml. The DNA band was visualized with the aid of a DARKREADER™ Transilluminator (Clare Chemical Research, Dolores, CO, USA) to avoid UV-induced mutations. The 1.6 kb DNA band was excised with a disposable razor blade and purified with an ULTRAFREE® DA spin cup (Millipore, Billerica, MA, USA) according to the manufacturer's instructions.

The purified PCR fragment was cloned into pCR®4Blunt-TOPO® (Invitrogen, Carlsbad, CA, USA) using a TOPO® Blunt Cloning Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. PCR clones containing the coding regions of interest were sequenced to Phred Q values of at least 40 to insure that there were no PCR induced errors. All sequence aligments were performed with Consed (University of Washington). One of the clones was determined to have the expected sequence and was selected and re-named CtPCR. The CtPCR clone containing the *C. thermophilum* cellobiohydrolase I coding region was digested with *Bsp* HI and gel purified as described above. This DNA fragment was then ligated into the *Nco* I restriction site of pAlLo2 with a Rapid Ligation Kit. Expression clones were confirmed by restriction digestion and sequenced to confirm that the junction vector-insert was correct. Plasmid DNA for transformation was prepared with a Midi-Prep Kit (QIAGEN Inc., Valencia, CA, USA). The final clone was re-named pAlLo4.

Aspergillus oryzae JaL250 protoplasts were prepared according to the method of Christensen *et al.*, 1988, *Bio/Technology* 6: 1419-1422. Eight micrograms of pAlLo4 (as well as pAlLo2 as a vector control) were used to transform *Aspergillus oryzae* JaL250 protoplasts. Twelve transformants were isolated to individual PDA plates and incubated for 5 days at 34°C. Confluent spore plates were washed with 5 ml of 0.01% TWEEN® 80 and the

spore suspension was used to inoculate 25 ml of MDU2BP medium in 125 ml glass shake flasks. Transformant cultures were incubated at 34°C with constant shaking at 200 rpm. At day five post-inoculation, cultures were centrifuged at 6000 *x g* and their supernatants collected. Five microliters of each supernatant were mixed with an equal volume of 2X loading buffer (10% beta-mercaptoethanol) and loaded onto a 1.5 mm 8%-16% Tris-glycine SDS-PAGE gel and stained with SIMPLY BLUE™ SafeStain (Invitrogen, Carlsbad, CA, USA). SDS-PAGE profiles of the culture broths showed that twelve out of twelve transformants had a new protein band of approximately 66 kDa. Transformant number 12 was selected and designated *A. oryzae* Jal250AlLo4.

Shake flask medium was composed per liter of 50 g of glucose, 2 g of $MgSO_4 \cdot 7H_2O$, 10 g of KH_2PO_4, 2 g of K_2SO_4, 0.5 g of $CaCl_2 \cdot 2H_2O$, 2 g of citric acid, 10 g of yeast extract, 0.5 g of AMG trace metals solution, and 2g of urea. AMG trace metals solution was composed per liter of 13.8 g of $FeSO_4 \cdot 7H_2O$, 14.3 g of $ZnSO_4 \cdot 7H_2O$, 8.5 g of $MnSO_4 \cdot H_2O$, 2.5 g of $CuSO_4 \cdot 5H_2O$, 0.5 g of $NiCl_2 \cdot 6H_2O$ and 3.0 g of citric acid monohydrate.

One hundred ml of shake flask medium was added to a 500 ml shake flask. The shake flask was inoculated with a glycerol spore stock of *A. oryzae* Jal250AlLo4 and incubated at 34°C on an orbital shaker at 200 rpm for 24 hours. Fifty ml of the shake flask broth was used to inoculate a fermentation vessel.

Fermentation batch medium was composed per liter of 25 g of sucrose, 2 g of $MgSO_4 \cdot 7H_2O$, 2 g of KH_2PO_4, 3 g of K_2SO_4, 5 g of $(NH_4)_2HPO_4$, 1 g of citric acid, 10 g of yeast extract, 0.5 g of AMG trace metals solution, and 0.55 g of pluronic antifoam. Fermentation feed medium was composed per liter of 320 g of maltose, 5 g of pluronic antifoam, and 1 g of citric acid monohydrate.

A total of 2 liters of the fermentation batch medium was added to a glass jacketed fermentor. The fermentation vessel was maintained at a temperature of 34°C and the pH was controlled at 7 for 180 hours using 10% NH_4OH and 10% H_3PO_4. Air was added to the vessel at a rate of 1 vvm and the broth was agitated by Rushton impeller rotating at 1100 rpm. Feed was started at a rate of 4 g per hour when the batch sucrose was consumed as indicated by a rise in the dissolved oxygen reading (at approximately 18-24 hours). At the end of the fermentation, whole broth was harvested from the vessel and centrifuged at 3000 x *g* to remove the biomass. The supernatant was sterile filtered and stored at 5 to 10°C.

A 350 ml (3.15 g total protein) aliquot of the filtered *A. oryzae* Jal250AlLo4 fermentation broth (AOC18-7) containing recombinant *Chaetomium thermophilum* Cel7A cellobiohydrolase I was concentrated and desalted, and then purified over a Q SEPHAROSE® Big Bead column (GE Healthcare, Piscataway, NJ, USA) in 20 mM Tris-HCl pH 8, over a linear 0 to 1 M NaCl gradient. Fractions were pooled based on SDS-PAGE, concentrated and buffer-exchanged to 25 mM Tris-HCl, pH 8. The purified cellobiohydrolase

l (approximately 800 mg total) was approximately 90% pure by SDS-PAGE. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit (Thermo Fischer Scientific, Waltham, MA, USA) in which bovine serum albumin was used as a protein standard.

Example 2: Preparation of *Myceliophthora thermophila* CBS 117.65 Cel7A cellobiohydrolase I

The *Myceliophthora thermophila* CBS 117.65 Cel7A cellobiohydrolase I (CBHI) gene (SEQ ID NO: 3 [DNA sequence] and SEQ ID NO: 4 [deduced amino acid sequence]) was isolated according to WO 2003/000941 and expressed in *Aspergillus oryzae* JaL250.

Two synthetic oligonucleotide primers, shown below, were designed to PCR amplify the full-length open reading frame from *Myceliophthora thermophila* CBS 117.65 encoding the Family Cel7A cellobiohydrolase I.

PCR Forward primer:

5'-ctcgcagtcgcagtcaag-3' (SEQ ID NO: 75)

PCR Reverse primer:

5'-cggtcaggttgcagtttag-3' (SEQ ID NO: 76)

Thirty picomoles of each of the primers above were used in an amplification reaction containing 50 ng of DNA consisting of a pool of *Myceliophtora thermophila* CBS 117.65 cDNA prepared according to U.S. Patent No. 6,242,237, 1X EXPAND™ PCR Buffer (Roche Diagnostics, Mannheim, Germany), 4 μl of 2.5 mM blend of dATP, dTTP, dGTP, and dCTP, 0.75 μl of EXPAND™ DNA Polymerase (Roche Diagnostics, Mannheim, Germany), in a final volume of 50 μl. The amplification of the fragment was performed in a thermocycler programmed for one cycle at 94°C for 5 minutes; and 35 cycles each at 94°C for 1 minute, 54°C for 1 minute, and 72°C for 2 minutes. After the 30 cycles, the reaction was incubated at 72°C for 10 minutes and then cooled at room temperature until further processed.

A 1.3 kb PCR product was isolated by 1% agarose gel electrophoresis using TBE buffer and 0.1 μg of ethidium bromide per ml. The 1.3 kb DNA band was excised with a disposable razor blade and purified using a JETSORB Gel Extraction Kit (Genomed GmbH, Löhne, Germany) according to the manufacturer's instructions.

The purified PCR fragment was cloned into pCR®4Blunt-TOPO® according to the manufacturer's instructions. PCR clones containing the coding regions of interest were sequenced. One of the clones having the expected sequence was selected and named pDAu27#15.

Two synthetic oligonucleotide primers containing a *Bsp* HI restriction site on the forward primer and a *Pac* I restriction site on the reverse primer, shown below, were

designed to PCR amplify the full-length open reading frame of the *Myceliophthora thermophila* CBS 117.65 Cel7A cellobiohydrolase I from pDAu27#15. A Rapid Ligation Kit was used to clone the fragment into pAlLo2 (WO 2004/099228).

PCR Forward primer:

5'-TC**ATGA**AGCAGTACCTCCAGTA-3' (SEQ ID NO: 77)

PCR Reverse primer:

5'-TTAATTAA**TTAGACGTTGACAGTCGAGC**-3' (SEQ ID NO: 78)

Bold letters represent coding sequence. The underlined sequence contains sequence identity to the *Bsp* HI and *Pac* I restriction sites.

Fifty picomoles of each of the primers above were used in a PCR reaction containing 50 ng of plasmid pDAu27#15 containing the *Myceliophthora thermophila* CBS 117.65 cellobiohydrolase I I gene, 1X *Pfx* Amplification Buffer (Invitrogen, Carlsbad, CA, USA), 6 µl of 10 mM blend of dATP, dTTP, dGTP, and dCTP, 2.5 units of PLATINUM® *Pfx* DNA Polymerase (Invitrogen, Carlsbad, CA, USA), 1 µl of 50 mM $MgSO_4$, and 2.5 µl of 10X pCRx Enhancer solution (Invitrogen, Carlsbad, CA, USA) in a final volume of 50 µl. A DNA ENGINE™ Thermal Cycler was used to amplify the fragment programmed for one cycle at 98°C for 2 minutes; and 35 cycles each at 94°C for 30 seconds, 58°C for 30 seconds, and 68°C for 1.5 minutes. After the 35 cycles, the reaction was incubated at 68°C for 10 minutes and then cooled at 10°C until further processed.

A 1.3 kb PCR reaction product was isolated on a 0.8% GTG® agarose gel using TAE buffer and 0.1 µg of ethidium bromide per ml. The DNA band was visualized with the aid of a DARKREADER™ Transilluminator to avoid UV-induced mutations. The 1.3 kb DNA band was excised with a disposable razor blade and purified with an ULTRAFREE® DA spin cup according to the manufacturer's instructions.

The purified PCR fragment was cloned into pCR®4Blunt-TOPO® according to the manufacturer's instructions. PCR clones containing the coding region of interest were sequenced to Phred Q values of at least 40 to insure that there were no PCR induced errors. All sequence aligments were performed with Consed (University of Washington). One of the clones that was shown to have the expected sequence was selected and re-named MtPCR. The MtPCR clone containing the *M. thermophila* cellobiohydrolase I coding region was double digested with *Bsp* HI and *Bss* SI and a 352 bp fragment was gel purified as described above. Another aliquot of MtPCR was also double digested with *Bss* SI and *Pac* I and a 1009 bp fragment was gel purified as described above. These DNA fragments were then ligated into pAlLo2 previously digested with *Nco* I and *Pac* I in a three-way ligation using a Rapid Ligation Kit. Expression clones were confirmed by restriction digestion and sequenced to confirm that the junction vector-insert was correct. Plasmid DNA for transformation was

prepared with a Midi-Prep Kit. The final clone was re-named pAlLo10.

Aspergillus oryzae JaL250 protoplasts were prepared according to the method of Christensen *et al.*, 1988, *supra*. Six micrograms of pAlLo10 (as well as pAlLo2 as a vector control) were used to transform *Aspergillus oryzae* JaL250 protoplasts. Eight transformants were isolated to individual PDA plates and incubated for five days at 34°C. Confluent spore plates were washed with 5 ml of 0.01% TWEEN® 80 and the spore suspension was used to inoculate 25 ml of MDU2BP medium in 125 ml glass shake flasks. Transformant cultures were incubated at 34°C with constant shaking at 200 rpm. At day five post-inoculation, cultures were centrifuged at 6000 *x g* and their supernatants collected. Five microliters of each supernatant were mixed with an equal volume of 2X loading buffer (10% beta-mercaptoethanol) and loaded onto a 1.5 mm 8%-16% Tris-glycine SDS-PAGE gel and stained with SIMPLY BLUE™ SafeStain. SDS-PAGE profiles of the culture broths showed that eight out of eight transformants had a new protein band of approximately 50 kDa. Transformant number 8 was selected for further studies and designated *A. oryzae* JaL250AlLo10.

A new fully confluent spore plate was prepared as described above. Spores were collected with 5 ml of an aqueous solution of 0.01% TWEEN® 80 and two more washes with MDU2BP medium to maximize the number of spores collected. The spore suspension was then used to inoculate 500 ml of MDU2BP medium in a two-liter Fernbach flask. The *A. oryzae* JaL250AlLo10 liquid culture was then incubated at 34°C with shaking at 200 rpm. At day five post-inoculation the culture broth was collected by filtration on a 500 milliliter, 75 mm Nylon filter unit with a pore size of 0.45 µm.

The culture filtrate was desalted and buffer exchanged in 20 mM Tris, 150 mM NaCl pH 8.5, using a HIPREP® 26/10 desalting column (GE Healthcare, Piscataway, NJ, USA) according to the manufacturer's instructions Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 3: Preparation of *Aspergillus fumigatus* NN055679 Cel7A cellobiohydrolase I

A tfasty search (Pearson *et al.*, 1997, Genomics 46:24-36) of the *Aspergillus fumigatus* partial genome sequence (The Institute for Genomic Research, Rockville, MD) was performed using as query a Cel7 cellobiohydrolase protein sequence from *Trichoderma reesei* (Accession No. P00725). Several genes were identified as putative Family GH7 homologs based upon a high degree of similarity to the query sequence at the amino acid level. One genomic region with significant identity to the query sequence was chosen for further study, and the corresponding gene was named *cel7A*.

Two synthetic oligonucleotide primers shown below were designed to PCR amplify

an *Aspergillus fumigatus* NN055679 *cel7A* cellobiohydrolase I gene (SEQ ID NO: 5 [DNA sequence] and SEQ ID NO: 6 [deduced amino acid sequence]) from genomic DNA of *Aspergillus fumigatus* prepared as described in WO 2005/047499.

Forward primer:

5'-gggcATGCTGGCCTCCACCTTCTCC-3' (SEQ ID NO: 79)

Reverse primer:

5'-gggttaattaaCTACAGGCACTGAGAGTAA-3' (SEQ ID NO: 80)

Upper case letters represent the coding sequence. The remainder of the sequence provides restriction endonuclease sites for *Sph* I and *Pac* I in the forward and reverse sequences, respectively. Using these primers, the *Aspergillus fumigatus cel7A* gene was amplified using standard PCR methods and the reaction product isolated by 1% agarose gel electrophoresis using TAE buffer and purified using a QIAQUICK® Gel Extraction Kit (QIAGEN Inc., Valencia, CA, USA) according to the manufacturer's instructions.

The fragment was digested with *Sph* I and *Pac* I and ligated into the expression vector pAlLo2 also digested with *Sph* I and *Pac* I according to standard procedures. The ligation products were transformed into *E. coli* XL10 SOLOPACK® cells (Stratagene, La Jolla, CA, USA) according to the manufacturer's instructions. An *E. coli* transformant containing a plasmid of the correct size was detected by restriction digestion and plasmid DNA was prepared using a BIOROBOT® 9600 (QIAGEN Inc., Valencia, CA, USA). DNA sequencing of the insert gene from this plasmid was performed with a Perkin-Elmer Applied Biosystems Model 377 XL Automated DNA Sequencer (Perkin-Elmer/Applied Biosystems, Inc., Foster City, CA, USA) using dye-terminator chemistry (Giesecke *et al.*, 1992, *Journal of Virology Methods* 38: 47-60) and primer walking strategy. Nucleotide sequence data were scrutinized for quality and all sequences were compared to each other with assistance of PHRED/PHRAP software (University of Washington, Seattle, WA, USA). The nucleotide sequence was shown to match the genomic sequence determined by TIGR (SEQ ID NO: 5 [DNA sequence] and SEQ ID NO: 6 [deduced amino acid sequence]). The resulting plasmid was named pEJG93.

Aspergillus oryzae JaL250 protoplasts were prepared according to the method of Christensen *et al.*, 1988, *supra*. Five μg of pEJG93 (as well as pAlLo2 as a vector control) was used to transform *Aspergillus oryzae* JaL250.

The transformation of *Aspergillus oryzae* JaL250 with pEJG93 yielded about 100 transformants. Ten transformants were isolated to individual PDA plates.

Confluent PDA plates of five of the ten transformants were washed with 5 ml of 0.01% TWEEN® 20 and inoculated separately into 25 ml of MDU2BP medium in 125 ml glass shake flasks and incubated at 34°C, 250 rpm. Five days after incubation, 0.5 μl of supernatant from each culture was analyzed using 8-16% Tris-Glycine SDS-PAGE gels

(Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. SDS-PAGE profiles of the cultures showed that one of the transformants had a major band of approximately 70 kDa. This transformant was named *Aspergillus oryzae* JaL250EJG93.

Five hundred ml of shake flask medium were added to a 2800 ml shake flask. The shake flask medium was composed of 45 g of maltose, 2 g of K_2HPO_4, 12 g of KH_2PO_4, 1 g of NaCl, 1 g of $MgSO_4 \cdot 7H_2O$, 7 g of yeast extract, 2 g of urea, and 0.5 ml of trace elements solution. The trace elements solution was composed per liter of 13.8 g of $FeSO_4 \cdot 7H_2O$, 14.3 g of $ZnSO_4 \cdot 7H_2O$, 8.5 g of $MnSO_4 \cdot H_2O$, 2.5 g of $CuSO_4 \cdot 5H_2O$, 0.5 g of $NiCl_2 \cdot 6H_2O$, 3 g of citric acid, and deionized water to 1 liter. Two shake flasks were inoculated with a suspension of a PDA plate of *Aspergillus oryzae* JaL250EJG93 with 0.01% TWEEN® 80 and incubated at 34°C on an orbital shaker at 200 rpm for 120 hours. The broth was filtered usinga 0.7 µm Whatman glass filter GF/F (Whatman, Piscataway, NJ, USA) followed by a 0.22 µm EXPRESS™ Plus Membrane (Millipore, Bedford, MA, USA).

Filtered broth was concentrated and buffer exchanged using a tangential flow concentrator (Pall Filtron, Northborough, MA, USA) equipped with a 10 kDa polyethersulfone membrane (Pall Filtron, Northborough, MA, USA) with 20 mM Tris-HCl pH 8.5. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 4: Preparation of *Thermoascus aurantiacus* CGMCC 0583 Cel7A cellobiohydrolase I

The *Thermoascus aurantiacus* CGMCC 0583 Cel7A cellobiohydrolase I (CBHI) gene (SEQ ID NO: 7 [DNA sequence] and SEQ ID NO: 8 [deduced amino acid sequence]) was isolated according to WO 2003/000941 and expressed in *Aspergillus oryzae* JaL250.

The fungal strain *Thermoascus aurantiacus* CGMCC 0583 was grown on an agar plate composed of 0.5% yeast extract, 1% glucose, and 2% agar for 3 days at 45°C. The fully grown culture was used to inoculate shake flasks containing liquid medium composed of 3% soymeal, 1.5% maltose, and 0.5% peptone. The flasks were incubated at 45°C for 48 hours with shaking. The mycelia were harvested by centrifugation of the culture broth at 8000 rpm, 4°C for 30 minutes, transferred into a clean plastic bag followed by immediate freezing in liquid nitrogen, and stored at -80°C before total RNA was isolated.

The frozen mycelia were grounded into a very fine powder with a sterilized mortar and pestle baked at 200°C for 24 hours. An RNEASY® Plant Mini Kit was used to isolate total RNA according to the manufacturer's instructions.

First strand cDNA synthesis from the total RNA was performed using a 3' RACE System for Rapid Amplification of cDNA Ends according to the manufacturer's instructions.

The first strand cDNA of 3' RACE was used as PCR template for PCR screening.

Two oligonucleotides shown below were used for PCR screening of cDNA of *Thermoascus aurantiacus* CGMCC 0583. The forward primer was derived from an alignment of conserved regions of cellobiohydrolase I genes and the reverse primer was provided by the 3' RACE System.

Forward primer:

5'-GGnACnGGnTA(t/c)TG(t/c)GA-3' (SEQ ID NO: 81)

Reverse primer:

5'-TCnA(a/g)CCAnA(a/g)CAT(a/g)TT-3' (SEQ ID NO: 82)

One hundred picomoles of the above primers were used in a PCR reaction composed of 2 µl of the first strand cDNA of 3' RACE, 5 µl of 10X *Taq* DNA polymerase buffer, 3 µl of 25 mM $MgCl_2$, 1 µl of 10 mM dNTP, and 2.5 units of *Taq* DNA polymerase in a final volume of 50 µl. The amplification was performed in a thermocycler programmed for 1 cycle at 95°C for 3 minutes; 30 cycles each at 95°C for 30 seconds, 50°C for 30 seconds, and 72°C for 50 seconds; and 1 cycle at 72°C for 10 minutes. The heat block then went to a 4°C soak cycle.

The reaction products were isolated by 1.0% agarose gel electrophoresis using TBE buffer where an approximately 0.65 kb product band was excised from the gel, and purified using a WIZARD® PCR Preps DNA Purification System according to the manufacturer's instructions. The PCR product was sequenced using a 377 DNA Analyzer. Sequencing showed that the 0.65 kb fragment was homologous to cellobiohydrolase I.

Two oligos were designed for 5' end cloning of *Thermoascus aurantiacus* CGMCC 0583 Cel7A cellobiohydrolase I by using a 5' RACE System for Rapid Amplification of cDNA Ends.

Primer 025AS1:

5'-GTAGAGATGCTGTTGGCT-3' (SEQ ID NO: 83)

Primer 025AS1.5:

5'-TCTCAGCGCAGCAGGAACCGT-3' (SEQ ID NO: 84)

The gene specific primer 025AS1 was used for first strand cDNA synthesis using the 5' RACE System according to the manufacturer's instructions. The first strand cDNA of 5' RACE was used as template for a PCR amplification composed of 5 µl of 10X *Taq* DNA polymerase buffer, 3 µl of 25 mM $MgCl_2$, 1 µl of 10 mM dNTP, 2 µl of 10 µM primer 025AS1.5, 2 µl of 10 µM primer AAP (Abridged Anchor Primer, provided by the kit), and 2.5 units of *Taq* DNA polymerase in a final volume of 50 µl. The amplification was performed in a thermocycler programmed for 1 cycle at 94°C for 2 minutes; 30 cycles each at 94°C for 40 seconds, 55°C for 40 seconds, and 72°C for 60 seconds; and 1 cycle at 72°C for 10 minutes. The heat block then went to a 4°C soak cycle.

The PCR products were isolated by 1.0% agarose gel electrophoresis using TBE buffer and purified using a WIZARD® PCR Preps DNA Purification System. A dominant DNA fragment at 0.8 kb was confirmed to be the 5' end of *Thermoascus aurantiacus* CGMCC 0583 Cel7A cellobiohydrolase I gene by sequencing using a 377 DNA Analyzer.

One forward primer, 1F shown below, was designed based on the sequence information of the 5' end cloning. Primer 1F was used for the full-length cloning of the *Thermoascus aurantiacus* CGMCC 0583 Cel7A cellobiohydrolase I gene together with primer AUAP (provided by the kit) as the reverse primer.

Primer 1F:

5'-AGCGACAGCAATAACAAT-3' (SEQ ID NO: 85)

Ten picomoles of the above 2 primers were used in a PCR reaction composed of the 4 µl of first strand cDNA of 3' RACE, 5 µl of 10X *Taq* DNA polymerase buffer, 3 µl of 25 mM $MgCl_2$1 µl of 10 mM dNTP, and 2.5 units of *Taq* DNA polymerase in a final volume of 50 µl. The amplification was performed in a thermocycler programmed for 1 cycle at 95°C for 2 minutes; 30 cycles each at 95°C for 40 seconds, 58°C for 40 seconds, and 72°C for 90 seconds; and 1 cycle at 72°C for 10 minutes. The heat block then went to a 4°C soak cycle.

The reaction products were isolated by 1.0% agarose gel electrophoresis using TBE buffer where an approximately 1.7 kb product band was excised from the gel, and purified using a WIZARD® PCR Preps DNA Purification System. The PCR fragment was then ligated to pGEM-T using a pGEM-T Vector System. The plasmid DNA was confirmed by sequencing using a 377 DNA Analyzer. The correct clone was designated pT002-5.

Two synthetic oligonucleotide primers shown below containing *Bsp* LU11I sites on the forward primer and *Pac* I at the reverse primer were designed to PCR amplify the full-length open reading frame of the *Thermoascus aurantiacus* CGMCC 0583 Cel7A cellobiohydrolase I gene. A Rapid Ligation Kit was used to clone the fragment into pAILo2.

PCR Forward primer:

5'-AC**ATGTATCAGCGCGCTCTTCTC**-3' (SEQ ID NO: 86)

PCR Reverse primer:

5'-TTAATTAA**TTAGTTGGCGGTGAAGGTCG**-3' (SEQ ID NO: 87)

Bold letters represent coding sequence. The underlined sequence contains sequence identity to the *Bsp* LU11I and *Pac* I restriction sites.

Fifty picomoles of each of the primers above were used in a PCR reaction containing 50 ng of plasmid pT002-5, 1X *Pwo* Amplification Buffer with $MgSO_4$, 4 µl of 10 mM blend of dATP, dTTP, dGTP, and dCTP, and 2.5 units of *Pwo* DNA Polymerase, in a final volume of 50 µl. A DNA ENGINE™ Thermal Cycler was used to amplify the fragment programmed for one cycle at 94°C for 2 minutes; and 25 cycles each at 94°C for 30 seconds, 59°C for 30

seconds, and 72°C for 1.5 minutes. After the 25 cycles, the reaction was incubated at 72°C for 10 minutes and then cooled at 10°C until further processed.

A 1.3 kb PCR reaction product was isolated on a 0.8% GTG® agarose gel using TAE buffer and 0.1 µg of ethidium bromide per ml. The DNA band was visualized with the aid of a DARKREADER™ Transilluminator to avoid UV-induced mutations. The 1.3 kb DNA band was excised with a disposable razor blade and purified with an ULTRAFREE® DA spin cup according to the manufacturer's instructions.

The purified PCR fragment was cloned into pCR®4Blunt-TOPO® according to the manufacturer's instructions. PCR clones containing the coding region of interest were sequenced to Phred Q values of at least 40 to insure that there were no PCR induced errors. All sequence aligments were performed with Consed (University of Washington). One of the clones that was shown to have the expected sequence was selected and re-named TaPCR. The TaPCR clone containing the *T. aurantiacus* cellobiohydrolase I coding region was double digested with the restriction enzymes *Bsp* LU11I and *Pac* I and gel purified as described above. This DNA fragment was then ligated into pAlLo2 previously digested with *Nco* I and *Pac* I using a Rapid Ligation Kit. Expression clones were confirmed by restriction digestion and sequenced to confirm that the junction vector-insert was correct. Plasmid DNA for transformation was prepared with a Midi-Prep Kit. The final clone was re-named pAlLo6.

Aspergillus oryzae JaL250 protoplasts were prepared according to the method of Christensen *et al.*, 1988, *supra*. Ten micrograms of pAlLo6 were used to transform the *Aspergillus oryzae* JaL250 protoplasts. Twelve transformants were isolated to individual PDA plates and incubated for 5 days at 34°C. Confluent spore plates were washed with 5 ml of 0.01% TWEEN® 80 and the spore suspension was used to inoculate 25 ml of MDU2BP medium in 125 ml glass shake flasks. Transformant cultures were incubated at 34°C with constant shaking at 200 rpm. At day five post-inoculation, cultures were centrifuged at 6000 *x g* and their supernatants collected. Five microliters of each supernatant were mixed with an equal volume of 2X loading buffer (10% beta-mercaptoethanol) and analyzed by SDS-PAGE using a 1.5 mm 8%-16% Tris-glycine SDS-PAGE gel and stained with SIMPLY BLUE™ SafeStain. SDS-PAGE profiles of the culture broths showed that eleven out of twelve transformants had a new protein band of approximately 60 kDa. Transformant number 12 was selected for further studies and designated *A. oryzae* JaL250AlLo6.

A spore stock suspension was prepared from a 5 day plate culture of *A. oryzae* JaL250AlLo6 by adding 10 ml of 0.1% TWEEN® 20 to the culture plate to release the spores. A shake flask culture was started by inoculating 100 µl of the spore stock to a 250 ml baffled flask containing 50 ml of M410 medium pH 6.0. The shake flask was grown at 34°C for 5 days with shaking a 250 rpm. The culture was filtered through a 0.2 µm pore filter device and the filtrate was recovered for protein purification.

A 50 ml volume of the filtrate was desalted and buffer exchanged in 20 mM sodium acetate pH 5.0 using an ECONO-PAC® 10-DG desalting column according to the manufacturer's instructions. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 5: Preparation of *Myceliophthora thermophila* CBS 117.65 Cel6A cellobiohydrolase II

The *Myceliophthora thermophila* CBS 117.65 Cel6A cellobiohydrolase II (SEQ ID NO: 9 [DNA sequence] and SEQ ID NO: 10 [deduced amino acid sequence]) was obtained according to the procedure described below.

One hundred ml of shake flask medium was added to a 500 ml shake flask. The shake flask medium was composed per liter of 15 g of glucose, 4 g of K_2HPO_4, 1 g of NaCl, 0.2 g of $MgSO_4 \cdot 7H_2O$, 2 g of MES free acid, 1 g of Bacto Peptone, 5 g of yeast extract, 2.5 g of citric acid, 0.2 g of $CaCl_2 \cdot 2H_2O$, 5 g of NH_4NO_3, and 1 ml of trace elements solution. The trace elements solution was composed per liter of 1.2 g of $FeSO_4 \cdot 7H_2O$, 10 g of $ZnSO_4 \cdot 7H_2O$, 0.7 g of $MnSO_4 \cdot H_2O$, 0.4 g of $CuSO_4 \cdot 5H_2O$, 0.4 g of $Na_2B_4O_7 \cdot 10H_2O$, and 0.8 g of $Na_2MoO_2 \cdot 2H_2O$. The shake flask was inoculated with two plugs from a solid plate culture of *Myceliophthora thermophila* strain CBS 117.65 and incubated at 45°C with shaking at 200 rpm for 48 hours. Fifty ml of the shake flask broth was used to inoculate a 2 liter fermentation vessel.

Fermentation batch medium was composed per liter of 5 g of yeast extract, 176 g of powdered cellulose, 2 g of glucose, 1 g of NaCl, 1 g of Bacto Peptone, 4 g of K_2HPO_4, 0.2 g of $CaCl_2 \cdot 2H_2O$, 0.2 g of $MgSO_4.7H_2O$, 2.5 g of citric acid, 5 g of NH_4NO_3, 1.8 ml of anti-foam, and 1 ml of trace elements solution (above). Fermentation feed medium was composed of water and antifoam.

A total of 1.8 liters of the fermentation batch medium was added to a two liter glass jacketed fermentor (Applikon Biotechnology, Schiedam, Netherlands). Fermentation feed medium was dosed at a rate of 4 g/l/hr for a period of 72 hours. The fermentation vessel was maintained at a temperature of 45°C and pH was controlled using an Applikon 1030 control system (Applikon Biotechnology, Schiedam, Netherlands) to a set-point of 5.6 +/- 0.1. Air was added to the vessel at a rate of 1 vvm and the broth was agitated by Rushton impeller rotating at 1100 to 1300 rpm. At the end of the fermentation, whole broth was harvested from the vessel and centrifuged at 3000 *x g* to remove the biomass.

The harvested broth obtained above was centrifuged in 500 ml bottles at 13,000 *x g* for 20 minutes at 4°C and then sterile filtered using a 0.22 µm polyethersulfone membrane (Millipore, Bedford, MA, USA). The filtered broth was concentrated and buffer exchanged with 20 mM Tris-HCl pH 8.5 using a tangential flow concentrator equipped with a 10 kDa

polyethersulfone membrane at approximately 20 psi. To decrease the amount of pigment, the concentrate was applied to a 60 ml Q SEPHAROSE™ Big Bead column equilibrated with 20 mM Tris-HCl pH 8.5, and step eluted with equilibration buffer containing 600 mM NaCl. Flow-through and eluate fractions were examined on 8-16% CRITERION® SDS-PAGE gels (Bio-Rad Laboratories, Inc., Hercules, CA, USA) stained with GELCODE® Blue Stain Reagent (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The flow-through fraction contained the *Myceliophthora thermophila* Cel6A cellobiohydrolase as judged by the presence of a band corresponding to the apparent molecular weight of the protein by SDS-PAGE (approximately 75 kDa).

The flow-through fraction was concentrated using an ultrafiltration device (Millipore, Bedford, MA, USA) equipped with a 10 kDa polyethersulfone membrane at 40 psi, 4°C and mixed with an equal volume of 20 mM Tris-HCl pH 7.5 containing 3.4 M ammonium sulfate for a final concentration of 1.7 M ammonium sulfate. The sample was filtered (0.2 µM syringe filter, polyethersulfone membrane, Whatman, Maidstone, United Kingdom) to remove particulate matter prior to loading onto a PHENYL SUPEROSE™ column (HR 16/10, GE Healthcare, Piscataway, NJ, USA) equilibrated with 1.7 M ammonium sulfate in 20 mM Tris-HCl pH 7.5. Bound proteins were eluted with a 12 column volume decreasing salt gradient of 1.7 M ammonium sulfate to 0 M ammonium sulfate in 20 mM Tris-HCl pH 7.5. Fractions were analyzed by 8-16% SDS-PAGE gel electrophoresis as described above, which revealed that the *Myceliophthora thermophila* Cel6A cellobiohydrolase eluted at the very end of the gradient (approximately 20 mM ammonium sulfate).

Fractions containing the Cel6A cellobiohydrolase II were pooled and diluted 10-fold in 20 mM Tris-HCl pH 9.0 (to lower the salt and raise the pH) and then applied to a 1 ml RESOURCE™ Q column (GE Healthcare, Piscataway, NJ, USA) equilibrated with 20 mM Tris-HCl pH 9.0. Bound proteins were eluted with a 20 column volume salt gradient from 0 mM to 550 mM NaCl in 20 mM Tris-HCl pH 9.0. *M. thermophila* Cel6A cellobiohydrolase II eluted as a single peak early in the gradient (~25 mM NaCl). The cellobiohydrolase II was >90% pure as judged by SDS-PAGE. Protein concentrations were determined using a BCA Protein Assay Kit (Pierce, Rockford, IL, USA) in which bovine serum albumin was used as a protein standard.

Example 6: Preparation of recombinant *Myceliophthora thermophila* CBS 202.75 Cel6A cellobiohydrolase II

Myceliophthora thermophila CBS 202.75 was grown in 100 ml of YEG medium in a baffled shake flask at 45°C for 2 days with shaking at 200 rpm. Mycelia were harvested by filtration using MIRACLOTH® (Calbiochem, La Jolla, CA, USA), washed twice in deionized

water, and frozen under liquid nitrogen. Frozen mycelia were ground, by mortar and pestle, to a fine powder, and total DNA was isolated using a DNEASY® Plant Maxi Kit (QIAGEN Inc., Valencia, CA, USA).

A full-length Family 6 cellobiohydrolase gene (*Cel6A*) was isolated from *Myceliophthora thermophila* CBS 202.75 using a GENOMEWALKER™ Universal Kit (Clontech Laboratories, Inc., Mountain View, CA, USA) according to the manufacturer's instructions. Briefly, total genomic DNA from *Myceliophthora thermophila* CBS 202.75 was digested separately with four different restriction enzymes (*Dra* I, *Eco* RV, *Pvu* II, and *Stu* I) that leave blunt ends. Each batch of digested genomic DNA was then ligated separately to the GENOMEWALKER™ Adaptor (Clontech Laboratories, Inc., Mountain View, CA, USA) to create four libraries. These libraries were then employed as templates in PCR reactions using two gene-specific primers shown below, one for primary PCR and one for secondary PCR. The primers were designed based on a partial Family 6 cellobiohydrolase gene (*Cel6A*) sequence from *Myceliophthora thermophila* (WO 2004/056981).

Primer MtCel6A-R4:

5′-ATTGGCAGCCCGGATCTGGGACAGAGTCTG-3′ (SEQ ID NO: 88)

Primer MtCel6A-R5:

5′-CCGGTCATGCTAGGAATGGCGAGATTGTGG-3′ (SEQ ID NO: 89)

The primary amplifications were composed of 1 μl (approximately 6 ng) of each library as template, 0.4 mM each of dATP, dTTP, dGTP, and dCTP, 10 pmol of Adaptor Primer 1 (Clontech Laboratories, Inc., Mountain View, CA, USA), 10 pmol of primer MtCel6A-R4, 1X ADVANTAGE® GC-Melt LA Buffer (Clontech Laboratories, Inc., Mountain View, CA, USA), and 1.25 units of ADVANTAGE® GC Genomic Polymerase Mix (Clontech Laboratories, Inc., Mountain View, CA, USA) in a final volume of 25 μl. The amplification reactions were incubated in an EPPENDORF® MASTERCYCLER® 5333 (Eppendorf Scientific, Inc., Westbury, NY, USA) programmed for pre-denaturing at 94°C for 1 minute; 7 cycles each at a denaturing temperature of 94°C for 30 seconds; annealing and elongation at 72°C for 5 minutes; and 32 cycles each at 67°C for 5 minutes.

The secondary ampliifications were composed of 1 μl of each primary PCR product as template, 0.4 mM each of dATP, dTTP, dGTP, and dCTP, 10 pmol of Adaptor Primer 2 (Clontech Laboratories, Inc., Mountain View, CA, USA), 10 pmol of primer MtCel6A-R5, 1X ADVANTAGE® GC-Melt LA Buffer, and 1.25 units of ADVANTAGE® GC Genomic Polymerase Mix in a final volume of 25 μl. The amplifications were incubated in an EPPENDORF® MASTERCYCLER® 5333 programmed for pre-denaturing at 94°C for 1 minute; 5 cycles each at a denaturing temperature of 94°C for 30 seconds; annealing and elongation at 72°C for 5 minutes; and 20 cycles at 67°C for 5 minutes.

The reaction products were isolated by 1.0% agarose gel electrophoresis using TAE buffer where a 3.5 kb product band from the *Eco* RV library was excised from the gel, purified using a QIAQUICK® Gel Extraction Kit according to the manufacturer's instructions, and sequenced.

DNA sequencing of the 3.5 kb PCR fragment was performed with a Perkin-Elmer Applied Biosystems Model 377 XL Automated DNA Sequencer (Perkin-Elmer/Applied Biosystems, Inc., Foster City, CA, USA) using dye-terminator chemistry (Giesecke *et al.*, 1992, *Journal of Virology Methods* 38: 47-60) and primer walking strategy. The following gene specific primers were used for sequencing:

MtCel6A-F2:

5'-GCTGTAAACTGCGAATGGGTTCAG-3' (SEQ ID NO: 90)

MtCel6A-F3:

5'-GGGTCCCACATGCTGCGCCT-3' (SEQ ID NO: 91)

MtCel6A-R8:

5'-AAAATTCACGAGACGCCGGG-3' (SEQ ID NO: 92)

Nucleotide sequence data were scrutinized for quality and all sequences were compared to each other with assistance of PHRED/PHRAP software (University of Washington, Seattle, WA, USA). The 3.5 kb sequence was compared and aligned with a partial Family 6 cellobiohydrolase gene (*Cel6A*) sequence from *Myceliophthora thermophila* (WO 2004/056981).

A gene model for the *Myceliophthora thermophila* sequence was constructed based on similarity of the encoded protein to homologous glycoside hydrolase Family 6 proteins from *Thielavia terrestris, Chaetomium thermophilum, Humicola insolens*, and *Trichoderma reesei*. The nucleotide sequence and deduced amino acid sequence of the *Myceliophthora thermophila* CBS 202.75 Cel6A cellobiohydrolase II gene are shown in SEQ ID NO: 11 and SEQ ID NO: 12, respectively. The genomic fragment encodes a polypeptide of 482 amino acids, interrupted by 3 introns of 96, 87, and 180 bp. The% G+C content of the gene and the mature coding sequence are 61.6% and 64%, respectively. Using the SignalP software program (Nielsen *et al.*, 1997, *Protein Engineering* 10: 1-6), a signal peptide of 17 residues was predicted. The predicted mature protein contains 465 amino acids with a molecular mass of 49.3 kDa.

Two synthetic oligonucleotide primers shown below were designed to PCR amplify the *Myceliophthora thermophila* cellobiohydrolase gene from the genomic DNA prepared above for construction of an *Aspergillus oryzae* expression vector. An IN-FUSION™ Cloning Kit (BD Biosciences, Palo Alto, CA, USA) was used to clone the fragment directly into the expression vector pAlLo2, without the need for restriction digestion and ligation.

MtCel6A-F4:

5'-ACTGGATTTACC**ATGGCCAAGAAGCTTTTCATCACC**-3' (SEQ ID NO: 93)

MtCel6A-R9:

5'-TCACCTCTAGTTAATTAA**TTAGAAGGGCGGGTTGGCGT**-3' (SEQ ID NO: 94)

Bold letters represent coding sequence. The remaining sequence is homologous to the insertion sites of pAlLo2.

Fifty picomoles of each of the primers above were used in a PCR reaction composed of 100 ng of *Myceliophthora thermophila* genomic DNA, 1X ADVANTAGE® GC-Melt LA Buffer, 0.4 mM each of dATP, dTTP, dGTP, and dCTP, and 1.25 units of ADVANTAGE® GC Genomic Polymerase Mix in a final volume of 25 µl. The amplification reaction was incubated in an EPPENDORF® MASTERCYCLER® 5333 programmed for 1 cycle at 94°C for 1 minutes; and 30 cycles each at 94°C for 30 seconds, 62°C for 30 seconds, and 72°C for 2 minutes. The heat block then went to a 4°C soak cycle.

The reaction products were isolated by 1.0% agarose gel electrophoresis using TAE buffer where a 1842 bp product band was excised from the gel, and purified using a QIAQUICK® Gel Extraction Kit according to the manufacturer's instructions.

Plasmid pAlLo2 was digested with *Nco* I and *Pac* I, isolated by 1.0% agarose gel electrophoresis using TAE buffer, and purified using a QIAQUICK® Gel Extraction Kit according to the manufacturer's instructions.

The gene fragment and the digested vector were ligated together using an IN-FUSION™ Cloning Kit resulting in pSMai180 in which transcription of the cellobiohydrolase gene was under the control of a NA2-tpi promoter (a modified promoter from the gene encoding neutral alpha-amylase in *Aspergillus niger* in which the untranslated leader has been replaced by an untranslated leader from the gene encoding triose phosphate isomerase in *Aspergillus nidulans*). The ligation reaction (50 µl) was composed of 1X IN-FUSION™ Buffer (BD Biosciences, Palo Alto, CA, USA), 1X BSA (BD Biosciences, Palo Alto, CA, USA), 1 µl of IN-FUSION™ enzyme (diluted 1:10) (BD Biosciences, Palo Alto, CA, USA), 100 ng of pAlLo2 digested with *Nco* I and *Pac* I, and 50 ng of the *Myceliophthora thermophila Cel6A* purified PCR product. The reaction was incubated at room temperature for 30 minutes. One µl of the reaction was used to transform *E. coli* XL10 SOLOPACK® Gold cells. An *E. coli* transformant containing pSMai180 was detected by restriction digestion and plasmid DNA was prepared using a BIOROBOT® 9600. The *Myceliophthora thermophila Cel6A* insert in pSMai180 was confirmed by DNA sequencing.

The same 1842 bp PCR fragment was cloned into pCR®2.1-TOPO® (Invitrogen, Carlsbad, CA, USA) using a TOPO® TA CLONING® Kit to generate pSMai182. The *Myceliophthora thermophila cel6A* gene insert in pSMai182 was confirmed by DNA sequencing. *E. coli* pSMai182 was deposited with the Agricultural Research Service Patent Culture Collection, Northern Regional Research Center, 1815 University Street, Peoria, Ill.,

61604, on Sept. 6, 2007.

The *Myceliophthora thermophila* Family 6 cellobiohydrolase *Cel6A* gene was expressed in *Aspergillus oryzae* JaL355. *A. oryzae* JaL355 (WO 2002/40694) protoplasts were prepared according to the method of Christensen *et al.*, 1988, *supra*. Three µg of pSMai180 were used to transform *Aspergillus oryzae* JaL355.

The transformation of *Aspergillus oryzae* JaL355 with pSMai180 yielded about 50 transformants. Twenty transformants were isolated to individual Minimal medium plates.

Confluent Minimal Medium plates of the 20 transformants were washed with 5 ml of 0.01% TWEEN® 20 and inoculated separately into 25 ml of MDU2BP medium in 125 ml glass shake flasks and incubated at 34°C, 250 rpm. After 5 days incubation, 5 µl of supernatant from each culture were analyzed using 8-16% CRITERION® SDS-PAGE gels and a CRITERION® Cell (Bio-Rad Laboratories, Inc., Hercules, CA, USA), according to the manufacturer's instructions. The resulting gel was stained with BIO-SAFE™ Coomassie Stain (Bio-Rad Laboratories, Inc., Hercules, CA, USA). SDS-PAGE profiles of the cultures showed that the majority of the transformants had a major band of approximately 70 kDa.

A confluent plate of one transformant, designated transformant 14, was washed with 10 ml of 0.01% TWEEN® 20 and inoculated into a 2 liter Fernbach flask containing 500 ml of MDU2BP medium to generate broth for characterization of the enzyme. The culture was harvested on day 5 and filtered using a 0.22 µm EXPRESS™ Plus Membrane.

The filtered broth was concentrated and buffer exchanged using a tangential flow concentrator equipped with a 10 kDa polyethersulfone membrane with 20 mM Tris-HCl pH 8.0. The concentrated and buffer exchanged broth was adjusted to 20 mM Tris-HCl pH 8.0-1.2 M $(NH_4)_2SO_4$ and applied to a Phenyl SUPEROSE™ column (HR 16/10) equilibrated with 20 mM Tris-HCl pH 8.0-1.2 M $(NH_4)_2SO_4$. Bound proteins were eluted with a linear gradient over 10 column volumes from 300 to 0 mM $(NH_4)_2SO_4$ in 20 mM Tris-HCl pH 8.0. SDS-PAGE of eluate fractions showed a major band at approximately 70 kDa. These fractions were then concentrated and buffer exchanged by centrifugal concentration using a VIVASPIN™ centrifugal concentrator (10 kDa polyethersulfone membrane, Sartorius, Göttingen, Germany) into 20 mM Tris-HCl pH 8.0. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 7: Preparation of *Thielavia terrestris* NRRL 8126 Cel6A cellobiohydrolase II (CBHII)

Thielavia terrestris NRRL 8126 Cel6A cellobiohydrolase II (SEQ ID NO: 13 [DNA sequence] and SEQ ID NO: 14 [deduced amino acid sequence]) was recombinantly

prepared according to WO 2006/074435 using *Trichoderma reesei* as a host.

Culture filtrate was desalted and buffer exchanged in 20 mM Tris–150 mM sodium chloride pH 8.5 using an ECONO-PAC® 10-DG desalting column according to the manufacturer's instructions. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 8: Preparation of *Trichophaea saccata* CBS 804.70 cellobiohydrolase II (CBHII)

The *Trichophaea saccata* CBS 804.70 cellobiohydrolase II (CBHII) (SEQ ID NO: 15 [DNA sequence] and SEQ ID NO: 16 [deduced amino acid sequence]) was prepared as described below.

Trichophaea saccata CBS 804.70 was inoculated onto a PDA plate and incubated for 7 days at 28°C. Several mycelia-PDA agar plugs were inoculated into 750 ml shake flasks containing 100 ml of MEX-1 medium. The flasks were incubated at 37°C for 9 days with shaking at 150 rpm. The fungal mycelia were harvested by filtration through MIRACLOTH® (Calbiochem, San Diego, CA, USA) before being frozen in liquid nitrogen. The mycelia were then pulverized into a powder by milling the frozen mycelia together with an equal volume of dry ice in a coffee grinder precooled with liquid nitrogen. The powder was transferred into a liquid nitrogen prechilled mortar and pestle and ground to a fine powder with a small amount of baked quartz sand. The powdered mycelial material was kept at -80°C until use.

Total RNA was prepared from the frozen, powdered mycelia of *Trichophaea saccata* CBS 804.70 by extraction with guanidium thiocyanate followed by ultracentrifugation through a 5.7 M CsCl cushion according to Chirgwin *et al.*, 1979, *Biochemistry* 18: 5294-5299. The polyA enriched RNA was isolated by oligo (dT)-cellulose affinity chromatography according to Aviv *et al.*, 1972, *Proc. Natl. Acad. Sci. USA* 69: 1408-1412.

Double stranded cDNA was synthesized according to the general methods of Gubler and Hoffman, 1983, *Gene* 25: 263-269; Sambrook, J., Fritsch, E.F., and Maniantis, T. *Molecular cloning: A Laboratory Manual*, 2nd ed., 1989, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; and Kofod *et al.*, 1994, *J. Biol. Chem.* 269: 29182-29189, using a polyA-*Not* I primer (Promega Corp., Madison, Wisconsin, USA). After synthesis, the cDNA was treated with mung bean nuclease, blunt ended with T4 DNA polymerase, and ligated to a 50-fold molar excess of *Eco* RI adaptors (Invitrogen Corp., Carlsbad, CA, USA). The cDNA was cleaved with *Not* I and the cDNA was size fractionated by 0.8% agarose gel electrophoresis using in 44 mM Tris base, 44 mM boric acid, 0.5 mM EDTA (TBE) buffer. The fraction of cDNA of 700 bp and larger was excised from the gel and purified using a GFX® PCR DNA and Gel Band Purification Kit (GE Healthcare Life Sciences, Piscataway, NJ, USA) according to the manufacturer's instructions.

The prepared cDNA was then directionally cloned by ligation into *Eco* RI-*Not* I cleaved pMHas5 (WO 03/044049) using a Rapid Ligation Kit (Roche Diagnostics GmbH, Penzberg, Germany) according to the manufacturer's instructions. The ligation mixture was electroporated into *E. coli* DH10B cells (Invitrogen Corp., Carlsbad, CA, USA) using a GENE PULSER® and Pulse Controller (Bio-Rad Laboratories, Inc., Hercules, CA, USA) at 50 µF, 25 mAmp, 1.8 kV with a 2 mm gap cuvette according to the manufacturer's procedure.

The electroporated cells were spread onto LB plates supplemented with 50 µg of kanamycin per ml. A cDNA plasmid pool was prepared from approximately 30,000 total transformants of the original cDNA-pMHas5 vector ligation. Plasmid DNA was prepared directly from the pool of colonies using a QIAPREP® Spin Midi/Maxiprep Kit (QIAGEN GmbH Corporation, Hilden, Germany). The cDNA library was designated SBL521-2.

A transposon containing plasmid designated pSigA4 was constructed from the pSigA2 transposon containing plasmid described in WO 01/77315 in order to create an improved version of the signal trapping transposon of pSigA2 with decreased selection background. The pSigA2 transposon contains a signal less beta-lactamase construct encoded on the transposon itself. PCR was used to create a deletion of the intact beta-lactamase gene found on the plasmid backbone using a proofreading PROOFSTART® DNA polymerase (QIAGEN GmbH Corporation, Hilden, Germany) and the following 5' phosphorylated primers (TAG Copenhagen, Denmark):

SigA2NotU-P:

5'-TCGCGATCCGTTTTCGCATTTATCGTGAAACGCT-3' (SEQ ID NO: 95)

SigA2NotD-P:

5'-CCGCAAACGCTGGTGAAAGTAAAAGATGCTGAA-3' (SEQ ID NO: 96)

The amplification reaction was composed of 1 µl of pSigA2 (10 ng/ µl), 5 µl of 10X ProofStart Buffer (QIAGEN GmbH Corporation, Hilden, Germany), 2.5 µl of dNTP mix (20 mM), 0.5 µl of SigA2NotU-P (10 mM), 0.5 µl of SigA2NotD-P (10 mM), 10 µl of Q solution (QIAGEN GmbH Corporation, Hilden, Germany), and 31.25 µl of deionized water. A DNA ENGINE™ Thermal Cycler (MJ Research Inc., Waltham, MA, USA) was used for the amplification programmed for 1 cycle at 95°C for 5 minutes; and 20 cycles each at 94°C for 30 seconds, 62°C for 30 seconds, and 72°C for 4 minutes.

A 3.9 kb PCR reaction product was isolated by 0.8% agarose gel electrophoresis using TAE buffer and 0.1 µg of ethidium bromide per ml. The DNA band was visualized with the aid of an Eagle Eye Imaging System (Stratagene, La Jolla, CA, USA) at 360 nm. The 3.9 kb DNA band was excised from the gel and purified by using a GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

The 3.9 kb fragment was self-ligated at 16°C overnight with 10 units of T4 DNA ligase (New England Biolabs, Inc., Beverly, MA, USA), 9 µl of the 3.9 kb PCR fragment, and

1 µl of 10X ligation buffer (New England Biolabs, Inc., Beverly, MA, USA). The ligation was heat inactivated for 10 minutes at 65°C and then digested with *Dpn* I at 37°C for 2 hours. After incubation, the digestion was purified using a GFX® PCR DNA and Gel Band Purification Kit.

The purified material was then transformed into *E. coli* TOP10 competent cells (Invitrogen Corp., Carlsbad, CA, USA) according to the manufacturer's instructions. The transformation mixture was plated onto LB plates supplemented with 25 µg of chloramphenicol per ml. Plasmid minipreps were prepared from several transformants and digested with *Bgl* II. One plasmid with the correct construction was chosen. The plasmid was designated pSigA4. Plasmid pSigA4 contains the *Bgl* II flanked transposon SigA2 identical to that disclosed in WO 01/77315.

A 60 µl sample of plasmid pSigA4 DNA (0.3 µg/µl) was digested with *Bgl* II and separated by 0.8% agarose gel electrophoresis using TAE buffer. A SigA2 transposon DNA band of 2 kb was eluted with 200 µl of EB buffer (QIAGEN GmbH Corporation, Hilden, Germany) and purified using a GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions and eluted in 200 µl of EB buffer. SigA2 was used for transposon assisted signal trapping.

A complete description of transposon assisted signal trapping can be found in WO 01/77315. A cDNA plasmid pool was prepared from 30,000 total transformants of the original cDNA-pMHas5 vector ligation. Plasmid DNA was prepared directly from a pool of colonies recovered from solid LB selective medium using a QIAPREP® Spin Midi/Maxiprep Kit. The plasmid pool was treated with transposon SigA2 and MuA transposase (Finnzymes OY, Espoo, Finland) according to the manufacturer's instructions.

For *in vitro* transposon tagging of the *Trichophaea saccata* CBS 804.70 cDNA library, 4 or 8 µl of SigA2 transposon containing approximately 2.6 µg of DNA were mixed with 1 µl of the plasmid DNA pool of the *Trichophaea saccata* CBS 804.70 cDNA library containing 2 µg of DNA, 2 µl of MuA transposase (0.22 µg/µl), and 5 µl of 5X buffer (Finnzymes OY, Espoo, Finland) in a total volume of 50 µl and incubated at 30°C for 3.5 hours followed by heat inactivation at 75°C for 10 minutes. The DNA was precipitated by addition of 5 µl of 3 M sodium acetate pH 5 and 110 µl of 96% ethanol and centrifuged for 30 minutes at 10,000 *x g*. The pellet was washed in 70% ethanol, air dried at room temperature, and resuspended in 10 µl of 10 mM Tris, pH 8, 1 mM EDTA (TE) buffer.

A 1.5 µl volume of the transposon tagged plasmid pool was electroporated into 20 µl of *E. coli* DH10B ultracompetent cells (Gibco-BRL, Gaithersburg MD, USA) according to the manufacturer's instructions using a GENE PULSER® and Pulse Controller (Bio-Rad Laboratories, Inc., Hercules, CA, USA) at 50 uF, 25 mAmp, 1.8 kV with a 2 mm gap cuvette according to the manufacturer's procedure.

The electroporated cells were incubated in SOC medium with shaking at 250 rpm for 2 hours at 28°C before being plated on the following selective media: LB medium supplemented with 50 µg of kanamycin per ml; LB medium supplemented with 50 µg of kanamycin per ml and 15 µg of chloramphencol per ml; and/or LB medium supplemented with 50 µg of kanamycin per ml, 15 µg of chloramphencol per ml, and 12.5 µg of ampicillin per ml.

From dilution plating of the electroporation onto LB medium supplemented with kanamycin and chloramphencol medium, it was determined that approximately 72,000 colonies were present containing a cDNA library plasmid with a SigA2 transposition per electroporation and that approximately 69 colonies were recovered under triple selection (LB, kanamycin, chorlamphenicol, ampicillin). Further electroporation and plating experiments were performed until 445 total colonies were recovered under triple selection. The colonies were miniprepped using a QIAPREP® 96 Turbo Miniprep Kit (QIAGEN GmbH Corporation, Hilden, Germany). Plasmids were sequenced with the transposon forward and reverse primers (primers A and B), shown below, according to the procedure disclosed in WO 2001/77315 (page 28)

Primer A:

5'-AGCGTTTGCGGCCGCGATCC-3' (SEQ ID NO: 97)

Primer B:

5'-TTATTCGGTCGAAAAGGATCC-3' (SEQ ID NO: 98)

The *Trichophaea saccata* Family GH6 cDNA encoding cellobiohydrolase was subcloned into the *Aspergillus* expression vector pMStr57 (WO 2004/032648) by PCR amplifying the protein coding sequence from the cDNA library SBL0521, described above, with the two synthetic oligonucleotide primers shown below.

Primer 848:

5'-ACACAACTGGGGATCCTCATCATGAAGAACTTCCTTCTGG-3' (SEQ ID NO: 99)

Primer 849:

5'-CCCTCTAGATCTCGAGTTACGTGAAGCTAGGATTAGCATT-3' (SEQ ID NO: 100)

The amplification was performed using IPROOF™ High Fidelity 2X Master Mix (Bio-Rad Laboratories, Inc., Hercules, CA, USA) following the manufacturer's instructions. The amplification reaction was composed of SBL0521 pool DNA as template, 25 pmol each of primers 848 and 849, and 25 µl of IPROOF™ High Fidelity 2X Master Mix in a final volume of 50 µl. The amplification was performed by pre-denaturing at 98°C for 2 minutes; 5 cycles each with denaturing at 98°C for 10 seconds, annealing at 65ºC for 10 seconds, and elongation at 72°C for 1 minute; and 25 cycles each with denaturing at 98°C for 10 seconds, and combined annealing extension at 72ºC for 1 minute. A final elongation was made at

72°C for 10 minutes.

A PCR product of 1.4 kb was separated from residual reaction components using a GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

The PCR fragment was cloned into *Bam* HI and *Xho* I digested pMStr57 using an IN-FUSION™ Dry-Down PCR Cloning Kit (Clontech Laboratories, Inc., Mountain View, CA, USA). Approximately 50 ng of PCR product and 200 ng of vector in a total volume of 10 µl were added to the IN-FUSION™ Dry-Down pellet. The reaction was performed according to the manufacturer's instructions. The *Trichophaea saccata* Family GH6 cellobiohydrolase encoding DNA of the resulting *Aspergillus* expression construct, pMStr179, was sequenced and the sequence agreed completely with the cellobiohydrolase coding sequence of SEQ ID NO: 16.

The same PCR fragment was cloned into the pCR®-BluntII-TOPO vector (Invitrogen, Life Technologies, Carlsbad, CA, USA) using a Zero Blunt TOPO PCR Cloning Kit, to generate pMStr199. The *Trichophaea saccata* Family GH6 cellobiohydrolase encoding DNA of pMStr199 was sequenced and the sequence agreed completely with the cellobiohydrolase coding sequence of SEQ ID NO: 1. *E. coli* strain NN059165, containing pMStr199, was deposited with the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM), Braunschweig, Germany, on February 24, 2010 and assigned the accession number DSM 23379.

The nucleotide sequence and deduced amino acid sequence of the *Trichophaea saccata* cellobiohydrolase cDNA are shown in SEQ ID NO: 1 and SEQ ID NO: 2, respectively. The coding sequence is 1344 bp including the stop codon. The encoded predicted protein is 447 amino acids. Using the SignalP program (Nielsen *et al.*, 1997, *Protein Engineering* 10: 1-6), a signal peptide of 16 residues was predicted. The predicted mature protein contains 431 amino acids with a predicted molecular mass of 45.3 kDa and an isoelectric pH of 5.06.

A comparative pairwise global alignment of amino acid sequences was determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *J. Mol. Biol.* 48: 443-453) as implemented in the Needle program of EMBOSS with gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 matrix. The alignment showed that the deduced amino acid sequence of the *Trichophaea saccata* cDNA encoding a Family GH6 polypeptide having cellobiohydrolase activity shares 64% identity (excluding gaps) to the deduced amino acid sequence of a cellobiohydrolase from *Aspergillus fumigatus* (GENESEQP:ABB80166).

The *Aspergillus oryzae* strain BECh2 (WO 2000/39322) was transformed with pMStr179 according to Christensen *et al.*, 1988, *Biotechnology* 6, 1419-1422 and WO

2004/032648. Ten transformants were cultured for 4 days at 30°C in 750 µl of DAP2C-1 medium (WO 2004/032648), in which 2% glucose was substituted for maltodextrin. Samples were monitored by SDS-PAGE using a CRITERION™ XT Precast 12% Bis-Tris gel (Bio-Rad Laboratories, Inc., Hercules, CA, USA) according to the manufacturer's instructions. LMW standards from an Amersham Low Molecular Weight Calibration Kit for SDS Electrophoresis (GE Healthcare UK Limited, Buckinghamshire, UK) were used as molecular weight markers. The gel was stained with INSTANTBLUE™ (Expedeon Protein Solutions, Cambridge, UK). Eight transformants produced a novel protein doublet in the range of 55-60 kDa.

Two of these transformants, designated *Aspergillus oryzae* MStr335 and MStr336, were isolated twice by dilution streaking conidia on selective medium (*amdS*) containing 0.01% TRITON® X-100 to limit colony size.

Spores from four confluent COVE N slants of *Aspergillus oryzae* MStr335 spores were collected with a solution of 0.01% TWEEN® 20 and used to inoculate 21 shake flasks each containing 150 ml of DAP2C-1 medium (WO 2004/032648) in which 2% glucose was substituted for maltodextrin. The flasks were incubated at 30°C with constant shaking at 200 rpm for 3 days. Fungal mycelia and spores were removed at harvesting by first filtering the fermentation broth through a sandwich of 3 glass microfiber filters with increasing particle retention sizes of 1.6 µm, 1.2 µm and 0.7 µm, and then filtering through a 0.45 µm filter. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 9: Preparation of *Aspergillus fumigatus* cellobiohydrolase II

Aspergillus fumigatus NN055679 cellobiohydrolase II (CBHII) (SEQ ID NO: 17 [DNA sequence] and SEQ ID NO: 18 [deduced amino acid sequence]) was prepared according to the following procedure.

Two synthetic oligonucleotide primers, shown below, were designed to PCR amplify the full-length open reading frame of the *Aspergillus fumigatus* Family 6A glycosyl hydrolase from genomic DNA. A TOPO Cloning kit was used to clone the PCR product. An IN-FUSION™ Cloning Kit was used to clone the fragment into pAILo2.

Forward primer:

5'-ACTGGATTTACC**ATGAAGCACCTTGCATCTTCCATCG**-3' (SEQ ID NO: 101)

Reverse primer:

5'-TCACCTCTAGTTAATTA**AAAGGACGGGTTAGCGT**-3' (SEQ ID NO: 102)

Bold letters represent coding sequence. The remaining sequence contains sequence identity compared with the insertion sites of pAILo2.

Fifty picomoles of each of the primers above were used in a PCR reaction containing 500 ng of *Aspergillus fumigatus* genomic DNA, 1X ThermoPol *Taq* reaction buffer (New

England Biolabs, Ipswich, MA, USA), 6 µl of 10 mM blend of dATP, dTTP, dGTP, and dCTP, 0.1 unit of *Taq* DNA Polymerase (New England Biolabs, Ipswich, MA, USA), in a final volume of 50 µl. An EPPENDORF® MASTERCYCLER® 5333 was used to amplify the fragment programmed for one cycle at 98°C for 2 minutes; and 35 cycles each at 96°C for 30 seconds, 61°C for 30 seconds, and 72°C for 2 minutes. After the 35 cycles, the reaction was incubated at 72°C for 10 minutes and then cooled at 10°C until further processed. To remove the A-tails produced by Taq the reaction was incubated for 10 minutes at 68°C in the presence of 1 unit of *Pfx* DNA polymerase (Invitrogen, Carlsbad, CA, USA).

A 1.3 kb PCR reaction product was isolated on a 0.8% GTG-agarose gel (Cambrex Bioproducts, East Rutherford, NJ, USA) using TAE buffer and 0.1 µg of ethidium bromide per ml. The DNA band was visualized with the aid of a DARK READER™ (Clare Chemical Research, Dolores, CO) to avoid UV-induced mutations. The 1.3 kb DNA band was excised with a disposable razor blade and purified with an Ultrafree-DA spin cup (Millipore, Billerica, MA) according to the manufacturer's instructions.

The purified 1.3 kb PCR product was cloned into the PCR4Blunt-TOPO vector (Invitrogen). Two microliters of the purified PCR product were mixed with one microliter of a 2M Sodium chloride solution and one microliter of the Topo vector. The reaction was incubated at room temperarature for 15 minutes and then two microliters of the Topo reaction were used to transform *E. coli* TOP10 competent cells according to the manufacturer's instructions. Two aliquots of 100 microliters each of the transformation reaction were spreaded onto two 150 mm 2xYT-Amp plates and incubated overnight at 37°C.

Eight recombinant colonies were used to inoculate liquid cultures containing three milliliters of LB supplemented with 100 µg of ampicillin per milliliter of media. Plasmid DNA was prepared from these cultures using a BIOROBOT® 9600. Clones were analyzed by restriction digest. Plasmid DNA from each clone was digested with the enzyme *Eco* RI according to the manufacturer instructions (NEB, Ipswich, MA, USA) and analyzed by agarose gel electrophoresis as above. Six out of eight clones had the expected restriction digest pattern from these, clones 2, 4, 5, 6, 7 and 8 were selected to be sequenced to confirm that there were no mutations in the cloned insert. Sequence analysis of their 5-prime and 3-prime ends indicated that clones 2, 6 and 7 had the correct sequence. These three clones were selected for re-cloning into pAlLo2. One microliter aliquot of each clone was mixed with 17 µl of diluted TE (1:10 dilution) and 1 µl of this mix was used to re-amplify the *Aspergillus fumigatus* glycosyl hydrolase 6A coding region.

Fifty picomoles of each of the primers above were used in a PCR reaction containing 1 µl of the diluted mix of clones 2, 6 and 7, 1X *Pfx* Amplification Buffer (Invitrogen, Carlsbad,

CA, USA), 6 µl of 10 mM blend of dATP, dTTP, dGTP, and dCTP, 2.5 units of PLATINUM® *Pfx* DNA Polymerase, 1 µl of 50 mM $MgSO_4$, in a final volume of 50 µl. An EPPENDORF® MASTERCYCLER® 5333 was used to amplify the fragment programmed for one cycle at 98°C for 2 minutes; and 35 cycles each at 94°C for 30 seconds, 61°C for 30 seconds, and 68°C for 1.5 minutes. After the 35 cycles, the reaction was incubated at 68°C for 10 minutes and then cooled at 10°C until further processed. A 1.3 kb PCR reaction product was isolated on a 0.8% GTG-agarose gel using TAE buffer and 0.1 µg of ethidium bromide per ml. The DNA band was visualized with the aid of a DARKREADER™ Transilluminator to avoid UV-induced mutations. The 1.0 kb DNA band was excised with a disposable razor blade and purified with an Ultrafree-DA spin cup (Millipore, Billerica, MA) according to the manufacturer's instructions.

The vector pAlLo2 was linearized by digestion with *Nco* I and *Pac* I (using conditions specified by the manufacturer). The fragment was purified by gel electrophoresis and ultrafiltration as described above. Cloning of the purified PCR fragment into the linearized and purified pAlLo2 vector was performed with an IN-FUSION™ Cloning Kit. The reaction (20 µl) contained 1X IN-FUSION™ Buffer, 1X BSA, 1 µl of IN-FUSION™ enzyme (diluted 1:10), 100 ng of pAlLo2 digested with *Nco* I and *Pac* I, and 50 ng of the *Aspergillus fumigatus* GH6A purified PCR product. The reaction was incubated at room temperature for 30 minutes. A 2 µl sample of the reaction was used to transform transform *E. coli* TOP10 competent cells according to the manufacturer's instructions. After the recovery period, two 100 µl aliquots from the transformation reaction were plated onto 150 mm 2X YT plates supplemented with 100 µg of ampicillin per ml. The plates were incubated overnight at 37°C. A set of eight putative recombinant clones was selected at random from the selection plates and plasmid DNA was prepared from each one using a BIOROBOT® 9600. Clones were analyzed by *Pst* I restriction digest. Seven out of eight clones had the expected restriction digest pattern. Clones 1. 2 and 3 were then sequenced to confirm that there were no mutations in the cloned insert. Clone #2 was selected and designated pAlLo33.

Aspergillus fumigatus cel6A (JaL355 ALLO33 Exp03191) was grown to obtain culture broth for the purification of a cellobiosehydrolase II.

Seven hundred and fifty ml of shake flask medium were added to a 2800 ml shake flask. The shake flask medium was composed per liter of 45 g of maltose, 2 g of K_2HPO_4, 12 g of KH_2PO_4, 1 g of NaCl, 1 g of $MgSO_4 \cdot 7H_2O$, 7 g of yeast extract, 2 g of urea, and 0.5 ml of trace elements solution. The trace elements solution was composed per liter of 13.8 g of $FeSO_4 \cdot 7H_2O$, 14.3 g of $ZnSO_4 \cdot 7H_2O$, 8.5 g of $MnSO_4 \cdot H_2O$, 2.5 g of $CuSO_4 \cdot 5H_2O$, 0.5 g of $NiCl_2 \cdot 6H_2O$, and 3 g of citric acid. Two shake flasks were inoculated by suspension of a PDA plate of *Aspergillus fumigatus* cel6A with 0.01% TWEEN® 20 and incubated at 34°C on an

orbital shaker at 200 rpm for 120 hours.

The broth was filtered using 0.7 µm glass filter GF/F (Whatman, Piscataway, NJ, USA) and then using a 0.22 µm EXPRESS™ Plus Membrane (Millipore, Bedford, MA, USA).

The filtered broth was concentrated and buffer exchanged using a tangential flow concentrator (Pall Filtron, Northborough, MA, USA) equipped with a 10 kDa polyethersulfone membrane (Pall Filtron, Northborough, MA, USA) with 20 mM Tris-HCl pH 8.0. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 10: Preparation of *Aspergillus terreus* ATCC 28865 Cel7 endoglucanase I

The *Aspergillus terreus* ATCC 28865 Cel7 endoglucanase I gene (SEQ ID NO: 19 [DNA sequence] and SEQ ID NO: 20 [deduced amino acid sequence]) was cloned and expressed in *Aspergillus oryzae* as described below.

Two synthetic oligonucleotide primers, shown below, were designed to PCR amplify the endoglucanase I gene from *Aspergillus terreus* ATCC 28865 genomic DNA. Genomic DNA was isolated using a FASTDNA® Spin Kit for Soil (MP Biomedicals, Solon, OH, USA).

Primer #226:

5'-TAACAATTGTCACCATGAATTCTCTTACAAAAAGCAT-3' (SEQ ID NO: 103)

Primer #227:

5'-TATGCGGCCGCAGTCTGCATGTGTTACGCACCT-3' (SEQ ID NO: 104)

The amplification reaction was composed of 1 µl of *Aspergillus terreus* ATCC 28865 genomic DNA, 12.5 µl of 2X REDDYMIX™ PCR Buffer (Thermo Fisher Scientific Inc., Waltham, MA, USA), 1 µl of primer #226 (5 µM), 1 µl of #227 (5 µM), and 9.5 µl of H_2O. The amplification reaction was incubated in a PTC-200 DNA ENGINE™ Thermal Cycler (MJ Research Inc., Waltham, MA, USA) programmed for 1 cycle at 94°C for 2 minutes; and 35 cycles each at 94°C for 15 seconds and 60°C for 1.5 minutes.

A 1.44 kb PCR reaction product was isolated by 1% agarose gel electrophoresis using TAE buffer and staining with SYBR® Safe DNA gel stain (Invitrogen Corp., Carlsbad, CA, USA). The DNA band was visualized with the aid of an EAGLE EYE® Imaging System (Stratagene, La Jolla, CA, USA) and a DARKREADER® Transilluminator. The 1.44 kb DNA band was excised from the gel and purified using a GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

The 1.44 kb fragment was cleaved with *Mfe* I and *Not* I and purified using a GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

The cleaved 1.44 kb fragment was then directionally cloned by ligation into *Eco* RI-*Not* I cleaved pXYG1051 (WO 2005/080559) using T4 ligase (Promega, Madison, WI, USA)

according to the manufacturer's instructions. The ligation mixture was transformed into *E. coli* TOP10F competent cells (Invitrogen Corp., Carlsbad, CA, USA) according to the manufacturer's instructions. The transformation mixture was plated onto LB plates supplemented with 100 µg of ampicillin per ml. Plasmid minipreps were prepared from several transformants and sequenced. One plasmid with the correct *Aspergillus terreus* GH7 coding sequence (SEQ ID NO: 13) was chosen. The plasmid was designated pXYG1051-NP003857. The expression vector pXYG1051 contains the same neutral amylase II (NA2) promoter derived from *Aspergillus niger*, and terminator elements as pCaHj483 (disclosed in Example 4 of WO 98/00529). Furthermore pXYG1051 has pUC18 derived sequences for selection and propagation in *E. coli*, and pDSY82 (disclosed in Example 4 of U.S. Patent No. 5,958,727) derived sequences for selection and expression in *Aspergillus* facilitated by the *pyrG* gene of *Aspergillus oryzae*, which encodes orotidine decarboxylase and is used to complement a *pyrG* mutant *Aspergillus* strain.

The expression plasmid pXYG1051-NP003857 was transformed into *Aspergillus oryzae* JaL355 as described in WO 98/00529. Transformants were purified on selection plates through single conidia prior to sporulating them on PDA plates. Production of the *Aspergillus terreus* GH7 polypeptide by the transformants was analyzed from culture supernatants of 1 ml 96 deep well stationary cultivations at 26°C in YP medium with 2% maltodextrin. Expression was verified by SDS-PAGE using a NUPAGE® NOVEX® 4-12% Bis-Tris Gel with 50 mM 2-(N-morpholino)ethanesulfonic acid (MES) buffer (Invitrogen Corporation, Carlsbad, CA, USA) by Coomassie blue staining. One transformant was selected for further work and designated *Aspergillus oryzae* 28.4.

For larger scale production, *Aspergillus oryzae* 28.4 spores were spread onto a PDA plate and incubated for five days at 37°C. The confluent spore plate was washed twice with 5 ml of 0.01% TWEEN® 20 to maximize the number of spores collected. The spore suspension was then used to inoculate twenty-five 500 ml flasks containing 100 ml of YPM medium. The culture was incubated at 30°C with constant shaking at 85 rpm. At day four post-inoculation, the culture broth was collected by filtration through a triple layer of glass microfiber filters of 1.6 µm, 1.2 µm, and 0.7 µm (Whatman, Piscataway, NJ, USA). Fresh culture broth from this transformant produced a band of GH7 protein of approximately 64 kDa. The identity of this band as the *Aspergillus terreus* GH7 polypeptide was verified by peptide sequencing using standard techniques.

Two liters of the filtered broth was concentrated to 400 ml and washed with 50 mM HEPES pH 7.0 using a SARTOFLOW® Alpha Plus Crossflow System with a 10 kDa cut-off (Sartorius Stedim Biotech S.A., Aubagne Cedex, France). Ammonium sulphate was added to a final concentration of 1 M and dissolved in the ultrafiltrate. The solution was loaded onto a Source 15 Phenyl XK 26/20 50 ml column (GE Healthcare, Hillerød, Denmark). After

loading the column was washed with 150 ml of 1 M ammonium sulphate and eluted with 1 column volume of 50% ethanol in a 0% to 100% gradient followed by 5 column volumes of 50% ethanol at a flow rate of 10 ml per minute. Fractions of 10 ml were collected and analyzed by SDS-PAGE. Fractions 3 to 8 were pooled and diluted to 1000 ml with 50 mM HEPES pH 7.0 before loading onto a Q SEPHAROSE® Fast Flow XK26/20 60 ml column (GE Healthcare, Hillerød, Denmark). After loading the column was washed 3 times with 60 ml of 50 mM HEPES pH 7.0 and eluded with 100 ml of 50 mM HEPES pH 7.0, 1 M NaCl at a flow rate of 10 ml per minute. Fractions of 10 ml were collected and analyzed by SDS-PAGE. The flow through and first wash were pooled and concentrated to 400 ml and washed with 50 mM HEPES pH 7.0 using a SARTOFLOW® Alpha plus Crossflow System with a 10 kDa cut-off. Further concentration was conducted using a VIVASPIN™ centrifugal concentrator according to the manufacturer's instructions to a final volume of 80 ml. The protein concentration was determined by A_{280}/A_{260} absorbance.

Example 11: Preparation of *Trichoderma reesei* RutC30 Cel5A endoglucanase II

The *Trichoderma reesei* RutC30 Cel5A endoglucanase II gene (SEQ ID NO: 21 [DNA sequence] and SEQ ID NO: 22 [deduced amino acid sequence]) was cloned and expressed in *Aspergillus oryzae* as described below.

Two synthetic oligonucleotide primers, shown below, were designed to PCR amplify the endoglucanase II gene from *Trichoderma reesei* RutC30 genomic DNA. Genomic DNA was isolated using a DNEASY® Plant Maxi Kit. An IN-FUSION™ PCR Cloning Kit was used to clone the fragment directly into pAILo2 (WO 2004/099228).

Forward primer:

5'-ACTGGATTTACC**ATGAACAAGTCCGTGGCTCCATTGCT**-3' (SEQ ID NO: 105)

Reverse primer:

5'-TCACCTCTAGTTAATTAA**CTACTTTCTTGCGAGACACG**-3' (SEQ ID NO: 106)

Bold letters represent coding sequence. The remaining sequence contains sequence identity to insertion sites of pAILo2.

Fifty picomoles of each of the primers above were used in a PCR reaction containing 200 ng of *Trichoderma reesei* genomic DNA, 1X *Pfx* Amplification Buffer (Invitrogen, Carlsbad, CA, USA), 6 µl of 10 mM blend of dATP, dTTP, dGTP, and dCTP, 2.5 units of PLATINUM® *Pfx* DNA polymerase, and 1 µl of 50 mM $MgSO_4$ in a final volume of 50 µl. The amplification reaction was incubated in an EPPENDORF® MASTERCYCLER® 5333 programmed for one cycle at 98°C for 2 minutes; and 35 cycles each at 94°C for 30 seconds, 61°C for 30 seconds, and 68°C for 1.5 minutes. After the 35 cycles, the reaction was incubated at 68°C for 10 minutes and then cooled at 10°C. A 1.5 kb PCR reaction product was isolated on a 0.8% GTG® agarose gel using TAE buffer and 0.1 µg of ethidium

bromide per ml. The DNA band was visualized with the aid of a DARKREADER™ Transilluminator. The 1.5 kb DNA band was excised with a disposable razor blade and purified using an ULTRAFREE® DA spin cup according to the manufacturer's instructions.

Plasmid pAlLo2 was linearized by digestion with *Nco* I and *Pac* I. The plasmid fragment was purified by gel electrophoresis and ultrafiltration as described above. Cloning of the purified PCR fragment into the linearized and purified pAlLo2 vector was performed using an IN-FUSION™ PCR Cloning Kit. The reaction (20 µl) contained 1X IN-FUSION™ Buffer, 1X BSA, 1 µl of IN-FUSION™ enzyme (diluted 1:10), 100 ng of pAlLo2 digested with *Nco* I and *Pac* I, and 100 ng of the *Trichoderma reesei* Cel5A endoglucanase II PCR product. The reaction was incubated at room temperature for 30 minutes. A 2 µl sample of the reaction was used to transform *E. coli* XL10 SOLOPACK® Gold cells according to the manufacturer's instructions. After a recovery period, two 100 µl aliquots from the transformation reaction were plated onto 150 mm 2X YT plates supplemented with 100 µg of ampicillin per ml. The plates were incubated overnight at 37°C. A set of 3 putative recombinant clones was recovered the selection plates and plasmid DNA was prepared from each one using a BIOROBOT® 9600. Clones were analyzed by *Pci* I/*Bsp* LU11 I restriction digestion. One clone with the expected restriction digestion pattern was then sequenced to confirm that there were no mutations in the cloned insert. Clone #3 was selected and designated pAlLo27.

Aspergillus oryzae JaL250 protoplasts were prepared according to the method of Christensen *et al.*, 1988, *supra*. Five micrograms of pAlLo27 (as well as pAlLo2 as a control) were used to transform *Aspergillus oryzae* JaL250 protoplasts.

The transformation of *Aspergillus oryzae* JaL250 with pAlLo27 yielded about 50 transformants. Eleven transformants were isolated to individual PDA plates and incubated for five days at 34°C.

Confluent spore plates were washed with 3 ml of 0.01% TWEEN® 80 and the spore suspension was used to inoculate 25 ml of MDU2BP medium in 125 ml glass shake flasks. Transformant cultures were incubated at 34°C with constant shaking at 200 rpm. At day five post-inoculation, cultures were centrifuged at 6000 *x g* and their supernatants collected. Five microliters of each supernatant were mixed with an equal volume of 2X loading buffer (10% beta-mercaptoethanol) and loaded onto a 1.5 mm 8%-16% Tris-glycine SDS-PAGE gel and stained with SIMPLYBLUE™ SafeStain (Invitrogen Corp., Carlsbad, CA, USA). SDS-PAGE profiles of the culture broths showed that ten out of eleven transformants produced a new protein band of approximately 45 kDa. Transformant number 1, designated *Aspergillus oryzae* JaL250AlLo27, was cultivated in a fermentor.

One hundred ml of shake flask medium were added to a 500 ml shake flask. The shake flask medium was composed per liter of 50 g of sucrose, 10 g of KH_2PO_4, 0.5 g of

$CaCl_2$, 2 g of $MgSO_4 \cdot 7H_2O$, 2 g of K_2SO_4, 2 g of urea, 10 g of yeast extract, 2 g of citric acid, and 0.5 ml of trace metals solution. The trace metals solution was composed per liter of 13.8 g of $FeSO_4 \cdot 7H_2O$, 14.3 g of $ZnSO_4 \cdot 7H_2O$, 8.5 g of $MnSO_4 \cdot H_2O$, 2.5 g of $CuSO_4 \cdot 5H_2O$, and 3 g of citric acid. The shake flask was inoculated with two plugs of *Aspergillus oryzae* JaL250AlLo27 from a PDA plate and incubated at 34°C on an orbital shaker at 200 rpm for 24 hours. Fifty ml of the shake flask broth was used to inoculate a 3 liter fermentation vessel.

A total of 1.8 liters of the fermentation batch medium was added to a three liter glass jacketed fermentor (Applikon Biotechnology, Schiedam, Netherlands). The fermentation batch medium was composed per liter of 10 g of yeast extract, 24 g of sucrose, 5 g of $(NH_4)_2SO_4$, 2 g of KH_2PO_4, 0.5 g of $CaCl_2 \cdot 2H_2O$, 2 g of $MgSO_4 \cdot 7H_2O$, 1 g of citric acid, 2 g of K_2SO_4, 0.5 ml of anti-foam, and 0.5 ml of trace metals solution. Trace metals solution was composed per liter of 13.8 g of $FeSO_4 \cdot 7H_2O$, 14.3 g of $ZnSO_4 \cdot 7H_2O$, 8.5 g of $MnSO_4 \cdot H_2O$, 2.5 g of $CuSO_4 \cdot 5H_2O$, and 3 g of citric acid. Fermentation feed medium was composed of maltose. Fermentation feed medium was dosed at a rate of 0 to 4.4 g/l/hr for a period of 185 hours. The fermentation vessel was maintained at a temperature of 34°C and pH was controlled using an Applikon 1030 control system (Applikon Biotechnology, Schiedam, Netherlands) to a set-point of 6.1 +/- 0.1. Air was added to the vessel at a rate of 1 vvm and the broth was agitated by a Rushton impeller rotating at 1100 to 1300 rpm. At the end of the fermentation, whole broth was harvested from the vessel and centrifuged at 3000 *x g* to remove the biomass. The supernatant was sterile filtered and stored at 5 to 10°C.

The supernatant was desalted and buffer-exchanged in 20 mM Bis-Tris pH 6.0 using a HIPREP® 26/10 desalting column according to the manufacturer's instructions. The buffer exchanged sample was loaded onto a MonoQ® column (GE Healthcare, Piscataway, NJ, USA) equilibrated with 20 mM Bis-Tris pH 6.0, and the bound protein was eluted with a linear gradient from 0 to 1000 mM sodium chloride. Protein fractions were pooled and buffer exchanged into 1.2 M $(NH_4)_2SO_4$-20 mM Tris-HCl pH 8.5. The sample was loaded onto a Phenyl SUPEROSE™ column (HR 16/10) equilibrated with 1.2 M $(NH_4)_2SO_4$-20 mM Tris-HCl pH 8.0. Bound proteins were eluted with a linear gradient over 20 column volumes from 1.2 to 0 M $(NH_4)_2SO_4$ in 20 mM Tris-HCl pH 8.5. The fractions were pooled, concentrated, and loaded onto a SUPERDEX® 75 HR 26/60 column (GE Healthcare, Piscataway, NJ, USA) equilibrated with 20 mM Tris-150 mM sodium chloride pH 8.5. Fractions were pooled and concentrated in 20 mM Tris-150 mM sodium chloride pH 8.5. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 12: Preparation of *Myceliophthora thermophila* CBS 202.75 Cel5A endoglucanase II

Myceliophthora thermophila CBS 202.75 Cel5A endoglucanase II (EGII) (SEQ ID NO: 23 [DNA sequence] and SEQ ID NO: 24 [deduced amino acid sequence]) was prepared recombinantly according to WO 2007/109441 using *Aspergillus oryza*e HowB104 as a host.

The culture filtrate was desalted and buffer-exchanged in 20 mM Tris pH 8.0 using a HIPREP® 26/10 desalting column according to the manufacturer's instructions. The buffer exchanged sample was applied to a MonoQ® column equilibrated with 20 mM Tris pH 8.0, and the bound protein was eluted with a gradient from 0 to 500 mM sodium chloride. Fractions were pooled and concentrated in 20 mM Tris pH 8.0. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 13: Preparation of *Thermoascus aurantiacus* CGMCC 0670 Cel5A endoglucanase II

Thermoascus aurantiacus CGMCC 0670 cDNA encoding a Cel5A endoglucanase II (SEQ ID NO: 25 [DNA sequence] and SEQ ID NO: 26 [deduced amino acid sequence]) was cloned according to the following procedure. The *T. aurantiacus* strain was grown in 80 ml of CBH1 medium (2.5% AVICEL®, 0.5% glucose, 0.14% $(NH_4)_2SO_4$) in 500 ml Erlenmeyer baffled flasks at 45°C for 3 days with shaking at 165 rpm. Mycelia were harvested by centrifugation at 7000 rpm for 30 minutes and stored at -80C before use for RNA extraction. RNA was isolated from 100 mg of mycelia using a RNEASY® Plant Mini Kit.

The cDNA for the *Thermoascus aurantiacus* endoglucanase was isolated by RT PCR using a 3' RACE system and a 5' RACE system and primers BG025-1, BG025-2, BG025-3, and BG025-4 shown below to the N-terminal amino acids.

Primer BG025-1:

5'-AA(T/C)GA(A/G)TC(T/C/A/G)GG(T/C/A/G)GC(T/C/A/G)GAATT-3' (SEQ ID NO: 107)

Primer BG025-2:

5'-AA(T/C)GA(A/G)TC(T/C/A/G)GG(T/C/A/G)GC(T/C/A/G)GAGTT-3' (SEQ ID NO: 108)

Primer BG025-3:

5'-AA(T/C)GA(A/G)AG(T/C)GG(T/C/A/G)GC(T/C/A/G)GAATT-3' (SEQ ID NO: 109)

Primer BG025-4:

5'-AA(T/C)GA(A/G)AG(T/C)GG(T/C/A/G)GC(T/C/A/G)GAGTT-3' (SEQ ID NO: 110)

The RT PCR products were ligated into plasmid pGEM-T using a pGEM-T Vector System and transformed into *E. coli* strain JM109. A single clone harboring a plasmid named pBGC1009 containing the endoglucanase cDNA was isolated.

PCR primers were designed to amplify the cDNA encoding the *T aurantiacus* endoglucanase from plasmid pBGC1009. Restriction enzyme sites *Bsp* HI and *Pac* I were incorporated for in-frame cloning into *Aspergillus oryzae* expression plasmid pBM120a (WO

2006/039541).

Primer 996261:

5'-GATCTCATGAAGCTCGGCTCTCTCGT-3' (SEQ ID NO: 111)

*BspH*I

Primer 996167:

5'-TTAATTAATCAAAGATACGGAGTCAAAATAGG-3' (SEQ ID NO: 112)

*Pac*I

The fragment of interest was amplified by PCR using an EXPAND™ High Fidelity PCR System. The PCR amplification reaction mixture contained 1 µl of 0.09 µg/µl pBGC1009, 1 µl of primer 996261 (50 pmol/µl), 1 µl of primer 996167 (50 pmol/µl), 5 µl of 10X PCR buffer with 15 mM $MgCl_2$, 1 µl of dNTP mix (10 mM each), 37.25 µl of water, and 0.75 µl (3.5 U/µl) of DNA polymerase mix. An EPPENDORF® MASTERCYCLER® thermocycler was used to amplify the fragment programmed for 1 cycle at 94°C for 2 minutes; 10 cycles each at 94°C for 15 seconds, 55°C for 30 seconds, 72°C for 1.5 minutes; 15 cycles each at 94°C for 15 seconds, 55°C for 30 seconds, and 72°C for 1.5 minutes plus 5 second elongation at each successive cycle; 1 cycle at 72°C for 7 minutes; and a 4°C hold.

The 1008 bp PCR product was purified by 1% agarose gel electrophoresis using TAE buffer, excised from the gel, and purified using a QIAQUICK® Gel Purification Kit (QIAGEN Inc., Valencia, CA, USA). The purified product was ligated directly into pCR®2.1-TOPO® according to the manufacturer's instructions. The resulting plasmid was named pBM124a.

Plasmid pBM124a was digested with *Bsp* HI and *Pac* I, purified by 1% agarose gel electrophoresis using TAE buffer, excised from the gel, and purified using a QIAQUICK® Gel Purification Kit. The plasmid fragment was ligated to the vector pBM120a, which was digested with *Nco* I and *Pac* I. The resulting expression plasmid was designated pBM123a. Plasmid pBM123a contains a duplicate NA2-TPI promoter driving expression of the *Thermoascus aurantiacus* endoglucanase cDNA clone, the AMG terminator, and *amdS* as a selectable marker.

Aspergillus oryzae BECh2 (WO 2000/139322) protoplasts were prepared according to the method of Christensen *et al.*, 1988, *supra*. Six µg of pBM123a were used to transform *Aspergillus oryzae* BECh2. Primary transformants were selected on COVE plates for 5 days. Transformants were spore purified twice prior to shake flask analysis.

Spores of the transformants were inoculated into 25 ml of MY25 medium in 125 ml shake flasks. The cultures were incubated at 34°C, 200 rpm on a platform shaker for five days. On day 3 and day 5, culture supernatants were harvested and clarified by centrifugation to remove mycelia. Twenty microliters of supernatant from three transformants were analyzed using a CRITERION® stain-free, 10-20% gradient SDS-PAGE gel (Bio-Rad

Laboratories, Inc., Hercules, CA, USA) according to the manufacturer's instructions. SDS-PAGE profiles of the cultures showed that all transformants had a new major band of approximately 32 kDa. One transformant was chosen and named EXP00858.

Plastic, non-baffled 500 ml shake flasks containing 100 ml of SY50 medium were inoculated with 0.1 ml of a spore stock of EXP00858, and incubated at 34°C, 200 rpm for 24 hours to produce a seed culture. Fifty ml of the seed culture was inoculated into a 2 liter fermentation tank containing 2 liters of medium composed per liter of 0.5 g of pluronic acid, 30 g of sucrose, 2 g of $MgSO_4 \cdot 7H_2O$, 2 g of anhydrous KH_2PO_4, 1 g of citric acid·, 2 g of $(NH_4)_2SO_4$, 1 g of K_2SO_4, 20 g of yeast extract, and 0.5 g of 200X AMG trace metals solution, pH 5.0. The fermentation was fed with a maltose feed. The pH was controlled using 5N H_3PO_4 and 15% NH_4OH and maintained at 5.0 and then raised to 5.25. Temperature was maintained 34.0 °C +/- 1.0 °C. Agitation was 1000 rpm. Airflow was 1.0 vvm.

A 200 ml volume of cell-free supernatant was diluted to 1 liter with deionized water. The pH was adjusted to 8 and the sample filter sterilized using a 0.22 µm polyethersulphone (PES) filter. The filter sterilized sample was loaded onto a 250 ml Q SEPHAROSE™ Fast Flow column (GE Healthcare, Piscataway, NJ, USA) pre-equilibrated with 25 mM Tris pH 8. The enzyme was eluted from the column with a 0 to 1 M NaOH gradient in the same buffer. The fractions containing beta-glucosidase activity were pooled (400 ml) and the enzyme concentration calculated from the theoretic extinction coefficient and the absorbance of the sample at 280 nm.

Example 14: Preparation of *Aspergillus fumigatus* NN055679 Cel3A beta-glucosidase

Aspergillus fumigatus NN055679 Cel3A beta-glucosidase (SEQ ID NO: 27 [DNA sequence] and SEQ ID NO: 28 [deduced amino acid sequence]) was recombinantly prepared according to WO 2005/047499 using *Trichoderma reesei* RutC30 as a host.

Filtered broth was concentrated and buffer exchanged using a tangential flow concentrator equipped with a 10 kDa polyethersulfone membrane with 20 mM Tris-HCl pH 8.5. The sample was loaded onto a Q SEPHAROSE® High Performance column (GE Healthcare, Piscataway, NJ, USA) equilibrated in 20 mM Tris pH 8.5, and bound proteins were eluted with a linear gradient from 0-600 mM sodium chloride. The fractions were concentrated and loaded onto a SUPERDEX® 75 HR 26/60 column equilibrated with 20 mM Tris-150 mM sodium chloride pH 8.5. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 15: Preparation of *Penicillium brasilianum* IBT 20888 Cel3A beta-glucosidase

Penicillium brasilianum IBT 20888 Cel3A beta-glucosidase (SEQ ID NO: 29 [DNA

sequence] and SEQ ID NO: 30 [deduced amino acid sequence]) was recombinantly prepared according to WO 2007/019442 using *Aspergillus oryzae* as a host.

Filtered broth was concentrated and buffer exchanged using a tangential flow concentrator equipped with a 10 kDa polyethersulfone membrane with 20 mM Tris-HCl pH 8.0. The sample was loaded onto a Q SEPHAROSE® High Performance column (GE Healthcare, Piscataway, NJ, USA) equilibrated in 20 mM Tris pH 8.0, and bound proteins were eluted with a linear gradient from 0-600 mM sodium chloride. The fractions were concentrated into 20 mM Tris pH 8.0. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 16: Preparation of *Aspergillus niger* IBT 10140 Cel3 beta-glucosidase

The *Aspergillus niger* IBT 10140 Cel3 beta-glucosidase gene (SEQ ID NO: 31 [DNA sequence] and SEQ ID NO: 32 [deduced amino acid sequence]) was isolated by PCR using two cloning primers GH3-9.1f and GH3-9.1r shown below, which were designed based on the publicly available *Aspergillus niger* Cel3 sequence (CAK48740.1) for direct cloning using an IN-FUSION™ Cloning Kit.

Primer GH3-9.1f:

acacaactggggatccaccatgaggttcacttcgatcgagg (SEQ ID NO: 113)

Primer GH3-9.1r:

agatctcgagaagcttaGTGAACAGTAGGCAGAGACGCCCG (SEQ ID NO: 114)

A PCR reaction was performed with genomic DNA prepared from *Aspergillus niger* strain NN005810 in order to amplify the full-length gene. The genomic DNA was isolated using a FASTDNA® Spin Kit (MP Biomedicals, Santa Ana, CA, USA). The PCR reaction was composed of 1 µl of genomic DNA, 0.75 µl of primer GH3-9.1f (10 µM), 0.75 µl of primer GH3-9.1r (10 µM), 3 µl of 5X HF buffer (Finnzymes Oy, Finland), 0.25 µl of 50 mM $MgCl_2$, 0.3 µl of 10 mM dNTP, 0.15 µl of PHUSION® DNA polymerase (Finnzymes Oy, Finland), and PCR-grade water up to 15 µl. The PCR reaction was performed using a DYAD® PCR machine (Bio-Rad Laboratories, Inc., Hercules, CA, USA) programmed for 2 minutes at 98°C followed by 10 touchdown cycles at 98°C for 15 seconds, 70°C (-1°C/cycle) for 30 seconds, and 72°C for 2 minutes 30 seconds; and 25 cycles each at 98°C for 15 seconds, 60°C for 30 seconds, 72°C for 2 minutes 30 seconds; and 5 minutes at 72°C.

The reaction products were isolated by 1.0% agarose gel electrophoresis using TAE buffer where an approximately 2.6 kb PCR product band was excised from the gel and purified using a GFX® PCR DNA and Gel Band Purification Kit according to manufacturer's instructions. DNA corresponding to the *A. niger* Cel3 beta-glucosidase gene was cloned into the expression vector pDAu109 (WO 2005/042735) linearized with *Bam* HI and *Hind* III,

using an IN-FUSION™ Dry-Down PCR Cloning Kit according to the manufacturer's instructions.

A 2.5 µl volume of the diluted ligation mixture was used to transform *E. coli* TOP10 chemically competent cells. Three colonies were selected on LB agar plates containing 100 µg of ampicillin per ml and cultivated overnight in 3 ml of LB medium supplemented with 100 µg of ampicillin per ml. Plasmid DNA was purified using an E.Z.N.A.® Plasmid Mini Kit (Omega Bio-Tek, Inc., Norcross, GA, USA) according to the manufacturer's instructions. The *A. niger* Cel3 beta-glucosidase gene sequence was verified by Sanger sequencing before heterologous expression.

Protoplasts of *Aspergillus oryzae* BECh2 (WO 2000/39322) were prepared as described in WO 95/02043. One hundred microliters of protoplast suspension were mixed with 2.5-15 µg of the *Aspergillus* expression vector and 250 µl of 10 mM $CaCl_2$-10 mM Tris-HCl pH 7.5-60% PEG 4000 (PEG 4000; Applichem, Omaha, NE, USA) (polyethylene glycol, molecular weight 4,000) were added and gently mixed. The mixture was incubated at 37°C for 30 minutes and the protoplasts were spread on COVE plates for transformant selection. After incubation for 4-7 days at 37°C, spores of sixteen transformants were picked up and inoculated into YPM medium. After 4 days cultivation at 30°C culture broth was analyzed in order to identified the best transformants based on their ability to produce *A. niger* Cel3 beta-glucosidase. The screening was based on intensity of the band corresponding to the heterologous expressed protein determined by SDS-PAGE and activity of the enzyme on 4-nitrophenyl-beta-D-glucopyranoside (pNPG) using an assay was modified from Hägerdal *et al.*, 1979, *Biotechnology and Bioengineering* 21: 345-355: 10 µl of culture broth was mixed with 90 µl of assay reagent containing 10 µl of 0.1% TWEEN®, 10 µl of 1 M sodium citrate pH 5, 4 µl of 100 mM pNPG substrate (Sigma Aldrich) solubilized in DMSO (0.4% final volume in stock solution), and filtered water. The assay was incubated for 30 minutes at 37°C and the absorbance was analyzed at 405 nm before and after addition of 100 µl of 1 M sodium carbonate pH 10. The highest absorbance values at 405 nm were correlated to the SDS-PAGE data for selection of the best transformant.

Spores of the best transformant were spread on COVE plates containing 0.01% TRITON® X-100 in order to isolate single colonies. The spreading was repeated twice in total on COVE sucrose medium (Cove, 1996, *Biochim. Biophys. Acta* 133: 51-56) containing 1 M sucrose and 10 mM sodium nitrate, supplemented with 10 mM acetamide and 15 mM CsCl. Fermentation was then performed in 250 ml shake flasks using YP medium containing 2% maltodextrin for 4 days at 30°C with shaking at 100 rpm.

A 2 liter volume of culture supernatant (EXP02895) was filtered with a 0.7 µm glass fiber filter and then sterile filtered using a 0.22 µm PES membrane (Nalgene Thermo Fisher Scientific, Rochester, NY, USA). The filtered supernatant was concentrated and diafiltered

using cross-flow Sartocon Slice Cassettes (non Cellulose) with 10 kDa cut-off (Sartorius Stedim Biotech S.A., Aubagne Cedex, France). The final volume was adjusted to 500 ml and pH adjusted to 4.5 by slowly adding dilute 10% acetic acid. The final ionic strength was under 4 MSi.

A 50 ml XK26 column (GE Healthcare, Hillerød, Denmark) was packed with Xpressline ProA (UpFront Chromatography A/S, Copenhagen, Denmark) equilibrated with 50 mM sodium acetate pH 4.5 buffer. The filtered supernatant was loaded onto the column using a P500 Pump (GE Health Care, Hillerød, Denmark) at a flow of 45 ml per minute and washed with 50 mM sodium acetate pH 4.5 buffer until all unbound material was eluted. The bound protein was eluted with 50 mM Tris pH 8 buffer using an ÄKTAexplorer System (GE Healthcare, Hillerød, Denmark). Fractions were collected and monitored by UV absorbance at 280 nm. The eluted protein were pooled and adjusted to pH 7 by slowly adding 0.5 M Tris base with a final ionic strength under 4 MSi.

A 50 ml Q SEPHAROSE® Fast Flow column was equilibrated with 50 mM HEPES pH 7 buffer (buffer A). The column was then washed to remove unbound material by washing with 50 mM HEPES pH 7 buffer until the UV absorbance of the wash was below 0.05 at 280 nm. The bound protein was eluted using a linear salt gradient of 0 to 1 M NaCl in 50 mM HEPES pH 7 buffer as buffer B (10 column volume) using an ÄKTAexplorer System. Purity of protein fractions was determined by SDS-PAGE analysis using a 4-20% Tris-Glycine Gel (Invitrogen Carlsbad, CA, USA) according to the manufacturer's instructions. Staining after electrophoresis was performed using INSTANT BLUE™ (Expedeon Ltd., Cambridgeshire, UK) according to the manufacturer's instructions. Fractions showing expected protein bands were pooled. Identification of the protein was determined by MS-Edman degradation using standard methods.

Example 17: Preparation of *Thermoascus aurantiacus* CGMCC 0583 GH61A polypeptide having cellulolytic enhancing activity

Thermoascus aurantiacus CGMCC 0583 GH61A polypeptide having cellulolytic enhancing activity (SEQ ID NO: 33 [DNA sequence] and SEQ ID NO: 34 [deduced amino acid sequence]) was recombinantly prepared according to WO 2005/074656 using *Aspergillus oryzae* JaL250 as a host. The recombinantly produced *Thermoascus aurantiacus* GH61A polypeptide was first concentrated by ultrafiltration using a 10 kDa membrane, buffer exchanged into 20 mM Tris-HCl pH 8.0, and then purified using a 100 ml Q SEPHAROSE® Big Beads column (GE Healthcare, Piscataway, NJ, USA) with 600 ml of a 0-600 mM NaCl linear gradient in the same buffer. Fractions of 10 ml were collected and pooled based on SDS-PAGE.

The pooled fractions (90 ml) were then further purified using a 20 ml MONO Q®

column (GE Healthcare, Piscataway, NJ, USA) with 500 ml of a 0-500 mM NaCl linear gradient in the same buffer. Fractions of 6 ml were collected and pooled based on SDS-PAGE. The pooled fractions (24 ml) were concentrated by ultrafiltration using a 10 kDa membrane, and chromatographed using a 320 ml SUPERDEX® 75 SEC column (GE Healthcare, Piscataway, NJ, USA) with isocratic elution of approximately 1.3 liters of 150 mM NaCl-20 mM Tris-HCl pH 8.0. Fractions of 20 ml were collected and pooled based on SDS-PAGE. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 18: Preparation of *Thielavia terrestris* NRRL 8126 GH61E polypeptide having cellulolytic enhancing activity

Thielavia terrestris NRRL 8126 GH61E polypeptide having cellulolytic enhancing activity (SEQ ID NO: 35 [DNA sequence] and SEQ ID NO: 36 [deduced amino acid sequence]) was recombinantly prepared according to U.S. Patent No. 7,361,495 using *Aspergillus oryzae* JaL250 as a host.

Filtered culture broth was desalted and buffer-exchanged into 20 mM sodium acetate-150 mM NaCl pH 5.0 using a HIPREP® 26/10 Desalting Column according to the manufacturer's instructions. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 19: Preparation of *Aspergillus fumigatus* NN051616 GH61B polypeptide having cellulolytic enhancing activity

A tblastn search (Altschul *et al.*, 1997, *Nucleic Acids Res.* 25: 3389-3402) of the *Aspergillus fumigatus* partial genome sequence (The Institute for Genomic Research, Rockville, MD) was performed using as query several known GH61 proteins including GH61A from *Thermoascus aurantiacus* (GeneSeqP Accession Number AEC05922). Several genes were identified as putative Family GH61 homologs based upon a high degree of similarity to the query sequences at the amino acid level. One genomic region of approximately 850 bp with greater than 70% identity to the *Thermoascus aurantiacus* GH61A sequence at the amino acid level was chosen for further study.

Aspergillus fumigatus NN051616 was grown and harvested as described in U.S. Patent No. 7,244,605. Frozen mycelia were ground, by mortar and pestle, to a fine powder and genomic DNA was isolated using a DNEASY® Plant Kit according to manufacturer's instructions.

Two synthetic oligonucleotide primers shown below were designed to PCR amplify the *Aspergillus fumigatus* Family GH61B protein gene from the genomic DNA. An IN-FUSION® Cloning Kit was used to clone the fragment directly into the expression vector

pAILo2, without the need for restriction digestion and ligation.

Forward primer:

5'-ACTGGATTTACC**ATGACTTTGTCCAAGATCACTTCCA**-3' (SEQ ID NO: 115)

Reverse primer:

5'-TCACCTCTAGTTAA**TTAAGCGTTGAACAGTGCAGGACCAG**-3' (SEQ ID NO: 116)

Bold letters represent coding sequence. The remaining sequence is homologous to the insertion sites of pAILo2.

Fifty picomoles of each of the primers above were used in a PCR reaction containing 204 ng of *Aspergillus fumigatus* genomic DNA, 1X *Pfx* Amplification Buffer (Invitrogen, Carlsbad, CA, USA), 1.5 μl of 10 mM blend of dATP, dTTP, dGTP, and dCTP, 2.5 units of PLATINUM® *Pfx* DNA Polymerase, and 1 μl of 50 mM $MgSO_4$ in a final volume of 50 μl. The amplification was performed using an EPPENDORF® MASTERCYCLER® 5333 epgradient S (Eppendorf Scientific, Inc., Westbury, NY, USA) programmed for one cycle at 94°C for 3 minutes; and 30 cycles each at 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 1 minutes. The heat block was then held at 72°C for 15 minutes followed by a 4°C soak cycle.

The reaction products were isolated by 1.0% agarose gel electrophoresis using TAE buffer where an approximately 850 bp product band was excised from the gel and purified using a MINELUTE® Gel Extraction Kit (QIAGEN Inc., Valencia, CA, USA) according to the manufacturer's instructions.

The fragment was then cloned into pAILo2 using an IN-FUSION® Cloning Kit. The vector was digested with *Nco* I and *Pac* I. The fragment was purified by gel electrophoresis as above and a QIAQUICK® Gel Purification Kit. The gene fragment and the digested vector were combined together in a reaction resulting in the expression plasmid pAG43 in which transcription of the Family GH61B protein gene was under the control of the NA2-tpi promoter. The recombination reaction (20 μl) was composed of 1X IN-FUSION® Buffer, 1X BSA, 1 μl of IN-FUSION® enzyme (diluted 1:10), 166 ng of pAILo2 digested with *Nco* I and *Pac* I, and 110 ng of the *Aspergillus fumigatus* GH61B protein purified PCR product. The reaction was incubated at 37°C for 15 minutes followed by 15 minutes at 50°C. The reaction was diluted with 40 μl of 10 mM Tris-0.1 M EDTA buffer and 2.5 μl of the diluted reaction was used to transform *E. coli* SOLOPACK® Gold cells. An *E. coli* transformant containing pAG43 (GH61B protein gene) was identified by restriction enzyme digestion and plasmid DNA was prepared using a BIOROBOT® 9600.

DNA sequencing of the 862 bp PCR fragment was performed with a Perkin-Elmer Applied Biosystems Model 377 XL Automated DNA Sequencer using dye-terminator chemistry (Giesecke *et al.*, 1992, *supra*) and primer walking strategy. The following vector specific primers were used for sequencing:

pAllo2 5 Seq:

5' TGTCCCTTGTCGATGCG 3' (SEQ ID NO: 117)

pAllo2 3 Seq:

5' CACATGACTTGGCTTCC 3' (SEQ ID NO: 118)

Nucleotide sequence data were scrutinized for quality and all sequences were compared to each other with assistance of PHRED/PHRAP software (University of Washington, Seattle, WA, USA).

A gene model for the *Aspergillus fumigatus* sequence was constructed based on similarity of the encoded protein to the *Thermoascus aurantiacus* GH61A protein (GeneSeqP Accession Number AEC05922). The nucleotide sequence and deduced amino acid sequence, are shown in SEQ ID NO: 37 and SEQ ID NO: 38, respectively. The genomic fragment encodes a polypeptide of 250 amino acids, interrupted by 2 introns of 53 and 56 bp. The% G+C content of the gene and the mature coding sequence are 53.9% and 57%, respectively. Using the SignalP software program (Nielsen *et al.*, 1997, *Protein Engineering* 10: 1-6), a signal peptide of 21 residues was predicted. The predicted mature protein contains 221 amino acids with a predicted molecular mass of 23.39 kDa.

Aspergillus oryzae JaL355 protoplasts were prepared according to the method of Christensen *et al.*, 1988, *supra*. Six μg of pAG43 were used to transform *Aspergillus oryzae* JaL355. Twenty-six transformants were isolated to individual PDA plates.

Confluent PDA plates of 24 transformants were each washed with 5 ml of 0.01% TWEEN® 20 and the spores were each collected. Eight μl of each spore stock was added to 1 ml of YPG, YPM, and M410 media separately in 24 well plates and incubated at 34°C. After 3 days of incubation, 7.5 μl of supernatant from four transformants were analyzed using a CRITERION® stain-free, 8-16% gradient SDS-PAGE gel according to the manufacturer's instructions. Based on this gel, M410 was chosen as the best medium. Five days after incubation, 7.5 μl of supernatant from each M410 culture was analyzed using a CRITERION® stain-free, 8-16% gradient SDS-PAGE gel. SDS-PAGE profiles of the cultures showed that several transformants had a new major band of approximately 25 kDa.

A confluent plate of one transformant (grown on PDA) was washed with 5 ml of 0.01% TWEEN® 20 and inoculated into four 500 ml Erlenmeyer flasks containing 100 ml of M410 medium to generate broth for characterization of the enzyme. The flasks were harvested on day 5 (300 ml), filtered using a 0.22 μm EXPRESS™ Plus Membrane, and stored at 4°C.

The filtered shake flask broth containing *Aspergillus fumigatus* GH61B polypeptide having cellulolytic enhancing activity was concentrated using a 10 kDa MWCO Amicon Ultra centrifuge concentrator (Millipore, Bedford, MA, USA) to approximately 10-fold smaller

volume. The concentrated filtrate was buffer-exchanged and desalted using a BIO-GEL® P6 desalting column (Bio-Rad Laboratories, Inc., Hercules, CA, USA) pre-equilibrated in 20 mM Tris-(hydroxymethyl)aminomethane (Sigma, St. Louis, MO, USA), pH 8.0, according to the manufacturer's instructions with the following exception: 3 ml of sample was loaded and eluted with 3 ml of buffer. Concentrated, desalted GH61B protein was quantified using a BCA protein assay using bovine serum albumin as a protein concentration standard. Quantification was performed in triplicate. Enzyme purity was confirmed using 8-16% gradient SDS-PAGE at 200 volts for 1 hour and staining with BIO-SAFE™ Coomassie Stain.

Example 20: Preparation of *Penicillium pinophilum* GH61 polypeptide having cellulolytic enhancing activity

Penicillium pinophilum GH61 polypeptide having cellulolytic enhancing activity SEQ ID NO: 39 [DNA sequence] and SEQ ID NO: 40 [deduced amino acid sequence]) was preaped according to the procedure described below.

Compost samples were collected from Yunnan, China on December 12, 2000. *Penicillium pinophilum* NN046877 was isolated using single spore isolation techniques on PDA plates at 45°C. *Penicillium pinophilum* strain NN046877 was inoculated onto a PDA plate and incubated for 4 days at 37°C in the darkness. Several mycelia-PDA plugs were inoculated into 500 ml shake flasks containing 100 ml of NNCYP-PCS medium. The flasks were incubated for 5 days at 37°C with shaking at 160 rpm. The mycelia were collected at day 4 and day 5. The mycelia from each day were frozen in liquid nitrogen and stored in a -80°C freezer until use.

The frozen mycelia were transferred into a liquid nitrogen prechilled mortar and pestle and ground to a fine powder. Total RNA was prepared from the powdered mycelia of each day by extraction with TRIZOL™ reagent (Invitrogen Corporation, Carlsbad, CA, USA). The polyA enriched RNA was isolated using a mTRAP™ Total Kit (Active Motif, Carlsbad, CA, USA).

Double stranded cDNA from each day was synthesized with a SMART™ cDNA library Construction Kit (Clontech Laboratories, Inc., Mountain View, CA, USA). The cDNA was cleaved with *Sfi* I and the cDNA was size fractionated by 0.8% agarose gel electrophoresis using TBE buffer. The fraction of cDNA of 500 bp and larger was excised from the gel and purified using a GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions. Then equal amounts of cDNA from day 4 and day 5 were pooled for library construction.

The pooled cDNA was then directionally cloned by ligation into *Sfi* I cleaved pMHas7 (WO 2009/037253) using T4 ligase (New England Biolabs, Inc., Beverly, MA, USA) according to the manufacturer's instructions. The ligation mixture was electroporated into *E.*

coli ELECTROMAX™ DH10B™ cells (Invitrogen Corp., Carlsbad, CA, USA) using a GENE PULSER® and Pulse Controller (Bio-Rad Laboratories, Inc., Hercules, CA, USA) at 25 uF, 25 mAmp, 1.8 kV with a 1 mm gap cuvette according to the manufacturer's procedure.

The electroporated cells were plated onto LB plates supplemented with 50 mg of kanamycin per liter. A cDNA plasmid pool was prepared from 60,000 total transformants of the original pMHas7 vector ligation. Plasmid DNA was prepared directly from the pool of colonies using a QIAGEN® Plasmid Kit (QIAGEN Inc., Valencia, CA, USA).

A transposon containing plasmid designated pSigA4 was constructed from the pSigA2 transposon containing plasmid described WO 2001/77315 in order to create an improved version of the signal trapping transposon of pSigA2 with decreased selection background. The pSigA2 transposon contains a signal less beta-lactamase construct encoded on the transposon itself. PCR was used to create a deletion of the intact beta lactamase gene found on the plasmid backbone using a proofreading *Pfu* Turbo polymerase PROOFSTART™ (QIAGEN GmbH Corporation, Hilden, Germany) and the following 5' phosphorylated primers (TAG Copenhagen, Denmark):

SigA2NotU-P:

5'-TCGCGATCCGTTTTCGCATTTATCGTGAAACGCT-3' (SEQ ID NO: 119)

SigA2NotD-P:

5'-CCGCAAACGCTGGTGAAAGTAAAAGATGCTGAA-3' (SEQ ID NO: 120)

The amplification reaction was composed of 1 µl of pSigA2 (10 ng/ µl), 5 µl of 10X PROOFSTART™ Buffer (QIAGEN GmbH Corporation, Hilden, Germany), 2.5 µl of dNTP mix (20 mM), 0.5 µl of SigA2NotU-P (10 mM), 0.5 µl of SigA2NotD-P (10 mM), 10 µl of Q solution (QIAGEN GmbH Corporation, Hilden, Germany), and 31.25 µl of deionized water. A DNA ENGINE™ Thermal Cycler (MJ Research Inc., Waltham, MA, USA) was used for amplification programmed for one cycle at 95°C for 5 minutes; and 20 cycles each at 94°C for 30 seconds, 62°C for 30 seconds, and 72°C for 4 minutes.

A 3.9 kb PCR reaction product was isolated by 0.8% agarose gel electrophoresis using TAE buffer and 0.1 µg of ethidium bromide per ml. The DNA band was visualized with the aid of an EAGLE EYE® Imaging System (Stratagene, La Jolla, CA, USA) at 360 nm. The 3.9 kb DNA band was excised from the gel and purified using a GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

The 3.9 kb fragment was self-ligated at 16°C overnight with 10 units of T4 DNA ligase (New England Biolabs, Inc., Beverly, MA, USA), 9 µl of the 3.9 kb PCR fragment, and 1 µl of 10X ligation buffer (New England Biolabs, Inc., Beverly, MA, USA). The ligation was heat inactivated for 10 minutes at 65°C and then digested with *Dpn* I at 37°C for 2 hours. After incubation, the digestion was purified using a GFX® PCR DNA and Gel Band Purification Kit.

The purified material was then transformed into *E. coli* TOP10 competent cells according to the manufacturer's instructions. The transformation mixture was plated onto LB plates supplemented with 25 µg of chloramphenicol per ml. Plasmid minipreps were prepared from several transformants and digested with *Bgl* II. One plasmid with the correct construction was chosen. The plasmid was designated pSigA4. Plasmid pSigA4 contains the *Bgl* II flanked transposon SigA2 identical to that disclosed in WO 2001/77315.

A 60 µl sample of plasmid pSigA4 DNA (0.3 µg/µl) was digested with *Bgl* II and separated by 0.8% agarose gel electrophoresis using TBE buffer. A SigA2 transposon DNA band of 2 kb was eluted with 200 µl of EB buffer (QIAGEN GmbH Corporation, Hilden, Germany) and purified using a GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions and eluted in 200 µl of EB buffer. SigA2 was used for transposon assisted signal trapping.

A complete description of transposon assisted signal trapping is described in WO 2001/77315. The plasmid pool was treated with transposon SigA2 and HYPERMU™ transposase (EPICENTRE Biotechnologies, Madison, WI, USA) according to the manufacturer's instructions.

For *in vitro* transposon tagging of the *Penicillium pinophilum* cDNA library, 2 µl of SigA2 transposon containing approximately 100 ng of DNA were mixed with 1 µl of the plasmid DNA pool of the *Penicillium pinophilum* cDNA library containing 1 µg of DNA, 1 µl of HYPERMU™ transposase, and 2 µl of 10X buffer (EPICENTRE Biotechnologies, Madison, WI, USA) in a total volume of 20 µl and incubated at 30°C for 3 hours followed by adding 2 µl of stop buffer (EPICENTRE Biotechnologies, Madison, WI, USA) and heat inactivation at 75°C for 10 minutes. The DNA was precipitated by addition of 2 µl of 3 M sodium acetate pH 5 and 55 µl of 96% ethanol and centrifuged for 30 minutes at 10,000 *x g*, 4°C. The pellet was washed in 70% ethanol, air dried at room temperature, and resuspended in 10 µl of deionized water.

A 2 µl volume of the transposon tagged plasmid pool was electroporated into 50 µl of *E. coli* ELECTROMAX™ DH10B™ cells (Invitrogen Corp., Carlsbad, CA, USA) according to the manufacturer's instructions using a GENE PULSER® and Pulse Controller at 25 uF, 25 mAmp, 1.8 kV with a 1 mm gap cuvette according to the manufacturer's procedure.

The electroporated cells were incubated in SOC medium with shaking at 225 rpm for 1 hour at 37°C before being plated onto the following selective media: LB medium supplemented with 50 µg of kanamycin per ml; LB medium supplemented with 50 µg of kanamycin per ml and 15 µg of chloramphencol per ml; and LB medium supplemented with 50 µg of kanamycin per ml, 15 µg of chloramphencol per ml, and 30 µg of ampicillin per ml.

From plating of the electroporation onto LB medium supplemented with kanamycin, chloramphencol and ampicillin, approximately 200 colonies per 50 µl were observed after 3

days at 30°C. All colonies were replica plated onto LB kanamycin, chloramphenicol, and ampicillin medium described above. Five hundred colonies were recovered under this selection condition. The DNA from each colony was sequenced with the transposon forward and reverse primers (primers A and B), shown below, according to the procedure disclosed in WO 2001/77315 (page 28).

Primer A:

5'-agcgtttgcggccgcgatcc-3' (SEQ ID NO: 121)

Primer B:

5'-ttattcggtcgaaaaggatcc-3' (SEQ ID NO: 122)

DNA sequences were obtained from SinoGenoMax Co., Ltd (Beijing, China). Primer A and primer B sequence reads for each plasmid were trimmed to remove vector and transposon sequence. The assembled sequences were grouped into contigs by using the program PhredPhrap (Ewing *et al.*, 1998, *Genome Research* 8: 175-185; Ewing and Green, 1998, *Genome Research* 8: 186-194). All contigs were subsequently compared to sequences available in standard public DNA and protein sequences databases (TrEMBL, SWALL, PDB, EnsemblPep, GeneSeqP) using the program BLASTX 2.0a19MP-WashU [14-Jul-1998] [Build linux-x86 18:51:44 30-Jul-1998] (Gish *et al.*, 1993, *Nat. Genet.* 3: 266-72). The family GH10 xylanase candidate was identified directly by analysis of the BlastX results.

Penicillium pinophilum NN046877 was grown on a PDA agar plate at 37°C for 4-5 days. Mycelia were collected directly from the agar plate into a sterilized mortar and frozen under liquid nitrogen. Frozen mycelia were ground, by mortar and pestle, to a fine powder, and genomic DNA was isolated using a DNEASY® Plant Mini Kit (QIAGEN Inc., Valencia, CA, USA).

Based on the *Penicillium pinophilum* GH10 xylanase gene information obtained as described above, oligonucleotide primers, shown below, were designed to amplify the GH61 gene from genomic DNA of *Penicillium pinophilum* GH10 NN046877. An IN-FUSION® CF Dry-down Cloning Kit was used to clone the fragment directly into the expression vector pPFJO355, without the need for restriction digestion and ligation.

Sense primer:

5'-ACACAACTGGGGATCCACCATGACTCTAGTAAAGGCTATTCTTTTAGC-3' (SEQ ID NO: 123)

Antisense primer:

5'-GTCACCCTCTAGATCTTCACAAACATTGGGAGTAGTATGG-3' (SEQ ID NO: 124)

Bold letters represented the coding sequence and the remaining sequence was homologous to insertion sites of pPFJO355.

The expression vector pPFJO355 contains the *Aspergillus oryzae* TAKA-amylase promoter, *Aspergillus niger* glucoamylase terminator elements, pUC19 derived sequences

for selection and propagation in *E. coli*, and an *Aspergillus nidulans pyrG* gene, which encodes an orotidine decarboxylase for selection of a transformant of a *pyrG* mutant *Aspergillus* strain.

Twenty picomoles of each of the primers above were used in a PCR reaction composed of *Penicillium sp.* NN051602 genomic DNA, 10 µl of 5X GC Buffer (Finnzymes Oy, Espoo, Finland), 1.5 µl of DMSO, 2.5 mM each of dATP, dTTP, dGTP, and dCTP, and 0.6 unit of PHUSION™ High-Fidelity DNA Polymerase (Finnzymes Oy, Espoo, Finland), in a final volume of 50 µl. The amplification was performed using a Peltier Thermal Cycler (MJ Research Inc., South San Francisco, CA, USA) programmed for denaturing at 98°C for 1 minutes; 5 cycles of denaturing at 98°C for 15 seconds, annealing at 56°C for 30 seconds, with a 1°C increase per cycle and elongation at 72°C for 75 seconds; and 25 cycles each at 98°C for 15 seconds, 65C for 30 seconds and 72°C for 75 seconds; and a final extension at 72°C for 10 minutes. The heat block then went to a 4°C soak cycle.

The reaction products were isolated by 1.0% agarose gel electrophoresis using TBE buffer where an approximately 1.4 kb product band was excised from the gel, and purified using an ILLUSTRA® GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

Plasmid pPFJO355 was digested with *Bam* I and *Bgl* II, isolated by 1.0% agarose gel electrophoresis using TBE buffer, and purified using an ILLUSTRA® GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

The gene fragment and the digested vector were ligated together using an IN-FUSION® CF Dry-down PCR Cloning Kit resulting in pPpin3 in which transcription of the *Penicillium pinophilum* GH10 xylanase gene was under the control of a promoter from the gene for *Aspergillus oryzae* alpha-amylase. In brief, 30 ng of pPFJO355 digested with *Bam* I and *Bgl* II, and 60 ng of the *Penicillium pinophilum* GH10 xylanase gene purified PCR product were added to a reaction vial and resuspended in a final volume of 10 µl with addition of deionized water. The reaction was incubated at 37°C for 15 minutes and then 50°C for 15 minutes. Three µl of the reaction were used to transform *E. coli* TOP10 competent cells. An *E. coli* transformant containing pPpin3 was detected by colony PCR and plasmid DNA was prepared using a QIAprep Spin Miniprep Kit (QIAGEN Inc., Valencia, CA, USA). The *Penicillium pinophilum* GH10 xylanase gene insert in pPpin3 was confirmed by DNA sequencing using a 3730XL DNA Analyzer (Applied Biosystems Inc, Foster City, CA, USA).

The same PCR fragment was cloned into vector pGEM-T using a pGEM-T Vector System to generate pGEM-T-Ppin3. The *Penicillium pinophilum* GH10 xylanase gene contained in pGEM-T-Ppin3 was confirmed by DNA sequencing using a 3730XL DNA Analyzer. *E. coli* strain T-Ppin3, containing pGEM-T-Ppin3, was deposited with the Deutsche

Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM), D-38124 Braunschweig, Germany on September 7, 2009, and assigned accession number DSM 22922.

DNA sequencing of the *Penicillium pinophilum* genomic clone encoding a GH10 polypeptide having xylanase activity was performed with an Applied Biosystems Model 3700 Automated DNA Sequencer using version 3.1 BIG-DYE™ terminator chemistry (Applied Biosystems, Inc., Foster City, CA, USA) and dGTP chemistry (Applied Biosystems, Inc., Foster City, CA, USA) and primer walking strategy. Nucleotide sequence data were scrutinized for quality and all sequences were compared to each other with assistance of PHRED/PHRAP software (University of Washington, Seattle, WA, USA).

The nucleotide sequence and deduced amino acid sequence of the *Penicillium pinophilum gh10* gene are shown in SEQ ID NO: 39 and SEQ ID NO: 40, respectively. The coding sequence is 1442 bp including the stop codon and is interrupted by three introns of 51 bp (199-249), 73 bp (383-455), and 94 bp (570-663). The encoded predicted protein is 407 amino acids. The%G+C of the coding sequence of the gene (including introns) is 47.99% G+C and the mature polypeptide coding sequence is 49.22%. Using the SignalP program (Nielsen *et al.*, 1997, *Protein Engineering* 10: 1-6), a signal peptide of 19 residues was predicted. The predicted mature protein contains 388 amino acids with a predicted molecular mass of 41.5 kDa and an isoelectric pH of 5.03.

A comparative pairwise global alignment of amino acid sequences was determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *J. Mol. Biol.* 48: 443-453) as implemented in the Needle program of EMBOSS with gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 matrix. The alignment showed that the deduced amino acid sequence of the *Penicillium pinophilum* gene encoding the GH10 polypeptide having xylanase activity shares 76% and 87% identity (excluding gaps) to the deduced amino acid sequence of a predicted GH10 family protein from *Talaromyces emersonii* (AAU99346) and *Penicillium marneffei* (B6QN64), respectively.

Aspergillus oryzae HowB101 (WO 95/35385 Example 1) protoplasts were prepared according to the method of Christensen *et al.*, 1988, *Bio/Technology* 6: 1419-1422. Three µg of pPpin3 were transformed into *Aspergillus oryzae* HowB101.

The transformation of *Aspergillus oryzae* HowB101 with pPpin3 yielded about 50 transformants. Twelve transformants were isolated to individual Minimal medium plates.

Four transformants were inoculated separately into 3 ml of YPM medium in a 24-well plate and incubated at 30°C with shaking at 150 rpm. After 3 days incubation, 20 µl of supernatant from each culture were analyzed by SDS-PAGE using a NUPAGE® NOVEX® 4-12% Bis-Tris Gel with MES buffer according to the manufacturer's instructions. The resulting gel was stained with INSTANT® Blue (Expedeon Ltd., Babraham Cambridge, UK). SDS-PAGE profiles of the cultures showed that the majority of the transformants had a

major band of approximately 55 kDa. The expression strain was designated *Aspergillus oryzae* EXP02765.

A slant of one transformant, designated transformant 2, was washed with 10 ml of YPM and inoculated into a 2 liter flask containing 400 ml of YPM medium to generate broth for characterization of the enzyme. The culture was harvested on day 3 and filtered using a 0.45 µm DURAPORE® Membrane (Millipore, Bedford, MA, USA).

A 1 liter volume of supernatant of the recombinant *Aspergillus oryzae* strain EXP02765 was precipitated with ammonium sulfate (80% saturation) and redissolved in 50 ml of 25 mM sodium acetate pH 4.3, and then dialyzed against the same buffer and filtered through a 0.45 µm filter. The solution was applied to a 40 ml Q SEPHAROSE™ Fast Flow column column equilibrated in 25 mM sodium acetate pH 4.3. The recombinant GH10 protein did not bind to the column. The fractions with xylanase activity were collected and evaluated by SDS-PAGE as described above. Fractions containing a band of approximately 55 kDa were pooled. The pooled solution was concentrated by ultrafiltration.

Example 21: Preparation of *Penicillium* sp. GH61 polypeptide having cellulolytic enhancing activity

Penicillium sp. GH61 polypeptide having cellulolytic enhancing activity SEQ ID NO: 41 [DNA sequence] and SEQ ID NO: 42 [deduced amino acid sequence]) according to the following procedure.

A compost sample was collected from Yunnan, China. *Penicillium sp.* NN051602 was isolated using single spore isolation techniques on PDA plates at 45°C. The *Penicillium* sp. strain was inoculated onto a PDA plate and incubated for 4 days at 45°C in the darkness. Several mycelia-PDA plugs were inoculated into 500 ml shake flasks containing 100 ml of NNCYP-PCS medium. The flasks were incubated for 6 days at 45°C with shaking at 160 rpm. The mycelia were collected at day 4, day 5, and day 6. Then the mycelia from each day were combined and frozen in liquid nitrogen, and then stored in a -80°C freezer until use.

The frozen mycelia were transferred into a liquid nitrogen prechilled mortar and pestle and ground to a fine powder. Total RNA was prepared from the powdered mycelia by extraction with TRIZOL® reagent and purified using a RNEASY® Mini Kit according to the manufacturer's protocol. Fifty micrograms of total RNA was submitted to sequencing as described above.

Total RNA enriched for polyA sequences with the mRNASeq protocol was sequenced using an ILLUMINA® GA2 system (Illumina, Inc., San Diego, CA, USA). The raw 36 base pair reads were assembled with an in-house developed assembler. The assembled sequences were analyzed using standard bioinformatics methods for gene finding and functional prediction. ESTscan 2.0 was used for gene prediction. NCBI blastall version

2.2.10 and HMMER version 2.1.1 were used to predict function based on structural homology. The Family GH61 candidate was identified directly by analysis of the Blast results.

Penicillium sp. NN051602 was grown on a PDA agar plate at 45°C for 3 days. Mycelia were collected directly from the agar plate into a sterilized mortar and frozen under liquid nitrogen. Frozen mycelia were ground, by mortar and pestle, to a fine powder, and genomic DNA was isolated using a DNEASY® Plant Mini Kit (QIAGEN Inc., Valencia, CA, USA).

Based on the ILLUMINA® sequencing information of the *Penicillium sp.* GH61 gene obtained as described above, oligonucleotide primers, shown below, were designed to amplify the GH61 gene from genomic DNA of *Penicillium sp.* NN051602. An IN-FUSION® CF Dry-down Cloning Kit (Clontech Laboratories, Inc., Mountain View, CA, USA) was used to clone the fragment directly into the expression vector pPFJO355, without the need for restriction digestion and ligation.

Sense primer:

5'-ACACAACTGGGGATCCACC**ATGCTGTCTTCGACGACTCGCA**-3' (SEQ ID NO: 125)

Antisense primer:

5'-GTCACCCTCTAGATCT**CGACTTCTTCTAGAACGTCGGCTCA**-3' (SEQ ID NO: 126)

Bold letters represented the coding sequence and the remaining sequence was homologous to insertion sites of pPFJO355.

Twenty picomoles of each of the primers above were used in a PCR reaction composed of *Penicillium sp.* NN051602 genomic DNA, 10 µl of 5X GC Buffer, 1.5 µl of DMSO, 2.5 mM each of dATP, dTTP, dGTP, and dCTP, and 0.6 unit of PHUSION™ High-Fidelity DNA Polymerase (Finnzymes Oy, Espoo, Finland) in a final volume of 50 µl. The amplification was performed using a Peltier Thermal Cycler programmed for denaturing at 98°C for 1 minutes; 5 cycles of denaturing at 98°C for 15 seconds, annealing at 63°C for 30 seconds, with a 1°C increase per cycle and elongation at 72°C for 60 seconds; and 25 cycles each at 98°C for 15 seconds and 72°C for 60 seconds; and a final extension at 72°C for 5 minutes. The heat block then went to a 4°C soak cycle.

The reaction products were isolated by 1.0% agarose gel electrophoresis using TBE buffer where an approximately 0.9 kb product band was excised from the gel, and purified using an ILLUSTRA® GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

Plasmid pPFJO355 was digested with *Bam* I and *Bgl* II, isolated by 1.0% agarose gel electrophoresis using TBE buffer, and purified using an ILLUSTRA® GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

The gene fragment and the digested vector were ligated together using an IN-

FUSION® CF Dry-down PCR Cloning Kit resulting in pGH61D23Y4 in which transcription of the *Penicillium sp.* GH61 gene was under the control of a promoter from the gene for *Aspergillus oryzae* alpha-amylase. In brief, 30 ng of pPFJO355 digested with *Bam* I and *Bgl* II, and 60 ng of the *Penicillium sp.* GH61 gene purified PCR product were added to a reaction vial and resuspended in a final volume of 10 µl with addition of deionized water. The reaction was incubated at 37°C for 15 minutes and then 50°C for 15 minutes. Three µl of the reaction were used to transform *E. coli* TOP10 competent cells. An *E. coli* transformant containing pGH61D23Y4 was detected by colony PCR and plasmid DNA was prepared using a QIAprep Spin Miniprep Kit (QIAGEN Inc., Valencia, CA, USA). The *Penicillium sp.* GH61 gene insert in pGH61D23Y4 was confirmed by DNA sequencing using a 3730XL DNA Analyzer.

The same PCR fragment was cloned into vector pGEM-T using a pGEM-T Vector System to generate pGEM-T-GH61D23Y4. The *Penicillium sp.* GH61 gene insert in pGEM-T-GH61D23Y4 was confirmed by DNA sequencing using a 3730XL DNA Analyzer. *E. coli* strain T-51602, containing pGEM-T-GH61D23Y4, was deposited with the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM), Mascheroder Weg 1 B, D-38124 Braunschweig, Germany on August 26, 2009 and assigned accession number DSM 22882.

DNA sequencing of the *Penicillium* sp. genomic clone encoding a GH61A polypeptide having cellulolytic-enhancing activity was performed with an Applied Biosystems Model 3700 Automated DNA Sequencer using version 3.1 BIG-DYE™ terminator chemistry (Applied Biosystems, Inc., Foster City, CA, USA) and dGTP chemistry (Applied Biosystems, Inc., Foster City, CA, USA) and primer walking strategy. Nucleotide sequence data were scrutinized for quality and all sequences were compared to each other with assistance of PHRED/PHRAP software (University of Washington, Seattle, WA, USA).

The nucleotide sequence and deduced amino acid sequence of the *Penicillium sp. gh61a* gene are shown in SEQ ID NO: 45 and SEQ ID NO: 46, respectvely. The coding sequence is 835 bp including the stop codon and is interrupted by one intron of 73 bp (114-186). The encoded predicted protein is 253 amino acids. The%G+C of the coding sequence of the gene (including introns) is 63.35% G+C and the mature polypeptide coding sequence is 64.62%. Using the SignalP program (Nielsen *et al.*, 1997, *Protein Engineering* 10: 1-6), a signal peptide of 25 residues was predicted. The predicted mature protein contains 228 amino acids with a predicted molecular mass of 24.33 kDa and an isoelectric pH of 4.17.

A comparative pairwise global alignment of amino acid sequences was determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *J. Mol. Biol.* 48: 443-453) as implemented in the Needle program of EMBOSS with gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 matrix. The alignment showed that the

deduced amino acid sequence of the *Penicillium* gene encoding the GH61A polypeptide having cellulolytic-enhancing activity shares 74% identity (excluding gaps) to the deduced amino acid sequence of a predicted GH61 family protein from *Thermoascus aurantiacus* (GENESEQP:AUM17198).

Aspergillus oryzae HowB101 (WO 95/35385 Example 1) protoplasts were prepared according to the method of Christensen *et al.*, 1988, *Bio/Technology* 6: 1419-1422. Three µg of pGH61D23Y4 were used to transform *Aspergillus oryzae* HowB101.

The transformation of *Aspergillus oryzae* HowB101 with pGH61D23Y4 yielded about 50 transformants. Twelve transformants were isolated to individual Minimal medium plates.

Six transformants were inoculated separately into 3 ml of YPM medium in a 24-well plate and incubated at 30°C, 150 rpm. After 3 days incubation, 20 µl of supernatant from each culture were analyzed on a NUPAGE® NOVEX® 4-12% Bis-Tris Gel with MES buffer according to the manufacturer's instructions. The resulting gel was stained with INSTANT® Blue (Expedeon Ltd., Babraham Cambridge, UK). SDS-PAGE profiles of the cultures showed that the majority of the transformants had a major band of approximately 45 kDa. The expression strain was designated as *Aspergillus oryzae* EXP03089.

A slant of one transformant, designated transformant 1, was washed with 10 ml of YPM medium and inoculated into a 2 liter flask containing 400 ml of YPM medium to generate broth for characterization of the enzyme. The culture was harvested on day 3 and filtered using a 0.45 µm DURAPORE Membrane (Millipore, Bedford, MA, USA).

A 400 ml volume of the filtered broth of the recombinant strain *Aspergillus oryzae* EXP03089 was precipitated with ammonium sulfate (80% saturation) and redissolved in 20 ml of 25 mM sodium acetate pH 5.0 buffer, and then dialyzed against the same buffer and filtered through a 0.45 µm filter. The solution was applied to a 30 ml Q SEPHAROSE® Fast Flow column (GE Healthcare, Buckinghamshire, UK) equilibrated in 25 mM sodium acetate pH 5.0. The recombinant GH61 protein was eluted with a linear NaCl gradient (0-0.4 M). Fractions eluted with 0.1-0.2 M NaCl were collected and dialyzed against the same equilibration buffer. The sample was further purified on a MONO Q® column (GE Healthcare, Buckinghamshire, UK) with a linear NaCl gradient (0-0.3 M). Fractions were evaluated by SDS-PAGE. Fractions containing a band of approximately 45 kDa were pooled. The pooled solution was concentrated by ultrafiltration.

Example 22: Preparation of *Thielavia terrestris* GH61N polypeptide having cellulolytic enhancing activity

Thielavia terrestris NRRL 8126 (SEQ ID NO: 43 [DNA sequence] and SEQ ID NO: 44 [deduced amino acid sequence]) was prepared according to the following procedure.

Genomic sequence information was generated by the U.S. Department of Energy

Joint Genome Institute (JGI). A preliminary assembly of the genome was downloaded from JGI and analyzed using the Pedant-Pro™ Sequence Analysis Suite (Biomax Informatics AG, Martinsried, Germany). Gene models constructed by the software were used as a starting point for detecting GH61 homologues in the genome. More precise gene models were constructed manually using multiple known GH61 protein sequences as a guide.

To generate genomic DNA for PCR amplification, *Thielavia terrestris* NRRL 8126 was grown in 50 ml of NNCYP medium supplemented with 1% glucose in a baffled shake flask at 42°C and 200 rpm for 24 hours. Mycelia were harvested by filtration, washed twice in TE (10 mM Tris-1 mM EDTA), and frozen under liquid nitrogen. A pea-size piece of frozen mycelia was suspended in 0.7 ml of 1% lithium dodecyl sulfate in TE and disrupted by agitation with an equal volume of 0.1 mm zirconia/silica beads (Biospec Products, Inc., Bartlesville, OK, USA) for 45 seconds in a FastPrep FP120 (ThermoSavant, Holbrook, NY, USA). Debris was removed by centrifugation at 13,000 *x g* for 10 minutes and the cleared supernatant was brought to 2.5 M ammonium acetate and incubated on ice for 20 minutes. After the incubation period, the nucleic acids were precipitated by addition of 2 volumes of ethanol. After centrifugation for 15 minutes in a microfuge at 4°C, the pellet was washed in 70% ethanol and air dried. The DNA was resuspended in 120 µl of 0.1X TE and incubated with 1 µl of DNase-free RNase A at 37°C for 20 minutes. Ammonium acetate was added to 2.5 M and the DNA was precipitated with 2 volumes of ethanol. The pellet was washed in 70% ethanol, air dried, and resuspended in TE buffer.

Two synthetic oligonucleotide primers shown below were designed to PCR amplify the *Thielavia terrestris* Family GH61N gene from the genomic DNA. An IN-FUSION™ Cloning Kit was used to clone the fragment directly into the expression vector, pAILo2 (WO 2005/074647), without the need for restriction digests and ligation.

Forward primer:

5'-ACTGGATTTACC**ATGCCTTCTTTCGCCTCCAA**-3' (SEQ ID NO: 127)

Reverse primer:

5'-TCACCTCTAGTTAATTAA**TCAGTTTGCCTCCTCAGCCC**-3' (SEQ ID NO: 128)

Bold letters represent coding sequence. The remaining sequence is homologous to the insertion sites of pAILo2.

Fifty picomoles of each of the primers above were used in a PCR reaction containing 1 µg of *Thielavia terrestris* genomic DNA, 1X ADVANTAGE® GC-Melt LA Buffer (BD Biosciences, Palo Alto, CA, USA), 1 µl of 10 mM blend of dATP, dTTP, dGTP, and dCTP, 1.25 units of ADVANTAGE® GC Genomic LA Polymerase Mix (BD Biosciences, Palo Alto, CA, USA), in a final volume of 25 µl. The amplification conditions were one cycle at 94°C for 1 minute; and 30 cycles each at 94°C for 30 seconds, 60.5°C for 30 seconds, and 72°C for 1

minute. The heat block was then held at 72°C for 5 minutes followed by a 4°C soak cycle.

The reaction products were isolated by 1.0% agarose gel electrophoresis using TAE buffer where an approximately 1.1 kb product band was excised from the gel and purified using a MINELUTE® Gel Extraction Kit (QIAGEN Inc., Valencia, CA, USA) according to the manufacturer's instructions.

The fragment was then cloned into pAILo2 using an IN-FUSION™ Cloning Kit. The vector was digested with *Nco* I and *Pac* I. The fragment was purified by 1.0% agarose gel electrophoresis using TAE buffer, excised from the gel, and purified using a QIAQUICK® Gel Extraction Kit (QIAGEN Inc., Valencia, CA, USA). The gene fragment and the digested vector were combined together in a reaction resulting in the expression plasmid pAG66, in which transcription of the Family GH61N gene was under the control of the NA2-tpi promoter (a modified promoter from the gene encoding neutral alpha-amylase in *Aspergillus niger* in which the untranslated leader has been replaced by an untranslated leader from the gene encoding triose phosphate isomerase in *Aspergillus nidulans*). The recombination reaction (20 μl) was composed of 1X IN-FUSION™ Buffer (BD Biosciences, Palo Alto, CA, USA), 1X BSA (BD Biosciences, Palo Alto, CA, USA), 1 μl of IN-FUSION™ enzyme (diluted 1:10) (BD Biosciences, Palo Alto, CA, USA), 186 ng of pAILo2 digested with *Nco* I and *Pac* I, and 96.6 ng of the *Thielavia terrestris* GH61N purified PCR product. The reaction was incubated at 37°C for 15 minutes followed by 15 minutes at 50°C. The reaction was diluted with 40 μl of TE buffer and 2.5 μl of the diluted reaction was used to transform *E. coli* TOP10 Competent cells. An *E. coli* transformant containing pAG66 (GH61N gene) was identified by restriction enzyme digestion and plasmid DNA was prepared using a BIOROBOT® 9600.

Aspergillus oryzae JaL355 protoplasts were prepared according to the method of Christensen *et al.*, 1988, *Bio/Technology* 6: 1419-1422. Five μg of pAG43 was used to transform *Aspergillus oryzae* JaL355. Three transformants were isolated to individual PDA plates.

Plugs were taken from the original transformation plate of each of the three transformants and added separately to 1 ml of M410 medium in 24 well plates, which were incubated at 34°C. Five days after incubation, 7.5 μl of supernatant from each culture was analyzed using CRITERION® stain-free, 8-16% gradient SDS-PAGE, (Bio-Rad Laboratories, Inc., Hercules, CA, USA) according to the manufacturer's instructions. SDS-PAGE profiles of the cultures showed that several transformants had new major bands of approximately 70 kDa and 35 kDa.

Confluent PDA plates of two of the transformants were washed with 5 ml of 0.01% TWEEN® 20 and inoculated into five 500 ml Erlenmeyer flask containing 100 ml of M410 medium and incubated to generate broth for characterization of the enzyme. The flasks were

harvested on days 3 and 5 and filtered using a 0.22 µm stericup suction filter (Millipore, Bedford, MA).

Example 23: Preparation of *Aspergillus aculeatus* CBS 101.43 GH10 xylanase II

Aspergillus aculeatus CBS 101.43 GH10 xylanase II (SEQ ID NO: 45 [DNA sequence] and SEQ ID NO: 46 [deduced amino acid sequence]) was purified from SHEARZYME® 2X-CDN01013. The sample was desalted and buffer-exchanged in 20 mM Bis-Tris pH 6.0 using a HIPREP® 26/10 desalting column according to the manufacturer's instructions. The buffer exchanged sample was applied to a Q SEPHAROSE® Big Beads column (64 ml) equilibrated with 20 mM Bis-Tris pH 6.0, and the bound protein was eluted with a gradient from 0 to 500 mM sodium chloride over 10 column volumes. Fractions were pooled and concentrated into 200 mM sodium chloride-20 mM Bis-Tris pH 6.0. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit with bovine serum albumin as a protein standard.

Example 24: Preparation of *Aspergillus fumigatus* NN055679 GH10 xylanase

Aspergillus fumigatus NN055679 GH10 xylanase (xyn3) (SEQ ID NO: 47 [DNA sequence] and SEQ ID NO: 48 [deduced amino acid sequence]) was prepared recombinantly according to WO 2006/078256 using *Aspergillus oryzae* BECh2 (WO 2000/39322) as a host.

The filtered broth was desalted and buffer-exchanged into 20 mM Tris -150 mM NaCl pH 8.5 using a HIPREP® 26/10 Desalting Column according to the manufacturer's instructions. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit with bovine serum albumin as a protein standard.

Example 25: Preparation of *Trichophaea saccata* CBS 804.70 GH10 xylanase

Trichophaea saccata CBS 804.70 was inoculated onto a PDA plate and incubated for 7 days at 28°C. Several mycelia-PDA agar plugs were inoculated into 750 ml shake flasks containing 100 ml of MEX-1 medium. The flasks were agitated at 150 rpm for 9 days at 37°C. The fungal mycelia were harvested by filtration through MIRACLOTH® (Calbiochem, San Diego, CA, USA) before being frozen in liquid nitrogen. The mycelia were then pulverized into a powder by milling the frozen mycelia together with an equal volume of dry ice in a coffee grinder precooled with liquid nitrogen. The powder was transferred into a liquid nitrogen prechilled mortor and pestle and ground to a fine powder with a small amount of baked quartz sand. The powdered mycelial material was kept at -80°C until use.

Total RNA was prepared from the frozen, powdered mycelium of *Trichophaea saccata* CBS 804.70 by extraction with guanidium thiocyanate followed by ultracentrifugation

through a 5.7 M CsCl cushion according to Chirgwin *et al.*, 1979, *Biochemistry* 18: 5294-5299. The polyA enriched RNA was isolated by oligo (dT)-cellulose affinity chromatography according to Aviv *et al.*, 1972, *Proc. Natl. Acad. Sci. USA* 69: 1408-1412.

Double stranded cDNA was synthesized according to the general methods of Gubler and Hoffman, 1983, *Gene* 25: 263-269; Sambrook, J., Fritsch, E.F., and Maniantis, T. Molecular cloning: A Laboratory Manual, 2nd ed., 1989, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; and Kofod *et al.*, 1994, *J. Biol. Chem.* 269: 29182-29189, using a polyA-*Not* I primer (Promega Corp., Madison, Wisconsin, USA). After synthesis, the cDNA was treated with mung bean nuclease, blunt ended with T4 DNA polymerase, and ligated to a 50-fold molar excess of *Eco* RI adaptors (Invitrogen Corp., Carlsbad, CA, USA). The cDNA was cleaved with *Not* I and the cDNA was size fractionated by 0.8% agarose gel electrophoresis using TBE buffer. The fraction of cDNA of 700 bp and larger was excised from the gel and purified using a GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

The directional, size-fractioned cDNA was ligated into *Eco* RI-*Not* I cleaved pYES 2.0 (Invitrogen, Carlsbad, CA, USA). The ligation reactions were performed by incubation at 16°C for 12 hours, then heating at 70°C for 20 minutes, and finally addition of 10 µl of water to each tube. One µl of each ligation mixture was electroporated into 40 µl of electrocompetent *E. coli* DH10B cells (Invitrogen Corp., Carlsbad, CA, USA) as described by Sambrook *et al.*, 1989, *supra*.

The *Trichophaea saccata* CBS 804.70 library was established in *E. coli* consisting of pools. Each pool was made by spreading transformed *E. coli* on LB ampicillin plates, yielding 15,000-30,000 colonies/plate after incubation at 37°C for 24 hours. Twenty ml of LB medium was added to the plate and the cells were suspended therein. The cell suspension was shaken in a 50 ml tube for 1 hour at 37°C.

Plasmid DNA from several of the library pools of *T. saccata* CBS 804.70 was isolated using a Midi Plasmid Kit (QIAGEN Inc., Valencia, CA, USA), according to the manufacturer's instructions, and stored at -20°C.

One µl aliquots of purified plasmid DNA from several of the library pools were transformed into *S. cerevisiae* W3124 by electroporation (Becker and Guarante, 1991, *Methods Enzymol.* 194: 182-187) and the transformants were plated onto SC-agar plates containing 2% glucose and incubated at 30°C. In total, 50-100 plates containing 250-400 yeast colonies were obtained from each pool. After 3-5 days of incubation, the SC agar plates were replica plated onto a set of 0.1% AZCL xylan (oat) SC-URA agar plates with galactose. The plates were incubated for 2-4 days at 30°C and xylanase positive colonies were identified as colonies surrounded by a blue halo. The positive clones were streak-purified and obtai **strain** CBS 521.95 ned as single colonies.

Xylanase-expressing yeast colonies were inoculated into 5 ml of YPD medium in 25 ml tubes. The tubes were shaken overnight at 30°C. One ml of the culture was centrifugated to pellet the yeast cells.

DNA was isolated according to WO 94/14953 and dissolved in 50 µl of water. The DNA was transformed into *E. coli* DH10B using standard procedures (Sambrook *et al.*, 1989, *supra*).

Plasmid DNA was isolated from the *E. coli* transformants using standard procedures (Sambrook *et al.*, 1989, *supra*). Plasmids were sequenced using both pYES primers as sequencing primers. One specific plasmid clone of 1283 bp designated TF12Xyl170 was found to encode a Family 10 glycoside hydrolase protein and was further characterized. More reliable sequence was obtained by further sequencing of the fragment using the specific primers shown below designed based on the initial sequence:

TF12Xyl170F1:

5'-TGAAATGGGATGCTACTGA-3' (SEQ ID NO: 129)

TF12Xyl170F2:

5'-CAACGACTACAACATCGAGG-3' (SEQ ID NO: 130)

TF12Xyl170R1:

5'-ATTTGCTGTCCACCAGTGAA-3' (SEQ ID NO: 131)

One plasmid matching the original cDNA sequence was designated pTF12Xyl170 and the *E. coli* strain containing this clone was designated *E. coli* pTF12Xyl170 and deposited on July 28, 2009, with the Agricultural Research Service Patent Culture Collection, Northern Regional Research Center, Peoria, IL, USA, and given the accession number NRRL B-50309.

The nucleotide sequence and deduced amino acid sequence of the *Trichophaea saccata gh10a* gene are shown in SEQ ID NO: 49 and SEQ ID NO: 50, respectively. The coding sequence is 1197 bp including the stop codon. The encoded predicted protein contains 398 amino acids. The%G+C of the coding region of the gene is 53.6% and the mature polypeptide coding region is also 53.6%. Using the SignalP program, version 3 (Nielsen *et al.*, 1997, *Protein Engineering* 10: 1-6), a signal peptide of 19 residues was predicted. The predicted mature protein contains 379 amino acids with a molecular mass of 40.4 kDa.

Analysis of the deduced amino acid sequence of the *gh10a* gene with the Interproscan program (Zdobnov and Apweiler, 2001, *Bioinformatics* 17: 847-848) showed that the GH10A protein contained the core sequence typical of a Family 10 glycoside hydrolase, extending from approximately amino acid residue 65 to residue 377 of the predicted mature polypeptide. The GH10A protein also contained the sequence signature of a type I fungal cellulose binding domain (CBMI). This sequence signature known as Prosite

Entry PS00562 (Sigrist *et al.*, 2002, *Brief Bioinform.* 3: 265-274) was present from amino acid residue 8 to residue 35 of the predicted mature polypeptide.

A comparative pairwise global alignment of amino acid sequences was determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *J. Mol. Biol.* 48: 443-453) as implemented in the Needle program of EMBOSS with gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 matrix. The alignment showed that the deduced amino acid sequence of the *Trichophaea saccata* gene encoding the GH10A mature polypeptide shared 62.6% and 62.0% identity (excluding gaps) to the deduced amino acid sequences of Family 10 glycoside hydrolase proteins from *Phanerochaete chrysosporium* and *Meripilus giganteus*, respectively (accession numbers UNIPROT:B7SIW2 and GENESEQP:AAW23327, respectively).

The *Trichophaea saccata* CBS 804.70 *gh10a* gene was excised from the pTF12xyl170 using *Bam* HI and *Xho* I, and ligated into the *Aspergillus* expression vector pDAu109 (WO 2005/042735), also digested with *Bam* HI and *Xho* I, using standard methods (Sambrook *et al.*, 1989, *supra*). The ligation reaction was transformed into *E. coli* TOP10 chemically competent cells according to the manufacturer's instructions. Eight colonies were grown overnight in LB ampicillin medium and plasmid DNA was isolated using a QIAprep Spin Miniprep Kit (QIAGEN Inc., Valencia, CA, USA) according to the manufacturer's directions. Plasmids containing the correct size inserts were sequenced to determine integrity and orientation of the insert. Plasmid pDAu81#5 was found to be error free and was therefore chosen for scale-up.

Protoplasts of *Aspergillus oryzae* BECH2 (WO 2000/39322) were prepared as described in WO 95/02043. *A. oryzae* BECh2 was constructed as described in WO 00139322. One hundred microliters of protoplast suspension were mixed with 5-25 µg of the *Aspergillus* expression vector pDAu81#5 in 10 µl of STC composed of 1.2 M sorbitol, 10 mM Tris-HCl, pH 7.5, 10 mM $CaCl_2$ (Christensen *et al.*, 1988, *Bio/Technology* 6: 1419-1422). The mixture was left at room temperature for 25 minutes. Two hundred microliters of 60% PEG 4000 (BDH, Poole, England) (polyethylene glycol, molecular weight 4,000), 10 mM $CaCl_2$, and 10 mM Tris-HCl pH 7.5 were added and gently mixed and finally 0.85 ml of the same solution was added and gently mixed. The mixture was left at room temperature for 25 minutes, centrifuged at 2,500 *x g* for 15 minutes, and the pellet was resuspended in 2 ml of 1.2 M sorbitol. This sedimentation process was repeated, and the protoplasts were spread on COVE plates. After incubation for 4-7 days at 37°C spores were picked and spread on COVE plates containing 0.01% TRITON® X-100 in order to isolate single colonies. The spreading was repeated twice more on COVE sucrose medium (Cove, 1996, *Biochim. Biophys. Acta* 133: 51-56) containing 1 M sucrose and 10 mM sodium nitrate.

Ten of the transformants were inoculated in 10 ml of YPG medium. After 3-4 days of

incubation at 30°C, 200 rpm, the supernatant was removed and analyzed by SDS-PAGE 10% Bis-Tris gels (Invitrogen, Carlsbad, CA, USA) as recommended by the manufacturer. Gels were stained with Coomassie blue and all isolates displayed a diffuse band between 35 and 45 kDa. These transformants were analyzed further for xylanase activity at pH 6.0 using a modified AZCL-arabinoxylan as substrate isolated from wheat (Megazyme, Wicklow, Ireland) in 0.2 M sodium phosphate pH 6.0 buffer containing 0.01% TRITON® X-100 according to the manufacturer's instructions. The transformant producing the highest level of activity was chosen for production of the xylanase.

The transformant producing the highest level of activity was grown using standard methods. The broth was filtered using Whatmann glass filters GF/D, GF/A, GF/C, GF/F (2.7 µm, 1.6 µm, 1.2 µm and 0.7 µm, respectively) (Whatman, Piscataway, NJ, USA) followed by filtration using a NALGENE® bottle top 0.45 µm filter (Thermo Fisher Scientific, Rochester, NY, USA).

Ammonia sulfate was added to the filtered broth to a final concentration of 3 M and the precipitate was collected after centrifugation at 10,000 x *g* for 30 minutes. The precipitate was dissolved in 10 mM Tris/HCl pH 8.0 and dialyzed against 10 mM Tris/HCl pH 8.0 overnight. The dialyzed preparation was applied to a 150 ml Q SEPHAROSE® Fast Flow column equilibrated with 10 mM Tris/HCl pH 8.0 and the enzyme was eluted with a 1050 ml (7 column volumes) linear salt gradient from 0 to 1 M NaCl in 10 mM Tris/HCl pH 8.0. Elution was followed at 280 nm and fractions were collected and assayed for xylanase activity using 0.2% AZCL-Arabinoxylan from wheat in 0.2 M sodium phosphate buffer pH 6.0 containing 0.01% TRITON® X-100. Fractions containing xylanase activity were pooled and stored at -20°C.

Example 26: Preparation of *Penicillium pinophilum* GH10 xylanase

Penicillium pinophilum GH10 xylanase (SEQ ID NO: 51 [DNA sequence] and SEQ ID NO: 52 [deduced amino acid sequence]) was prepared according to the following procedure.

Compost samples were collected from Yunnan, China on December 12, 2000. *Penicillium pinophilum* NN046877 was isolated using single spore isolation techniques on PDA plates at 45°C. *Penicillium pinophilum* strain NN046877 was inoculated onto a PDA plate and incubated for 4 days at 37°C in the darkness. Several mycelia-PDA plugs were inoculated into 500 ml shake flasks containing 100 ml of NNCYP-PCS medium. The flasks were incubated for 5 days at 37°C with shaking at 160 rpm. The mycelia were collected at day 4 and day 5. The mycelia from each day were frozen in liquid nitrogen and stored in a -80°C freezer until use.

The frozen mycelia were transferred into a liquid nitrogen prechilled mortar and pestle and ground to a fine powder. Total RNA was prepared from the powdered mycelia of

each day by extraction with TRIZOL™ reagent. The polyA enriched RNA was isolated using a mTRAP™ Total Kit.

Double stranded cDNA from each day was synthesized with a SMART™ cDNA library Construction Kit (Clontech Laboratories, Inc., Mountain View, CA, USA). The cDNA was cleaved with *Sfi* I and the cDNA was size fractionated by 0.8% agarose gel electrophoresis using TBE buffer. The fraction of cDNA of 500 bp and larger was excised from the gel and purified using a GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions. Then equal amounts of cDNA from day 4 and day 5 were pooled for library construction.

The pooled cDNA was then directionally cloned by ligation into *Sfi* I cleaved pMHas7 (WO 2009/037253) using T4 ligase (New England Biolabs, Inc., Beverly, MA, USA) according to the manufacturer's instructions. The ligation mixture was electroporated into *E. coli* ELECTROMAX™ DH10B™ cells (Invitrogen Corp., Carlsbad, CA, USA) using a GENE PULSER® and Pulse Controller (Bio-Rad Laboratories, Inc., Hercules, CA, USA) at 25 uF, 25 mAmp, 1.8 kV with a 1 mm gap cuvette according to the manufacturer's procedure.

The electroporated cells were plated onto LB plates supplemented with 50 mg of kanamycin per liter. A cDNA plasmid pool was prepared from 60,000 total transformants of the original pMHas7 vector ligation. Plasmid DNA was prepared directly from the pool of colonies using a QIAGEN® Plasmid Kit (QIAGEN Inc., Valencia, CA, USA).

A transposon containing plasmid designated pSigA4 was constructed from the pSigA2 transposon containing plasmid described WO 2001/77315 in order to create an improved version of the signal trapping transposon of pSigA2 with decreased selection background. The pSigA2 transposon contains a signal less beta-lactamase construct encoded on the transposon itself. PCR was used to create a deletion of the intact beta lactamase gene found on the plasmid backbone using a proofreading *Pfu* Turbo polymerase PROOFSTART™ (QIAGEN GmbH Corporation, Hilden, Germany) and the following 5' phosphorylated primers (TAG Copenhagen, Denmark):

SigA2NotU-P:

5'-TCGCGATCCGTTTTCGCATTTATCGTGAAACGCT-3' (SEQ ID NO: 132)

SigA2NotD-P:

5'-CCGCAAACGCTGGTGAAAGTAAAAGATGCTGAA-3' (SEQ ID NO: 133)

The amplification reaction was composed of 1 µl of pSigA2 (10 ng/ µl), 5 µl of 10X PROOFSTART™ Buffer (QIAGEN GmbH Corporation, Hilden, Germany), 2.5 µl of dNTP mix (20 mM), 0.5 µl of SigA2NotU-P (10 mM), 0.5 µl of SigA2NotD-P (10 mM), 10 µl of Q solution (QIAGEN GmbH Corporation, Hilden, Germany), and 31.25 µl of deionized water. A DNA ENGINE™ Thermal Cycler (MJ Research Inc., Waltham, MA, USA) was used for amplification programmed for one cycle at 95°C for 5 minutes; and 20 cycles each at 94°C

for 30 seconds, 62°C for 30 seconds, and 72°C for 4 minutes.

A 3.9 kb PCR reaction product was isolated by 0.8% agarose gel electrophoresis using TAE buffer and 0.1 µg of ethidium bromide per ml. The DNA band was visualized with the aid of an EAGLE EYE® Imaging System (Stratagene, La Jolla, CA, USA) at 360 nm. The 3.9 kb DNA band was excised from the gel and purified using a GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

The 3.9 kb fragment was self-ligated at 16°C overnight with 10 units of T4 DNA ligase (New England Biolabs, Inc., Beverly, MA, USA), 9 µl of the 3.9 kb PCR fragment, and 1 µl of 10X ligation buffer (New England Biolabs, Inc., Beverly, MA, USA). The ligation was heat inactivated for 10 minutes at 65°C and then digested with *Dpn* I at 37°C for 2 hours. After incubation, the digestion was purified using a GFX® PCR DNA and Gel Band Purification Kit.

The purified material was then transformed into *E. coli* TOP10 competent cells according to the manufacturer's instructions. The transformation mixture was plated onto LB plates supplemented with 25 µg of chloramphenicol per ml. Plasmid minipreps were prepared from several transformants and digested with *Bgl* II. One plasmid with the correct construction was chosen. The plasmid was designated pSigA4. Plasmid pSigA4 contains the *Bgl* II flanked transposon SigA2 identical to that disclosed in WO 2001/77315.

A 60 µl sample of plasmid pSigA4 DNA (0.3 µg/µl) was digested with *Bgl* II and separated by 0.8% agarose gel electrophoresis using TBE buffer. A SigA2 transposon DNA band of 2 kb was eluted with 200 µl of EB buffer (QIAGEN GmbH Corporation, Hilden, Germany) and purified using a GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions and eluted in 200 µl of EB buffer. SigA2 was used for transposon assisted signal trapping.

A complete description of transposon assisted signal trapping is described in WO 2001/77315. The plasmid pool was treated with transposon SigA2 and HYPERMU™ transposase (EPICENTRE Biotechnologies, Madison, WI, USA) according to the manufacturer's instructions.

For *in vitro* transposon tagging of the *Penicillium pinophilum* cDNA library, 2 µl of SigA2 transposon containing approximately 100 ng of DNA were mixed with 1 µl of the plasmid DNA pool of the *Penicillium pinophilum* cDNA library containing 1 µg of DNA, 1 µl of HYPERMU™ transposase, and 2 µl of 10X buffer (EPICENTRE Biotechnologies, Madison, WI, USA) in a total volume of 20 µl and incubated at 30°C for 3 hours followed by adding 2 µl of stop buffer (EPICENTRE Biotechnologies, Madison, WI, USA) and heat inactivation at 75°C for 10 minutes. The DNA was precipitated by addition of 2 µl of 3 M sodium acetate pH 5 and 55 µl of 96% ethanol and centrifuged for 30 minutes at 10,000 *x g*, 4°C. The pellet was washed in 70% ethanol, air dried at room temperature, and resuspended in 10 µl of

deionized water.

A 2 µl volume of the transposon tagged plasmid pool was electroporated into 50 µl of *E. coli* ELECTROMAX™ DH10B™ cells (Invitrogen Corp., Carlsbad, CA, USA) according to the manufacturer's instructions using a GENE PULSER® and Pulse Controller at 25 uF, 25 mAmp, 1.8 kV with a 1 mm gap cuvette according to the manufacturer's procedure.

The electroporated cells were incubated in SOC medium with shaking at 225 rpm for 1 hour at 37°C before being plated onto the following selective media: LB medium supplemented with 50 µg of kanamycin per ml; LB medium supplemented with 50 µg of kanamycin per ml and 15 µg of chloramphencol per ml; and LB medium supplemented with 50 µg of kanamycin per ml, 15 µg of chloramphencol per ml, and 30 µg of ampicillin per ml.

From plating of the electroporation onto LB medium supplemented with kanamycin, chloramphencol and ampicillin, approximately 200 colonies per 50 µl were observed after 3 days at 30°C. All colonies were replica plated onto LB kanamycin, chloramphenicol, and ampicillin medium described above. Five hundred colonies were recovered under this selection condition. The DNA from each colony was sequenced with the transposon forward and reverse primers (primers A and B), shown below, according to the procedure disclosed in WO 2001/77315 (page 28).

Primer A:

5'-agcgtttgcggccgcgatcc-3' (SEQ ID NO: 134)

Primer B:

5'-ttattcggtcgaaaaggatcc-3' (SEQ ID NO: 135)

DNA sequences were obtained from SinoGenoMax Co., Ltd (Beijing, China). Primer A and primer B sequence reads for each plasmid were trimmed to remove vector and transposon sequence. The assembled sequences were grouped into contigs by using the program PhredPhrap (Ewing *et al.*, 1998, *Genome Research* 8: 175-185; Ewing and Green, 1998, *Genome Research* 8: 186-194). All contigs were subsequently compared to sequences available in standard public DNA and protein sequences databases (TrEMBL, SWALL, PDB, EnsemblPep, GeneSeqP) using the program BLASTX 2.0a19MP-WashU [14-Jul-1998] [Build linux-x86 18:51:44 30-Jul-1998] (Gish *et al.*, 1993, *Nat. Genet.* 3: 266-72). The family GH10 xylanase candidate was identified directly by analysis of the BlastX results.

Penicillium pinophilum NN046877 was grown on a PDA agar plate at 37°C for 4-5 days. Mycelia were collected directly from the agar plate into a sterilized mortar and frozen under liquid nitrogen. Frozen mycelia were ground, by mortar and pestle, to a fine powder, and genomic DNA was isolated using a DNEASY® Plant Mini Kit (QIAGEN Inc., Valencia, CA, USA).

Based on the *Penicillium pinophilum* GH10 xylanase gene information obtained as

described above, oligonucleotide primers, shown below, were designed to amplify the GH61 gene from genomic DNA of *Penicillium pinophilum* GH10 NN046877. An IN-FUSION® CF Dry-down Cloning Kit (Clontech Laboratories, Inc., Mountain View, CA, USA) was used to clone the fragment directly into the expression vector pPFJO355, without the need for restriction digestion and ligation.

Sense primer:

5'-ACACAACTGGGGATCCACCATGACTCTAGTAAAGGCTATTCTTTTAGC-3' (SEQ ID NO: 136)

Antisense primer:

5'-GTCACCCTCTAGATCTTCACAAACATTGGGAGTAGTATGG-3' (SEQ ID NO: 137)

Bold letters represented the coding sequence and the remaining sequence was homologous to insertion sites of pPFJO355.

Twenty picomoles of each of the primers above were used in a PCR reaction composed of *Penicillium sp.* NN051602 genomic DNA, 10 µl of 5X GC Buffer, 1.5 µl of DMSO, 2.5 mM each of dATP, dTTP, dGTP, and dCTP, and 0.6 unit of PHUSION™ High-Fidelity DNA Polymerase, in a final volume of 50 µl. The amplification was performed using a Peltier Thermal Cycler programmed for denaturing at 98°C for 1 minutes; 5 cycles of denaturing at 98°C for 15 seconds, annealing at 56°C for 30 seconds, with a 1°C increase per cycle and elongation at 72°C for 75 seconds; and 25 cycles each at 98°C for 15 seconds, 65C for 30 seconds and 72°C for 75 seconds; and a final extension at 72°C for 10 minutes. The heat block then went to a 4°C soak cycle.

The reaction products were isolated by 1.0% agarose gel electrophoresis using TBE buffer where an approximately 1.4 kb product band was excised from the gel, and purified using an ILLUSTRA® GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

Plasmid pPFJO355 was digested with *Bam* I and *Bgl* II, isolated by 1.0% agarose gel electrophoresis using TBE buffer, and purified using an ILLUSTRA® GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

The gene fragment and the digested vector were ligated together using an IN-FUSION® CF Dry-down PCR Cloning Kit resulting in pPpin3 in which transcription of the *Penicillium pinophilum* GH10 xylanase gene was under the control of a promoter from the gene for *Aspergillus oryzae* alpha-amylase. In brief, 30 ng of pPFJO355 digested with *Bam* I and *Bgl* II, and 60 ng of the *Penicillium pinophilum* GH10 xylanase gene purified PCR product were added to a reaction vial and resuspended in a final volume of 10 µl with addition of deionized water. The reaction was incubated at 37°C for 15 minutes and then 50°C for 15 minutes. Three µl of the reaction were used to transform *E. coli* TOP10 competent cells. An *E. coli* transformant containing pPpin3 was detected by colony PCR and

plasmid DNA was prepared using a QIAprep Spin Miniprep Kit (QIAGEN Inc., Valencia, CA, USA). The *Penicillium pinophilum* GH10 xylanase gene insert in pPpin3 was confirmed by DNA sequencing using a 3730XL DNA Analyzer.

The same PCR fragment was cloned into vector pGEM-T using a pGEM-T Vector System to generate pGEM-T-Ppin3. The *Penicillium pinophilum* GH10 xylanase gene contained in pGEM-T-Ppin3 was confirmed by DNA sequencing using a 3730XL DNA Analyzer. *E. coli* strain T-Ppin3, containing pGEM-T-Ppin3, was deposited with the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM), D-38124 Braunschweig, Germany on September 7, 2009, and assigned accession number DSM 22922.

DNA sequencing of the *Penicillium pinophilum* genomic clone encoding a GH10 polypeptide having xylanase activity was performed with an Applied Biosystems Model 3700 Automated DNA Sequencer using version 3.1 BIG-DYE™ terminator chemistry (Applied Biosystems, Inc., Foster City, CA, USA) and dGTP chemistry (Applied Biosystems, Inc., Foster City, CA, USA) and primer walking strategy. Nucleotide sequence data were scrutinized for quality and all sequences were compared to each other with assistance of PHRED/PHRAP software (University of Washington, Seattle, WA, USA).

The nucleotide sequence and deduced amino acid sequence of the *Penicillium pinophilum gh10* gene are shown in SEQ ID NO: 51 and SEQ ID NO: 52, respectively. The coding sequence is 1442 bp including the stop codon and is interrupted by three introns of 51 bp (199-249), 73 bp (383-455), and 94 bp (570-663). The encoded predicted protein is 407 amino acids. The%G+C of the coding sequence of the gene (including introns) is 47.99% G+C and the mature polypeptide coding sequence is 49.22%. Using the SignalP program (Nielsen *et al.*, 1997, *Protein Engineering* 10: 1-6), a signal peptide of 19 residues was predicted. The predicted mature protein contains 388 amino acids with a predicted molecular mass of 41.5 kDa and an isoelectric pH of 5.03.

A comparative pairwise global alignment of amino acid sequences was determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, *J. Mol. Biol.* 48: 443-453) as implemented in the Needle program of EMBOSS with gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 matrix. The alignment showed that the deduced amino acid sequence of the *Penicillium pinophilum* gene encoding the GH10 polypeptide having xylanase activity shares 76% and 87% identity (excluding gaps) to the deduced amino acid sequence of a predicted GH10 family protein from *Talaromyces emersonii* (AAU99346) and *Penicillium marneffei* (B6QN64), respectively.

Aspergillus oryzae HowB101 (WO 95/35385 Example 1) protoplasts were prepared according to the method of Christensen *et al.*, 1988, *Bio/Technology* 6: 1419-1422. Three µg of pPpin3 were transformed into *Aspergillus oryzae* HowB101.

The transformation of *Aspergillus oryzae* HowB101 with pPpin3 yielded about 50

transformants. Twelve transformants were isolated to individual Minimal medium plates.

Four transformants were inoculated separately into 3 ml of YPM medium in a 24-well plate and incubated at 30°C with shaking at 150 rpm. After 3 days incubation, 20 µl of supernatant from each culture were analyzed by SDS-PAGE using a NUPAGE® NOVEX® 4-12% Bis-Tris Gel with MES buffer according to the manufacturer's instructions. The resulting gel was stained with INSTANT® Blue (Expedeon Ltd., Babraham Cambridge, UK). SDS-PAGE profiles of the cultures showed that the majority of the transformants had a major band of approximately 55 kDa. The expression strain was designated *Aspergillus oryzae* EXP02765.

A slant of one transformant, designated transformant 2, was washed with 10 ml of YPM and inoculated into a 2 liter flask containing 400 ml of YPM medium to generate broth for characterization of the enzyme. The culture was harvested on day 3 and filtered using a 0.45 µm DURAPORE® Membrane (Millipore, Bedford, MA, USA).

A 1 liter volume of supernatant of the recombinant *Aspergillus oryzae* strain EXP02765 was precipitated with ammonium sulfate (80% saturation) and redissolved in 50 ml of 25 mM sodium acetate pH 4.3, and then dialyzed against the same buffer and filtered through a 0.45 µm filter. The solution was applied to a 40 ml Q SEPHAROSE™ Fast Flow column column equilibrated in 25 mM sodium acetate pH 4.3. The recombinant GH10 protein did not bind to the column. The fractions with xylanase activity were collected and evaluated by SDS-PAGE as described above. Fractions containing a band of approximately 55 kDa were pooled. The pooled solution was concentrated by ultrafiltration.

Example 27: Preparation of *Thielavia terrestris* GH10E xylanase

Thielavia terrestris NRRL 8126 GH10E xylanase (SEQ ID NO: 53 [DNA sequence] and SEQ ID NO: 54 [deduced amino acid sequence]) was prepared according to the following procedure.

Two synthetic oligonucleotide primers shown below were designed to PCR amplify the *Thielavia terrestris gh10e* gene from genomic DNA. An InFusion Cloning Kit (Clontech, Mountain View, CA) was used to clone the fragment directly into the expression vector, pAILo2 (WO 2005/074647).

Forward primer:

5'-ACTGGATTTACC**ATGGCCCTCAAATCGCTCCTGTTG**-3' (SEQ ID NO: 138)

Reverse primer:

5'-TCACCTCTAGTTAATTAA**TTACAAGCACTGAGAGTA**-3' (SEQ ID NO: 139)

Bold letters represent coding sequence. The remaining sequence is homologous to the insertion sites of pAILo2.

Fifty picomoles of each of the primers above were used in a PCR reaction containing

2 µg of *Thielavia terrestris* genomic DNA, 1X *Pfx* Amplification Buffer (Invitrogen, Carlsbad, CA, USA), 2X PCR_x Enhancer solution (Invitrogen, Carlsbad, CA, USA), 1.5 µl of 10 mM blend of dATP, dTTP, dGTP, and dCTP, 2.5 units of PLATINUM® *Pfx* DNA Polymerase, and 1 µl of 50 mM $MgSO_4$ in a final volume of 50 µl. The amplification conditions were one cycle at 94°C for 2 minutes; and 30 cycles each at 94°C for 15 seconds, 59.5°C for 30 seconds, and 68°C for 150 seconds. The heat block was then held at 68°C for 7 minutes followed by a 4°C soak cycle.

The reaction products were isolated on a 1.0% agarose gel using TAE buffer and an approximately 1.2 kb product band was excised from the gel and purified using a MINELUTE® Gel Extraction Kit (QIAGEN Inc., Valencia, CA, USA) according to the manufacturer's instructions.

The fragment was then cloned into pAlLo2 using an IN-FUSION™ Cloning Kit. The vector was digested with *Nco* I and *Pac* I (using conditions specified by the manufacturer). The fragment was purified by gel electrophoresis as above and a QIAQUICK® Gel Extraction Kit. The gene fragment and the digested vector were combined together in a reaction resulting in the expression plasmid pAG29, in which transcription of the *gh10e* gene was under the control of the NA2-tpi promoter (a hybrid of the promoters from the genes for *Aspergillus niger* neutral alpha-amylase and *Aspergillus nidulans* triose phosphate isomerase). The recombination reaction (10 µl) was composed of 1X InFusion Buffer (Clontech, Mountain View, CA, USA), 1X BSA (Clontech, Mountain View, CA, USA), 0.5 µl of InFusion enzyme (diluted 1:10) (Clontech, Mountain View, CA, USA) , 93 ng of pAlLo2 digested with *Nco* I and *Pac* I, and 1 µl of the *Thielavia terrestris gh10e* purified PCR product. The reaction was incubated at 37°C for 15 minutes followed by 15 minutes at 50°C. The reaction was diluted with 40 µl of TE buffer and 2.5 µl of the diluted reaction was used to transform *E. coli* SOLOPACK® Gold cells.

Plasmid DNA was prepared using a BIOROBOT® 9600 and a restriction enzyme digest performed. Putative pAG29 clones were digested with *Pst* I. The plasmid DNA from these clones was then sequenced to identify clones without PCR induced errors. Sequencing reactions contained 1.5 µl of plasmid DNA, 4.5 µl of water, and 4 µl of sequencing master-mix containing 1 µl of 5X sequencing buffer (Millipore, Billerica, MA, USA), 1 µl of BIGDYE™ terminator (Applied Biosystems, Inc., Foster City, CA, USA), 1 µl of water and one of the following primers at 3.2 pmoles per reaction.

pAlLo2 5'

5'-TGTCCCTTGTCGATGCG-3' (SEQ ID NO: 140)

pAlLo2 3'

5'-CACATGACTTGGCTTCC-3' (SEQ ID NO: 141)

Aspergillus oryzae JaL355 protoplasts were prepared according to the method of Christensen *et al.*, 1988, *Bio/Technology* 6: 1419-1422. Five μg of pAG29 was used to transform *Aspergillus oryzae* JaL355. Twenty-four transformants were isolated to individual PDA plates.

Confluent PDA plates of 24 transformants were washed with 5 ml of 0.01% TWEEN® 20 and spores collected. Eight μl of each spore stock was added to 1ml of YPG, YPM, and M410 separately in 24 well plates and incubated at 34°C. Three days after incubation, 7.5 μl of supernatant from selected culture was analyzed using Criterion stain-free, 8-16% gradient SDS-PAGE, (BioRad, Hercules, CA) according to the manufacturer's instructions. SDS-PAGE profiles of the cultures showed that several transformants had a new major band of approximately 50 kDa and the best expression in M410. After a total of six days of incubation, all M410 cultures were sampled as described above and analyzed using Criterion stain-free, 8-16% gradient SDS-PAGE gel, (BioRad, Hercules, CA) at which point the transformant exhibiting the best expression was selected.

A confluent PDA plate of the top transformant was washed with 5 ml of 0.01% TWEEN® 20 and inoculated into five 500 ml Erlenmeyer flask containing 100 ml of M410 medium to generate broth for characterization of the enzyme. The flasks were harvested on day 5. Broths were filtered using a 0.22 μm stericup suction filter (Millipore, Bedford, MA).

Example 28: Preparation of *Thermobifida fusca* GH11 xylanase

A linear integration vector-system was used for the expression cloning of a *Thermobifida fusca* DSM 22883 GH11 xylanase gene (SEQ ID NO: 55 [DNA sequence] and SEQ ID NO: 56 [deduced amino acid sequence]). The linear integration construct was a PCR fusion product made by fusion of each gene between two *Bacillus subtils* homologous chromosomal regions along with a strong promoter and a chloramphenicol resistance marker. The fusion was made by SOE PCR (Horton *et al.*, 1989, *Gene* 77: 61-68). The SOE PCR method is also described in WO 2003/095658. Each gene was expressed under the control of a triple promoter system (as described in WO 99/43835), consisting of the promoters from *Bacillus licheniformis* alpha-amylase gene (*amyL*), Bacillus amyloliquefaciens alpha-amylase gene (*amyQ*), and the *Bacillus thuringiensis cryIIIA* promoter including stabilizing sequence. The gene coding for chloramphenicol acetyl-transferase was used as marker (Diderichsen *et al.*, 1993, *Plasmid* 30: 312). The final gene construct was integrated into the *Bacillus* chromosome by homologous recombination into the pectate lyase locus.

The GH11 xylanase gene (SEQ ID NO: 55 [DNA sequence] and SEQ ID NO: 56 [deduced amino acid sequence]) was isolated from *Thermobifida fusca* DSM 22883 (NN018438) by a polymerase chain reaction (PCR1) using the primers shown in the table

below. The primers are based on the protein sequence UNIPROT:Q5RZ98. *Thermobifida fusca* DSM 22883 was isolated from a soil sample obtained from Oahu, Hawaii in 2001. The xylanase gene was cloned as a full-length gene and as a truncated gene. The genes were designed to contain a C-terminal HQHQHQH tag to ease purification but the His-tag was not used for the purification. The forward primer Ocs3 was designed so the gene was amplified from the start codon (ATG) and has 24 bases overhang (shown in italic in the table below). This overhang is complementary to part of one of the two linear vector fragments and is used when the PCR fragment and the vector fragments are assembled (described below). The reverse primer Ocs1 was designed to amplify the truncated version of the gene while the reverse primer Ocs2 was designed to amplify the full-length gene. Both Ocs1 and Ocs2 carry an overhang consisting of 24 bp encoding a HQHQHQH-tag and a stop codon (the overhang is shown in italic in the table below). This overhang is complementary to part of one of the two linear vector fragments and is used when the PCR fragment and the vector fragments are assembled (described below).

A PCR fragment was isolated containing the full-length xylanase gene and the short 24 bp flanking DNA sequences included in the primers as overhang. Another PCR fragment was isolated containing the truncated xylanase gene and the same short 24 bp flanking DNA sequences.

For each gene construct 3 fragments were PCR amplified: the gene fragment from genomic DNA from the *Thermobifida fusca* (NN018438), the upstream flanking fragment was amplified with primers 260558 and iMB1361Uni1, and the downstream flanking fragment was amplified with primers 260559 and oth432 from genomic DNA of the strain iMB1361 (described in patent application WO 2003095658). All primers used are listed in Table below.

Amplification of	**SPECIFIC PRIMER FORWARD**	**SPECIFIC PRIMER REVERSE**
Full-length gene	OCS3: 5'-***CTGAAAAAAAGGAGAGGAT AAAGA***ATGAACCATGCCCCCG CCA-3' (SEQ ID NO: 142)	OCS2: 5'-***CTAATGCTGGTGTTGGTGC TGATG***GTTGGCGCTGCAGGA CACCGT-3' (SEQ ID NO: 143)
Truncated gene	OCS3: 5'-***CTGAAAAAAAGGAGAGGAT AAAGA***ATGAACCATGCCCCCG CCA 3' (SEQ ID NO: 142)	OCS1: 5'-***CTAATGCTGGTGTTGGTGC TGATG***GGGGTTGTCACCGCC GCT-3' (SEQ ID NO: 144)

Upstream flanking fragment	260558: 5'-GAGTATCGCCAGTAAGGGG CG 3' (SEQ ID NO: 145)	iMB1361Uni1: 5'-TCTTTATCCTCTCCTTTTTTT CAGAGCTC 3' (SEQ ID NO: 146)
Downstream flanking fragment	oth432: 5'-CATCAGCACCAACACCAGCA TCCGTAATCGCATGTTCAATCC GCTCCATA 3' (SEQ ID NO: 147)	260559: 5'-GCAGCCCTAAAATCGCATAA AGC-3' (SEQ ID NO: 148)

The gene fragment was amplified using a proofreading polymerase PHUSION™ DNA Polymerase according to the manufacturer's instructions with the addition of 2% DMSO. The two flanking DNA fragments were amplified with an EXPAND™ High Fidelity PCR System according to the manufacturer's recommendations. The PCR conditions were as follows: one cycle at 94°C for 2 minutes; 10 cycles each at 94°C for 15 seconds, 50°C for 45 seconds, 68°C for 4 minutes; 20 cycles each at 94°C for 15 seconds, 50°C for 45 seconds, and 68°C for 4 minutes (+20 seconds extension pr cycle); and one cycle at 68°C for 10 minutes The 3 PCR fragments were subjected to a subsequent splicing by overlap extension (SOE) PCR reaction to assemble the 3 fragments into one linear vector construct. This was performed by mixing the 3 fragments in equal molar ratios and a new PCR reaction was run under the following conditions: one cycle at 94°C for 2 minutes; 10 cycles each at 94°C for 15 seconds, 50°C for 45 seconds, and 68°C for 5 minutes; 10 cycles each at 94°C for 15 seconds, 50°C for 45 seconds, and 68°C for 8 minutes; 15 cycles each at 94°C for 15 seconds, 50°C for 45 seconds, and 68°C for 8 minutes (in addition 20 seconds extra per cycle). After the first cycle the two end primers 260558 and 260559 were added (20 pMol of each). Two µl of the PCR product was transformed into *Bacillus subtilis.* Transformants were selected on LB plates supplemented with 6 µg of chloramphenicol per ml. The full-length xylanase coding sequence was integrated by homologous recombination into the genome of the *Bacillus subtilis* host TH1 (*amy*-, spo-, *apr*-, *npr*-; WO 2005/038024). The truncated xylanase coding sequence was integrated by homologous recombination into the genome of the *Bacillus subtilis* host PL2317 (*amy*-, spo-, *apr*-, *npr*-, *xyl*-).

Transformants were then screened for their ability to produce large amounts of active xylanase. The screening was based on intensity of the band corresponding to the heterologous expressed protein by SDS-PAGE analysis and activity of the enzyme on LB agar plates containing AZCL-xylan (Megazyme International Ireland, Ltd., Wicklow, Ireland).

One transformant, *Bacillus subtilis* EXP01687, was isolated which contained the full-length xylanase gene expressed in the host strain *Bacillus subtilis* TH1. The full-length xylanase gene sequence of *Bacillus subtilis* EXP01687 was confirmed by Sanger

sequencing. The protein sequence differs by 2 amino acids from UNIPROT:Q5RZ98.

Another transformant, *Bacillus subtilis* EXP01672, was isolated which contains the truncated xylanase gene expressed in the host strain *Bacillus subtilis* PL2317. The truncated xylanase gene sequence of *Bacillus subtilis* EXP01672 was confirmed by Sanger sequencing to encode the secretion signal and mature amino acid sequence to proline at position 236 of SEQ ID NO: 56. The protein sequence differs by 2 amino acids from UNIPROT:Q5RZ98.

Bacillus subtilis EXP01687 was grown in 500 ml baffled Erlenmeyer flasks containing Cal-18 medium supplemented with 34 mg of chloramphenicol per liter for 2 days at 37°C with shaking at 200 rpm. The enzyme was purified from the culture supernatant according to the protocol described below.

In step 1, the whole culture (800 ml) was centrifuged at 17,600 x *g* for 30 minutes and then filtered through a SEITZ-EKS filter (Pall Seitzschenk Filtersystems GmbH, Bad Kreuznach, Germany). Sodium chloride was added to the filtered sample to 50 mM NaCl and the pH was adjusted to pH 7.5. The sample (750 ml) was applied to a 20 ml Ni SEPHAROSE® 6 Fast Flow column (GE Healthcare, Piscataway, NJ, USA) equilibrated with 50 mM Na_2HPO_4, 50 mM NaCl pH 7.5, and the bound protein was eluted with a 5 column volume gradient to 100% 50 mM Na_2HPO_4, 500 mM imidazol pH 7.5. The enzyme was found in the effluent by SDS-PAGE analysis revealing a protein of the correct size in the effluent and nothing of the correct size in the eluent. This result was confirmed by activity measurement using AZCL-arabinoxylan (wheat) as substrate as described above.

In step 2, ammonium sulfate was added to the effluent (of step 1) to 1 M and the pH adjusted to 7.5. The sample (800 ml) was applied to a 60 ml TOYOPEARL® Phenyl-650M column (TOSOH Corporation, Tokyo, Japan) equilibrated with 1 M ammonium sulfate pH 7.5. The bound protein was eluted with Milli-Q® ultrapure water (Millipore, Billerica, MA, USA). Fractions with A_{280} were pooled (55 ml). The pooled fractions were desalted and buffer-exchanged with 25 mM acetic acid pH 4.5 using a HIPREP® 26/10 desalting column according to the manufacturer's instructions.

In step 3, the buffer exchanged sample of step 2 (110 ml) was diluted 2.5-fold with Milli-Q® ultrapure water and applied to a 10 ml SP SEPHAROSE® Fast Flow column (GE Healthcare, Piscataway, NJ, USA) equilibrated with 25 mM acetic acid pH 4.5. The bound protein was eluted with a five column volume gradient from 0 to 500 mM sodium chloride in 25 mM acetic acid pH 4.5. Based on SDS-PAGE analysis and A_{280} and A_{260} fractions were pooled (10 ml).

In step 4, the pooled fractions of step 3 were diluted 25-fold with Milli-Q® ultrapure water and the pH was adjusted to pH 6.0 and applied to 10 ml SP SEPHAROSE® Fast Flow column equilibrated with 5 mM succinic acid pH 6.0. The bound protein was eluted with a ten

column volume gradient from 0 to 500 mM sodium chloride in 5 mM succinic acid pH 6.0. Based on SDS-PAGE analysis and A_{280} and A_{260} fractions were pooled (12 ml). Separate portions of the pooled fractions were then subjected to three different steps (steps 5(a), 5(b), and 5(c)) described below.

In step 5(a), 6 ml of the pooled fractions of step 4 were diluted to 25 ml with Milli-Q® ultrapure water and the pH was adjusted to pH 9.0 and applied to a 1 ml RESOURCE™ Q column (GE Healthcare, Piscataway, NJ, USA) equilibrated with 25 mM boric acid pH 9.0. The bound protein was eluted with a ten column volume gradient from 0 to 500 mM sodium chloride in 25 mM boric acid pH 9.0. Based on A_{280} and A_{260}, the protein was in the effluent (35 ml). This sample was concentrated using an AMICON® ultrafiltration cell equipped with a 5 kDa cut-off membrane (Millipore, Billerica, MA, USA).

In step 5(b), 3 ml of the pool of step 4 were diluted to 30 ml with Milli-Q® ultrapure water and pH was adjusted to pH 9.6 and applied to a 1 ml RESOURCE™ Q column equilibrated with 12.5 mM boric acid pH 9.7. The bound protein was eluted with a ten column volume gradient from 0 to 500 mM sodium chloride in 12.5 mM boric acid pH 9.7. Based on A_{280} and A_{260} and SDS-PAGE analysis, the protein was in the effluent (36 ml).

In step 5(c), one quarter of the pool of step 4 was diluted with Milli-Q® ultrapure water and the pH was adjusted to pH 8.0 and applied to a 1 ml RESOURCE™ Q column equilibrated with 25 mM borate pH 8.0. The bound protein was eluted with a ten column volume gradient from 0 to 500 mM sodium chloride in 25 mM borate pH 8.0. Based on A_{280} and A_{260} and SDS-PAGE analysis, the protein was in the effluent (25 ml).

In step 6, the sample from step 5(a), the effluent from step 5(b), and the effluent from step 5(c) were pooled and diluted with 25 mM acetic acid pH 4.5, and the pH was adjusted to pH 4.58. The pooled sample (100 ml) was applied to a 1 ml HITRAP® SP Fast Flow column (GE Healthcare, Piscataway, NJ, USA) equilibrated with 25 mM acetic acid pH 4.5. The bound protein was eluted with one column volume gradient from 0 to 500 mM sodium chloride in 25 mM acetic acid pH 4.5. Based on SDS-PAGE analysis and A_{280} and A_{260} fractions were pooled (3.5 ml). The MW of the purified xylanase was 20-25 kDa based on SDS-PAGE analysis.

Example 29: Preparation of *Trichoderma reesei* RutC30 GH3 beta-xylosidase

A *Trichoderma reesei* RutC30 beta-xylosidase gene (SEQ ID NO: 57 [DNA sequence] and SEQ ID NO: 58 [deduced amino acid sequence]) was isolated by screening a Lambda ZAP®-CMR XR Library prepared from *T. reesei* RutC30 genomic DNA using a Lambda ZAP®-CMR XR Library Construction Kit (Stratagene, La Jolla, CA, USA) according to the manufacturer's instructions. *T. reesei* RutC30 genomic DNA was prepared using standard methods. A DNA segment encoding 2300 bp of the *T. reesei* beta-xylosidase was

amplified using the PCR primers shown below.

Forward Primer:

5'-gtgaataacgcagctcttctcg-3' (SEQ ID NO: 149)

Reverse Primer:

5'-ccttaattaattatgcgtcaggtgt-3' (SEQ ID NO: 150)

Primer 994768 was designed to amplify from the first base after the beta-xylosidase start site and primer 994769 was designed with a *Pac* I site at the 5' end.

Fifty picomoles of each of the primers above were used in a PCR reaction consisting of 50 ng of plasmid DNA from the lamda zap library, 1 μl of 10 mM blend of dATP, dTTP, dGTP, and dCTP, 5 μl of 10X PLATINUM® *Pfx* DNA Polymerase Buffer, and 1 unit of PLATINUM® *Pfx* DNA Polymerase, in a final volume of 50 μl. An EPPENDORF® MASTERCYCLER® 5333 was used to amplify the DNA fragment programmed for one cycle at 95°C for 3 minutes; and 30 cycles each at 94°C for 45 seconds, 55°C for 60 seconds, and 72°C for 1 minute 30 seconds. After the 30 cycles, the reaction was incubated at 72°C for 10 minutes and then cooled to 4°C until further processing.

A 2.3 kb PCR product was purified by 1% agarose gel electrophoresis using TAE buffer, excised from the gel, and purified using a QIAQUICK® Gel Extraction Kit. The 2.3 kb PCR product was then digested with *Pac* I to facilitate insertion into pAlLo1 (WO 2004/099228).

The pAlLo1 vector was digested with *Nco* I and then filled in using T4 polymerase (Roche, Nutley, NJ, USA) according to manufacturer's instructions. A second enzyme, *Pac* I, was then used to digest the 5' end of pAlLo1 and the reaction was purified by agarose gel electrophoresis as described above to isolate a 6.9 kb vector fragment.

The 2.3 kb beta-xylosidase fragment was then ligated into the 6.9 kb vector fragment and transformed into *E. coli* XL1-Blue Subcloning Competent Cells (Invitrogen, Carlsbad, CA, USA) according to manufacturer's instructions. Transformants were screened using restriction digest analysis in order to identify those with the correct insert. A new expression vector, pSaMe04, was confirmed by sequencing using an ABI3700 (Applied Biosystems, Foster City, CA) and dye terminator chemistry (Giesecke *et al.*, 1992, *Journal of Virology Methods* 38: 47-60).

Two synthetic oligonucleotide primers shown below were designed to PCR amplify the *Trichoderma reesei* beta-xylosidase gene from pSaMe04 to construct a *Trichoderma* expression vector. An IN-FUSION™ Cloning Kit was used to clone the fragment directly into the expression vector pMJ09 (WO 2005/056772), without the need for restriction digestion and ligation.

TrBXYL-F (ID 064491):

5'-CGGACTGCGCACC**ATGGTGAATAACGCAGCTCT**-3' (SEQ ID NO: 151)

TrBXYL-R (ID 064492):

5'-TCGCCACGGAGCTTA**TTATGCGTCAGGTGTAGCAT**-3' (SEQ ID NO: 152)

Bold letters represent coding sequence. The remaining sequence is homologous to the insertion sites of pMJ09.

Fifty picomoles of each of the primers above were used in a PCR reaction composed of 50 ng of pSaMe04, 1 µl of 10 mM blend of dATP, dTTP, dGTP, and dCTP, 5 µl of 10X ACCUTAQ™ DNA Polymerase Buffer (Sigma-Aldrich, St. Louis, MO, USA), and 5 units of ACCUTAQ™ DNA Polymerase (Sigma-Aldrich, St. Louis, MO, USA), in a final volume of 50 µl. An EPPENDORF® MASTERCYCLER® 5333 was used to amplify the DNA fragment programmed for one cycle at 95°C for 3 minutes; and 30 cycles each at 94°C for 45 seconds, 55°C for 60 seconds, and 72°C for 1 minute 30 seconds. After the 30 cycles, the reaction was incubated at 72°C for 10 minutes and then cooled to 4°C until further processing.

The reaction products were isolated by 1.0% agarose gel electrophoresis using TAE buffer where a 1.2 kb product band was excised from the gel and purified using a QIAQUICK® Gel Extraction Kit according to the manufacturer's instructions.

The 1.2 kb fragment was then cloned into pMJ09 using an IN-FUSION™ Cloning Kit. The vector was digested with *Nco* I and *Pac* I and purified by agarose gel electrophoresis as described above. The gene fragment and the digested vector were ligated together in a reaction resulting in the expression plasmid pSaMe-TrBXYL in which transcription of the beta-xylosidase gene was under the control of the *T. reesei cbh1* gene promoter. The ligation reaction (50 µl) was composed of 1X IN-FUSION™ Buffer, 1X BSA, 1 µl of IN-FUSION™ enzyme (diluted 1:10), 100 ng of pMJ09 digested with *Nco* I and *Pac* I, and 100 ng of the *Trichoderma reesei* beta-xylosidase purified PCR product. The reaction was incubated at room temperature for 30 minutes. One µl of the reaction was used to transform *E. coli* XL10 SOLOPACK® Gold cells. An *E. coli* transformant containing pSaMe-TrBXYL was detected by restriction enzyme digestion and plasmid DNA was prepared using a BIOROBOT® 9600. DNA sequencing of the *Trichoderma reesei* beta-xylosidase gene from pSaMe-TrBXYL was performed using dye-terminator chemistry (Giesecke *et al.*, 1992, *supra*) and primer walking strategy.

Plasmid pSaMe-AaXYL was constructed to comprise the *Trichoderma reesei* cellobiohydrolase I gene promoter and terminator and the *Aspergillus aculeatus* GH10 xylanase coding sequence.

Cloning of the *Aspergillus aculeatus* xylanase followed the overall expression cloning protocol as outlined in H. Dalbøge *et al.*, 1994, *Mol. Gen. Genet.* 243: 253-260.

RNA was isolated from *Aspergillus aculeatus* CBS 101.43 mycelium. Poly(A)$^+$ RNA was isolated from total RNA by chromatography on oligo(dT)-cellulose. Double-stranded cDNA was synthesized as described by Maniatis *et al.* (Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, 1982). After synthesis the cDNA was treated with mung bean nuclease, blunt-ended with T4 DNA polymerase, and ligated to non-palindromic *Bst* XI adaptors (Invitrogen, Carlsbad, CA, USA). The cDNA was size fractionated by 1% agarose gel electrophoresis using TAE buffer where fragments ranging from 600 bp to 4000 bp were used in the library construction. The DNA was ligated into *Bst* XI-digested pYES 2.0 between the GAL1 promoter and the iso-1-cytochrome c terminator and transformed into *Escherichia coli* MC1061 cells (Stratagene, La Jolla, CA, USA. The library was plated onto LB plates and incubated overnight at 37°C. The colonies were scraped from the plates and resuspended in LB medium supplemented with 100 µg of ampicillin per ml. Plasmid DNA was isolated using a Plasmid Midi Kit (QIAGEN Inc., Valenicia, CA, USA). The purified plasmid DNA was pooled.

The purified plasmid DNA mixture was transformed into *Saccharomyces cerevisiae* W3124 cells (MATa; ura 3-52; leu 2-3, 112; his 3-D200; pep 4-1137; prc1::HIS3; prb1:: LEU2; cir+; van den Hazel *et al.*, 1992, *Eur. J. Biochem.* 207: 277-283). Cultivation, transformation and media were as described by Guthrie *et al.*, 1991, *Meth. Enzymol.* Vol 194, Academic Press. The transformed cells were plated onto synthetic complete agar containing 2% glucose for 3 days at 30°C. After 3 days the colonies were replica plated to SC medium with 2% galactose and incubated for 4 days at 30°C. Xylanase expressing colonies were identified by 1% agarose overlay with 0.1% AZCL-Birch-Xylan at pH 4.5 (Dalbøge, 2006, *FEMS Microbiology Reviews* 21: 29-42). Colonies expressing xylanase activity were surrounded by a blue zone. Plasmid DNA, rescued from the positive colonies, contained a DNA insert of approximately 1.3 kb. Sequencing of the isolated gene fragment revealed a 1218 bp open reading frame encoding a polypeptide with a theoretical molecular weight of 43.0 kDa. The cDNA fragment was subcloned into the *Aspergillus* expression vector pHD464 (Dalbøge and Heldt-Hansen, 1994, *Mol. Gen. Genet.* 243, 253–260) digested with *Bam* HI and *Xho* I by cutting the clone with *Bam* HI and *Xho* I and isolating the 1.2 kb cDNA insert (Christgau *et al.*, 1996, *Biochem. J.* 319: 705-712) to generate plasmid pA2X2.

The *Aspergillus aculeatus* GH10 xylanase coding sequence was PCR amplified using plasmid pA2x2 as template and primers 153505 and 153506 shown below using standard methods to yield an approximately 1.2 kb fragment. The 1.2 kb fragment was digested with *Bam* HI and *Xho* I (introduced in the PCR primers) and cloned into vector pCaHj527 (WO 2004/099228). The resulting plasmid was designated pMT2155 in which the cDNA was under transcriptional control of the neutral amylase II (NA2) promoter from *A. niger* and the AMG terminator from *A. niger*.

Primer 153505:

5'-TCTTGGATCCACCATGGTCGGACTGCTTTCAATCACC-3' (SEQ ID NO: 153)

Primer 153506:

5'-TTAACTCGAGTCACAGACACTGCGAGTAATAGTC-3' (SEQ ID NO: 154)

Two synthetic oligonucleotide primers shown below were designed to PCR amplify the *Aspergillus aculeatus* GH10 gene from plasmid pMT2155 and introduce flanking regions for insertion into expression vector pMJ09 (WO 2005/056772). Bold letters represent coding sequence and the remaining sequence is homologous to the insertion sites of pMJ09.

Forward Primer:

5'-cggactgcgcacc**atggtcggactgctttcaat**-3' (SEQ ID NO: 155)

Reverse Primer:

5'-tcgccacggagctta**tcacagacactgcgagtaat**-3' (SEQ ID NO: 156)

Fifty picomoles of each of the primers above were used in a PCR reaction consisting of 50 ng of pMT2155, 1 μl of 10 mM blend of dATP, dTTP, dGTP, and dCTP, 5 μl of 10X ACCUTAQ™ DNA Polymerase Buffer, and 5 units of ACCUTAQ™ DNA Polymerase, in a final volume of 50 μl. An EPPENDORF® MASTERCYCLER® 5333 was used to amplify the DNA fragment programmed for one cycle at 95°C for 3 minutes; and 30 cycles each at 94°C for 45 seconds, 55°C for 60 seconds, and 72°C for 1 minute 30 seconds. After the 30 cycles, the reaction was incubated at 72°C for 10 minutes and then cooled to 4°C until further processing.

The reaction products were isolated on a 1.0% agarose gel using TAE buffer where a 1.2 kb product band was excised from the gel and purified using a QIAquick Gel Extraction Kit according to the manufacturer's instructions.

The fragment was then cloned into pMJ09 using an IN-FUSION™ Cloning Kit. The vector was digested with *Nco* I and *Pac* I and purified by agarose gel electrophoresis as described above. The 1.2 kb gene fragment and the digested vector were ligated together in a reaction resulting in the expression plasmid pSaMe-AaXYL in which transcription of the Family GH10 gene was under the control of the *T. reesei* cbh1 promoter. The ligation reaction (50 ul) was composed of 1X IN-FUSION™ Buffer, 1X BSA, 1 μl of IN-FUSION™ enzyme (diluted 1:10), 100 ng of pAlLo2 digested with *Nco* I and *Pac* I, and 100 ng of the *Aspergillus aculeatus* GH10 xylanase purified PCR product. The reaction was incubated at room temperature for 30 minutes. One μl of the reaction was used to transform *E. coli* XL10 SOLOPACK® Gold cells according to the manufacturer. An *E. coli* transformant containing pSaMe-AaGH10 was detected by restriction enzyme digestion and plasmid DNA was prepared using a BIOROBOT® 9600. DNA sequencing of the *Aspergillus aculeatus* GH10 gene from pSaMe-AaXYL was performed using dye-terminator chemistry (Giesecke *et al.*,

1992, *supra*) and primer walking strategy.

Plasmids pSaMe-AaXYL encoding the *Aspergillus aculeatus* GH10 endoglucanase and pSaMe-TrBXYL encoding the *Trichoderma reesei* beta-xylosidase were co-transformed into *Trichoderma reesei* RutC30 by PEG-mediated transformation (Penttila *et al.*, 1987, *Gene* 61 155-164) to generate *T. reesei* strain SaMe-BXX13. Each plasmid contained the *Aspergillus nidulans amdS* gene to enable transformants to grow on acetamide as the sole nitrogen source.

Trichoderma reesei RutC30 was cultivated at 27°C and 90 rpm in 25 ml of YP medium supplemented with 2% (w/v) glucose and 10 mM uridine for 17 hours. Mycelia were collected by filtration using a Vacuum Driven Disposable Filtration System (Millipore, Bedford, MA, USA) and washed twice with deionized water and twice with 1.2 M sorbitol. Protoplasts were generated by suspending the washed mycelia in 20 ml of 1.2 M sorbitol containing 15 mg of GLUCANEX™ (Novozymes A/S, Bagsvaerd, Denmark) per ml and 0.36 units of chitinase (Sigma Chemical Co., St. Louis, MO, USA) per ml and incubating for 15-25 minutes at 34°C with gentle shaking at 90 rpm. Protoplasts were collected by centrifuging for 7 minutes at 400 *x g* and washed twice with cold 1.2 M sorbitol. The protoplasts were counted using a haemacytometer and re-suspended in STC to a final concentration of 1 X 10^8 protoplasts per ml. Excess protoplasts were stored in a Cryo 1°C Freezing Container (Nalgene, Rochester, NY, USA) at -80°C.

Approximately 4 μg of plasmids pSaMe-AaXYL and pSaMe-TRBXYL were digested with *Pme* I and added to 100 μl of protoplast solution and mixed gently, followed by 250 μl of 10 mM $CaCl_2$-10 mM Tris-HCl pH 7.5-60% PEG 4000, mixed, and incubated at room temperature for 30 minutes. STC (3 ml) was then added and mixed and the transformation solution was plated onto COVE plates using *Aspergillus nidulans amdS* selection. The plates were incubated at 28°C for 5-7 days. Transformants were sub-cultured onto COVE2 plates and grown at 28°C.

Over 40 transformants were subcultured onto fresh plates containing acetamide and allowed to sporulate for 7 days at 28°C.

The *Trichoderma reesei* transformants were cultivated in 125 ml baffled shake flasks containing 25 ml of cellulase-inducing medium at pH 6.0 by inoculating spores of the transformants and incubating at 28°C and 200 rpm for 7 days. *Trichoderma reesei* RutC30 was run as a control. Culture broth samples were removed at day 5. One ml of each culture broth was centrifuged at 15,700 *x g* for 5 minutes in a micro-centrifuge and the supernatants transferred to new tubes.

SDS-PAGE was performed using CRITERION® Tris-HCl (5% resolving) gels (Bio-Rad Laboratories, Inc.) with a CRITERION® System. Five μl of day 7 supernatants (see

above) were suspended in 2X concentration of Laemmli Sample Buffer (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and boiled in the presence of 5% beta-mercaptoethanol for 3 minutes. The supernatant samples were loaded onto a polyacrylamide gel and subjected to electrophoresis with 1X Tris/Glycine/SDS as running buffer (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The resulting gel was stained with BIO-SAFE™ Coomassie Stain. The transformant showing the highest expression of both the *A. aculeatus* GH10 xylanase and the *T. reesei* beta-xylosidase based on the protein gel was designated *T. reesei* SaMe-BXX13.

Trichoderma reesei SaMe-BXX13 was cultivated in 500 ml baffled shake flasks containing 250 ml of cellulase-inducing medium at pH 6.0 inoculated with spores of *T. reesei* SaMe-BXX13. Shake flasks were incubated at 28°C at 200 rpm for five days. The culture broth was then filtered using an 0.22 µm EXPRESS™ Plus Membrane.

The filtered broth was concentrated and buffer exchanged using a tangential flow concentrator equipped with a 10 kDa polyethersulfone membrane to pH 4.0 with acetic acid. Sample was loaded onto a SP SEPHAROSE® column equilibrated in 50 mM sodium acetate pH 4.0, eluting bound proteins with a gradient of 0-1000 mM sodium chloride. Fractions were buffer exchanged into 20 mM sodium phosphate pH 7.0 using a tangential flow concentrator and applied to a Phenyl SUPEROSE™ column (HR 16/10) equilibrated with 1.5 M $(NH_4)_2SO_4$-20 mM sodium phosphate pH 7.0. Bound proteins were eluted with a linear gradient over 20 column volumes from 1.5 to 0 M $(NH_4)_2SO_4$ in 20 mM Tris-HCl pH 7.0. The protein fractions were buffer exchanged into 20 mM TEA HCl pH 7.5 using a tangential flow concentrator. Sample was applied to a MonoQ® column, equilibrated in 20 mM TEA HCl pH 7.5, eluting bound proteins with a gradient from 0-300 mM sodium chloride. Buffer of final protein fractions was 20 mM TEA-100 mM sodium chloride pH 7.5. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 30: Preparation of *Talaromyces emersonii* CBS 393.64 GH3 beta-xylosidase

Talaromyces emersonii CBS 393.64 (NN005049) beta-xylosidase (SEQ ID NO: 59 [DNA sequence] and SEQ ID NO: 60 [deduced amino acid sequence]) was prepared recombinantly according to Rasmussen *et al.*, 2006, *Biotechnology and Bioengineering* 94: 869-876 using *Aspergillus oryzae* JaL355 as a host (WO 2003/070956).

The *Talaromyces emersonii* beta-xylosidase was purified according to Rasmussen *et al.*, 2006, *supra*.

Example 31: Preparation of *Trichoderma reesei* RutC30 Cel7B endoglucanase I

Trichoderma reesei RutC30 Cel7B endoglucanase I (EGI) (SEQ ID NO: 61 [DNA sequence] and SEQ ID NO: 62 [deduced amino acid sequence]) was prepared recombinantly according to WO 2005/067531 using *Aspergillus oryzae* JaL250 as a host.

The harvested broth was centrifuged in 500 ml bottles at 13,000 x *g* for 20 minutes at 4°C and then sterile filtered using a 0.22 µm polyethersulfone membrane (Millipore, Bedford, MA, USA). The filtered broth was concentrated and buffer exchanged with 20 mM Tris-HCl pH 8.5 using a tangential flow concentrator equipped with a 10 kDa polyethersulfone membrane. The sample was loaded onto a Q SEPHAROSE® High Performance column equilibrated with 20 mM Tris-HCl pH 8.5, and step eluted with equilibration buffer containing 600 mM NaCl. Flow-through and eluate fractions were analyzed by SGS-PAGE gel analysis using a CRITERION™ stain-free imaging system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The eluate fractions containing *Trichoderma reesei* Cel7B EGI were pooled, concentrated and buffer exchanged into 20 mM Tris-HCl pH 8.5. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 32: Preparation of *Trichoderma reesei* RutC30 Cel7A cellobiohydrolase I

Trichoderma reesei RutC30 Cel7A cellobiohydrolase I (CBHI) (SEQ ID NO: 63 [DNA sequence] and SEQ ID NO: 64 [deduced amino acid sequence]) was prepared as described by Ding and Xu, 2004, "Productive cellulase adsorption on cellulose" in Lignocellulose Biodegradation (Saha, B. C. ed.), Symposium Series 889, pp. 154–169, American Chemical Society, Washington, DC. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 33: Preparation of *Trichoderma reesei* RutC30 Cel6A cellobiohydrolase II

The *Trichoderma reesei* RutC30 Cel6A cellobiohydrolase II gene (SEQ ID NO: 65 [DNA sequence] and SEQ ID NO: 66 [deduced amino acid sequence]) was isolated from *Trichoderma reesei* RutC30 as described in WO 2005/056772.

The *Trichoderma reesei* Cel6A cellobiohydrolase II gene was expressed in *Fusarium venenatum* using pEJG61 as an expression vector according to the procedures described in U.S. Published Application No. 20060156437. Fermentation was performed as described in U.S. Published Application No. 20060156437.

Filtered broth was desalted and buffer-exchanged into 20 mM sodium acetate-150 mM NaCl pH 5.0 using a HIPREP® 26/10 Desalting Column according to the manufacturer's instructions. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 34: Pretreated corn stover hydrolysis assay

Corn stover was pretreated at the U.S. Department of Energy National Renewable Energy Laboratory (NREL) using 1.4 wt% sulfuric acid at 165°C and 107 psi for 8 minutes. The water-insoluble solids in the pretreated corn stover (PCS) contained 56.5% cellulose, 4.6% hemicellulose and 28.4% lignin. Cellulose and hemicellulose were determined by a two–stage sulfuric acid hydrolysis with subsequent analysis of sugars by high performance liquid chromatography using NREL Standard Analytical Procedure #002. Lignin was determined gravimetrically after hydrolyzing the cellulose and hemicellulose fractions with sulfuric acid using NREL Standard Analytical Procedure #003.

Unmilled, unwashed PCS (whole slurry PCS) was prepared by adjusting the pH of PCS to 5.0 by addition of 10 M NaOH with extensive mixing, and then autoclaving for 20 minutes at 120°C. The dry weight of the whole slurry PCS was 29%. The PCS was used unwashed or washed with water. Milled unwashed PCS (dry weight 32.35%) was prepared by milling whole slurry PCS in a Cosmos ICMG 40 wet multi-utility grinder (EssEmm Corporation, Tamil Nadu, India). Milled washed PCS (dry weight 32.35%) was prepared in the same manner, with subsequent washing with deionized water and decanting off the supernatant fraction repeatedly.

The hydrolysis of PCS was conducted using 2.2 ml deep-well plates (Axygen, Union City, CA, USA) in a total reaction volume of 1.0 ml. The hydrolysis was performed with 50 mg of PCS (insoluble solids in case of unwashed PCS and total solids in case of washed PCS) per ml of 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate and various protein loadings of various enzyme compositions (expressed as mg protein per gram of cellulose). Enzyme compositions were prepared and then added simultaneously to all wells in a volume ranging from 50 µl to 200 µl, for a final volume of 1 ml in each reaction. The plate was then sealed using an ALPS-300™ plate heat sealer (Abgene, Epsom, United Kingdom), mixed thoroughly, and incubated at a specific temperature for 72 hours. All experiments reported were performed in triplicate.

Following hydrolysis, samples were filtered using a 0.45 µm MULTISCREEN® 96-well filter plate (Millipore, Bedford, MA, USA) and filtrates analyzed for sugar content as described below. When not used immediately, filtered aliquots were frozen at -20°C. The sugar concentrations of samples diluted in 0.005 M H_2SO_4 were measured using a 4.6 x 250 mm AMINEX® HPX-87H column (Bio-Rad Laboratories, Inc., Hercules, CA, USA) by elution with 0.05% w/w benzoic acid-0.005 M H_2SO_4 at 65°C at a flow rate of 0.6 ml per minute, and quantitation by integration of the glucose, cellobiose, and xylose signals from refractive index detection (CHEMSTATION®, AGILENT® 1100 HPLC, Agilent Technologies, Santa Clara, CA, USA) calibrated by pure sugar samples. The resultant glucose and cellobiose equivalents were used to calculate the percentage of cellulose conversion for each reaction.

Glucose, cellobiose, and xylose were measured individually. Measured sugar concentrations were adjusted for the appropriate dilution factor. In case of unwashed PCS, the net concentrations of enzymatically-produced sugars were determined by adjusting the measured sugar concentrations for corresponding background sugar concentrations in unwashed PCS at zero time point. All HPLC data processing was performed using MICROSOFT EXCEL™ software (Microsoft, Richland, WA, USA).

The degree of cellulose conversion to glucose was calculated using the following equation:% conversion = glucose concentration / glucose concentration in a limit digest. To calculate total conversion the glucose and cellobiose values were combined. Cellobiose concentration was multiplied by 1.053 in order to convert to glucose equivalents and added to the glucose concentration. The degree of total cellulose conversion was calculated using the following equation:

% conversion = [glucose concentration + 1.053 x (cellobiose concentration)] / [(glucose concentration + 1.053 x (cellobiose concentration) in a limit digest].

The 1.053 factor for cellobiose takes into account the increase in mass when cellobiose is converted to glucose. In order to calculate% conversion, a 100% conversion point was set based on a cellulase control (50-100 mg of *Trichoderma reesei* cellulase per gram cellulose), and all values were divided by this number and then multiplied by 100. Triplicate data points were averaged and standard deviation was calculated.

Example 35: Evaluation of several cellulolytic proteins replacing CBHI, CBHII, and EGII components in a reconstituted *Trichoderma reesei*-based enzyme composition for improved performance at 50ºC, 55ºC, and 60ºC

Several cellulolytic proteins were tested in various combinations at 50°C, 55°C, and 60°C against a reconstituted *Trichoderma reesei*-based enzyme composition that included four major *Trichoderma reesei* cellulases (45% *Trichoderma reesei* Cel7A CBHI, 25% *Trichoderma reesei* Cel6A CBHII, 5% *Trichoderma reesei* Cel7B EGI, 5% *Trichoderma reesei* Cel5A EGII), a beta-glucosidase (10% *Aspergillus fumigatus* Cel3A beta-glucosidase), and a Family 61 polypeptide having cellulolytic enhancing activity (10% *Thermoascus aurantiacus* GH61A polypeptide).

The evaluated enzymes included *Chaetomium thermophilum* Cel7A CBHI, *Myceliophthora thermophila* Cel7A CBHI, *Myceliophthora thermophila* Cel6A CBHII, and *Myceliophthora thermophila* Cel5A EGII. All enzyme compositions contained 45% CBHI, 25% CBHII, 5% *Trichoderma reesei* Cel7B EGI, 5% EGII, 10% *Aspergillus fumigatus* beta-glucosidase, and 10% *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity. All enzyme compositions, including the *Trichoderma reesei*-based composition, were applied at the same dosage of 5 mg protein per g cellulose.

The assay was performed as described in Example 34. The 1 ml reactions with 5% milled washed PCS were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

The results as shown in Figure 1 demonstrated that the best enzyme composition for cellulose hydrolysis included *Chaetomium thermophilum* Cel7A CBHI, *Myceliophthora thermophila* Cel6A CBHII, *Trichoderma reesei* Cel7B EGI, *Myceliophthora thermophila* Cel5A EGII, *Aspergillus fumigatus* beta-glucosidase, and *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity. Replacement of the *Trichoderma reesei* Cel7A CBHI, Cel6A CBHII, and Cel5A EGII with *Chaetomium thermophilum* Cel7A CBHI, *Myceliophthora thermophila* Cel6A CBHII, and *Myceliophthora thermophila* Cel5A EGII significantly improved the degree of cellulose conversion to glucose at 60°C (from 50% to 65%). The improved composition hydrolyzed milled washed PCS almost as efficiently at 60°C (65% cellulose conversion to glucose) as the *Trichoderma reesei*–based composition at 50°C (68%).

Example 36: Evaluation of GH61 polypeptides having cellulolytic enhancing activity for the ability to enhance PCS-hydrolyzing activity of a high-temperature enzyme composition at 50°C, 55°C, and 60°C

Thermoascus aurantiacus GH61A polypeptide having cellulolytic enhancing activity and *Thielavia terrestris* GH61E polypeptide having cellulolytic enhancing activity, prepared as described herein, were tested at 10% total protein addition for the ability to stimulate a high-temperature cellulase composition at 50°C, 55°C, and 60°C using milled washed PCS. The compositions consisting of 45% *Chaetomium thermophilum* Cel7A CBHI, 25% *Myceliophthora thermophila* Cel6A CBHII, 5% *Trichoderma reesei* Cel7B EGI, 5% *Myceliophthora thermophila* Cel5A EGII, 10% *Aspergillus fumigatus* beta-glucosidase, and 10% GH61 polypeptide having cellulolytic enhancing activity were used for hydrolysis of milled washed PCS at 5 mg protein per g cellulose, and the results were compared with the results for a high-temperature enzyme composition without a GH61 polypeptide, which was used at 4.5 mg protein per g cellulose.

The assay was performed as described in Example 34. The 1 ml reactions with 5% milled washed PCS were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

The results shown in Figure 2 demonstrated that the *Thermoascus aurantiacus* GH61A and *Thielavia terrestris* GH61E polypeptides were able to significanty enhance PCS-hydrolyzing activity of the high-temperature enzyme composition at all temperatures from

50°C to 60°C, and that the stimulating effect was more pronounced at higher temperatures. At 60°C, a composition without a GH61 polypeptide showed 48% conversion of cellulose to glucose. For comparison, compositions that included *Thermoascus aurantiacus* GH61A or *Thielavia terrestris* GH61E polypeptides showed 70% and 68% conversion of cellulose to glucose, respectively.

Example 37: Evaluation of a binary composition of *Thermoascus aurantiacus* GH61A and *Thielavia terrestris* GH61E GH61 polypeptides having cellulolytic enhancing activity for the ability to enhance PCS-hydrolyzing activity of a high-temperature enzyme composition at 50°C, 55°C, and 60°C

The boosting performance of a binary composition comprising equal amounts (on a protein basis) of *Thermoascus aurantiacus* GH61A and *Thielavia terrestris* GH61E GH61 polypeptides having cellulolytic enhancing activity was compared with the boosting performance of the individual GH61 proteins alone at equivalent total protein loading at 50°C, 55°C, and 60°C. The high-temperature enzyme compositions included 45% *Chaetomium thermophilum* Cel7A CBHI, 25% *Myceliophthora thermophila* Cel6A CBHII, 5% *Trichoderma reesei* Cel7B EGI, 5% *Myceliophthora thermophila* Cel5A EGII, 10% *Aspergillus fumigatus* beta-glucosidase, and 10% GH61 component (either an individual polypeptide or a binary composition). The total protein loading in hydrolysis reactions was 5 mg protein per g cellulose.

The assay was performed as described in Example 34. The 1 ml reactions with 5% milled washed PCS were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

The results shown in Figure 3 demonstrated that the binary combination of the two GH61 polypeptides having cellulolytic enhancing activity, *Thermoascus aurantiacus* GH61A and *Thielavia terrestris* GH61E, provided greater enhancement than either of the GH61 proteins alone. The effect was especially pronounced at 60°C.

Example 38: Evaluation of binary compositions containing different ratios of *Thermoascus aurantiacus* GH61A and *Thielavia terrestris* GH61E GH61 polypeptides having cellulolytic enhancing activity for the ability to enhance PCS-hydrolyzing activity of a high-temperature enzyme composition at 60°C

After determining that a 1:1 composition of *Thermoascus aurantiacus* GH61A and *Thielavia terrestris* GH61E GH61 polypeptides having cellulolytic enhancing activity performed better in enhancing the PCS-hydrolyzing activity of a high-temperature enzyme composition than either protein alone, the effect was analyzed in more detail by examining

different ratios of the *Thermoascus aurantiacus* GH61A and *Thielavia terrestris* GH61E polypeptides. The various binary compositions of the GH61 polypeptides were added at 0.5 mg protein per g cellulose to a high-temperature enzyme composition (4.5 mg protein per g cellulose) so that the final mixture consisted of 45% *Chaetomium thermophilum* Cel7A CBHI, 25% *Myceliophthora thermophila* Cel6A CBHII, 5% *Trichoderma reesei* Cel7B EGI, 5% *Myceliophthora thermophila* Cel5A EGII, 10% *Penicillium brasilianum* beta-glucosidase, and 10% GH61 component (either an individual GH61 polypeptide or a binary composition).

The assay was performed as described in Example 34. The 1 ml reactions with 5% milled washed PCS were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

The results are shown in Table 1 and Figure 4. All binary compositions of *Thermoascus aurantiacus* GH61A and *Thielavia terrestris* GH61E GH61 polypeptides having cellulolytic enhancing activity provided significantly better enhancement of PCS hydrolysis by the high-temperature enzyme composition than either protein alone. The best performance was obtained for binary GH61 compositions that included at least 20% of either *Thermoascus aurantiacus* GH61A or *Thielavia terrestris* GH61E polypeptide having cellulolytic enhancing activity.

Table 1. Addition of binary GH61 polypeptide compositions (0.5 mg protein per g cellulose) to a high-temperature enzyme composition (4.5 mg protein per g cellulose) at 60°C.

mg protein per g cellulose		% of total GH61 addition		% Conversion
Thermoascus aurantiacus GH61A	*Thielavia terrestris* GH61E	*Thermoascus aurantiacus* GH61A	*Thielavia terrestris* GH61E	
0.00	0.00	0	0	47.9
0.00	0.50	0	100	71.0
0.05	0.45	10	90	79.0
0.10	**0.40**	**20**	**80**	**81.3**
0.15	**0.35**	**30**	**70**	**81.8**
0.20	**0.30**	**40**	**60**	**82.0**
0.25	**0.25**	**50**	**50**	**82.7**
0.30	**0.20**	**60**	**40**	**81.8**
0.35	**0.15**	**70**	**30**	**81.2**
0.40	**0.10**	**80**	**20**	**80.6**
0.45	0.05	90	10	78.8

mg protein per g cellulose		% of total GH61 addition		% Conversion
Thermoascus aurantiacus GH61A	*Thielavia terrestris* GH61E	*Thermoascus aurantiacus* GH61A	*Thielavia terrestris* GH61E	
0.50	0.00	100	0	72.8

Example 39: Evaluation of different levels of a binary 1:1 composition comprising *Thermoascus aurantiacus* GH61A and *Thielavia terrestris* GH61E GH61 polypeptides having cellulolytic enhancing activity for the ability to enhance PCS-hydrolyzing activity of a high-temperature enzyme composition at 60°C

The ability of *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, *Thielavia terrestris* GH61E polypeptide having cellulolytic enhancing activity, or their binary 1:1 composition (on a protein basis) to stimulate a high-temperature enzyme composition was examined by adding different GH61 protein loadings (0.125, 0.25, 0.5, 1.0, 1.5 mg protein per g cellulose) to a constant loading of the high-temperature enzyme composition (4.5 mg per g cellulose) at 60°C. The high-temperature enzyme composition contained 45% *Chaetomium thermophilum* Cel7A CBHI, 25% *Myceliophthora thermophila* Cel6A CBHII, 5% *Trichoderma reesei* Cel7B EGI, 5% *Myceliophthora thermophila* Cel5A EGII, and 10% *Penicillium brasilianum* Cel3A beta-glucosidase.

The assay was performed as described in Example 34. The 1 ml reactions with 5% milled washed PCS were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

The results shown in Figure 5 demonstrated that at equivalent protein loadings, the 1:1 compositions of *Thermoascus aurantiacus* GH61A and *Thielavia terrestris* GH61E GH61 polypeptides having cellulolytic enhancing activity provided greater PCS hydrolysis enhancement than either of the GH61 proteins alone. The effect was especially pronounced at relatively high additions of the GH61 proteins. The effect saturated when the level of the GH61 binary composition reached approximately 20% of the total protein loading.

Example 40: Effect of *Thermobifida fusca* GH11 xylanase on saccharification of milled washed PCS by a high-temperature enzyme composition at 50-65ºC

The ability of *Thermobifida fusca* GH11 xylanase (0.5 mg protein per g cellulose) to stimulate saccharification of milled washed PCS by a high-temperature enzyme composition (5 mg protein per g cellulose) was examined at 50°C, 55°C, 60°C, and 65°C. For comparison, the high-temperature enzyme composition without a supplemental xylanase was tested at 5.0, 5.5, and 6.0 mg protein per g cellulose. The high-temperature enzyme

composition included 45% *Chaetomium thermophilum* Cel7A CBHI, 25% *Myceliophthora thermophila* Cel6A CBHII, 5% *Trichoderma reesei* Cel7B EGI, 5% *Myceliophthora thermophila* Cel5A EGII, 10% *Penicillium brasilianum* Cel3A beta-glucosidase, and 10% *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity.

The assay was performed as described in Example 34. The 1 ml reactions with 5% milled washed PCS were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

The results shown in Figure 6 demonstrated that addition of *Thermobifida fusca* GH11 xylanase significantly improved performance of the high-temperature enzyme composition at all temperatures from 50°C to 65°C, increasing the cellulose conversion to glucose by 4-7% after 72 hours of hydrolysis.

Example 41: Replacement of *Chaetomium thermophilum* Cel7A cellobiohydrolase I in a high-temperature enzyme composition with various thermostable CBHI proteins at 50-65°C

The ability of several thermostable CBHI proteins to replace *Chaetomium thermophilum* Cel7A CBHI in a high-temperature enzyme composition (4 mg total protein per g cellulose) was tested at 50°C, 55°C, 60°C, and 65°C. The high-temperature enzyme composition included 45% *Chaetomium thermophilum* Cel7A CBHI, 25% *Myceliophthora thermophila* Cel6A CBHII, 10% *Myceliophthora thermophila* Cel5A EGII, 5% *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, 5% *Thielavia terrestris* GH61E polypeptide having cellulolytic enhancing activity, and 10% *Penicillium brasilianum* Cel3A beta-glucosidase.

The following recombinant CBHI cellulases from an *Aspergillus oryzae* expression host were tested as a replacement for *Chaetomium thermophilum* Cel7A CBHI: *Myceliophthora thermophila* Cel7A, *Aspergillus fumigatus* Cel7A, and *Thermoascus aurantiacus* Cel7A.

The assay was performed as described in Example 34. The 1 ml reactions with 5% milled washed PCS were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

As shown in Figure 7, the replacement of *Chaetomium thermophilum* Cel7A CBHI with *Aspergillus fumigatus* Cel7A CBHI gave the best hydrolysis results, providing an almost 6% improvement of the final hydrolysis yield at 60°C. Surprisingly, a native *Thermoascus aurantiacus* Cel7A CBHI, which contained no CBM, also showed a remarkably good performance at elevated temperatures.

Example 42: Comparison of high-temperature enzyme compositions containing *Aspergillus fumigatus* Cel7A CBHI or *Chaetomium thermophilum* Cel7A CBHI with *Trichoderma reesei*-based cellulase SaMe-MF268 (XCL-533) at 50°C and 60°C

Two high-temperature enzyme compositions that included either *Aspergillus fumigatus* Cel7A CBHI or *Chaetomium thermophilum* Cel7A CBHI were tested in comparison with *Trichoderma reesei*-based cellulase composition SaMe-MF268 (XCL-533) at four protein loadings (3.5, 4.0, 4.5, and 5.0 mg protein per g cellulose) and two temperatures (50°C and 60°C) using milled washed PCS as a substrate The high-temperature enzyme compositions included 45% Cel7A CBHI, 25% *Myceliophthora thermophila* Cel6A CBHII, 5% *Trichoderma reesei* Cel7B EGI, 5% *Myceliophthora thermophila* Cel5A EGII, 5% *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, 5% *Thielavia terrestris* GH61E polypeptide having cellulolytic enhancing activity, 7.5% *Penicillium brasilianum* GH3A beta-glucosidase, and 2.5% *Thermobifida fusca* GH11 xylanase. The *Trichoderma reesei*-based enzyme composition SaMe-MF268 (XCL-533) was obtained as described in WO 2008/151079. The composition comprises a *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, a beta-glucosidase fusion protein comprising the *Humicola insolens* endoglucanase V core polypeptide fused to the wild-type *Aspergillus oryzae* beta-glucosidase, a *Trichoderma reesei* Cel7A cellobiohydrolase I, a *Trichoderma reesei* Cel6A cellobiohydrolase II, a *Trichoderma reesei* Cel7B endoglucanase I, a *Trichoderma reesei* Cel5A endoglucanase II, a *Trichoderma reesei* Cel45A endoglucanase V, and a *Trichoderma reesei* Cel12A endoglucanase III.

The assay was performed as described in Example 34. The 1 ml reactions with 5% milled washed PCS were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

Protein dose profiles for the *Aspergillus fumigatus* Cel7A CBHI-based composition in comparison with the *Chaetomium thermophilum* Cel7A CBHI-based composition and the *Trichoderma reesei*-based cellulase composition XCL-533 are shown in Figure 8. The *Trichoderma reesei*-based cellulase composition XCL-533 showed poor performance at 60°C, while both high-temperature enzyme compositions were significantly activated by the temperature increase from 50°C to 60°C. The high-temperature enzyme composition that included *Aspergillus fumigatus* Cel7A CBHI performed at 60°C as well as the *Trichoderma reesei*-based cellulase composition XCL-533 performed at its optimum temperature of 50°C, requiring approximately 3.5 mg protein per g cellulose to achieve 80% conversion of cellulose to glucose in 72 hours. For the *Chaetomium thermophilum* Cel7A CBHI-based

composition, the protein loading required to achieve the same degree of cellulose conversion at 60°C was 4.5 mg protein per g cellulose.

Example 43: Hydrolysis time-course for *Aspergillus fumigatus* Cel7A CBHI-based high-temperature enzyme composition in comparison with *Trichoderma reesei*-based cellulase composition SaMe-MF268 at 50°C and 60°C

Hydrolysis performance of the *Aspergillus fumigatus* Cel7A CBHI-based high-temperature enzyme composition and the *Trichoderma reesei*-based cellulase composition SaMe-MF268 (XCL-533) was compared over a longer incubation time (five days) at 50°C and 60°C using milled washed PCS as a substrate. The *Aspergillus fumigatus* Cel7A CBHI-based enzyme composition included 45% *Aspergillus fumigatus* Cel7A CBHI, 25% *Myceliophthora thermophila* Cel6A CBHII, 5% *Trichoderma reesei* Cel7B EGI, 5% *Myceliophthora thermophila* Cel5A EGII, 5% *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, 5% *Thielavia terrestris* GH61E polypeptide having cellulolytic enhancing activity, 7.5% *Penicillium brasilianum* GH3A beta-glucosidase, and 2.5% *Thermobifida fusca* GH11 xylanase. The *Aspergillus fumigatus* Cel7A CBHI-based enzyme composition and the *Trichoderma reesei*-based cellulase composition XCL-533 were tested at four different protein loadings, 2.0, 3.0, 3.5, and 4.0 mg protein per g cellulose.

The assay was performed as described in Example 34. The 1 ml reactions with 5% milled washed PCS were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

The time-course hydrolysis results for one of the four tested protein loadings (2 mg protein per g cellulose) are shown in Figure 9. Similar trends were obtained for the three other protein loadings (data not shown). The *Trichoderma reesei*-based cellulase composition XCL-533 showed significantly reduced performance at 60°C, while the *Aspergillus fumigatus* Cel7A CBHI-based enzyme composition was significantly activated by the temperature increase from 50°C to 60°C.

Comparison of the *Aspergillus fumigatus* Cel7A CBHI-based enzyme composition at 60°C and the *Trichoderma reesei*-based cellulase composition XCL-533 at 50°C showed that the high-temperature composition performed better than the *Trichoderma reesei*-based cellulase composition XCL-533 during the initial three days, and similarly to the *Trichoderma reesei*-based cellulase composition XCL-533 during the last two days of hydrolysis. Comparison of the *Aspergillus fumigatus* Cel7A CBHI-based enzyme composition and the *Trichoderma reesei*-based cellulase composition XCL-533 at the same temperature of 50°C showed slower but steadier rates of glucose accumulation for the high-temperature

composition in comparison with the *Trichoderma reesei*-based cellulase composition XCL-533, resulting in a better performance of the high-temperature composition in a long-term hydrolysis at 50°C (5 days).

Example 44: Evaluation of four xylanases for synergy with the *Aspergillus fumigatus* Cel7A CBH I-based high-temperature enzyme composition at 50°C, 55°C, and 60°C

Aspergillus aculeatus GH10 xylanase II, *Aspergillus fumigatus* GH10 xyn3 xylanase, *Trichophaea saccata* GH10 xylanase, and *Thermobifida fusca* GH11 xylanase were assayed for synergy with a high-temperature enzyme composition containing Cel7A CBHI from *Aspergillus fumigatus* at 50°C, 55°C, and 60°C using milled washed PCS as a substrate. The xylanases were added at 10% (0.35 mg protein per g cellulose) to a constant loading of the high-temperature enzyme composition (3.5 mg protein per g cellulose). The high-temperature enzyme composition included 45% *Aspergillus fumigatus* Cel7A CBHI, 25% *Myceliophthora thermophila* Cel6A CBHII, 5% *Trichoderma reesei* Cel7B EGI, 5% *Myceliophthora thermophila* Cel5A EGII, 5% *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, 5% *Thielavia terrestris* GH61E polypeptide having cellulolytic enhancing activity, and 10% *Penicillium brasilianum* Cel3A beta-glucosidase.

The assay was performed as described in Example 34. The 1 ml reactions with 5% milled washed PCS were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

The results shown in Figure 10 demonstrated a considerable synergy between the xylanases and the high-temperature enzyme composition. At an equivalent protein loading (3.85 mg protein per g cellulose), all enzyme compositions that included xylanases achieved a significantly higher degree of cellulose conversion compared to the non-supplemented high-temperature enzyme composition. GH10 xylanases from *Aspergillus fumigatus* (xyn3) and *Trichophaea saccata* showed better performance than *Aspergillus aculeatus* GH10 xylanase II and *Thermobifida fusca* GH11 xylanase. The addition of the top two xylanases to the high-temperature enzyme composition resulted in an additional 11-16% conversion of cellulose to glucose in 72 hours compared to the non-supplemented enzyme composition (3.85 mg protein per g cellulose).

Example 45: Evaluation of *Aspergillus fumigatus* GH10 xylanase xyn3, *Trichophaea saccata* GH10 xylanase, and *Thermobifida fusca* GH11 xylanase for synergy with a high-temperature enzyme composition at 60°C

The ability of *Aspergillus fumigatus* GH10 xyn3 xylanase, *Trichophaea saccata* GH10 xylanase, and *Thermobifida fusca* GH11 xylanase to synergize with a high-temperature

enzyme composition containing *Aspergillus fumigatus* Cel7A CBHI was further examined by adding different levels of each xylanase (1.25%, 2.5%, 5%, 10%, and 20%) to a constant loading of the high-temperature enzyme composition (3 mg protein per g cellulose) at 60°C using washed milled PCS as a substrate. The high-temperature enzyme composition included 45% *Aspergillus fumigatus* Cel7A CBHI, 25% *Myceliophthora thermophila* Cel6A CBHII, 5% *Trichoderma reesei* Cel7B EGI, 5% *Myceliophthora thermophila* Cel5A EGII, 5% *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, 5% *Thielavia terrestris* GH61E polypeptide having cellulolytic enhancing activity, and 10% *Penicillium brasilianum* Cel3A beta-glucosidase.

The assay was performed as described in Example 34. The 1 ml reactions with 5% milled washed PCS were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis. The results are shown in Figure 11.

Aspergillus fumigatus GH10 xyn3 and *Trichophaea saccata* GH10 xylanases performed similarly and showed better enhancement of PCS hydrolysis than *Thermobifida fusca* GH11 xylanase. The high-temperature enzyme composition supplemented with the GH10 xylanase from *Aspergillus fumigatus* or *Trichophaea saccata* significantly outperformed the *Trichoderma reesei*-based cellulase composition SaMe-MF268 (XCL-533) after 72 hours of incubation with milled washed PCS. The optimal addition level was about 5% for the *Aspergillus fumigatus* GH10 xyn3 and *Trichophaea saccata* GH10 xylanases and about 10% for the *Thermobifida fusca* GH11 xylanase. As shown in Figure 11, the addition of either *Aspergillus fumigatus* GH10 xyn3 or *Trichophaea saccata* GH10 xylanase to the high-temperature composition at a level of only 5% at 60°C enhanced the cellulose conversion to glucose from 65% to 83%. An equivalent loading of the *Trichoderma reesei*-based cellulase composition XCL-533 (3.15 mg protein per g cellulose) yielded 73% conversion of cellulose to glucose at 50°C.

Example 46: Comparison of *Aspergillus fumigatus* Cel7A-based high-temperature enzyme composition containing *Aspergillus fumigatus* GH10 xyn3 xylanase with *Trichoderma reesei*-based cellulase SaMe-MF268

A high-temperature enzyme composition containing *Aspergillus fumigatus* GH10 xyn3 xylanase and the *Trichoderma reesei*-based cellulase composition SaMe-MF268 (XCL-533) were tested at three different protein loadings, 2.0, 3.0, and 4.0 mg protein per g cellulose, and the protein loading profile of the high-temperature enzyme composition at 60°C was compared with the protein loading profile of the *Trichoderma reesei*-based cellulase composition XCL-533 at 50°C. The high-temperature enzyme composition contained 45% *Aspergillus fumigatus* Cel7A CBHI, 25% *Myceliophthora thermophila* Cel6A

CBHII, 5% *Trichoderma reesei* Cel7B EGI, 5% *Myceliophthora thermophila* Cel5A EGII, 5% *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, 5% *Thielavia terrestris* GH61E polypeptide having cellulolytic enhancing activity, 5% *Penicillium brasilianum* Cel3A beta-glucosidase, and 5% *Aspergillus fumigatus* GH10 xyn3.

The assay was performed as described in Example 34. The 1 ml reactions with 5% milled washed PCS were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis. The results are shown in Figure 12.

The experiment confirmed that the improved high-temperature enzyme composition containing *Aspergillus fumigatus* GH10 xyn3 xylanase significantly surpassed the performance of the *Trichoderma reesei*-based cellulase composition XCL-533. At 60°C, the high-temperature enzyme composition required a significantly lower protein loading (2.75 mg protein per g cellulose) than the *Trichoderma reesei*-based cellulase composition XCL-533 at 50°C (3.80 mg protein per g cellulose) to hydrolyze 80% of cellulose to glucose in 72 hours.

Example 47: Comparison of high-temperature enzyme compositions containing *Aspergillus fumigatus* GH10 xyn3 or *Trichophaea saccata* GH10 xylanase with *Trichoderma reesei*-based cellulase SaMe-MF268 in hydrolysis of washed and unwashed PCS

Protein loading profiles of the improved high-temperature enzyme compositions containing either GH10 xylanase from *Aspergillus fumigatus* (xyn 3) or GH10 xylanase from *Trichophaea saccata* were compared with protein loading profiles of the *Trichoderma reesei*-based cellulase composition SaMe-MF268 (XCL-533) using milled washed and milled unwashed PCS. The high-temperature enzyme compositions included 45% *Aspergillus fumigatus* Cel7A CBHI, 25% *Myceliophthora thermophila* Cel6A CBHII, 5% *Trichoderma reesei* Cel7B EGI, 5% *Myceliophthora thermophila* Cel5A EGII, 5% *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, 5% *Thielavia terrestris* GH61E polypeptide having cellulolytic enhancing activity, 5% *Penicillium brasilianum* Cel3A beta-glucosidase, and 5% GH10 xylanase. The high-temperature enzyme compositions and the *Trichoderma reesei*-based cellulase composition XCL-533 were tested at five different protein loadings, 2.0, 3.0, 4.0, 5.0, and 6.0 mg protein per g cellulose. All reactions with the high-temperature enzyme compositions were performed at 60°C, while all reactions with the *Trichoderma reesei*-based cellulase composition XCL-533 were performed at 50°C.

The assay was performed as described in Example 34. The 1 ml reactions with milled washed or milled unwashed PCS were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. Washed PCS was used at 5%

total solids, whereas unwashed PCS was used at 5% insoluble solids. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

The results are shown in Table 2 and Figures 13A and 13B. The high-temperature enzyme compositions containing *Aspergillus fumigatus* GH10 xyn 3 xylanase or *Trichophaea saccata* GH10 xylanase showed similar performance, requiring similar protein loadings to achieve the same levels of cellulose conversion to glucose. The improved high-temperature enzyme compositions containing *Aspergillus fumigatus* GH10 xyn 3 xylanase or *Trichophaea saccata* GH10 xylanase outperformed the *Trichoderma reesei*-based cellulase composition XCL-533 on both washed (A) and unwashed (B) PCS.

Table 2. Protein loadings required for reaching 80% cellulose conversion of washed and unwashed PCS (mg protein per g cellulose). Temperature: 60°C for high-temperature enzyme compositions, 50°C for XCL-533.

Composition	Washed PCS	Unwashed PCS
XCL-533	3.6	4.9
High-temperature composition with *Aspergillus fumigatus* GH10 xyn3 xylanase	2.6	4.2
High-temperature composition with *Trichophaea saccata* GH10 xylanase	2.5	4.2

Example 48: Effect of *Trichoderma reesei* GH3 and *Talaromyces emersonii* GH3 beta-xylosidases on saccharification of milled unwashed PCS by a high-temperature enzyme composition at 60ºC

The ability of two beta-xylosidases, *Trichoderma reesei* GH3 beta-xylosidase and *Talaromyces emersonii* GH3 beta-xylosidase, to enhance hydrolysis of milled unwashed PCS by a high-temperature enzyme composition was evaluated at 60°C as described in Example 34. The 1 ml reactions with milled unwashed PCS (5% insoluble solids) were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis. The results are shown in Figures 14A and 14B.

The high-temperature enzyme composition included 45% *Aspergillus fumigatus* Cel7A CBHI, 25% *Myceliophthora thermophila* Cel6A CBHII, 5% *Trichoderma reesei* Cel7B EGI, 5% *Myceliophthora thermophila* Cel5A EGII, 5% *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, 5% *Thielavia terrestris* GH61E polypeptide having cellulolytic enhancing activity, 5% *Aspergillus fumigatus* GH10 xyn3 xylanase, and 5% *Penicillium brasilianum* Cel3A beta glucosidase. The high-temperature enzyme

composition (3 mg total protein per g cellulose) was supplemented with *Trichoderma reesei* GH3 beta-xylosidase and *Talaromyces emersonii* GH3 beta-xylosidase at 1%, 2%, 3.5%, 5%, and 10% replacement levels, and the hydrolysis results were compared with the results for the high-temperature enzyme composition containing no beta-xylosidase (0% replacement level).

As shown in Figure 14A, the inclusion of *Trichoderma reesei* GH3 beta-xylosidase and *Talaromyces emersonii* GH3 beta-xylosidase in the high-temperature enzyme composition increased the level of enzymatically-produced monomeric xylose from approximately 0.5 g/L (0% beta-xylosidase replacement) to approximately 2 g/L. The optimal replacement levels were 3.5% for *Trichoderma reesei* GH3 beta-xylosidase and 1-2% for *Talaromyces emersonii* GH3 beta-xylosidase. As shown in Figure 14B, the combined yield of glucose and cellobiose was increased by an additional 1.5% for a mixture containing 3.5% *Trichoderma reesei* GH3 beta-xylosidase and by an additional 2.5% for a mixture containing 2% *Talaromyces emersonii* GH3 beta-xylosidase.

These results demonstrated that both beta-xylosidases provided a small but significant benefit by increasing the degree of cellulose and hemicellulose conversion of milled unwashed PCS to soluble sugars (glucose, cellobiose and xylose). *Talaromyces emersonii* GH3 beta-xylosidase appeared to be slightly more active than *Trichoderma reesei* GH3 beta-xylosidase at 60°C, increasing the monomeric xylose level by approximately 1.5 g/L (Figure 14A) and the cellulose conversion by an additional 2.5% (Figure 14B) at a very low replacement level (1-2%) of the high-temperature enzyme composition.

Example 49: Evaluation of four cellobiohydrolases I replacing a CBHI component in a high-temperature enzyme composition in saccharification of milled unwashed PCS at 50-65°C

The ability of four cellobiohydrolase I proteins to replace a CBHI component in a high-temperature enzyme composition (3 mg total protein per g cellulose) was tested at 50°C, 55°C, 60°C, and 65°C using milled unwashed PCS as a substrate. The high-temperature enzyme composition included 35% CBHI, 25% *Myceliophthora thermophila* Cel6A CBHII, 15% *Thermoascus aurantiacus* Cel5A EGII, 15% *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, 5% *Aspergillus fumigatus* Cel3A beta-glucosidase, and 5% *Aspergillus fumigatus* GH10 xyn3 xylanase.

The following CBHI cellulases were tested in the high-temperature enzyme composition: *Trichoderma reesei* Cel7A CBHI, *Chaetomium thermophilum* Cel7A CBHI, *Aspergillus fumigatus* Cel7A CBHI, and *Thermoascus aurantiacus* Cel7A CBHI.

The assay was performed as described in Example 34. The 1 ml reactions with milled unwashed PCS (5% insoluble solids) were conducted for 72 hours in 50 mM sodium

acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

As shown in Figure 15, *Aspergillus fumigatus* Cel7A performed better than other cellobiohydrolases I at all temperatures from 50°C to 65°C. *Chaetomium thermophilum* Cel7A also performed well in the entire range of temperatures, but the degree of cellulose conversion to glucose was lower compared to *Aspergillus fumigatus* Cel7A. *Trichoderma reesei* Cel7A did not perform well at temperatures above 55°C. *Thermoascus aurantiacus* Cel7A, which contained no CBM, showed a remarkably good performance at 60°C and 65°C, but was less efficient in hydrolyzing the cellulose in unwashed PCS at lower temperatures (50°C and 55°C).

Example 50: Evaluation of four cellobiohydrolases II replacing a CBHII component in a high-temperature enzyme composition in saccharification of milled unwashed PCS at 50-65°C

The ability of several cellobiohydrolase II proteins to replace a CBHII component in a high-temperature enzyme composition (3 mg total protein per g cellulose) was tested at 50°C, 55°C, 60°C, and 65°C using milled unwashed PCS as a substrate. The high-temperature enzyme composition included 35% *Aspergillus fumigatus* Cel7A CBHI, 25% CBHII, 15% *Thermoascus aurantiacus* Cel5A EGII, 15% *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, 5% *Aspergillus fumigatus* Cel3A beta-glucosidase, and 5% *Aspergillus fumigatus* GH10 xyn3 xylanase.

The following CBHII cellulases were tested in the high-temperature enzyme composition: *Myceliophthora thermophila* Cel6A CBHII, *Thielavia terrestris* Cel6A CBHII, *Aspergillus fumigatus* Cel6A CBHII, and *Trichophaea saccata* Cel6A CBHII.

The assay was performed as described in Example 34. The 1 ml reactions with milled unwashed PCS (5% insoluble solids) were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

As shown in Figure 16, CBHII from *Aspergillus fumigatus* performed about the same as CBHII from *Myceliophthora thermophila* at all temperatures, showing only a slightly lower hydrolysis at 60-65°C. *Thielavia terrestris* Cel6A CBHII had high thermostability and performed well in the entire range of temperatures, but the degree of cellulose conversion to glucose was lower compared to *Myceliophthora thermophila* Cel6A CBHII. *Trichophaea saccata* Cel6A CBHII had high activity at 50-55°C, but did not perform well at higher temperatures.

Example 51: Evaluation of two endoglucanases I replacing an endoglucanase

component in a high-temperature enzyme composition in saccharification of milled unwashed PCS at 50-65°C

The ability of two endoglucanase I proteins to replace an endoglucanase component in a high-temperature enzyme composition (3 mg total protein per g cellulose) was tested at 50°C, 55°C, 60°C, and 65°C using milled unwashed PCS as a substrate. The high-temperature enzyme composition included 35% *Aspergillus fumigatus* Cel7A CBHI, 25% *Myceliophthora thermophila* Cel6A CBHII, 15% EG cellulase, 15% *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, 5% *Aspergillus fumigatus* Cel3A beta-glucosidase, and 5% *Aspergillus fumigatus* GH10 xyn3 xylanase.

The following EGIs were tested in the high-temperature enzyme composition: *Trichoderma reesei* Cel7B EGI and *Aspergillus terreus* Cel7 EGI.

The assay was performed as described in Example 34. The 1 ml reactions with milled unwashed PCS (5% insoluble solids) were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

As shown in Figure 17, the high-temperature enzyme composition containing *Aspergillus terreus* Cel7 EGI performed significantly better than the high-temeperature enzyme composition containing *Trichoderma reesei* Cel7B EGI within this temperature range.

Example 52: Evaluation of three endoglucanases II replacing an endoglucanase component in a high-temperature enzyme composition in saccharification of milled unwashed PCS at 50-65°C

The ability of three endoglucanase II proteins to replace an endoglucanase component in a high-temperature enzyme composition (3 mg total protein per g cellulose) was tested at 50°C, 55°C, 60°C, and 65°C using milled unwashed PCS as a substrate. The high-temperature enzyme composition included 35% *Aspergillus fumigatus* Cel7A CBHI, 25% *Myceliophthora thermophila* Cel6A CBHII, 15% EG cellulase, 15% *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, 5% *Aspergillus fumigatus* Cel3A beta-glucosidase, and 5% *Aspergillus fumigatus* GH10 xyn3 xylanase.

The following EGIIs were tested in the high-temperature enzyme composition: *Trichoderma reesei* Cel5A EGII, *Myceliophthora thermophila* Cel5A EGII, and *Thermoascus aurantiacus* Cel5A EGII.

The assay was performed as described in Example 34. The 1 ml reactions with milled unwashed PCS (5% insoluble solids) were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

As shown in Figure 18, all endoglucanase II proteins performed similarly within this temperature range, with *Myceliophthora thermophila* Cel5A EGII slightly outperforming *Trichoderma reesei* Cel5A EGII and *Thermoascus aurantiacus* Cel5A EGII.

Example 53: Evaluation of three beta-glucosidases replacing a beta-glucosidase component in a high-temperature enzyme composition in saccharification of milled unwashed PCS at 50-65°C

In a first experiment, three beta-glucosidases, including *Aspergillus fumigatus* Cel3A beta-glucosidase, *Penicillium brasilianum* Cel3A beta-glucosidase, and *Aspergillus niger* Cel3 beta-glucosidase, were evaluated in a high-temperature enzyme composition at 50°C, 55°C, and 60°C using milled unwashed PCS as a substrate. The high-temperature enzyme composition included 45% *Aspergillus fumigatus* Cel7A CBHI, 25% *Thielavia terrestris* Cel6A CBHII, 5% *Trichoderma reesei* Cel7B EGI, 5% *Thermoascus aurantiacus* Cel5A EGII, 5% *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, 5% *Thielavia terrestris* GH61E polypeptide having cellulolytic enhancing activity, 5% *Aspergillus fumigatus* GH10 xyn3 xylanase, and 5% beta-glucosidase. The high-temperature enzyme composition was used at 3.0 mg total protein per g cellulose.

The assay was performed as described in Example 34. The 1 ml reactions with milled unwashed PCS (5% insoluble solids) were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

The results shown in Figure 19 demonstrated that all three beta-glucosidases performed about the same at 50°C and 55°C, whereas *Aspergillus niger* Cel3 beta-glucosidase showed slightly lower hydrolysis at 60°C than the other two beta-glucosidases.

In a second experiment, *Aspergillus fumigatus* Cel3A beta-glucosidase and *Penicillium brasilianum* Cel3A beta-glucosidase were compared in a high-temperature enzyme composition at four temperatures, 50°C, 55°C, 60°C, and 65°C, using milled unwashed PCS as a substrate. The high-temperature enzyme composition included 35% *Aspergillus fumigatus* Cel7A CBHI, 25% *Myceliophthora thermophila* Cel6A CBHII, 15% *Thermoascus aurantiacus* Cel5A EGII, 15% *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, 5% beta-glucosidase, and 5% *Aspergillus fumigatus* GH10 xyn3 xylanase. The high-temperature enzyme composition was used at 3.0 mg total protein per g cellulose.

The assay was performed as described in Example 34. The 1 ml reactions with milled unwashed PCS (5% insoluble solids) were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

The results shown in Figure 20 demonstrated that Cel3A beta-glucosidases from *Aspergillus fumigatus* and *Penicillium brasilianum* performed about the same within this temperature range.

Example 54: Evaluation of six xylanases replacing a xylanase component in a high-temperature enzyme composition in saccharification of milled unwashed PCS at 50-65°C

The ability of six xylanases to replace a xylanase component in a high-temperature enzyme composition (3 mg total protein per g cellulose) was tested at 50°C, 55°C, 60°C, and 65°C using milled unwashed PCS as a substrate. The high-temperature enzyme composition included 35% *Aspergillus fumigatus* Cel7A CBHI, 25% *Myceliophthora thermophila* Cel6A CBHII, 15% *Thermoascus aurantiacus* Cel5A EGII, 15% *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, 5% *Aspergillus fumigatus* Cel3A beta-glucosidase, and 5% xylanase.

The following xylanases were tested in the high-temperature enzyme composition: *Aspergillus aculeatus* GH10 xyn II xylanase, *Aspergillus fumigatus* GH10 xyn3, *Trichophaea saccata* GH10 xylanase, *Thermobifida fusca* GH11 xylanase, *Penicillium pinophilum* GH10 xylanase, and *Thielavia terrestris* GH10E xylanase.

The assay was performed as described in Example 34. The 1 ml reactions with milled unwashed PCS (5% insoluble solids) were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

As shown in Figure 21, GH10 xylanase from *Penicillium pinophilum* was superior to GH10 xylanases from *Aspergillus fumigatus* and *Trichophaea saccata* at all temperatures from 50°C to 65°C. The *Aspergillus aculeatus* GH10 and *Thielavia terrestris* GH10E xylanases performed about the same as the *Aspergillus fumigatus* GH10 and *Trichophaea saccata* GH10 xylanases at 50°C and 55°C, but did not perform well at higher temperatures (60°C and 65°C). The *Thermobifida fusca* GH11 performed relatively well at 60°C and 65°C, but overall the degree of cellulose conversion to glucose was lower compared to other xylanases.

Example 55: Evaluation of the ability of four GH61 polypeptides having cellulolytic enhancing activity to enhance PCS-hydrolyzing activity of a high-temperature enzyme composition at 50-65°C using milled unwashed PCS

The ability of four GH61 polypeptides having cellulolytic enhancing activity, *Thermoascus aurantiacus* GH61A, *Thielavia terrestris* GH61E, *Penicillium pinophilum* GH61, and *Aspergillus fumigatus* GH61B, to enhance the PCS-hydrolyzing activity of a high-

temperature enzyme composition was evaluated using milled unwashed PCS at 50°C, 55°C, 60°C, and 65°C. The high-temperature enzyme composition included 35% *Aspergillus fumigatus* Cel7A CBHI, 25% *Myceliophthora thermophila* Cel6A CBHII, 15% *Thermoascus aurantiacus* Cel5A EGII, 15% GH61 polypeptide having cellulolytic enhancing activity, 5% *Aspergillus fumigatus* Cel3A beta-glucosidase, and 5% *Aspergillus fumigatus* GH10 xyn3 xylanase. The high-temperature enzyme composition was used at 3 mg total protein per g cellulose.

The assay was performed as described in Example 34. The 1 ml reactions with milled unwashed PCS (5% insoluble solids) were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

As shown in Figure 22, all four GH61 polypeptides showed a significant cellulase-enhancing activity, with *Thermoascus aurantiacus* GH61A polypeptide being the most efficient enhancer among the four within this temperature range. High-temperature enzyme compositions containing *Thermoascus aurantiacus* GH61A and *Thielavia terrestris* GH61E polypeptides performed better at higher temperatures (60°C and 65°C) than high-temperature enzyme compositions containing *Penicillium pinophilum* GH61 and *Aspergillus fumigatus* GH61B polypeptides.

Example 56: Evaluation of the ability of three GH61 polypeptides having cellulolytic enhancing activity to enhance PCS-hydrolyzing activity of a high-temperature enzyme composition at 50-65°C using milled unwashed PCS

The ability of three GH61 polypeptides having cellulolytic enhancing activity, *Thermoascus aurantiacus* GH61A, *Thielavia terrestris* GH61N, and *Penicillium sp* GH61A, to enhance the PCS-hydrolyzing activity of a high-temperature enzyme composition was evaluated using milled unwashed PCS at 50°C, 55°C, 60°C, and 65°C. The high-temperature enzyme composition included 45% *Aspergillus fumigatus* Cel7A CBHI, 25% *Myceliophthora thermophila* Cel6A CBHII, 10% *Myceliophthora thermophila* Cel5A EGII, 10% GH61 polypeptide, 5% *Aspergillus fumigatus* Cel3A beta-glucosidase, and 5% *Aspergillus fumigatus* GH10 xyn3 xylanase. The results for the enzyme compositions containing GH61 polypeptides (3 mg total protein per g cellulose) were compared with the results for a similar enzyme composition to which no GH61 polypeptide was added (2.7 mg total protein per g cellulose).

The assay was performed as described in Example 34. The 1 ml reactions with milled unwashed PCS (5% insoluble solids) were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

As shown in Figure 23, all three GH61 polypeptides showed a significant cellulase-enhancing activity, with *Thermoascus aurantiacus* GH61A polypeptide being the most efficient enhancer at 55-65°C, and *Thielavia terrestris* GH61N polypeptide slightly outperforming *Thermoascus aurantiacus* GH61A polypeptide at 50°C. The high-temperature enzyme composition containing *Penicillium* sp. GH61A polypeptide performed almost as well as the high-temperature enzyme composition containing *Thermoascus aurantiacus* GH61A polypeptide at all four temperatures (50-65°C). *Thielavia terrestris* GH61N polypeptide performed well at 50°C and 55°C, but showed a significant decline in performance at higher temperatures (60°C and 65°C).

Example 57: Hydrolysis of milled unwashed PCS by *Trichoderma reesei*-based XCL-602 cellulase at different replacement levels by *Aspergillus fumigatus* Cel7A cellobiohydrolase I, *Myceliophthora thermophila* Cel6A cellobiohydrolase II, or their binary compositions at 50-60°C

A *Trichoderma reesei* strain 981-08-D4-based cellulase containing *Aspergillus fumigatus* beta-glucosidase and *Thermoascus aurantiacus* GH61A polypeptide, designated *Trichoderma reesei*-based XCL-602 cellulase, was tested in 1-ml hydrolysis reactions at 50°C, 55°C, and 60°C using milled unwashed PCS as a substrate. The *Trichoderma reesei*-based XCL-602 cellulase was used alone or in mixtures with *Aspergillus fumigatus* Cel7A CBHI (10%, 20%, 30% or 40% of total protein), *Myceliophthora thermophila* Cel6A CBHII (10% or 20% of total protein), or both *Aspergillus fumigatus* Cel7A CBHI and *Myceliophthora thermophila* Cel6A CBHII (10%/10%, 10%/20%, 20%/10%, 20%/20%, 30%/10%, 30%/20%, 40%/20% of total protein). The level of *Thermoascus aurantiacus* GH61A polypeptide in all *Trichoderma reesei* XCL-602 compositions was maintained constant at 8% of total protein. *Trichoderma reesei*-based enzyme composition SaMe-MF268 (XCL-533) was also included in the experiment. The non-replaced *Trichoderma reesei*-based XCL-602 cellulase and *Trichoderma reesei*-based XCL-533 cellulase and various *Trichoderma reesei* XCL-602 compositions containing *Aspergillus fumigatus* Cel7A CBHI and/or *Myceliophthora thermophila* Cel6A CBHII were used at 3 mg total protein per g cellulose.

The assay was performed as described in Example 34. The 1 ml reactions with 50 mg of insoluble PCS solids per ml were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

The results shown in Figure 24 demonstrated that non-replaced *Trichoderma reesei*-based XCL-602 cellulase performed about the same as non-replaced *Trichoderma reesei*-based XCL-533 cellulase at all three temperatures. At 50°C and 55°C, the replacement of 10%, 20%, or 30% protein in XCL-602 cellulase by *Aspergillus fumigatus* Cel7A CBHI or the

replacement of 10% or 20% protein in XCL-602 cellulase by *Myceliophthora thermophila* Cel6A CBHII did not have a significant effect on the degree of cellulose conversion to glucose after 72 hours of hydrolysis. The replacement of 40% protein in the *Trichoderma reesei*-based XCL-602 cellulase by *Aspergillus fumigatus* Cel7A CBHI had a negative effect on the degree of cellulose conversion to glucose at 50°C and 55°C. At 60°C, the replacement of protein in the *Trichoderma reesei*-based XCL-602 cellulase by *Aspergillus fumigatus* Cel7A CBHI (10-40% of total protein) or *Myceliophthora thermophila* Cel6A CBHII (10-20% of total protein) significantly improved the hydrolysis over non-replaced *Trichoderma reesei*-based XCL-602 cellulase at an equivalent protein loading (3 mg protein per g cellulose). Higher replacement levels by *Aspergillus fumigatus* Cel7A CBHI or *Myceliophthora thermophila* Cel6A CBHII provided a greater hydrolysis enhancement over non-replaced XCL-602 cellulase at 60°C.

For the *Trichoderma reesei*-based XCL-602 cellulase compositions that included both *Aspergillus fumigatus* Cel7A CBHI and *Myceliophthora thermophila* Cel6A CBHII, the optimal replacement levels were 10-20% *Aspergillus fumigatus* Cel7A CBHI / 10-20% *Myceliophthora thermophila* Cel6A CBHII at 50°C and 55°C, and 40% *Aspergillus fumigatus* Cel7A CBHI / 20% *Myceliophthora thermophila* Cel6A CBHII at 60°C.

Example 58: Hydrolysis of milled unwashed PCS by *Trichoderma reesei*-based XCL-602 cellulase containing *Aspergillus fumigatus* Cel7A cellobiohydrolase I and *Myceliophthora thermophila* Cel6A cellobiohydrolase II, and additionally supplemented by *Aspergillus fumigatus* GH10 xyn 3 and/or *Thielavia terrestris* GH61E at 50-60°C

The *Trichoderma reesei*-based XCL-602 cellulase containing *Aspergillus fumigatus* Cel7A cellobiohydrolase I and *Myceliophthora thermophila* Cel6A cellobiohydrolase II was tested in 1-ml hydrolysis reactions at 50°C, 55°C and 60°C using milled unwashed PCS as a substrate. The *Trichoderma reesei*-based XCL-602 cellulase was used alone (3.0 mg protein per g cellulose) or with replacement by different binary compositions consisting of *Aspergillus fumigatus* Cel7A CBHI and *Myceliophthora thermophila* Cel6A CBHII (10%/10% or 40%/20% of total protein) to a total protein loading of 3 mg protein per g cellulose. The level of *Thermoascus aurantiacus* GH61A polypeptide in all *Trichoderma reesei* XCL-602-based compositions containing *Aspergillus fumigatus* Cel7A CBHI and *Myceliophthora thermophila* Cel6A CBHII was maintained constant at 8% of total protein. The *Trichoderma reesei* XCL-602-based compositions containing *Aspergillus fumigatus* Cel7A CBHI and *Myceliophthora thermophila* Cel6A CBHII (3 mg protein per g cellulose) were additionally supplemented with 5% *Aspergillus fumigatus* GH10 xyn 3 xylanase (0.15 mg protein per g cellulose), 5% *Thielavia terrestris* GH61E polypeptide (0.15 mg protein per g cellulose), or

with a binary composition consisting of 5% *Aspergillus fumigatus* GH10 xyn 3 xylanase and 5% *Thielavia terrestris* GH61E polypeptide (0.30 mg protein per g cellulose). For comparison, the non-replaced *Trichoderma reesei*-based XCL-602 cellulase was used alone at 3.15 mg protein per g cellulose and 3.3 mg protein per g cellulose.

The assay was performed as described in Example 34. The 1 ml reactions with 50 mg of insoluble PCS solids per ml were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

The results shown in Figures 25A and 25B demonstrated that the addition of 5% *Aspergillus fumigatus* GH10 xyn3 xylanase and/or 5% *Thielavia terrestris* GH61E polypeptide to the *Trichoderma reesei* XCL-602-based compositions containing *Aspergillus fumigatus* Cel7A CBHI and *Myceliophthora thermophila* Cel6A CBHII significantly improved the hydrolysis performance at all three temperatures, with a very large improvement obtained when both the *Aspergillus fumigatus* GH10 xyn3 xylanase and the *Thielavia terrestris* GH61E polypeptide were added together. The best XCL-602-based composition in this experiment (XCL-602 with 10% replacement by *Aspergillus fumigatus* Cel7A CBHI and 10% replacement by *Myceliophthora thermophila* Cel6A CBHII, and with additional supplementation by 5% *Aspergillus fumigatus* GH10 xyn3 xylanase and 5% *Thielavia terrestris* GH61E polypeptide) required 3.3 mg protein per g cellulose to achieve 82% conversion of cellulose to glucose in milled unwashed PCS after 72 hours of hydrolysis at 55°C. This represents a 1.5X reduction in protein loading compared to *Trichoderma reesei* XCL-533 (SaMe-MF268), which required 4.9 mg protein per g cellulose to achieve 80% conversion of cellulose to glucose in milled unwashed PCS after 72 hours of hydrolysis at 50°C (Table 2).

Exaple 59: Hydrolysis of milled unwashed PCS by *Trichoderma reesei*-based XCL-602 cellulase compositions containing different replacement levels of *Trichoderma reesei*-based XCL-592 cellulase at 50-60°C

The *Trichoderma reesei*-based XCL-602 cellulase was tested alone (3.0 mg protein per g cellulose) or in mixtures with *Trichoderma reesei* RutC30-based cellulase containing *Aspergillus aculeatus* GH10 xylanase, designated as *Trichoderma reesei*-based XCL-592 cellulase. The *Trichoderma reesei*-based XCL-592 cellulase replaced 5%, 10%, 15%, 20%, or 25% of protein in the *Trichoderma reesei*-based XCL-602 cellulase to a total protein loading of 3 mg protein per g cellulose. The non-replaced *Trichoderma reesei*-based XCL-602 cellulase and XCL-602-based enzyme compositions were tested in 1-ml hydrolysis reactions at 50°C, 55°C and 60°C using milled unwashed PCS as a substrate. For comparison, *Trichoderma reesei*-based XCL-533 cellulase was tested at 4.5 mg protein per

g cellulose.

The assay was performed as described in Example 34. The 1 ml reactions with 50 mg of insoluble PCS solids per ml were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

The results shown in Figure 26 demonstrated that the optimal replacement level of *Trichoderma reesei*-based XCL-602 cellulase by *Trichoderma reesei*-based XCL-592 cellulase at 50°C and 55°C was between 10% and 15% of total protein. At 50°C and 55°C, the best *Trichoderma reesei*-based XCL-602 cellulase compositions performed significantly better than *Trichoderma reesei*-based XCL-602 cellulase alone at an equivalent protein loading (3 mg protein per g cellulose), but were not able to reach the performance level obtained with *Trichoderma reesei*-based XCL-533 cellulase at 4.5 mg protein per g cellulose.

Example 60: Comparison of *Thermoascus aurantiacus* GH61A and *Thielavia terrestris* GH61E GH61 polypeptides having cellulolytic enhancing activity replacing 5% of protein in *Trichoderma reesei*-based XCL-602 cellulase or XCL-602-based enzyme composition in hydrolysis of milled unwashed PCS at 50-60°C

The *Trichoderma reesei*-based XCL-602 cellulase was tested alone and with 5% replacement by *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity or *Thielavia terrestris* GH61E polypeptide having cellulolytic enhancing activity at 50-60°C using milled unwashed PCS as a substrate. In addition, the *Trichoderma reesei*-based XCL-602 cellulase composition containing 30% *Aspergillus fumigatus* Cel7A CBHI, 20% *Myceliophthora thermophila* Cel6A CBHI,I and 5% *Thermoascus aurantiacus* GH61A polypeptide was tested in comparison with a similar composition containing *Thielavia terrestris* GH61E polypeptide instead of *Thermoascus aurantiacus* GH61A polypeptide. All compositions were tested at 3 mg protein per g cellulose.

The assay was performed as described in Example 34. The 1 ml reactions with 50 mg of insoluble PCS solids per ml were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

As shown in Figure 27A, replacement of 5% protein in *Trichoderma reesei*-based XCL-602 cellulase by *Thielavia terrestris* GH61E polypeptide did not provide an advantage over the equivalent replacement by *Thermoascus aurantiacus* GH61A polypeptide. Similarly, as shown in Figure 27B, the replacement of 5% protein in *Trichoderma reesei*-based XCL-602 cellulase by *Thielavia terrestris* GH61E polypeptide along with the 30% replacement by *Aspergillus fumigatus* Cel7A CBHI and 20% replacement by *Myceliophthora thermophila* Cel6A CBHII, did not provide an advantage over the equivalent replacement by

Thermoascus aurantiacus GH61A polypeptide. Each GH61 polypeptide was tested at six different replacement levels ranging from 5% to 20%, and the conclusion was consistent for all replacement levels (data not shown). The results indicated that the *Thielavia terrestris* GH61E polypeptide did not synergize with the *Thermoascus aurantiacus* GH61A polypeptide under these conditions.

Example 61: Comparison of XCL-602-based enzyme compositions containing different replacement levels of *Thermoascus aurantiacus* GH61A or *Thielavia terrestris* GH61E GH61 polypeptides having cellulolytic enhancing activity in hydrolysis of milled unwashed PCS at 50-60°C

Thermoascus aurantiacus GH61A and *Thielavia terrestris* GH61E GH61 polypeptides having cellulolytic enhancing activity were tested separately at six different replacement levels (5%, 7.5%, 10%, 12.5%, 15%, and 20%) in different *Trichoderma reesei*-based XCL-602 cellulase compositions at 50-60°C using milled unwashed PCS as a substrate. In one case, the *Trichoderma reesei*-based XCL-602 cellulase compositions comprised different replacement levels of a GH61 polypeptide along with a 5% replacement by *Aspergillus fumigatus* GH10 xyn3 xylanase. In another case, *Trichoderma reesei*-based XCL-602 cellulase compositions comprised different replacement levels of a GH61 polypeptide along with a 30% replacement by *Aspergillus fumigatus* Cel7A CBHI, a 20% replacement by *Myceliophthora thermophila* Cel6A CBHII, and a 5% replacement by *Aspergillus fumigatus* GH10 xyn3 xylanase. All enzyme compositions were tested at 3 mg total protein per g cellulose. For comparison, *Trichoderma reesei*-based XCL-533 cellulase was tested at 4.5 mg protein per g cellulose.

The assay was performed as described in Example 34. The 1 ml reactions with 50 mg of insoluble PCS solids per ml were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

The results shown in Table 3 demonstrated that the optimal replacement level for both GH61 polypeptides at 50°C and 55°C was 10-12.5% of the total protein in the *Trichoderma reesei*-based XCL-602 cellulase compositions. At 60°C, the optimal replacement level for both GH61 polypeptides was 20% of the total protein in the *Trichoderma reesei*-based XCL-602 cellulase compositions. The equivalent replacement levels of the *Thermoascus aurantiacus* GH61A and *Thielavia terrestris* GH61E polypeptides in similar *Trichoderma reesei*-based XCL-602 cellulase compositions provided a similar enhancement of the cellulose hydrolysis in milled unwashed PCS at 50-60°C.

Table 3. Comparison of *Trichoderma reesei*-based XCL-602 cellulase compositions

containing different replacement levels of *Thermoascus aurantiacus* GH61A or *Thielavia terrestris* GH61E polypeptides in hydrolysis of milled unwashed PCS at 50-60°C.

GH61 replacement level	50°C	55°C	60°C
XCL-602 with 5% replacement by *Aspergillus fumigatus* GH10 xyn3 xylanase and 0-20% replacement by a GH61 polypeptide (3 mg total protein per g cellulose)			
0% *T. aurantiacus* GH61A	67%	66%	42%
5% *T. aurantiacus* GH61A	69%	69%	46%
7.5% *T. aurantiacus* GH61A	69%	69%	47%
10% *T. aurantiacus* GH61A	69%	69%	49%
12.5% *T. aurantiacus* GH61A	69%	69%	49%
15% *T. aurantiacus* GH61A	69%	69%	49%
20% *T. aurantiacus* GH61A	66%	69%	51%
0% *T. terrestris* GH61E	67%	66%	42%
5% *T. terrestris* GH61E	68%	69%	45%
7.5% *T. terrestris* GH61E	68%	68%	46%
10% *T. terrestris* GH61E	68%	68%	47%
12.5% *T. terrestris* GH61E	65%	68%	48%
15% *T. terrestris* GH61E	64%	67%	48%
20% *T. terrestris* GH61E	62%	65%	49%
XCL-602 with 30% replacement by *Aspergillus fumigatus* Cel7A CBHI, 20% replacement by *Myceliophthora thermophila* Cel6A CBHII, 5% replacement by *Aspergillus fumigatus* GH10 xyn3 xylanase and 0-20% replacement by a GH61 polypeptide (3 mg total protein per g cellulose)			
0% *T. aurantiacus* GH61A	64%	68%	58%
5% *T. aurantiacus* GH61A	70%	75%	66%
7.5% *T. aurantiacus* GH61A	72%	75%	67%
10% *T. aurantiacus* GH61A	72%	76%	68%
12.5% *T. aurantiacus* GH61A	71%	76%	70%
15% *T. aurantiacus* GH61A	71%	76%	69%
20% *T. aurantiacus* GH61A	68%	72%	71%
0% *T. terrestris* GH61E	64%	68%	58%
5% *T. terrestris* GH61E	68%	72%	65%
7.5% *T. terrestris* GH61E	69%	73%	65%
10% *T. terrestris* GH61E	69%	74%	69%
12.5% *T. terrestris* GH61E	67%	73%	68%

GH61 replacement level	50°C	55°C	60°C
15% *T. terrestris* GH61E	66%	74%	69%
20% *T. terrestris* GH61E	65%	72%	69%

Example 62: Hydrolysis of milled unwashed PCS by non-replaced XCL-602 and various XCL-602-based enzyme compositions (3 mg protein per g cellulose) in comparison with *Trichoderma reesei*-based XCL-533 cellulase (4.5 mg protein per g cellulose) at 50-60°C

Example 61 was repeated using the same *Trichoderma reesei*-based XCL-602 cellulase compositions except that only one GH61 polypeptide (*Thermoascus aurantiacus* GH61A) was tested at a single replacement level (10% of total protein). The *Trichoderma reesei*-based XCL-602 cellulase and all *Trichoderma reesei*-based XCL-602 cellulase compositions were used at 3 mg total protein per g cellulose.

The assay was performed as described in Example 34. The 1 ml reactions with 50 mg of insoluble PCS solids per ml were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

The results shown in Figure 28A demonstrated that the replacement of *Trichoderma reesei*-based XCL-602 cellulase with a composition comprising 5% *Aspergillus fumiga*tus GH10 xyn3 xylanase and 10% *Thermoascus aurantiacus* GH61A polypeptide to a total protein loading of 3 mg protein per g cellulose has significantly improved the hydrolysis yield over the non-replaced *Trichoderma reesei*-based XCL-602 cellulase at all three temperatures. As shown in Figure 27B, the enhancement was even more pronounced when the *Trichoderma reesei*-based XCL-602 cellulase was additionally replaced with 30% *Aspergillus fumigatus* Cel7A CBHI and 20% *Myceliophthora thermophila* Cel6A CBHII. The results shown in Figure 28B demonstrated that the replacement of *Trichoderma reesei*-based XCL-602 cellulase with 30% *Aspergillus fumigatus* Cel7A CBHI and 20% *Myceliophthora thermophila* Cel6A CBHII increased the degree of cellulose conversion to glucose after 72 hours of hydrolysis at 60°C from 37% to 48%. Additional 5% replacement by *Aspergillus fumigatus* GH10 xyn3 xylanase increased the degree of cellulose conversion to glucose to 58%, and additional 10% replacement by *Thermoascus aurantiacus* GH61A polypeptide increased the degree of cellulose conversion to glucose to 68% compared to 37% obtained with non-replaced *Trichoderma reesei*-based XCL-602 cellulase. At 3 mg protein per g cellulose, the best XCL-602-based enzyme composition comprising the XCL-602 cellulase with 30% replacement by *Aspergillus fumigatus* Cel7A CBHI, 20% replacement by *Myceliophthora thermophila* Cel6A CBHII, 5% replacement by *Aspergillus*

fumigatus GH10 xyn3 xylanase and 10% replacement by *Thermoascus aurantiacus* GH61A polypeptide, was capable of achieving the same cellulose conversion of milled unwashed PCS at 55°C (76%) as non-replaced *Trichoderma reesei*-based XCL-602 cellulase at 4.5 mg protein per g cellulose and 50°C - a 1.5-fold reduction in protein loading.

Example 63: Preparation of *Penicillium emersonii* strain NN051602 GH7 cellobiohydrolase I

The *Penicillium emersonii* strain NN051602 Cel7 cellobiohydrolase I (SEQ ID NO: 157 [DNA sequence] and SEQ ID NO: 158 [deduced amino acid sequence]) was obtained according to the procedure described below.

Penicillium emersonii was grown on a PDA agar plate at 45°C for 3 days. Mycelia were collected directly from the agar plate into a sterilized mortar and frozen under liquid nitrogen. Frozen mycelia were ground, by mortar and pestle, to a fine powder, and genomic DNA was isolated using a DNeasy® Plant Mini Kit (QIAGEN Inc., Valencia, CA, USA).

Oligonucleotide primers, shown below, were designed to amplify the GH7 cellobiohydrolase I gene from genomic DNA of *Penicillium emersonii*. An IN-FUSION™ CF Dry-down Cloning Kit (Clontech Laboratories, Inc., Mountain View, CA, USA) was used to clone the fragment directly into the expression vector pPFJO355, without the need for restriction digestion and ligation.

Sense primer:

5'-ACACAACTGGGGATCCACC **atgcttcgacgggctcttc**-3' (SEQ ID NO: 207)

Antisense primer:

5'-GTCACCCTCTAGATCT **CGCAGAGCAACTTCCGTCTACTTC**-3' (SEQ ID NO: 208)

Bold letters represented the coding sequence (for the sense primer) or the downstream sequence of the coding region (for the antisense primer). The remaining sequence was homologous to the insertion sites of pPFJO355.

Twenty picomoles of each of the primers above were used in a PCR reaction composed of *Penicillium emersonii* genomic DNA, 10 µl of 5X GC Buffer, 1.5 µl of DMSO, 2.5 mM each of dATP, dTTP, dGTP, and dCTP, and 0.6 unit of Phusion™ High-Fidelity DNA Polymerase in a final volume of 50 µl. The amplification was performed using a Peltier Thermal Cycler programmed for denaturing at 98°C for 1 minute; 8 cycles of denaturing at 98°C for 15 seconds, annealing at 65°C for 30 seconds, with 1°C decrease per cycle and elongation at 72°C for 80 seconds; and another 23 cycles each at 98°C for 15 seconds, 66°C for 30 seconds and 72C for 75 seconds; final extension at 72°C for 7 minutes. The heat block then went to a 4°C soak cycle.

The reaction products were isolated by 1.0% agarose gel electrophoresis using 90mM Tris-borate and 1 mM EDTA (TBE) buffer where an approximately 1.4 kb product

band was excised from the gel, and purified using an ILLUSTRA® GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

Plasmid pPFJO355 was digested with *Bam* I and *Bgl* II, isolated by 1.0% agarose gel electrophoresis using TBE buffer, and purified using an ILLUSTRA® GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

The gene fragment and the digested vector were ligated together using an IN-FUSION™ CF Dry-down PCR Cloning resulting in pGH7_ZY209383 in which transcription of the *Penicillium emersonii* GH7 cellobiohydrolase I gene was under the control of the *Aspergillus oryzae* TAKA amylase promoter from the gene for *Aspergillus oryzae* alpha-amylase. The cloning operation was according to the manufacturer's instruction. In brief, 30 ng of pPFJO355 digested with *Bam* I and *Bgl* II, and 60 ng of the *Penicillium emersonii* GH7 cellobiohydrolase I gene purified PCR product were added to the reaction vial and resuspended the powder in a final volume of 10ul with addition of deionized water. The reaction was incubated at 37°C for 15 minutes and then 50°C for 15 minutes. Five µl of the reaction were used to transform *E. coli* TOP10 competent cells. An *E. coli* transformant containing pGH7_ZY209383 was detected by colony PCR and plasmid DNA was prepared using a QIAprep Spin Miniprep Kit (QIAGEN Inc., Valencia, CA, USA). The *Penicillium emersonii* GH7 cellobiohydrolase I gene insert in pGH7_ZY209383 was confirmed by DNA sequencing using a 3730XL DNA Analyzer.

Aspergillus oryzae HowB101 (WO 95/35385) protoplasts were prepared according to the method of Christensen *et al.*, 1988, *Bio/Technology* 6: 1419-1422 and transformed with 3 µg of pGH7_ZY209383. The transformation yielded about 50 transformants. Twelve transformants were isolated to individual Minimal medium plates.

Four transformants were inoculated separately into 3 ml of YPM medium in a 24-well plate and incubated at 30°C, 150 rpm. After 3 days incubation, 20 µl of supernatant from each culture were analyzed by SDS-PAGE using a NUPAGE® NOVEX® 4-12% Bis-Tris Gel with MES buffer according to the manufacturer's instructions. The resulting gel was stained with INSTANT® Blue. SDS-PAGE profiles of the cultures showed that the majority of the transformants had a major smeary band of approximately 50 kDa. The expression strain was designated as *A. oryzae* EXP03477.

A slant of *A. oryzae* EXP03477 was washed with 10 ml of YPM medium and inoculated into a 2 liter flask containing 400 ml of YPM medium to generate broth for characterization of the enzyme. The culture was harvested on day 3 and filtered using a 0.45 µm DURAPORE Membrane (Millipore, Bedford, MA, USA).

A 1600 ml volume of filtered broth of *A. oryzae* EXP03477 was precipitated with ammonium sulfate (80% saturation), re-dissolved in 100 ml of 25 mM Bis-Tris pH 6.5 buffer, dialyzed against the same buffer, and filtered through a 0.45 µm filter; the final volume was

200 ml. The solution was applied to a 40 ml Q Sepharose® Fast Flow column equilibrated with 25 mM Bis-Tris pH 6.5, and the proteins were eluted with a linear NaCl gradient (0–0.4 M). Fractions with activity against PASC were collected and applied to a 40 ml Phenyl Sepharose™ HIC column (GE Healthcare, Piscataway, NJ, USA) equilibrated in 20 mM PBS with 1.8 M $(NH_4)_2SO_4$ pH 7 buffer, and the proteins were eluted with 20 mM PBS pH 7. Fractions from the column with activity toward phosphoric acid swollen cellulose (PASC) as substrate were analyzed by SDS-PAGE using a NUPAGE® NOVEX® 4-12% Bis-Tris Gel with MES buffer. Fractions with the correct molecular weight were pooled. Then the pooled solution was concentrated by ultrafiltration. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 64: Preparation of *Penicillium pinophilium* strain NN046877 GH7 cellobiohydrolase I

The *Penicillium pinophilium* strain NN046877 Cel7 cellobiohydrolase II (SEQ ID NO: 159 [DNA sequence] and SEQ ID NO: 160 [deduced amino acid sequence]) was obtained according to the procedure described below.

Penicillium pinophilum was grown on a PDA agar plate at 37°C for 4-5 days. Mycelia were collected directly from the agar plate into a sterilized mortar and frozen under liquid nitrogen. Frozen mycelia were ground, by mortar and pestle, to a fine powder, and genomic DNA was isolated using a DNeasy® Plant Mini Kit.

Oligonucleotide primers, shown below, were designed to amplify the GH7 cellobiohydrolase I gene from the genomic DNA of *Penicillium pinophilum*. An IN-FUSION™ CF Dry-down Cloning Kit (Clontech Laboratories, Inc., Mountain View, CA, USA) was used to clone the fragment directly into the expression vector pPFJO355, without the need for restriction digestion and ligation.

Sense primer:

5'-**ACACAACTGGGGATCCACC**ATGTCTGCCTTGAACTCTTTC-3' (SEQ ID NO: 209)

Antisense primer:

5'-**GTCACCCTCTAGATCT**TCACAAACATTGAGAGTAGTAAGGGTT-3' (SEQ ID NO: 210)

Bold letters represented the coding sequence and the remaining sequence was homologous to the insertion sites of pPFJO355.

Twenty picomoles of each of the primers above were used in a PCR reaction composed of *Penicillium pinophilum* genomic DNA, 10 µl of 5X GC Buffer, 1.5 µl of DMSO, 2.5 mM each of dATP, dTTP, dGTP, and dCTP, and 0.6 unit of Phusion™ High-Fidelity DNA Polymerase in a final volume of 50 µl. The amplification was performed using a Peltier Thermal Cycler programmed for denaturing at 98°C for 1 minute; 5 cycles of denaturing at

98°C for 15 seconds, annealing at 56°C for 30 seconds, with 1°C increasing per cycle and elongation at 72°C for 75 seconds; and another 25 cycles each at 98°C for 15 seconds, 65C for 30 seconds and 72°C for 75 seconds; final extension at 72°C for 10 minutes. The heat block then went to a 4°C soak cycle.

The reaction products were isolated by 1.0% agarose gel electrophoresis using 90mM Tris-borate and 1 mM EDTA (TBE) buffer where an approximately 1.6kb product band was excised from the gel, and purified using an ILLUSTRA® GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

Plasmid pPFJO355 was digested with *Bam* I and *Bgl* II, isolated by 1.0% agarose gel electrophoresis using TBE buffer, and purified using an ILLUSTRA® GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

The gene fragment and the digested vector were ligated together using an IN-FUSION™ CF Dry-down PCR Cloning resulting in pPpin6 in which transcription of the *Penicillium pinophilum* GH7 cellobiohydrolase I gene was under the control of the *Aspergillus oryzae* TAKA amylase promoter. In brief, 30 ng of pPFJO355 digested with *Bam* I and *Bgl* II, and 100 ng of the *Penicillium pinophilum* GH7 cellobiohydrolase I gene purified PCR product were added to a reaction vial and resuspended in a final volume of 10 µl with addition of deionized water. The reaction was incubated at 37°C for 15 minutes and then 50°C for 15 minutes. Three µl of the reaction were used to transform *E. coli* TOP10 competent cells. An *E. coli* transformant containing pPpin6 was detected by colony PCR and plasmid DNA was prepared using a QIAprep Spin Miniprep Kit (QIAGEN Inc., Valencia, CA, USA). The *Penicillium pinophilum* GH7 cellobiohydrolase I gene insert in pPpin6 was confirmed by DNA sequencing using a 3730XL DNA Analyzer.

Aspergillus oryzae HowB101 (WO 95/35385) protoplasts were prepared according to the method of Christensen *et al.*, 1988, *Bio/Technology* 6: 1419-1422 and transformed with 3 µg of pPpin6. The transformation yielded about 50 transformants. Four transformants were isolated to individual Minimal medium plates.

Four transformants were inoculated separately into 3 ml of YPM medium in a 24-well plate and incubated at 30°C, 150 rpm. After 3 days incubation, 20 µl of supernatant from each culture were analyzed by SDS-PAGE using a NUPAGE® NOVEX® 4-12% Bis-Tris Gel with MES buffer according to the manufacturer's instructions. The resulting gel was stained with INSTANT® Blue. SDS-PAGE profiles of the cultures showed that the majority of the transformants had a major smear band of approximately 60-90 kDa. The expression strain was designated as *A. oryzae* EXP02768.

A slant of *A. oryzae* EXP02768 was washed with 10 ml of YPM medium and inoculated into a 2 liter flask containing 400 ml of YPM medium to generate broth for characterization of the enzyme. The culture was harvested on day 3 and filtered using a 0.45

µm DURAPORE Membrane (Millipore, Bedford, MA, USA).

A 1600 ml volume of the filtered broth of *A. oryzae* EXP02768 was precipitated with ammonium sulfate (80% saturation), re-dissolved in 100 ml of 25 mM Bis-Tris pH 6.0, dialyzed against the same buffer, and filtered through a 0.45 µm filter; the final volume was 200 ml. The solution was applied to a 40 ml Q Sepharose® Fast Flow column equilibrated in 25 mM Bis-Tris pH 6.0, and the proteins were eluted with a linear NaCl gradient (0–0.4 M). Fractions from the column with activity against pNP-β-D-lactopyranoside were analyzed by SDS-PAGE using a NUPAGE® NOVEX® 4-12% Bis-Tris Gel with MES buffer. Fractions with the correct molecular weight were pooled and concentrated by ultrafiltration. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 65: Preparation of *Aspergillus terreus* ATCC 28865 GH7 cellobiohydrolase I

The *Aspergillus terreus* ATCC 28865 GH7 cellobiohydrolase I (SEQ ID NO: 161 [DNA sequence] and SEQ ID NO: 162 [deduced amino acid sequence]) was obtained according to the procedure described below.

Two synthetic oligonucleotide primers, shown below, were designed to PCR amplify the cellobiohydrolase I gene from *Aspergillus terreus* ATCC 28865 genomic DNA. Genomic DNA was isolated using a FastDNA spin for Soil Kit (MP Biomedicals, OH, USA).

Primer #222:

5'-TAAGAATTCACCATGCCTTCCACCTACGA-3' (SEQ ID NO: 211)

Primer #302:

5'-TATGCGGCCGCATTCTCCTAGACACCCCGCAT-3' (SEQ ID NO: 212)

The amplification reaction was composed of 1 µl of *Aspergillus terreus* genomic DNA, 12.5 µl of 2X REDDYMIX™ PCR Buffer (Thermo Fisher Scientific Inc., Waltham, MA, USA), 1 µl of 5 µM primer #222, 1 µl of 5 µM primer #302, and 9.5 µl of H_2O. The amplification reaction was incubated in a PTC-200 DNA ENGINE™ Thermal Cycler programmed for 1 cycle at 94°C for 2 minutes; and 35 cycles each at 94°C for 15 seconds and 60°C for 1.5 minutes.

A 1.7 kb PCR reaction product was isolated by 1% agarose gel electrophoresis using TAE buffer and staining with SYBR Safe DNA gel stain (Invitrogen Corp., Carlsbad, CA, USA). The DNA band was visualized with the aid of an Eagle Eye Imaging System and a DarkReader Transilluminator (Clare Chemical Research, Dolores, CO, USA). The 1.7 kb DNA band was excised from the gel and purified using a GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

The 1.7 kb fragment was cleaved with *EcoR* I and *Not* I and purified using a GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

The cleaved 1.7 kb fragment was then directionally cloned by ligation into *Eco* RI-*Not* I cleaved pXYG1051 (WO 2005/080559) using T4 ligase (Promega, Madison, WI, USA) according to the manufacturer's instructions. The ligation mixture was transformed into *E. coli* TOP10F competent cells according to the manufacturer's instructions. The transformation mixture was plated onto LB plates supplemented with 100 µg of ampicillin per ml. Plasmid minipreps were prepared from several transformants and sequenced. One plasmid with the correct *Aspergillus terreus* GH7 coding sequence was chosen. The plasmid was designated pXYG1051-NP003568.

The expression plasmid pXYG1051-NP003568 was transformed into *Aspergillus oryzae* JaL355 as described in WO 98/00529. Transformants were purified on selection plates through single conidia prior to sporulating them on PDA plates. Production of the *Aspergillus terreus* GH7 polypeptide by the transformants was analyzed from culture supernatants of 1 ml 96 deep well stationary cultivations at 26°C in YP medium with 2% maltodextrin. Expression was verified by SDS-PAGE using a NUPAGE® NOVEX® 4-12% Bis-Tris Gel with MES buffer and Coomassie blue staining. One transformant was selected for further work and designated *Aspergillus oryzae* 64.1.

For larger scale production, *Aspergillus oryzae* 64.1 spores were spread onto a PDA plate and incubated for five days at 37°C. The confluent spore plate was washed twice with 5 ml of 0.01% TWEEN® 20 to maximize the number of spores collected. The spore suspension was then used to inoculate twenty-five 500 ml flasks containing 100 ml of YPG medium. The culture was incubated at 30°C with constant shaking at 120 rpm. At day four post-inoculation, the culture broth was collected by filtration through a triple layer of Whatman glass microfiber filters of 1.6 µm, 1.2 µm, and 0.7 µm. Fresh culture broth from this transformant produced a band of GH7 protein of approximately 55 kDa. The identity of this band as the *Aspergillus terreus* GH7 polypeptide was verified by peptide sequencing.

Two liters of the filtered broth were concentrated to 400 ml and washed with 50 mM HEPES pH 7.0 using a SARTOFLOW® Alpha ultrafiltration system with a 10 kDa MW-CO (Sartorius Stedim Biotech S.A., Aubagne Cedex, France). Ammonium sulphate was added to a final concentration of 1 M and dissolved in the ultrafiltrate. The solution was applied on a Source 15 Phenyl XK 26/20 50 ml column (GE Healthcare, Hillerød, Denmark). After application the column was washed with 150 ml of 1 M ammonium sulphate and eluded with 1 column volume of 50% ethanol in a 0% to 100% gradient followed by 5 column volumes of 50% ethanol at a flow rate of 10 ml/minute. Fractions of 10 ml were collected and analyzed by SDS-PAGE. Fraction 3 to 8 were pooled and diluted to 1000 ml with 50 mM HEPES pH 7.0 before application on a Q Sepharose® Fast Flow column XK26/20 60 ml column (GE Healthcare, Hillerød, Denmark). After application the column was washed 3 times with 60 ml of 50 mM HEPES pH 7.0 and eluded with 100 ml of 50 mM HEPES pH 7.0, 1 M NaCl at a

flow rate of 10 ml/minute. Fractions of 10 ml were collected and analyzed by SDS-PAGE. The flow-through and first wash were pooled and concentrated to 400 ml and washed with 50 mM HEPES pH 7.0 using the ultrafiltration system described above. Further concentration was conducted using a VIVASPIN™ centrifugal concentrator according to the manufacturer's instructions to a final volume of 80 ml. The protein concentration was determined by A280/A260 absorbance. Protein concentration was also determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 66: Preparation of *Neosartorya fischeri* strain NRRL 181 GH7 cellobiohydrolase I

The *Neosartorya fischeri* NRRL 181 GH7 cellobiohydrolase I (SEQ ID NO: 163 [DNA sequence] and SEQ ID NO: 164 [deduced amino acid sequence]) was obtained according to the procedure described below.

Two synthetic oligonucleotide primers, shown below, were designed to PCR amplify the cellobiohydrolase I gene from *Neosartorya fischeri* genomic DNA. Genomic DNA was isolated using a FastDNA Spin for Soil Kit.

Primer #374:

5'-TAAGAATTCACCATGCCTTCCACCTACGA-3' (SEQ ID NO: 213)

Primer #375:

5'-TATGCGGCCGCATTCTCCTAGACACCCCGCAT-3' (SEQ ID NO: 214)

The amplification reaction was composed of 1 µl of *Neosartorya fischeri* genomic DNA, 12.5 µl of 2X REDDYMIX™ PCR Buffer, 1 µl of 5 µM primer #374, 1 µl of 5 µM primer #375, and 9.5 µl of H_2O. The amplification reaction was incubated in a PTC-200 DNA ENGINE™ Thermal Cycler programmed for 1 cycle at 94°C for 2 minutes; and 35 cycles each at 94°C for 15 seconds and 60°C for 1.5 minutes.

A 1.6 kb PCR reaction product was isolated by 1% agarose gel electrophoresis using TAE buffer and staining with SYBR Safe DNA gel stain. The DNA band was visualized with the aid of an Eagle Eye Imaging System and a DarkReader Transilluminator. The 1.6 kb DNA band was excised from the gel and purified using a GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

The 1.6 kb fragment was cleaved with *EcoR* I and *Not* I and purified using a GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

The cleaved 1.6 kb fragment was then directionally cloned by ligation into *Eco* RI-*Not* I cleaved pXYG1051 (WO 2005/080559) using T4 ligase according to the manufacturer's instructions. The ligation mixture was transformed into *E. coli* TOP10F competent cells according to the manufacturer's instructions. The transformation mixture was plated onto LB

plates supplemented with 100 µg of ampicillin per ml. Plasmid minipreps were prepared from several transformants and sequenced. One plasmid with the correct *Neosartorya fischeri* GH7 cellobiohydrolase coding sequence was chosen. The plasmid was designated pXYG1051-NP003786.

The expression plasmid pXYG1051-NP003786 was transformed into *Aspergillus oryzae* JaL355 as described in WO 98/00529. Transformants were purified on selection plates to single conidia prior to sporulating them on PDA plates. Production of the *Neosartorya fischeri* GH7 cellobiohydrolase by the transformants was analyzed from culture supernatants of 1 ml 96 deep well stationary cultivations at 26°C in YP medium with 2% maltodextrin. Expression was verified by SDS-PAGE using a NUPAGE® NOVEX® 4-12% Bis-Tris Gel with MES buffer and Coomassie blue staining. One transformant was selected for further work and designated *Aspergillus oryzae* 92.7.

For larger scale production, *Aspergillus oryzae* 92.7 spores were spread onto a PDA plate and incubated for five days at 37°C. The confluent spore plate was washed twice with 5 ml of 0.01% TWEEN® 20 to maximize the number of spores collected. The spore suspension was then used to inoculate twenty-five 500 ml flasks containing 100 ml of YPM medium. The culture was incubated at 26°C with constant shaking at 120 rpm. At day five post-inoculation, the culture broth was collected by filtration through a triple layer of Whatman glass microfiber filters of 1.6 µm, 1.2 µm, and 0.7 µm. Fresh culture broth from this transformant produced a band of GH7 protein of approximately 70 kDa. The identity of this band as the *Neosartorya fischeri* GH7 cellobiohydrolase was verified by peptide sequencing.

Two liters of the filtered broth were concentrated to 400 ml and washed with 50 mM HEPES pH 7.0 using a SARTOFLOW® Alpha ultrafiltration system with a 10 kDa MW-CO. Ammonium sulphate was added to a final concentration of 1 M and dissolved in the ultrafiltrate. The solution was applied to a Source 15 Phenyl XK 26/20 50 ml column. After application the column was washed with 150 ml of 1 M ammonium sulphate and eluded with 1 column volume of 50% ethanol in a 0% to 100% gradient followed by 5 column volumes of 50% ethanol at a flow rate of 10 ml/minute. Fractions of 10 ml were collected and analyzed by SDS-PAGE. Fraction 3 to 8 were pooled and diluted to 1000 ml with 50 mM HEPES pH 7.0 before application to a Q Sepharose® Fast Flow XK26/20 60 ml column. After application the column was washed 3 times with 60 ml of 50 mM HEPES pH 7.0 and eluded with 100 ml of 50 mM HEPES pH 7.0, 1 M NaCl at a flow rate of 10 ml/minute. Fractions of 10 ml were collected and analyzed by SDS-PAGE. The flow-through and first wash were pooled and concentrated to 400 ml and washed with 50 mM HEPES pH 7.0 using a SARTOFLOW® Alpha ultrafiltration system with a 10 kDa MW-CO. Further concentration was conducted using a VIVASPIN™ centrifugal concentrator according to the manufacturer's instructions to a final volume of 80 ml. The protein concentration determined

by A280/A260 absorbance. Protein concentration was also determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 67: Preparation of *Aspergillus nidulans* strain FGSCA4 GH7 cellobiohydrolase I

The *Aspergillus nidulans* strain FGSCA4 GH7 cellobiohydrolase I (SEQ ID NO: 165 [DNA sequence] and SEQ ID NO: 166 [deduced amino acid sequence]) was obtained according to the procedure described below.

Two synthetic oligonucleotide primers, shown below, were designed to PCR amplify the cellobiohydrolase I gene from *Aspergillus nidulans* genomic DNA. Genomic DNA was isolated using a FastDNA Spin for Soil Kit.

Primer #376:

5'-TAACAATTGACCATGGCATCTTCATTCCAGTTGTA-3' (SEQ ID NO: 215)

Primer #377:

5'-TATGCGGCCGCGTCTCCCATTTACGACCCACCA-3' (SEQ ID NO: 216)

The amplification reaction was composed of 1 µl of *Aspergillus nidulans* genomic DNA, 12.5 µl of 2X REDDYMIX™ PCR Buffer, 1 µl of 5 µM primer #374, 1 µl of 5 µM primer #375, and 9.5 µl of H_2O. The amplification reaction was incubated in a PTC-200 DNA ENGINE™ Thermal Cycler programmed for 1 cycle at 94°C for 2 minutes; and 35 cycles each at 94°C for 15 seconds and 60°C for 1.5 minutes.

A 1.6 kb PCR reaction product was isolated by 1% agarose gel electrophoresis using TAE buffer and staining with SYBR Safe DNA gel stain. The DNA band was visualized with the aid of an Eagle Eye Imaging System and a DarkReader Transilluminator. The 1.6 kb DNA band was excised from the gel and purified using a GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

The 1.6 kb fragment was cleaved with *Mfe* I and *Not* I and purified using a GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

The cleaved 1.6 kb fragment was then directionally cloned by ligation into *Eco* RI-*Not* I cleaved pXYG1051 (WO 2005/080559) using T4 ligase according to the manufacturer's instructions. The ligation mixture was transformed into *E. coli* TOP10F competent cells according to the manufacturer's instructions. The transformation mixture was plated onto LB plates supplemented with 100 µg of ampicillin per ml. Plasmid minipreps were prepared from several transformants and sequenced. One plasmid with the *Aspergillus nidulans* GH7 coding sequence was chosen. Two mutations introduced during PCR were identified which result in a change of Leu 7 to Trp and of Glu 436 to Gly relative to the public sequence Q8NK02. The plasmid was designated pXYG1051-NP003787.

The expression plasmid pXYG1051-NP003787 was transformed into *Aspergillus*

oryzae JaL355 as described in WO 98/00529. Transformants were purified on selection plates to single conidia prior to sporulating them on PDA plates. Production of the *Aspergillus nidulans* GH7 cellobiohydrolase by the transformants was analyzed from culture supernatants of 1 ml 96 deep well stationary cultivations at 26°C in YP medium with 2% maltodextrin. Expression was verified by SDS-PAGE using a NUPAGE® NOVEX® 4-12% Bis-Tris Gel with MES buffer and Coomassie blue staining. One transformant was selected for further work and designated *Aspergillus oryzae* 70.5.

For larger scale production, *Aspergillus oryzae* 70.5 spores were spread onto a PDA plate and incubated for five days at 37°C. The confluent spore plate was washed twice with 5 ml of 0.01% TWEEN® 20 to maximize the number of spores collected. The spore suspension was then used to inoculate twenty-five 500 ml flasks containing 100 ml of YPM medium. The culture was incubated at 26°C with constant shaking at 120 rpm. At day six post-inoculation, the culture broth was collected by filtration through a triple layer of Whatman glass microfiber filters of 1.6 µm, 1.2 µm, and 0.7 µm. Fresh culture broth from this transformant produced a band of GH7 protein of approximately 54 kDa. The identity of this band as the *Aspergillus nidulans* GH7 cellobiohydrolase was verified by peptide sequencing.

Two liters of the filtered broth were concentrated to 400 ml and washed with 50 mM HEPES pH 7.0 using a SARTOFLOW® Alpha ultrafiltration system with a 10 kDa MW-CO. Ammonium sulphate was added to a final concentration of 1 M and dissolved in the ultrafiltrate. The solution was applied on a Source 15 Phenyl XK 26/20 50 ml column. After application the column was washed with 150 ml of 1 M ammonium sulphate and eluded with 1 column volume of 50% ethanol in a 0% to 100% gradient followed by 5 column volumes of 50% ethanol at a flow rate of 10 ml/minute. Fractions of 10 ml were collected and analyzed by SDS-PAGE. Fraction 3 to 8 were pooled and diluted to 1000 ml with 50 mM HEPES pH 7.0 before application on a Q Sepharose® Fast Flow XK26/20 60 ml column. After application the column was washed 3 times with 60 ml of 50 mM HEPES pH 7.0 and eluded with 100 ml of 50 mM HEPES pH 7.0, 1 M NaCl at a flow rate of 10 ml/minute. Fractions of 10 ml were collected and analyzed by SDS-PAGE. The flow-through and first wash were pooled and concentrated to 400 ml and washed with 50 mM HEPES pH 7.0 using a SARTOFLOW® Alpha ultrafiltration system with a 10 kDa MW-CO. Further concentration was conducted using a VIVASPIN™ centrifugal concentrator according to the manufacturer's instructions to a final volume of 80 ml. The protein concentration determined by A280/A260 absorbance. Protein concentration was also determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 68: Preparation of a *Fennellia nivea* strain NN046949 GH6 cellobiohydrolase II

The *Fennellia nivea* strain NN046949 GH6 cellobiohydrolase II (SEQ ID NO: 167 [DNA sequence] and SEQ ID NO: 168 [deduced amino acid sequence]) was obtained according to the procedure described below.

Fennellia nivea strain NN046949 was isolated from a soil from Yunnan, China by directly plating the soil sample onto a PDA plate followed by incubation at 37°C for 5 days. The strain was then purified by transferring the mycelia onto a YG agar plate. The *Fennellia nivea* strain NN046949 was identified as *Fennellia nivea* based on both morphological and molecular (ITS sequencing) characterization.

Fennellia nivea strain NN046949 was inoculated onto a PDA plate and incubated for 4 days at 37°C in the darkness. Several mycelia-PDA plugs were inoculated into 500 ml shake flasks containing 100 ml of NNCYP-PCS medium. The flasks were incubated for 6 days at 37°C with shaking at 160 rpm. The mycelia were collected at 4, 5, and 6 days, and each frozen in liquid nitrogen and stored in a -80°C freezer until use.

The frozen *F. nivea* mycelia were mixed and transferred into a liquid nitrogen prechilled mortar and pestle and ground to a fine powder. Total RNA was prepared from the powdered mycelia of each day by extraction with TRIZOL® reagent and purified using a RNEASY® Plant Mini Kit according to the manufacturer's protocol. The polyA enriched RNA was isolated using a mTRAP™ Total Kit. Eighty-seven µgs of total RNA was submitted for sequencing as described in Example 3.

Total RNA enriched for polyA sequences with the mRNASeq protocol were sequenced using an ILLUMINA® GA2 System (Illumina, Inc., San Diego, CA, USA). The raw 75 base pair reads were assembled and the assembled sequences were analyzed using standard bioinformatics methods for gene finding and functional prediction. Briefly, ESTscan 2.0 was used for gene prediction. NCBI blastall version 2.2.10 and HMMER version 2.1.1 were used to predict function based on structural homology. The Family GH6 cellobiohydrolase was identified directly by analysis of the Blast results.

Fennellia nivea strain NN046949 was grown on PDA agar plate at 37°C for 3 days. Mycelia were collected directly from the agar plate into a sterilized motar and frozen under liquid nitrogen. Frozen mycelia were ground, by mortar and pestle, to a fine powder, and genomic DNA was isolated using a DNEASY® Plant Mini Kit (QIAGEN Inc., Valencia, CA, USA).

Based on the *F. nivea* GH6 cellobiohydrolase gene sequence obtained in Example 3, oligonucleotide primers, shown below, were designed to amplify the gene from genomic DNA of *Fennellia nivea*. An IN-FUSION™ CF Dry-down PCR Cloning Kit was used to clone the fragment directly into the expression vector pPFJO355, without the need for restriction digestion and ligation.

Sense primer:

5'-ACACAACTGGGGATCCACC**ATGGGACGGGTTTCTTCTCTTG**-3' (SEQ ID NO: 217)
Antisense primer:
5'-GTCACCCTCTAGATCT**AAGAACACCCCGCAAAGAAAGTC**-3' (SEQ ID NO: 218)
Bold letters represent the coding sequence for the sense primer or the reverse compliment sequence downstream of the stop codon for the antisense primer. The remaining sequence is homologous to the insertion sites of pPFJO355.

Twenty picomoles of each of the primers above were used in a PCR reaction composed of *Fennellia nivea* genomic DNA, 10 µl of 5X GC Buffer, 1.5 µl of DMSO, 2 µl of 2.5 mM each of dATP, dTTP, dGTP, and dCTP, and 1 unit of Phusion™ High-Fidelity DNA Polymerase in a final volume of 50 µl. The amplification was performed using an Peltier Thermal Cycler programmed for denaturing at 98°C for 1 minutes; 5 cycles of denaturing at 98°C for 15 seconds, annealing at 70°C for 30 seconds, with 1°C decreasing per cycle and elongation at 72°C for 30 seconds; 25 cycles each at 98°C for 15 seconds and 72°C for 90 seconds; and a final extension at 72°C for 10 minutes. The heat block then went to a 4°C soak cycle.

The reaction products were isolated by 1.0% agarose gel electrophoresis using TBE buffer where an approximately 1.8 kb product band was excised from the gel, and purified using an ILLUSTRA™ GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare, Buckinghamshire, UK) according to the manufacturer's instructions.

Plasmid pPFJO355 was digested with *Bam* I and *Bgl* II, isolated by 1.0% agarose gel electrophoresis using TBE buffer, and purified using an ILLUSTRA™ GFX™ PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions. The gene fragment and the digested vector were ligated together using an IN-FUSION™ Dry Down PCR Cloning Kit resulting in pCBHII46949-2 in which transcription of the *Fennellia nivea* GH6 cellobiohydrolase gene was under the control of the *Aspergillus oryzae* TAKA alpha-amylase promoter. In brief, 30 ng of pPFJO355 digested with *Bam* I and *Bgl* II, and 50 ng of the *F. nivea* GH6 cellobiohydrolase gene purified PCR product were added to a reaction vial and resuspended in a final volume of 10 µl with addition of deionized water. The reaction was incubated at 37°C for 15 minutes and then 50°C for 15 minutes. Three µl of the reaction were used to transform *E. coli* TOP10 competent cells. An *E. coli* transformant containing pCBHII46949-2 was detected by colony PCR and plasmid DNA was prepared using a QIAprep Spin Miniprep Kit (QIAGEN Inc., Valencia, CA, USA). The *F. nivea* GH6 cellobiohydrolase gene insert in pCBHII46949-2 was confirmed by DNA sequencing using a 3730XL DNA Analyzer.

The same gene fragment was then incubated in 10X *Taq* DNA polymerase mix (TIANGEN Biotech (Beijing) Co. Ltd., Beijing, China) at 72°C for 20 minutes to add adenine to the 3' end of each nucleotide strand. Then the gene fragment was ligated to pGEM-T

vector using a pGEM-T Vector System to generate pGEM-T-CBHII46949-2. The *Fennellia nivea* cellobiohydrolase gene insert in pGEM-T-CBHII46949-2 was confirmed by DNA sequencing using a 3730XL DNA Analyzer. *E. coli* strain T-CBHII46949-2, containing pGEM-T-CBHII46949-2, was deposited on October 28, 2010 with the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Mascheroder Weg 1 B, D-38124 Braunschweig, Germany assigned the accession number DSM 24143.

Nucleotide sequence data were scrutinized for quality and all sequences were compared to each other with assistance of PHRED/PHRAP software (University of Washington, Seattle, WA, USA).

The nucleotide sequence and deduced amino acid sequence of the *F. nivea* cellobiohydrolase gene are shown in SEQ ID NO: 167 and SEQ ID NO: 168, respectively. The genomic fragment encodes a polypeptide of 469 amino acids, interrupted by 7 predicted introns of 49 bp (nucleotides 77-125), 247 bp (nucleotides 195-241), 46 bp (nucleotides 570-615), 55 bp (nucleotides 870-924), 50 bp (nucleotides 1063-1112), 46 bp (nucleotides 1371-1416), and 49 bp (nucleotides 1659-1707). The% G+C content of the full-length coding sequence and the mature coding sequence are 57.65% and 60.24%, respectively. Using the SignalP software program (Nielsen *et al.*, 1997, *Protein Engineering* 10: 1-6), a signal peptide of 18 residues was predicted. The predicted mature protein contains 451 amino acids with a predicted molecular mass of 48.77 kDa and an isoelectric point of 5.17. Amino acids 112 to 469 are indicative of a Family 6 glycosyl hydrolase. Based on the deduced amino acid sequence, the cellobiohydrolase appears to fall into the cellobiohydrolase Family GH6 according to Coutinho and Henrissat, 1999, *supra*. Amino acids 22 to 50 are indicative of a CBM domain and amino acids 58 to 111 a linker region.

Aspergillus oryzae HowB101 (WO 95/35385) protoplasts were prepared according to the method of Christensen *et al.*, 1988, *Bio/Technology* 6: 1419-1422 and transformed with 3 µg of pCBHII46949-2. The transformation yielded about 50 transformants. Four transformants were isolated to individual Minimal medium plates.

Four transformants were inoculated separately into 3 ml of YPM medium in 24-well plate and incubated at 30°C, 150 rpm. After 3 days incubation, 20 µl of supernatant from each culture were analyzed by SDS-PAGE using a NUPAGE® NOVEX® 4-12% Bis-Tris Gel with MES buffer according to the manufacturer's instructions. The resulting gel was stained with INSTANTBLUE™ (Expedeon Ltd., Babraham Cambridge, UK). SDS-PAGE profiles of the cultures showed that the majority of the transformants had a band of approximately 60 kDa. One transformant was chosen as the expression strain and designated *Aspergillus oryzae* EXP03324.

A slant of *Aspergillus oryzae* EXP03324 was washed with 10 ml of YPM medium and inoculated into 4 2-liter flasks, containing 400 ml of YPM medium for each, to generate broth

for characterization of the enzyme. The culture was harvested on day 3 by filtering the culture against MIRACLOTH® (CALBIOCHEM, Inc. La Jolla, CA, USA). The filtered culture broth was then again filtered using a 0.45 µm DURAPORE Membrane (Millipore, Bedford, MA, USA).

A 1600 ml volume of the *Aspergillus oryzae* EXP03324 filtered broth was precipitated with ammonium sulfate (80% saturation), re-dissolved in 100 ml 25 mM Bis-Tris pH 6.5 buffer, dialyzed against the same buffer, and filtered through a 0.45 µm filter; the final volume was 200 ml. The solution was applied to a 40 ml Q Sepharose® Fast Flow column equilibrated in 25 mM Bis-Tris pH 6.5 buffer and the proteins were eluted with a linear 0–0.4 M NaCl gradient. Fractions from the column were analyzed by SDS-PAGE using a NUPAGE® NOVEX® 4-12% Bis-Tris Gel with MES buffer. Fractions with a molecular weight of 60 kDa were pooled. Then the pooled solution was concentrated by ultrafiltration and assayed for cellobiohydrolase activity using phosphoric acid swollen cellulose (PASC) as substrate. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

A PASC stock slurry solution was prepared by moistening 5 g of AVICEL® (JRS Pharma GmbH & Co., Rosenberg, Germany) with water, followed by the addition of 150 ml of ice cold 85% *o*-phosphoric acid. The suspension was slowly stirred in an ice-bath for 1 hour. Then 500 ml of ice cold acetone were added while stirring. The slurry was filtered using MIRACLOTH® and then washed three times with 100 ml of ice-cold acetone (drained as dry as possible after each wash). Finally, the filtered slurry was washed twice with 500 ml of water, and again drained as dry as possible after each wash. The PASC was mixed with deionized water to a total volume of 500 ml to a concentration of 10 g/liter, blended to homogeneity using an ULTRA-TURRAX® Homogenizer (Cole-Parmer, Vernon Hills, IL, USA), and stored in a refrigerator for up to one month.

The PASC stock solution was diluted with 50 mM sodium acetate pH 5.0 buffer to a concentration of 2 g/liter, and used as the substrate. To 150 µl of PASC stock solution, 20 µl of enzyme sample were added and the reaction mixture was incubated for 60 minutes with shaking at 850 rpm. At the end of the incubation, 50 µl of 2% NaOH were added to stop the reaction. The reaction mixture was centrifuged at 1,000 x *g*. The released sugars were measured by first mixing 10 µl of the reaction mixture with 90 µl of 0.4% NaOH, followed by 50 µl of 1.5% *p*-hydroxybenzoic acid hydrazide in 2% NaOH (PHBAH, Sigma Chemical Co., St. Loius, MO, USA). The mixture was boiled at 100°C for 5 minutes, and then 100 µl were transferred to a microtiter plate for an absorbance reading at 410 nm using a Spectra Max M2 (Molecular Devices, Sunnyvale, CA, USA). Blanks were made by omitting PASC in the hydrolysis step, and by replacing the hydrolysate with buffer in the sugar determination step.

The assay results demonstrated that the purified enzyme possessed

cellobiohydrolase activity.

Example 69: Preparation of *Penicillium emersonii* strain NN051602 GH6A cellobiohydrolase II

Penicillium emersonii strain NN051602 GH6A cellobiohydrolase II (SEQ ID NO: 169 [DNA sequence] and SEQ ID NO: 170 [deduced amino acid sequence]) was obtained according to the procedure described below.

Penicillium emersonii was grown on a PDA agar plate at 45°C for 3 days. Mycelia were collected directly from the agar plate into a sterilized mortar and frozen under liquid nitrogen. Frozen mycelia were ground, by mortar and pestle, to a fine powder, and genomic DNA was isolated using a DNeasy® Plant Mini Kit (QIAGEN Inc., Valencia, CA, USA).

Oligonucleotide primers, shown below, were designed to amplify the GH6 cellobiohydrolase II gene from genomic DNA of *Penicillium emersonii*. An IN-FUSION™ CF Dry-down Cloning Kit was used to clone the fragment directly into the expression vector pPFJO355, without the need for restriction digestion and ligation.

Sense primer:

5'-ACACAACTGGGGATCCACC**ATGCGGAATCTTCTTGCTCTTGC**-3' (SEQ ID NO: 219)

Antisense primer:

5'-GTCACCCTCTAGATCT**CTAGAACAGCGGGTTAGCATTCGTG**-3' (SEQ ID NO: 220)

Bold letters represented the coding sequence. The remaining sequence was homologous to the insertion sites of pPFJO355.

Twenty picomoles of each of the primers above were used in a PCR reaction composed of *Penicillium emersonii* genomic DNA, 10 µl of 5X HF Buffer, 1.5 µl of DMSO, 5 mM each of dATP, dTTP, dGTP, and dCTP, and 0.6 unit of Phusion™ High-Fidelity DNA Polymerase in a final volume of 50 µl. The amplification was performed using a Peltier Thermal Cycler programmed for denaturing at 98°C for 1 minutes; 8 cycles of denaturing at 98°C for 15 seconds, annealing at 66°C for 30 seconds, with a 1°C decrease per cycle and elongation at 72°C for 70 seconds; and another 25 cycles each at 98°C for 15 seconds, 62°C for 30 seconds and 72°C for 80 seconds; final extension at 72°C for 5 minutes. The heat block then went to a 4°C soak cycle.

The reaction products were isolated by 1.0% agarose gel electrophoresis using TBE buffer where an approximately 1.8 kb product band was excised from the gel, and purified using an ILLUSTRA® GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

Plasmid pPFJO355 was digested with *Bam* I and *Bgl* II, isolated by 1.0% agarose gel electrophoresis using TBE buffer, and purified using an ILLUSTRA® GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

The gene fragment and the digested vector were ligated together using an IN-FUSION™ CF Dry-down PCR Cloning resulting in pCBHII51602 in which transcription of the *Penicillium emersonii* GH6 cellobiohydrolase II gene was under the control of the *Aspergillus oryzae* TAKA amylase promoter. In brief, 30 ng of pPFJO355, digested with *Bam* I and *Bgl* II, and 60 ng of the *Penicillium emersonii* GH6 cellobiohydrolase II gene purified PCR product were added to a reaction vial and resuspended in a final volume of 10 µl with addition of deionized water. The reaction was incubated at 37°C for 15 minutes and then 50°C for 15 minutes. Three µl of the reaction were used to transform *E. coli* TOP10 competent cells. An *E. coli* transformant containing pCBHII51602 was detected by colony PCR and plasmid DNA was prepared using a QIAprep Spin Miniprep Kit (QIAGEN Inc., Valencia, CA, USA). The *Penicillium emersonii* GH6 cellobiohydrolase II gene insert in pCBHII51602 was confirmed by DNA sequencing using a 3730XL DNA Analyzer.

Aspergillus oryzae HowB101 (WO 95/35385) protoplasts were prepared according to the method of Christensen *et al.*, 1988, *Bio/Technology* 6: 1419-1422 and transformed with 3 µg of pCBHII51602. The transformation yielded about 50 transformants. Twelve transformants were isolated to individual Minimal medium plates.

Six transformants were inoculated separately into 3 ml of YPM medium in 24-well plate and incubated at 30°C, 150 rpm. After 3 days incubation, 20 µl of supernatant from each culture were analyzed by SDS-PAGE using a NUPAGE® NOVEX® 4-12% Bis-Tris Gel with MES buffer according to the manufacturer's instructions. The resulting gel was stained with INSTANT® Blue. SDS-PAGE profiles of the cultures showed that the majority of the transformants had a major smeary band of approximately 62 kDa. The expression strain was designated as *A. oryzae* EXP03259.

A slant of *A. oryzae* EXP03259 was washed with 10 ml of YPM medium and inoculated into a 2 liter flask containing 400 ml of YPM medium to generate broth for characterization of the enzyme. The culture was harvested on day 3 and filtered using a 0.45 µm DURAPORE Membrane.

A 1600 ml volume of filtered broth of *A. oryzae* EXP03259 was precipitated with ammonium sulfate (80% saturation), re-dissolved in 100 ml 2of 5 mM Bis-Tris pH 6.0, dialyzed against the same buffer, and filtered through a 0.45 µm filter; the final volume was 200 ml. The solution was applied to a 40 ml Q Sepharose® Fast Flow column equilibrated with 25 mM Bis-Tris pH 6.0, and the proteins were eluted with a linear NaCl gradient (0–0.4 M). Fractions from the column with activity against PASC were analyzed by SDS-PAGE using a NUPAGE® NOVEX® 4-12% Bis-Tris Gel with MES buffer. Fractions with the correct molecular weight were pooled and concentrated by ultrafiltration. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 70: Preparation of *Penicillium pinophilum* strain NN046877 GH6A cellobiohydrolase II

The *Penicillium pinophilum* strain NN046877 GH6A cellobiohydrolase II (SEQ ID NO: 171 [DNA sequence] and SEQ ID NO: 172 [deduced amino acid sequence]) was obtained according to the procedure described below.

Penicillium pinophilum was grown on a PDA agar plate at 37°C for 4-5 days. Mycelia were collected directly from the agar plate into a sterilized mortar and frozen under liquid nitrogen. Frozen mycelia were ground, by mortar and pestle, to a fine powder, and genomic DNA was isolated using a DNeasy® Plant Mini Kit (QIAGEN Inc., Valencia, CA, USA).

Oligonucleotide primers, shown below, were designed to amplify the GH6 cellobiohydrolase II gene from genomic DNA of *Penicillium pinophilum*. An IN-FUSION™ CF Dry-down Cloning Kit was used to clone the fragment directly into the expression vector pPFJO355, without the need for restriction digestion and ligation.

Sense primer:

5'-**ACACAACTGGGGATCCACC**ATGTTGCGATATCTTTCCACC-3' (SEQ ID NO: 221)

Antisense primer:

5'-**GTCACCCTCTAGATCT**TCATCTAGACCAAAGCTGGGTTG-3' (SEQ ID NO: 222)

Bold letters represented the coding sequence and the remaining sequence was homologous to the insertion sites of pPFJO355.

Twenty picomoles of each of the primers above were used in a PCR reaction composed of *Penicillium pinophilum* genomic DNA, 10 µl of 5X GC Buffer, 1.5 µl of DMSO, 2.5 mM each of dATP, dTTP, dGTP, and dCTP, and 0.6 unit of Phusion™ High-Fidelity DNA Polymerase in a final volume of 50 µl. The amplification was performed using a Peltier Thermal Cycler programmed for denaturing at 98°C for 1 minutes; 5 cycles of denaturing at 98°C for 15 seconds, annealing at 56°C for 30 seconds, with a 1°C increase per cycle and elongation at 72°C for 75 seconds; and another 25 cycles each at 98°C for 15 seconds, 65C for 30 seconds and 72°C for 75 seconds; final extension at 72°C for 10 minutes. The heat block then went to a 4°C soak cycle.

The reaction products were isolated by 1.0% agarose gel electrophoresis using TBE buffer where an approximately 1.7 kb product band was excised from the gel, and purified using an ILLUSTRA® GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

Plasmid pPFJO355 was digested with *Bam* I and *Bgl* II, isolated by 1.0% agarose gel electrophoresis using TBE buffer, and purified using an ILLUSTRA® GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

The gene fragment and the digested vector were ligated together using an IN-

FUSION™ CF Dry-down PCR Cloning resulting in pPpin12 in which transcription of the *Penicillium pinophilum* GH6 cellobiohydrolase II gene was under the control of the *Aspergillus oryzae* TAKA amylase promoter. In brief, 30 ng of pPFJO355, digested with *Bam* I and *Bgl* II, and 60 ng of the *Penicillium pinophilum* GH6 cellobiohydrolase II gene purified PCR product were added to a reaction vial and resuspended in a final volume of 10ul with addition of deionized water. The reaction was incubated at 37°C for 15 minutes and then 50°C for 15 minutes. Three µl of the reaction were used to transform *E. coli* TOP10 competent cells. An *E. coli* transformant containing pPpin12 was detected by colony PCR and plasmid DNA was prepared using a QIAprep Spin Miniprep Kit. The *Penicillium pinophilum* GH6 cellobiohydrolase II gene insert in pPpin12 was confirmed by DNA sequencing using a 3730XL DNA Analyzer.

Aspergillus oryzae HowB101 (WO9535385) protoplasts were prepared according to the method of Christensen *et al.*, 1988, *Bio/Technology* 6: 1419-1422 and transformed with 3 µg of pPpin12. The transformation yielded about 50 transformants. Four transformants were isolated to individual Minimal medium plates.

Four transformants were inoculated separately into 3 ml of YPM medium in 24-well plate and incubated at 30°C, 150 rpm. After 3 days incubation, 20 µl of supernatant from each culture were analyzed by SDS-PAGE using a NUPAGE® NOVEX® 4-12% Bis-Tris Gel with MES buffer according to the manufacturer's instructions. The resulting gel was stained with INSTANT® Blue. SDS-PAGE profiles of the cultures showed that the majority of the transformants had a major band of approximately 65 kDa. The expression strain was designated *A. oryzae* EXP02774.

A slant of *A. oryzae* EXP02774 was washed with 10 ml of YPM mediumand inoculated into a 2 liter flask containing 400 ml of YPM medium to generate broth for characterization of the enzyme. The culture was harvested on day 3 and filtered using a 0.45 µm DURAPORE Membrane.

A 1600 ml volume of the filtered broth of *A. oryzae* EXP02774 was precipitated with ammonium sulfate (80% saturation), re-dissolved in 100 ml 25 mM Bis-Tris pH 6.0, dialyzed against the same buffer, and filtered through a 0.45 µm filter; the final volume was 200 ml. The solution was applied to a 40 ml Q Sepharose® Fast Flow column equilibrated in 25 mM Bis-Tris pH 6.0, and the proteins were eluted with a linear NaCl gradient (0–0.4 M). Fractions from the column with activity against PASC were collected and applied to a 40 ml HITRAP® SP Fast Flow column equilibrated in 25 mM Bis-Tris pH 6.0, and the proteins were eluted with a linear NaCl gradient (0–0.4 M). Fractions from the column with activity against PASC were analyzed by SDS-PAGE using a NUPAGE® NOVEX® 4-12% Bis-Tris Gel with MES buffer. Fractions with the correct molecular weight were pooled and concentrated by ultrafiltration. Protein concentration was determined using a Microplate BCA™ Protein Assay

Kit in which bovine serum albumin was used as a protein standard.

Example 71: Preparation of *Aspergillus fumigatus* strain NN051616 GH5 endoglucanase II

The *Aspergillus fumigatus* strain NN051616 GH5 endoglucanase II (SEQ ID NO: 173 [DNA sequence] and SEQ ID NO: 174 [deduced amino acid sequence]) was obtained according to the procedure described below.

Two synthetic oligonucleotide primers shown below were designed to PCR amplify the *Aspergillus fumigatus* Family GH5 gene from the genomic DNA. An IN-FUSION™ Cloning Kit was used to clone the fragment directly into the expression vector, pAILo2 (WO 2005/074647), without the need for restriction digests and ligation.

Forward primer:

5'-ACTGGATTTACC**ATGAAATTCGGTAGCATTGTGCTC**-3' (SEQ ID NO: 223)

Reverse primer:

5'-TCACCTCTAGTTAATTAA**TCAACCCAGGTAGGGCTCCAAGATG**-3' (SEQ ID NO: 224)

Bold letters represent coding sequence. The remaining sequence is homologous to the insertion sites of pAILo2.

Fifteen picomoles of each of the primers above were used in a PCR reaction containing 200 ng of *Aspergillus fumigatus* genomic DNA, 1X Pfx Amplification Buffer (Invitrogen, Carlsbad, CA, USA), 1mM $MgSO_4$, 1.5 μl of 10 mM blend of dATP, dTTP, dGTP, and dCTP, 2.5 units of *Pfx* DNA polymerase (Invitrogen, Carlsbad, CA, USA), in a final volume of 50 μl. The amplification conditions were one cycle at 98°C for 3 minutes; and 30 cycles each at 98°C for 30 seconds, 57°C for 30 seconds, and 72°C for 75 seconds. The heat block was then held at 72°C for 15 minutes followed by a 4°C soak cycle.

The reaction products were isolated on a 1.0% agarose gel using TAE buffer and a 2.4 kb product band was excised from the gel and purified using a MinElute® Gel Extraction Kit (QIAGEN Inc., Valencia, CA) according to the manufacturer's instructions.

The fragment was then cloned into pAILo2 using an IN-FUSION™ Cloning Kit. The vector was digested with *Nco* I and *Pac* I. The fragment was purified by gel electrophoresis and a QIAquick Kit (QIAGEN Inc., Valencia, CA, USA). The gene fragment and the digested vector were combined together in a reaction resulting in the expression plasmid pAG10, in which transcription of the *Aspergillus fumigatus* Family GH5 gene was under the control of the NA2-tpi promoter (a hybrid of the promoters from the genes for *Aspergillus niger* neutral alpha-amylase and *Aspergillus oryzae* triose phosphate isomerase). The recombination reaction (20 μl) was composed of 1X I IN-FUSION™ Buffer (Clontech, Mountain View, CA) , 1X BSA (Clontech, Mountain View, CA) , 1 μl of IN-FUSION™ enzyme (diluted 1:10)

(Clontech, Mountain View, CA) , 180 ng of pAlLo2 digested with *Nco* I and *Pac* I, and 80 ng of the *Aspergillus fumigatus* beta-xylosidase purified PCR product. The reaction was incubated at ambient temperature for 30 minutes. The reaction was diluted with 40 µl of TE buffer and 2.5 µl of the diluted reaction was used to transform *E. coli* SOLOPACK® Gold cells. An *E. coli* transformant containing pAG10 (*Aspergillus fumigatus* Family GH5 gene) was identified by restriction enzyme digestion and plasmid DNA was prepared using a QIAGEN BioRobot 9600. The pAG10 plasmid construct was sequenced using an Applied Biosystems 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) to verify the sequence.

Aspergillus oryzae JaL355 protoplasts were prepared according to the method of Christensen *et al.*, 1988, *Bio/Technology* 6: 1419-1422 and transformed with 5 µg of pAG10. Three transformants were isolated to individual PDA plates.

Plugs taken from the original transformation plate of each of the three transformants were added to 1 ml of M410 medium separately in 24 well plates, which were incubated at 34°C. After five days of incubation, 7.5 µl of supernatant from each culture was analyzed using CRITERION® stain-free, 8-16% gradient SDS-PAGE, (Bio-Rad Laboratories, Inc., Hercules, CA, USA) according to the manufacturer's instructions. SDS-PAGE profiles of the cultures showed that the transformants had a new major band of approximately 35 kDa.

Confluent PDA plate of the highest expressing transformant was washed with 5 ml of 0.01% TWEEN® 20 and inoculated into three 500 ml Erlenmeyer flasks, each containing 100 ml of M410 medium. Inoculated flasks were incubated with shaking for 3 days at 34°C. The broths were pooled and filtered through a 0.22 µm stericup suction filter (Millipore, Bedford, MA, USA).

A 35 ml volume of filtered broth was buffer exchanged into 50 mM sodium acetate pH 5.0 using a 400 ml Sephadex G-25 column (GE Healthcare, United Kingdom) according to the manufacturer's instructions. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 72: Preparation of *Neosartorya fischeri* strain NRRL 181 GH5 endoglucanase II

The *Neosartorya fischeri* NRRL 181 GH5 endoglucanase II (SEQ ID NO: 175 [DNA sequence] and SEQ ID NO: 176 [deduced amino acid sequence]) was obtained according to the procedure described below.

Two synthetic oligonucleotide primers, shown below, were designed to PCR amplify the endoglucanase gene from *Neosartorya fischeri* NRRL 181 genomic DNA. Genomic DNA was isolated using a FastDNA spin for Soil Kit.

Primer #350:

5'-TAAGAATTCACCATGAAGGCTTCGACTATTATCTGTGCA-3' (SEQ ID NO: 225)

Primer#358:

5'-TATGCGGCCGCACGGCAATCCAAGTCATTCAA-3' (SEQ ID NO: 226)

The amplification reaction was composed of 1 µl of *Neosartorya fischeri* genomic DNA, 12.5 µl of 2X REDDYMIX™ PCR Buffer, 1 µl of 5 µM primer #374, 1 µl of 5 µM primer #375, and 9.5 µl of H_2O. The amplification reaction was incubated in a PTC-200 DNA ENGINE™ Thermal Cycler programmed for 1 cycle at 94°C for 2 minutes; and 35 cycles each at 94°C for 15 seconds and 60°C for 1.5 minutes.

A 1.4 kb PCR reaction product was isolated by 1% agarose gel electrophoresis using TAE buffer and staining with SYBR Safe DNA gel stain. The DNA band was visualized with the aid of an Eagle Eye Imaging System and a DarkReader Transilluminator. The 1.4 kb DNA band was excised from the gel and purified using a GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

The 1.4 kb fragment was cleaved with *EcoR* I and *Not* I and purified using a GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

The cleaved 1.4 kb fragment was then directionally cloned by ligation into *Eco* RI-*Not* I cleaved pXYG1051 (WO 2005/080559) using T4 ligase according to the manufacturer's instructions. The ligation mixture was transformed into *E. coli* TOP10F competent cells according to the manufacturer's instructions. The transformation mixture was plated onto LB plates supplemented with 100 µg of ampicillin per ml. Plasmid minipreps were prepared from several transformants and sequenced. One plasmid with the correct *Neosartorya fischeri* GH5 coding sequence was chosen.

The expression plasmid pXYG1051-NP003772 was transformed into *Aspergillus oryzae* JaL355 as described in WO 98/00529. Transformants were purified on selection plates to single conidia prior to sporulating them on PDA plates. Production of the *Neosartorya fischeri* GH5 polypeptide by the transformants was analyzed from culture supernatants of 1 ml 96 deep well stationary cultivations at 26°C in YP medium with 2% maltodextrin. Expression was verified SDS-PAGE using a NUPAGE® NOVEX® 4-12% Bis-Tris Gel with MES buffer and Coomassie blue staining. One transformant was selected for further work and designated *Aspergillus oryzae* 83.3.

For larger scale production, *Aspergillus oryzae* 83.3 spores were spread onto a PDA plate and incubated for five days at 37°C. The confluent spore plate was washed twice with 5 ml of 0.01% TWEEN® 20 to maximize the number of spores collected. The spore suspension was then used to inoculate twenty-five 500 ml flasks containing 100 ml of YPG medium. The culture was incubated at 26°C with constant shaking at 120 rpm. At day five post-inoculation, the culture broth was collected by filtration through a triple layer of

Whatman glass microfiber filters of 1.6 µm, 1.2 µm, and 0.7 µm. Fresh culture broth from this transformant produced a band of GH7 protein of approximately 46 kDa. The identity of this band as the *Neosartorya fischeri* GH5 polypeptide was verified by peptide sequencing.

Two liters of the filtered broth were concentrated to 400 ml and washed with 50 mM HEPES pH 7.0 using a SARTOFLOW® Alpha ultrafiltration system with a 10 kDa MW-CO. Ammonium sulphate was added to a final concentration of 1 M and dissolved in the ultrafiltrate. The solution was applied on a Source 15 Phenyl XK 26/20 50 ml column. After application the column was washed with 150 ml of 1 M ammonium sulphate and eluded with 1 column volume of 50% ethanol in a 0% to 100% gradient followed by 5 column volumes of 50% ethanol at a flow rate of 10 ml/minute. Fractions of 10 ml were collected and analyzed by SDS-PAGE. Fraction 3 to 8 were pooled and diluted to 1000 ml with 50 mM HEPES pH 7.0 before application on a Q Sepharose® Fast Flow column XK26/20 60 ml column. After application the column was washed 3 times with 60 ml of 50 mM HEPES pH 7.0 and eluded with 100 ml of 50 mM HEPES pH 7.0, 1 M NaCl at a flow rate of 10 ml/minute. Fractions of 10 ml were collected and analyzed by SDS-PAGE. The flow-through and first wash were pooled and concentrated to 400 ml and washed with 50 mM HEPES pH 7.0 using the ultrafiltration system described above. Further concentration was conducted using a VIVASPIN™ centrifugal concentrator according to the manufacturer's instructions to a final volume of 80 ml. The protein concentration was determined by A280/A260 absorbance. Protein concentration was also determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 73: Preparation of *Aspergillus aculeatus* strain WDCM190 GH3 beta-glucosidase

The *Aspergillus aculeatus* strain WDCM190 GH3 beta-glucosidase (SEQ ID NO: 177 [DNA sequence] and SEQ ID NO: 178 [deduced amino acid sequence]) was obtained according to the procedure described below.

To generate genomic DNA for PCR amplification, *Aspergillus aculeatus* WDCM190 was propagated on PDA agar plates by growing at 26°C for 7 days. Spores harvested from the PDA plates were inoculated into 25 ml of YP+2% glucose medium in a baffled shake flask and incubated at 26°C for 48 hours with agitation at 200 rpm.

Genomic DNA was isolated according to a modified FastDNA® SPIN protocol (Qbiogene, Inc., Carlsbad, CA, USA). Briefly, a FastDNA® SPIN Kit for Soil (Qbiogene, Inc., Carlsbad, CA, USA) was used in a FastPrep® 24 Homogenization System (MP Biosciences, Santa Ana, CA, USA). Two ml of fungal material were harvested by centrifugation at 14,000 x *g* for 2 minutes. The supernatant was removed and the pellet resuspended in 500 µl of deionized water. The suspension was transferred to a Lysing Matrix E FastPrep® tube

(Qbiogene, Inc., Carlsbad, CA, USA) and 790 µl of sodium phosphate buffer and 100 µl of MT buffer from the FastDNA® SPIN Kit were added to the tube. The sample was then secured in a FastPrep® Instrument (Qbiogene, Inc., Carlsbad, CA, USA) and processed for 60 seconds at a speed of 5.5 m/sec. The sample was then centrifuged at 14000 x *g* for two minutes and the supernatant transferred to a clean EPPENDORF® tube. A 250 µl volume of PPS reagent from the FastDNA® SPIN Kit was added and then the sample was mixed gently by inversion. The sample was again centrifuged at 14000 x *g* for 5 minutes. The supernatant was transferred to a 15 ml tube followed by 1 ml of Binding Matrix suspension from the FastDNA® SPIN Kit and then mixed by inversion for two minutes. The sample was placed in a stationary tube rack and the silica matrix was allowed to settle for 3 minutes. A 500 µl volume of the supernatant was removed and discarded and then the remaining sample was resuspended in the matrix. The sample was then transferred to a SPIN filter tube from the FastDNA® SPIN Kit and centrifuged at 14000 x *g* for 1 minute. The catch tube was emptied and the remaining matrix suspension added to the SPIN filter tube. The sample was again centrifuged (14000 x *g*, 1 minute). A 500 µl volume of SEWS-M solution from the FastDNA® SPIN Kit was added to the SPIN filter tube and the sample was centrifuged at the same speed for 1 minute. The catch tube was emptied and the SPIN filter replaced in the catch tube. The unit was centrifuged at 14000 x *g* for 2 minutes to "dry" the matrix of residual SEWS-M wash solution. The SPIN filter was placed in a fresh catch tube and allowed to air dry for 5 minutes at room temperature. The matrix was gently resuspended in 100 µl of DES (DNase/Pyrogen free water) with a pipette tip. The unit was centrifuged (14000 x *g*, 1 minute) to elute the genomic DNA followed by elution with 100 µl of 10 mM Tris, 0.1 mM EDTA, pH 8.0 by renewed centrifugation at 14000 x *g* for 1 minute and the eluates were combined. The concentration of the DNA harvested from the catch tube was measured by a UV spectrophotometer at 260 nm.

The *Aspergillus aculeatus* Cel3 beta-glucosidase gene was isolated by PCR using two cloning primers GH3-8f and GH3-8r shown below, which were designed based on the publicly available *Aspergillus aculeatus* Cel3 mRNA sequence (Genbank D64088.1) for direct cloning by IN-FUSION™ strategy.

Primer GH3-8f:

5'-acacaactggggatccaccatgaagctcagttggcttgaggcgg-3' (SEQ ID NO: 227)

Primer GH3-8r:

5'-agatctcgagaagcttattgcaccttcgggagcgccgcgtgaag-3' (SEQ ID NO: 228)

A PCR reaction was performed with genomic DNA prepared from *Aspergillus aculeatus* strain (IAM2445; WDCM190) in order to amplify the full-length gene. The PCR reaction was composed of 1 µl of genomic DNA, 0.75 µl of primer GH3-8f (10 µM), 0.75 µl of primer GH3-8r (10 µM), 3 µl of 5X HF buffer, 0.25 µl of 50 mM $MgCl_2$, 0.3 µl of 10 mM dNTP,

0.15 µl of PHUSION® DNA polymerase, and PCR-grade water up to 15 µl. The PCR reaction was performed using a DYAD® PCR machine programmed for 2 minutes at 98°C followed by 10 touchdown cycles at 98°C for 15 seconds, 70°C (-1°C/cycle) for 30 seconds, and 72°C for 2 minutes 30 seconds; and 25 cycles each at 98°C for 15 seconds, 60°C for 30 seconds, 72°C for 2 minutes 30 seconds, and 5 minutes at 72°C.

The reaction products were isolated by 1.0% agarose gel electrophoresis using TAE buffer where an approximately 2.9 kb PCR product band was excised from the gel and purified using a GFX® PCR DNA and Gel Band Purification Kit according to manufacturer's instructions. DNA corresponding to the *Aspergillus aculeatus* Cel3 beta-glucosidase gene was cloned into the expression vector pDAu109 (WO 2005042735) linearized with *Bam* HI and *Hind* III, using an IN-FUSION™ Dry-Down PCR Cloning Kit according to the manufacturer's instructions.

A 2.5 µl volume of the diluted ligation mixture was used to transform *E. coli* TOP10 chemically competent cells. Three colonies were selected on LB agar plates containing 100 µg of ampicillin per ml and cultivated overnight in 3 ml of LB medium supplemented with 100 µg of ampicillin per ml. Plasmid DNA was purified using an E.Z.N.A.® Plasmid Mini Kit according to the manufacturer's instructions. The *Aspergillus aculeatus* Cel3 beta-glucosidase gene sequence was verified by Sanger sequencing before heterologous expression.

The coding sequence is 2940 bp including the stop codon and is interrupted by 6 introns of 73 bp (nucleotides 58 to 130), 52 bp (nucleotides 274 to 325), 57 bp (nucleotides 371 to 427), 61 bp (nucleotides 481 to 541), 64 bp (nucleotides 1734 to 1797), and 50 bp (nucleotides 2657 to 2706). The encoded predicted protein is 860 amino acids. Using the SignalP program (Nielsen *et al.*, 1997, *Protein Engineering* 10: 1-6), a signal peptide of 19 residues was predicted. The predicted mature protein contains 841 amino acids.

Protoplasts of *Aspergillus oryzae* BECh2 (WO 2000/39322) were prepared as described in WO 95/02043. One hundred microliters of protoplast suspension were mixed with 2.5-15 µg of the *Aspergillus* expression vector and 250 µl of 60% PEG 4000 (Applichem Inc. Omaha, NE, USA) (polyethylene glycol, molecular weight 4,000), 10 mM $CaCl_2$, and 10 mM Tris-HCl pH 7.5 were added and gently mixed. The mixture was incubated at 37°C for 30 minutes and the protoplasts were spread on COVE sucrose (1 M) plates supplemented with 10 mM acetamide and 15 mM CsCl for transformant selection. After incubation for 4-7 days at 37°C spores of several transformants were seeded on YP-2% maltodextrin medium. After 4 days cultivation at 30°C culture broth was analyzed in order to identify the best transformants based on their ability to produce a large amount of active *Aspergillus aculeatus* Cel3 beta-glucosidase. The screening was based on intensity of the band corresponding to the heterologous expressed protein determined by SDS-PAGE and activity

of the enzyme on 4-nitrophenyl-beta-D-glucopyranoside (pNPG) as described in Example 16 herein.

Spores of the best transformant designated were spread on COVE plates containing 0.01% TRITON® X-100 in order to isolate single colonies. The spreading was repeated twice in total on COVE sucrose medium (Cove, 1996, *Biochim. Biophys. Acta* 133: 51-56) containing 1 M sucrose and 10 mM sodium nitrate, supplemented with 10 mM acetamide and 15 mM CsCl. Fermentation was then carried out in 250 ml shake flasks using YP-2% maltodextrin medium for 4 days at 30°C with shaking at 100 rpm. The broth was filtered using standard methods. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 74: Preparation of *Aspergillus kawashii* strain IFO 4308 GH3 beta-glucosidase

The *Aspergillus kawashii* strain IFO 4308 GH3 beta-glucosidase (SEQ ID NO: 179 [DNA sequence] and SEQ ID NO: 180 [deduced amino acid sequence]) was obtained according to the procedure described below.

To generate genomic DNA for PCR amplification, the fungi were propagated on PDA agar plates by growing at 26°C for 7 days. Spores harvested from the PDA plates were used to inoculate 25 ml of YP+2% glucose medium in a baffled shake flask and incubated at 26°C for 48 hours with agitation at 200 rpm.

Genomic DNA was isolated according to the procedure described in Example 73.

The *Aspergillus kawachii* beta-glucosidase gene was isolated by PCR using two cloning primers GH3-33f and GH3-33r shown below, which were designed based on the publicly available *Aspergillus kawachii* full-length sequence (GenBank AB003470.1) for direct cloning by IN-FUSION™ strategy.

Primer GH3-33f:

acacaactggggatccaccatgaggttcactttgattgaggcgg (SEQ ID NO: 229)

Primer GH3-33r:

agatctcgagaagcttaGTGAACAGTAGGCAGAGACGCCCGGAGC (SEQ ID NO: 230)

A PCR reaction was performed with the genomic DNA prepared from *Aspergillus kawachii* IFO 4308 in order to amplify the full-length gene. The PCR reaction was composed of 1 µl of genomic DNA, 0.75 µl of primer GH3-33f (10 µM), 0.75 µl of primer GH3-33r (10 µM), 3 µl of 5X HF buffer, 0.25 µl of 50 mM $MgCl_2$, 0.3 µl of 10 mM dNTP, 0.15 µl of PHUSION® DNA polymerase, and PCR-grade water up to 15 µl. The PCR reaction was performed using a DYAD® PCR machine programmed for 2 minutes at 98°C followed by 10 touchdown cycles at 98°C for 15 seconds, 70°C (-1°C/cycle) for 30 seconds, and 72°C for 2

minutes 30 seconds; and 25 cycles each at 98°C for 15 seconds, 60°C for 30 seconds, 72°C for 2 minutes 30 seconds, and 5 minutes at 72°C.

The reaction products were isolated by 1.0% agarose gel electrophoresis using TAE buffer where an approximately 2.9 kb PCR product band was excised from the gel and purified using a GFX® PCR DNA and Gel Band Purification Kit according to manufacturer's instructions. DNA corresponding to the *Aspergillus kawachii* beta-glucosidase gene was cloned into the expression vector pDAu109 (WO 2005042735) linearized with *Bam* HI and *Hind* III, using an IN-FUSION™ Dry-Down PCR Cloning Kit according to the manufacturer's instructions.

A 2.5 µl volume of the diluted ligation mixture was used to transform *E. coli* TOP10 chemically competent cells. Three colonies were selected on LB agar plates containing 100 µg of ampicillin per ml and cultivated overnight in 3 ml of LB medium supplemented with 100 µg of ampicillin per ml. Plasmid DNA was purified using an E.Z.N.A.® Plasmid Mini Kit according to the manufacturer's instructions. The *Aspergillus kawachii* beta-glucosidase gene sequence was verified by Sanger sequencing before heterologous expression.

The coding sequence is 2935 bp including the stop codon and is interrupted by 6 introns of 92 bp (nucleotides 58 to 149), 48 bp (nucleotides 293 to 340), 54 bp (nucleotides 386 to 439), 51 bp (nucleotides 493 to 543), 57 bp (nucleotides 1736 to 1792), and 50 bp (nucleotides 2652 to 2701). The encoded predicted protein is 860 amino acids. Using the SignalP program (Nielsen *et al.*, 1997, *Protein Engineering* 10: 1-6), a signal peptide of 19 residues was predicted. The predicted mature protein contains 841 amino acids.

Protoplasts of *Aspergillus oryzae* BECh2 (WO 2000/39322) were prepared as described in WO 95/02043. One hundred microliters of protoplast suspension were mixed with 2.5-15 µg of the *Aspergillus* expression vector and 250 µl of 60% PEG 4000, 10 mM $CaCl_2$, and 10 mM Tris-HCl pH 7.5 were added and gently mixed. The mixture was incubated at 37°C for 30 minutes and the protoplasts were spread on COVE sucrose (1 M) plates supplemented with 10 mM acetamide and 15 mM CsCl for transformant selection. After incubation for 4-7 days at 37°C spores of several transformants were seeded on YP-2% maltodextrin medium. After 4 days cultivation at 30°C culture broth was analyzed in order to identify the best transformants based on their ability to produce a large amount of active *Aspergillus kawachii* beta-glucosidase. The screening was based on intensity of the band corresponding to the heterologous expressed protein determined by SDS-PAGE and activity of the enzyme on 4-nitrophenyl-beta-D-glucopyranoside (pNPG) as described in Example 16 herein.

Spores of the best transformant were spread on COVE plates containing 0.01% TRITON® X-100 in order to isolate single colonies. The spreading was repeated twice in total on COVE sucrose medium (Cove, 1996, *Biochim. Biophys. Acta* 133: 51-56) containing

1 M sucrose and 10 mM sodium nitrate, supplemented with 10 mM acetamide and 15 mM CsCl. Fermentation was then carried out in 250 ml shake flasks using YP-2% maltodextrin medium for 4 days at 30°C with shaking at 100 rpm. The broth was filtered using standard methods. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 75: Preparation of *Aspergillus clavatus* strain NRRL 1 GH3 beta-glucosidase

The *Aspergillus clavatus* strain NRRL 1 GH3 beta-glucosidase (SEQ ID NO: 181 [DNA sequence] and SEQ ID NO: 182 [deduced amino acid sequence]) was obtained according to the procedure described below.

Genomic DNA was isolated according to the procedure described in Example 73.

The *Aspergillus clavatus* beta-glucosidase gene was isolated by PCR using two cloning primers, GH3-10f and GH3-10r, shown below, which were designed based on the publicly available *Aspergillus clavatus* partial mRNA sequence (XM_001269581) for direct cloning by IN-FUSION™ strategy.

Primer GH3-10f:

acacaactggggatccaccATGAGGTTCAGCTGGCTTGAGGTCG (SEQ ID NO: 231)

Primer GH3-10r:

agatctcgagaagcttaCTGTACCCGGGGCAGAGGTGCTCTC (SEQ ID NO: 232)

A PCR reaction was performed with the genomic DNA prepared from *Aspergillus clavatus* NRRL1 in order to amplify the full-length gene. The PCR reaction was composed of 1 µl of genomic DNA, 0.75 µl of primer GH3-10f (10 µM), 0.75 µl of primer GH3-10r (10 µM), 3 µl of 5X HF buffer, 0.25 µl of 50 mM $MgCl_2$, 0.3 µl of 10 mM dNTP, 0.15 µl of PHUSION® DNA polymerase, and PCR-grade water up to 15 µl. The PCR reaction was performed using a DYAD® PCR machine programmed for 2 minutes at 98°C followed by 10 touchdown cycles at 98°C for 15 seconds, 70°C (-1°C/cycle) for 30 seconds, and 72°C for 2 minutes 30 seconds; and 25 cycles each at 98°C for 15 seconds, 60°C for 30 seconds, 72°C for 2 minutes 30 seconds, and 5 minutes at 72°C.

The reaction products were isolated by 1.0% agarose gel electrophoresis using TAE buffer where an approximately 3.0 kb PCR product band was excised from the gel and purified using a GFX® PCR DNA and Gel Band Purification Kit according to manufacturer's instructions. DNA corresponding to the *Aspergillus clavatus* beta-glucosidase gene was cloned into the expression vector pDAu109 (WO 2005042735) linearized with *Bam* HI and *Hind* III, using an IN-FUSION™ Dry-Down PCR Cloning Kit according to the manufacturer's instructions.

A 2.5 µl volume of the diluted ligation mixture was used to transform *E. coli* TOP10 chemically competent cells. Three colonies were selected on LB agar plates containing 100

µg of ampicillin per ml and cultivated overnight in 3 ml of LB medium supplemented with 100 µg of ampicillin per ml. Plasmid DNA was purified using an E.Z.N.A.® Plasmid Mini Kit according to the manufacturer's instructions. The *Aspergillus clavatus* beta-glucosidase gene sequence was verified by Sanger sequencing before heterologous expression.

The coding sequence is 3062 bp including the stop codon and is interrupted by 8 introns of 67 bp (nucleotides 58 to 124), 61 bp (nucleotides 265 to 325), 62 bp (nucleotides 371 to 432), 65 bp (nucleotides 489 to 553), 50 bp (nucleotides 948 to 997), 53 bp (nucleotides 1021 to 1073), 61 bp (nucleotides 1849 to 1909), and 60 bp (nucleotides 2769 to 2828). The encoded predicted protein is 860 amino acids. Using the SignalP program (Nielsen *et al.*, 1997, *Protein Engineering* 10: 1-6), a signal peptide of 18 residues was predicted. The predicted mature protein contains 842 amino acids.

Protoplasts of *Aspergillus oryzae* BECh2 (WO 2000/39322) were prepared as described in WO 95/02043. One hundred microliters of protoplast suspension were mixed with 2.5-15 µg of the *Aspergillus* expression vector and 250 µl of 60% PEG 4000, 10 mM $CaCl_2$, and 10 mM Tris-HCl pH 7.5 were added and gently mixed. The mixture was incubated at 37°C for 30 minutes and the protoplasts were spread on COVE sucrose (1 M) plates supplemented with 10 mM acetamide and 15 mM CsCl for transformant selection. After incubation for 4-7 days at 37°C spores of several transformants were seeded on YP-2% maltodextrin medium. After 4 days cultivation at 30°C culture broth was analyzed in order to identify the best transformants based on their ability to produce a large amount of active *Aspergillus clavatus* beta-glycosidase. The screening was based on intensity of the band corresponding to the heterologous expressed protein determined by SDS-PAGE and activity of the enzyme on 4-nitrophenyl-beta-D-glucopyranoside (pNPG) as described in Example 16.

Spores of the best transformant were spread on COVE plates containing 0.01% TRITON® X-100 in order to isolate single colonies. The spreading was repeated twice in total on COVE sucrose medium (Cove, 1996, *Biochim. Biophys. Acta* 133: 51-56) containing 1 M sucrose and 10 mM sodium nitrate, supplemented with 10 mM acetamide and 15 mM CsCl. Fermentation was then carried out in 250 ml shake flasks using YP-2% maltodextrin medium for 4 days at 30°C with shaking at 100 rpm. The broth was filtered using standard methods. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 76: Preparation of *Thielavia terrestris* NRRL 8126 GH3 beta-glucosidase

The *Thielavia terrestris* GH3 beta-glucosidase (SEQ ID NO: 183 [DNA sequence] and SEQ ID NO: 184 [deduced amino acid sequence]) was obtained according to the procedure described below.

Three agarose plugs from culture of *Thielavia terrestris* NRRL 8126 grown on a PDA plate were inoculated into 100 ml of NNCYP medium supplemented with 1.5% glucose and incubated for 25 hours at 42°C and 200 rpm on an orbital shaker. Fifty ml of this culture was used to inoculate 1.8 liter of NNCYP medium supplemented with 0.4% glucose and 52 g of powdered cellulose per liter and was incubated at 42°C. The pH was controlled at 5.0 by the addition of 15% ammonium hydroxide or 5 N phosphoric acid, as needed.

The fermentations were run at 42°C with minimum dissolved oxygen at 25% at a 1.0 VVM air flow and an agitation at 1100 rpm. Feed medium was delivered into a 2 liter fermentation vessel at 0 hours with a feed rate of 6.0–8.0 g/hour for 120 hours. Pooled cultures were centrifuged at 3000 *x g* for 10 minutes and the supernatant was filtered through a disposable filtering unit with a glass fiber prefilter (Nalgene, Rochester NY, USA). The filtrate was cooled to 4°C for storage.

A 0.3 ml aliquot of the filtrate was precipitated with 10% trichloroacetic acid (TCA) - 80% acetone for 20 minutes on ice. The suspension was centrifuged for 10 minutes at 13,000 *x g*. The supernatant was removed and the protein pellet remaining was rinsed with cold acetone. The protein pellet was dissolved in 30 µl of 1X lithium dodecyl sulfate (LDS) SDS-PAGE loading buffer with 50 mM dithiothreitol (DTT) and heated at 80°C for 10 minutes. A 15 µl sample was separated by SDS-PAGE using a 7 cm 4-12% NuPAGE Bis-Tris SDS-PAGE gradient gel and 2-(N-morpholino)ethanesulfonic acid (MES) running buffer. The SDS-PAGE was run under reducing conditions according to the manufacturer's recommended protocol (Invitrogen, Carlsbad, CA, USA). The gel was removed from the cassette and rinsed 3 times with deionized water for at least 5 minutes each and stained with Bio-Safe Coomassie Stain (Bio-Rad Laboratories, Inc., Hercules, CA, USA) for 1 hour followed by destaining with doubly-distilled water for more than 30 minutes. Protein bands observed at approximately 95 kD was excised and reduced with 50 µl of 10 mM DTT in 100 mM ammonium bicarbonate for 30 minutes. Following reduction, the gel pieces were alkylated with 50 µl of 55 mM iodoacetamide in 100 mM ammonium bicarbonate for 20 minutes. The dried gel pieces were allowed to re-hydrate in a trypsin digestion solution (6 ng/µl sequencing grade trypsin in 50 mM ammonium bicarbonate) for 30 minutes at room temperature, followed by an 8 hour digestion at 40°C. Each of the reaction steps described was followed by numerous washes and pre-washes with the desired solutions. Fifty µl of acetonitrile was used to de-hydrate the gel pieces between reactions and they were air-dried between steps. Peptides were extracted twice with 1% formic acid/2% acetonitrile in HPLC grade water for 30 minutes. Peptide extraction solutions were transferred to a 96 well PCR type microtiter plate that had been cooled to 10–15°C. Microtiter plates containing the recovered peptide solutions were sealed to prevent evaporation and stored at 4°C until mass

spectrometry analysis could be performed.

For de-novo peptide sequencing by tandem mass spectrometry, a Q-Tof *micro*™, a hybrid orthogonal quadrupole time-of-flight mass spectrometer (Waters Micromass® MS Technologies, Milford, MA, USA) was used for LC/MS/MS analysis. The Q-Tof *micro*™ was fitted with an Ultimate™ capillary and nano-flow HPLC system which had been coupled with a FAMOS micro autosampler and a Switchos II column switching device (LCPackings, San Francisco, CA, USA) for concentrating and desalting samples. Samples were loaded onto a guard column (300 μm ID X 5 cm, C18 pepmap) fitted in the injection loop and washed with 0.1% formic acid in water at 40 μl/minute for 2 minutes using the Switchos II pump. Peptides were separated on a 75 μm ID x 15 cm, C18, 3 μm, 100Å PepMap™ (LC Packings, San Francisco, CA, USA) nanoflow fused capillary column at a flow rate of 175 nl/minute from a split flow of 175 μl/minute using a NAN-75 calibrator (Dionex, Sunnyvale, CA, USA). A step elution gradient of 5% to 80% acetonitrile in 0.1% formic acid was applied over a 45 minute interval. The column eluent was monitored at 215 nm and introduced into the Q-Tof *micro*™ through an electrospray ion source fitted with the nanospray interface. The Q-Tof *micro*™ is fully microprocessor controlled using Masslynx™ software version 3.5 (Waters Micromass® MS Technologies, Milford, MA, USA). Data was acquired in survey scan mode and from a mass range of m/z 400 to 1990 with the switching criteria for MS to MS/MS to include an ion intensity of greater than 10.0 counts per second and charge states of +2, +3, and +4. Analysis spectra of up to 4 co-eluting species with a scan time of 1.9 seconds and inter-scan time of 0.1 seconds could be obtained. A cone voltage of 65 volts was typically used and the collision energy was programmed to be varied according to the mass and charge state of the eluting peptide and in the range of 10 – 60 volts. The acquired spectra were combined, smoothed and centered in an automated fashion and a peak list generated. This peak list was searched against selected public and private databases using ProteinLynx™ Global Server 1.1 software (Waters Micromass® MS Technologies, Milford, MA). Results from the ProteinLynx™ searches were evaluated and un-identified proteins were analyzed further by evaluating the MS/MS spectrums of each ion of interest and de-novo sequence was determined by identifying the y and b ion series and matching mass differences to the appropriate amino acid.

Peptide sequences obtained from *de novo* sequencing by mass spectrometry were obtained from several multiply charged ions for the approximately 95 kDa polypeptide gel band.

A doubly charged tryptic peptide ion of 524.76 m/z sequence was determined to be Ser-Pro-Phe-Thr-Trp-Gly-Pro-Thr-Arg (amino acids 607 to 615 of SEQ ID NO: 184). A second doubly charged tryptic peptide ion of 709.91 partial sequence was determined to be

Gly-Val-Asn-Val-[Ile/Leu]-[Ile-Leu]-Gly-[Ile/Leu]-Gly-Pro (amino acids 148 to 155 of SEQ ID NO: 184). A second doubly charged tryptic peptide ion of 745.38 partial sequence was determined to be Pro-Pro-His-Ala-Thr-Asp (amino acids 747 to 752 of SEQ ID NO: 184). A third doubly charged tryptic peptide ion of 808.92 m/z sequence was determined to be Tyr-Glu-Ser-[Ile/Leu]-[Ile/Leu]-Ser-Asn-Tyr-Ala-Thr-Ser-Qln-[Ile/Leu]-Lys (amino acids 487 to 499 of SEQ ID NO: 184). A fourth doubly charged tryptic peptide ion of 1023.96 a partial sequence was determined to be Phe-Asn-Ser-Gly-Phe-Pro-Ser-Gly-Gln-Thr-Ala-Ala-Ala-Thr-Phe-Asp-Arg (amino acids 114 to 130 of SEQ ID NO: 184).

To generate genomic DNA for PCR amplification, *Thielavia terrestris* NRRL 8126 was grown in 50 ml of NNCYP medium supplemented with 1% glucose in a baffled shake flask at 42°C and 200 rpm for 24 hours. Mycelia were harvested by filtration, washed twice in TE (10 mM Tris-1 mM EDTA), and frozen under liquid nitrogen. A pea-size piece of frozen mycelia was suspended in 0.7 ml of 1% lithium dodecyl sulfate in TE and disrupted by agitation with an equal volume of 0.1 mm zirconia/silica beads (Biospec Products, Inc., Bartlesville, OK, USA) for 45 seconds in a FastPrep FP120 (ThermoSavant, Holbrook, NY, USA). Debris was removed by centrifugation at 13,000 *x g* for 10 minutes and the cleared supernatant was brought to 2.5 M ammonium acetate and incubated on ice for 20 minutes. After the incubation period, the nucleic acids were precipitated by addition of 2 volumes of ethanol. After centrifugation for 15 minutes in a microfuge at 4°C, the pellet was washed in 70% ethanol and air dried. The DNA was resuspended in 120 µl of 0.1X TE and incubated with 1 µl of DNase-free RNase A at 37°C for 20 minutes. Ammonium acetate was added to 2.5 M and the DNA was precipitated with 2 volumes of ethanol. The pellet was washed in 70% ethanol, air dried, and resuspended in TE buffer.

A low redundancy draft sequence of the *Thielavia terrestris* NRRL 8126 genome was generated by the Joint Genome Center (JGI), Walnut Creek, CA, USA, using the whole genome shotgun method according to Martinez *et al.*, 2008, *Nature Biotechnol.* 26: 553-560. Shotgun sequencing reads (approximately 18307) were assembled into contigs using the Phrap assembler (Ewing and Green, 1998, *Genome Res.* 8: 186-194).

A tblastn search (Altschul *et al.*, 1997, *Nucleic Acids Res.* 25: 3389-3402) of the assembled contigs was carried out using as query a beta-glucosidase protein sequence from *Neurospora crassa (*UniProt accession number q7rwp2*)*. A translated amino acid sequence with greater than 73.8% identity to the query sequence was identified.

The sequence was searched against public databases using blastp (Altschul *et al.*, 1997, *supra*) and was found to be 80.1% identical to a beta-glucosidase from *Podospora anserina* (UniProt accession number B2AVE8).

The nucleotide sequence and deduced amino acid sequence of the *Thielavia terrestris* beta-glucosidase gene are shown in SEQ ID NO: 183 and SEQ ID NO: 184,

respectively. The coding sequence is 3032 bp including the stop codon and is interrupted by three introns of 295 bp, 57 bp, and 61 bp. The encoded predicted protein is 872 amino acids. Using the SignalP program (Nielsen *et al.*, 1997, *Protein Engineering* 10: 1-6), a signal peptide of 18 residues was predicted. The predicted mature protein contains 854 amino acids with a predicted molecular mass of 93 kDa and an isoelectric point of 5.57.

Thielavia terrestris NRRL 8126 was grown for 3 days on PDA plates at 45°C. Mycelia were scraped from the plates and approximately 100 mg of mycelia (wet weight) were used to inoculate 500 ml shake flasks containing 100 ml of either MY50 medium or 1X Vogel's medium supplemented with 2% microcrystalline cellulose (AVICEL®). Cultures were grown for 2, 3, 4 and 5 days at 45°C with vigorous shaking. The mycelia were harvested by filtration through MIRACLOTH® (EMD Chemicals Inc., Gibbstown, NJ, USA), and quick frozen in liquid nitrogen.

To isolate total RNA frozen mycelia of *Thielavia terrestris* NRRL 8126 were ground in an electric coffee grinder. The ground material was mixed 1:1 v/v with 20 ml of FENAZOL™ (Ambion, Inc., Austin, TX, USA) in a 50 ml tube. Once the mycelia were suspended, they were extracted with chloroform and three times with a mixture of phenol-chloroform-isoamyl alcohol 25:24:1 v/v/v. From the resulting aqueous phase, the RNA was precipitated by adding 1/10 volume of 3 M sodium acetate pH 5.2 and 1.25 volumes of isopropanol. The precipitated RNA was recovered by centrifugation at 12,000 *x g* for 30 minutes at 4°C. The final pellet was washed with cold 70% ethanol, air dried, and resuspended in 500 ml of diethylpyrocarbonate treated water (DEPC-water).

RNA samples were pooled and the quality and quantity of the purified RNA was assessed with an AGILENT® 2100 Bioanalyzer (Agilent Technologies, Inc., Palo Alto, CA, USA). A total of 630 µg of RNA was isolated.

Poly A+ RNA was isolated from total RNA using an Absolutely mRNA™ Purification Kit (Stratagene, La Jolla, CA, USA) according to the manufacturer's instructions. cDNA synthesis and cloning was performed according to a procedure based on the SuperScript™ Plasmid System with Gateway® Technology for cDNA Synthesis and Cloning (Invitrogen, Carsbad, CA, USA). 1-2 µg of poly A+ RNA, reverse transcriptase SuperScript II (Invitrogen, Carsbad, CA, USA), and oligo dT-*Not* I primer 5'-GACTAGTTCTAGATCGCGAGCGGCCGCCCTTTTTTTTTTTTTTTVN-3' (SEQ ID NO: 233) were used to synthesize first strand cDNA. Second strand synthesis was performed with *E. coli* DNA ligase, polymerase I, and RNase H followed by end repair using T4 DNA polymerase. The *Sal* I adaptor (5'-TCGACCCACGCGTCCG-3' [SEQ ID NO: 234] and 5'-CGGACGCGTGGG-3' [SEQ ID NO: 235]) was ligated to the cDNA, digested with *Not* I, and subsequently size selected (>2 kb) by 1.1% agarose gel electrophoresis using TAE buffer. The cDNA inserts were directionally ligated into *Sal* I and *Not* I digested vector pCMVsport6

(Invitrogen, Carsbad, CA, USA). The ligation was transformed into ElectroMAX™ DH10B™ T1 cells (Invitrogen, Carsbad, CA, USA).

Library quality was first assessed by randomly selecting 24 clones and PCR amplifying the cDNA inserts with the primers M13-F and M13-R shown below to determine the fraction of insertless clones.

Primer M13-F:

5'-GTAAAACGACGGCCAGT-3' (SEQ iD NO: 236)

Primer M13-R:

5'-AGGAAACAGCTATGACCAT-3' (SEQ iD NO: 237)

Colonies from each library were plated onto LB plates at a density of approximately 1000 colonies per plate. Plates were grown at 37°C for 18 hours and then individual colonies were picked and each used to inoculate a well containing LB medium supplemented with 100 µg of ampicillin per ml in a 384 well plate (Nunc, Rochester, NY, USA). Clones were grown at 37°C for 18 hours. Plasmid DNA for sequencing was produced by rolling circle amplification using a Templiphi™ Kit (GE Healthcare, Piscataway, NJ, USA). Subclone inserts were sequenced from both ends using primers complimentary to the flanking vector sequence as shown below and BigDye® terminator chemistry using a 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

Forward primer:

5'-ATTTAGGTGACACTATAGAA-3' (SEQ iD NO: 238)

Reverse primer:

5'-TAATACGACTCACTATAGGG-3' (SEQ ID NO: 239)

A clone showing 67.4% identity at the nucleotide level to a beta-glucosidase from *Aspergillus oryzae* (U.S. Published Application No. 2005233423) was identified.

Two synthetic oligonucleotide primers shown below were designed to PCR amplify the *Thielavia terrestris* Family GH3B beta-glucosidase gene from the cDNA clone. An IN-FUSION® Cloning Kit (BD Biosciences, Palo Alto, CA, USA) was used to clone the fragment directly into the expression vector pAlLo2 (WO 2005/074647), without the need for restriction digestion and ligation.

Forward primer:

5'-ACTGGATTTACC**ATGAAGCCTGCCATTGTGCT**-3' (SEQ ID NO: 240)

Reverse primer:

5'-TCACCTCTAGTTAATTAA**TCACGGCAACTCAATGCTCA**-3' (SEQ ID NO: 241)

Bold letters represent coding sequence. The remaining sequence is homologous to the insertion sites of pAlLo2.

Fifty picomoles of each of the primers above were used in a PCR reaction containing 200 ng of plasmid cDNA, 1X 2X Advantage GC-Melt LA Buffer (Clonetech Laboratories, Inc.,

Mountain View, CA, USA), 1 μl of 10 mM blend of dATP, dTTP, dGTP, and dCTP, and 1.25 units of Advantage GC Genomic LA Polymerase Mix (Clonetech Laboratories, Inc., Mountain View, CA, USA) in a final volume of 50 μl. The amplification was performed using an EPPENDORF® MASTERCYCLER® 5333 epgradient S (Eppendorf Scientific, Inc., Westbury, NY, USA) programmed for one cycle at 94°C for 1 minute; and 30 cycles each at 94°C for 30 seconds, 60.5°C for 30 seconds, and 72°C for 3 minutes. The heat block was then held at 72°C for 15 minutes followed by a 4°C soak cycle.

The reaction products were isolated by 1.0% agarose gel electrophoresis using TAE buffer where an approximately 2.6 kb product band was excised from the gel and purified using a MINELUTE® Gel Extraction Kit (QIAGEN Inc., Valencia, CA, USA) according to the manufacturer's instructions.

The fragment was then cloned into pAlLo2 using an IN-FUSION® Cloning Kit. The vector was digested with *Nco* I and *Pac* I. The fragment was purified by gel electrophoresis as above and a QIAQUICK® Gel Purification Kit (QIAGEN Inc., Valencia, CA, USA). The gene fragment and the digested vector were combined together in a reaction resulting in the expression plasmid pAG81, in which transcription of the Family GH3B protein gene was under the control of the NA2-tpi promoter (a hybrid of the promoters from the genes for *Aspergillus niger* neutral alpha-amylase and *Aspergillus nidulans* triose phosphate isomerase). The recombination reaction (10 μl) was composed of 1X IN-FUSION® Buffer (BD Biosciences, Palo Alto, CA, USA), 1X BSA (BD Biosciences, Palo Alto, CA, USA), 1 μl of IN-FUSION® enzyme (diluted 1:10) (BD Biosciences, Palo Alto, CA, USA), 108 ng of pAlLo2 digested with *Nco* I and *Pac* I, and 94 ng of the *Thielavia terrestris* GH3B protein purified PCR product. The reaction was incubated at 37°C for 15 minutes followed by 15 minutes at 50°C. The reaction was diluted with 40 μl of 10 mM Tris-0.1 M EDTA buffer and 2.5 μl of the diluted reaction was used to transform *E. coli* TOP10 Competent cells. An *E. coli* transformant containing pAG81 (GH3B protein gene) was identified by restriction enzyme digestion and plasmid DNA was prepared using a BIOROBOT® 9600. The plasmid construct was sequenced using an Applied Biosystems 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) to verify the gene sequence.

Aspergillus oryzae JaL355 protoplasts were prepared according to the method of Christensen *et al.*, 1988, *Bio/Technology* 6: 1419-1422, which were transformed with 5 μg of pAG81. Twenty-six transformants were isolated to individual PDA plates. A small plug from each transformant was used to inoculate 1 ml of M410 media in a 24 well plate and incubated at 34°C. After 5 days of incubation, 7.5 μl of supernatant from four transformants was analyzed using a CRITERION® stain-free, 8-16% gradient SDS-PAGE gel (Bio-Rad Laboratories, Inc., Hercules, CA, USA) according to the manufacturer's instructions. SDS-

PAGE profiles of the cultures showed that several transformants had a new major band of approximately 150 kDa.

A confluent PDA plate of the top transformant (was washed with 5 ml of 0.01% TWEEN® 20 and inoculated into 500 ml flasks each containing 100 ml of M410 medium to generate broth for characterization of the enzyme. The flasks were harvested on day 5, filtered using a 0.22 µm stericup suction filter (Millipore, Bedford, MA, USA), and stored at 4°C.

A 45 ml aliquot of shake flask broth prepared was concentrated ten-fold using a VIVASPIN™ centrifugal concentrator with a molecular weight cut-off of 10 kDa and buffer-exchanged into 50 mM sodium acetate of pH 5. A 4 ml aliquot of the buffer-exchanged, concentrated broth was then diluted to a 10 ml volume with 1 M Tris-HCl pH 8 and water to a concentration of 20 mM Tris-HCl, and the pH adjusted to a final value of 8 using 2 N sodium hydroxide. The resulting material was then purified using a MONO Q™ 5/5 column (GE Healthcare, Piscataway, NJ, USA) equilibrated with 20 mM Tris-HCl buffer pH 7.8. Unbound material was washed from the column with 2 ml equilibration buffer, and then the column was eluted with a linear gradient from 0 – 500 mM sodium chloride in the equilibration buffer. Fractions of 0.3 ml were collected. Unbound material collected during column loading and unbound material washed from the column were pooled for a total volume of 12 ml. Five µl of the fractions, including the pooled unbound material, showing UV absorbance at 280 nm were analyzed using a CRITERION STAIN FREE™ 8–16% Tris-HCl SDS-PAGE gel (Bio-Rad Laboratories, Inc., Hercules, CA, USA) according to the manufacturer's instructions. Precision Plus Protein™ unstained standards (Bio-Rad Laboratories, Inc., Hercules, CA, USA) were used as molecular weight markers. The gel was removed from the cassette and imaged using a CRITERION STAIN FREE™ IMAGER (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The beta-glucosidase was identified in the pooled unbound material as a band at 150 kDa by SDS-PAGE. The 12 ml of pooled unbound material was concentrated as described above to a final volume of 2 ml. The concentrated material was purified using a HILOAD™ 16/60 SUPERDEX™ 75 PREP GRADE column (GE Healthcare, Piscataway, NJ, USA) equilibrated with 20 mM Tris.HCl buffer of pH 7.8 containing 150 mM sodium chloride, and eluted with the equilibration buffer. Fractions of 3 ml were collected. Column fractions were analyzed by SDS-PAGE as described above. Fractions containing the purified beta-glucosidase, identified by a band at 150 kDa by SDS-PAGE, were pooled for a total volume of 6 ml. The pooled material was concentrated using a VIVASPIN™ centrifugal concentrator with a molecular weight cut-off of 5 kDa (GE Healthcare, Piscataway, NJ, USA) to a final volume of 0.4 ml. Protein concentration was determined using the Bio-Rad Protein Assay (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

The enzyme activity of the purified beta-glucosidase was measured using *p*-

nitrophenyl-beta-D-glucopyranoside as substrate. A *p*-nitrophenyl-beta-D-glucopyranoside stock solution was made by dissolving *p*-nitrophenyl-beta-D-glucopyranoside in dimethylsulfoxide (DMSO) to constitute a 0.1 M solution. Before assay, a sample of the stock solution was diluted 100-fold in 100 mM sodium acetate pH 5 containing 0.01% TWEEN® 20 to a 1 mM solution. A 100 µl volume of 1 mM *p*-nitrophenyl-beta-D-glucopyranoside was mixed with each dilution of the enzyme for a 120 µl total volume, and then incubated at 40°C for 20 minutes. Substrate alone, enzyme alone, and buffer alone were run as controls. *p*-Nitrophenol standard solutions of 0.40, 0.25, 0.20, 0.10, 0.05, and 0.02 mM were prepared by diluting a 10 mM stock solution in 100 mM sodium acetate pH 5 containing 0.01% TWEEN® 20. At 20 minutes, 50 µl of 1.0 M sodium carbonate buffer pH 10 was added to each well (including samples, substrate control, enzyme control, reagent control, and standards), mixed, and the absorbance at 405 nm immediately measured on a SPECTRAMAX™ 340 PC plate reader (Molecular Devices, Sunnyvale, CA, USA). The activity measured was 84 units per mg of protein. One unit of activity was defined as the amount of enzyme capable of releasing 1 µmole of *p*-nitrophenolate anion per minute at pH 5, 40°C.

The enzyme activity of the purified beta-glucosidase was also measured with cellobiose. A 2 mg/ml stock solution of cellobiose was prepared in 50 mM sodium acetate pH 5 containing 0.01% TWEEN® 20, and 100 µl were combined with each enzyme dilution for a total volume of 150 µl and incubated at 50°C for 30 minutes. Substrate alone, enzyme alone, and buffer alone were run as controls. Glucose standard solutions of 1.0, 0.50, 0.25, 0.125, 0.063, and 0.031 mg/ml were prepared in 50 mM sodium acetate pH 5. containing 0.01% TWEEN® 20. At 30 minutes, 50 µl of 0.5 M sodium hydroxide solution was added to each well (including samples, substrate control, enzyme control, reagent control, and standards), mixed, and then 20 µl of the mixture was transferred to a Costar® EIA/RIA 96-well plate (Corning Incorporated, Corning, NY, USA) and combined with 200 µl of GLUCOSE OXIDASE REAGENT (Pointe Scientific, Inc., Canton, MI, USA), and allowed to stand at 25°C for 20 minutes and then the absorbance at 500 nm was measured on a SPECTRAMAX™ 340 PC plate reader (Molecular Devices, Sunnyvale, CA, USA). The activity measured was 264 units per mg of protein. One unit of activity was defined as the amount of enzyme capable of releasing 1 µmole of glucose per minute at pH 5, 50°C.

Example 77: Preparation of *Penicillium oxalicum* strain IBT5387 GH3 beta-glucosidase

The *Penicillium oxalicum* strain IBT5387 (Technical University of Denmark; NN005786) GH3 beta-glucosidase (SEQ ID NO: 185 [DNA sequence] and SEQ ID NO: 186 [deduced amino acid sequence]) was obtained according to the procedure described below.

Aspergillus oryzae BECh2 (WO 2000/39322) was used as a host cell for expressing the *P. oxalicum* strain IBT5387 Family GH3 beta-glucosidase gene.

A set of degenerate primers shown below were designed according to the strategy described by Rose *et al.*, 1998, *Nucleic Acids Research* 26: 1628-1635, for cloning a gene encoding a beta-glucosidase (EC 3.2.1.21) belonging to Family GH3.

GH3scree.f1:

atgaccctggccgaaaaagtcaacytnacnacngg (SEQ ID NO: 242)

GH3scree.f2:

ggtggccggaactgggaaggcttctsnccngaycc (SEQ ID NO: 243)

GH3scree.f5:

gagctgggcttccagggctttgtnatgwsngaytgg (SEQ ID NO: 244)

GH3scree.f6:

agcgctttggccggcctcgayatgwsnatgcc (SEQ ID NO: 245)

GH3scree.r1:

atcccagttgctcaggtcccknckngt (SEQ ID NO: 246)

GH3scree.r2:

aaaggttgtgtagctcagnccrtgnccraaytc (SEQ ID NO: 247)

GH3scree.r3:

gtcaaagtggcggtagtcgatraanacnccytc (SEQ ID NO: 248)

GH3scree.r4:

ggtgggcgagttgccgacggggttgactctgccrtanar (SEQ ID NO: 249)

GH3scree.r5:

gccgggcagaccggcccagaggatggcngtnacrttngg (SEQ ID NO: 250)

GH3scree.r6:

caggacggggccaaccgagtgaatgacnacdatngtrtt (SEQ ID NO: 251)

PCR screening of *Penicillium oxalicum* strain IBT5387 was performed using two successive PCRs. The forward primers (GH3scree.f1, GH3scree.f2, GH3scree.f5, or GH3scree.f6) (0.33 µl of a 10 mM stock) were combined with the reverse primers (GH3scree.r1, GH3scree.r2, GH3scree.r3, GH3scree.r4, GH3scree.r5, or GH3scree.r6) (0.33 µl of a 10 mM stock) in a 10 µl mixture containing 0.33 µl of *P. oxalicum* genomic DNA and 5 µl of REDDYMIX™ Extensor PCR Master Mix 1 (ABgene Ltd., Surrey, United Kingdom). *P. oxalicum* genomic DNA was obtained according to the procedure described in the FastDNA® SPIN Kit (Q-BIOgene, Carlsbad, CA, USA). The PCR reaction was performed using a DYAD® Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA) programmed for one cycle at 94°C for 2 minutes; 9 cycles each at 94°C for 15 seconds, 63°C for 30 seconds with a decrease of 1°C for each cycle, and 68°C for 1 minutes 45 seconds; 24 cycles each at 94°C for 15 seconds, 55°C for 30 seconds, and 68°C for 1

minutes 45 seconds; and extension at 68°C for 7 minutes.

PCR products obtained during the first PCR were re-amplified with their corresponding primers by transferring 0.5 µl of the first PCR reaction to a second 20 µl mixture containing the same concentration of primers, dNTPs, DNA polymerase, and buffer as the first PCR reaction. The second PCR was performed using a DYAD® Thermal Cycler programmed for one cycle at 94°C for 2 minutes; 24 cycles each at 94°C for 15 seconds, 58°C for 30 seconds, and 68°C for 1 minutes 45 seconds; and an extension at 68°C for 7 minutes.

PCR products obtained during the second amplification were analyzed by 1% agarose gel electrophoresis using TAE buffer. Single bands ranging from 2000 to 800 nucleotides in size were excised from the gel and purified using a GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions. Purified DNA samples were directly sequenced with primers used for amplification. Sequences were assembled in SeqMan v7.2.1 (DNA star, Madison, WI, USA) into a contig that was used for designing primers shown below, based on recommendations of length and temperature described in a GENE WALKING SPEEDUP™ Kit (Seegene, Inc., Seoul, Korea).

5786GH3-51TSP1f:

CACCAACACCGGCAATCTAGC (SEQ ID NO: 252)

5786GH3-51TSP2f:

GGTGACGAGGTTGTCCAACTGTACG (SEQ ID NO: 253)

5786GH3-51TSP1r:

CTTGAAGCCAAGGCGAGG (SEQ ID NO: 254)

5786GH3-51TSP2r:

TCCGGTATTTCCTACACATGGTCC (SEQ ID NO: 255)

GeneWalking was based on the protocol from the GENE WALKING SPEEDUP™ Kit with some minor differences. Only two PCR amplifications were carried out and in both cases, the REDDYMIX™ Extensor PCR Master Mix 1 was used in place of the enzyme mix present in the Kit. GeneWalking PCR 1 was performed in a total volume of 15 µl by mixing 1.2 µl of primer 1 to 4 (2.5 mM) from the GENE WALKING SPEEDUP™ Kit with 0.3 µl of primer 5786GH3-51TSP1f or primer 5786GH3-51TSP1r (10 mM) in the presence of 7.5 µl of REDDYMIX™ Extensor PCR Master Mix 1, and 0.5 µl of *P. oxalicum* genomic DNA. The PCR was performed using a DYAD® Thermal Cycler programmed for one cycle at 94°C for 3 minutes followed by 1 minute at 42°C and 2 minutes at 68°C; 30 cycles each at 94°C for 30 seconds, 58°C for 30 seconds, and 68°C for 1 minute and 40 seconds; and elongation at 68°C for 7 minutes. A 0.5 µl aliquot of the amplification reaction was transferred to a second PCR tube containing a 20 µl mixture composed of 10 µl of REDDYMIX™ Extensor PCR Master Mix 1, 1 µl of primer 5 (10 mM) from the Kit, 1 µl of primers T5786GH3-51SP2f or

5786GH3-51TSP2r (10 mM). The amplification was performed in a DYAD® Thermal Cycler programmed for denaturation at 94°C for 3 minutes; 35 cycles each at 94°C for 30 seconds, 58°C for 30 seconds, and 68°C for 1 minute and 40 seconds; and elongation at 68°C for 7 minutes.

The PCR products were analyzed by 1% agarose gel electrophoresis in TAE buffer. Single bands ranging from 700 to 1200 nucleotides in size were excised from the gel and purified using a GFX® PCR DNA and Gel Band Purification Kit according to manufacturer's instructions. Purified DNA samples were directly sequenced with the primers used for amplification.

Based on blastx analyses, the start and the stop codons of the gene were identified and the primers shown below were designed for cloning the gene into the expression vector pDAu109 (WO 2005042735) using an IN-FUSION™ Dry-Down PCR Cloning Kit.

5786GH3-51r1:

agatctcgagaagcttaCCATGACTCCAATCGCGCGCTCAAGG (SEQ ID NO: 256)

5786GH3-51f1:

acacaactggggatccaccATGAGGAGCTCAACGACGGTTCTGGCC (SEQ ID NO: 257)

The *P. oxalicum* beta-glucosidase gene was amplified by PCR using the two cloning primers described above with *P. oxalicum* strain IBT5387 genomic DNA. The PCR was composed of 1 µl of *P. oxalicum* genomic DNA, 0.75 µl of primer 5786GH3-51f1 (10 µM), 0.75 µl of primer 5786GH3-51r1 (10 µM), 3 µl of 5X HF buffer, 0.25 µl of 50 mM $MgCl_2$, 0.3 µl of 10 mM dNTP, 0.15 µl of PHUSION® DNA polymerase, and PCR-grade water to 15 µl. The amplification reaction was performed using a DYAD® Thermal Cycler programmed for 2 minutes at 98°C followed by 10 touchdown cycles each at 98°C for 15 seconds, 70°C (-1°C/cycle) for 30 seconds, and 72°C for 2 minutes and 30 seconds; and 25 cycles each at 98°C for 15 seconds, 60°C for 30 seconds, 72°C for 2 minutes and 30 seconds, and 5 minutes at 72°C.

The reaction product was isolated by 1.0% agarose gel electrophoresis using TAE buffer where an approximately 2.8 kb PCR product band was excised from the gel and purified using a GFX® PCR DNA and Gel Band Purification Kit according to manufacturer's instructions. DNA corresponding to the *Penicillium oxalicum* beta-glucosidase gene was cloned into the expression vector pDAu109 (WO 2005042735) linearized with *Bam* HI and *Hind* III, using an IN-FUSION™ Dry-Down PCR Cloning Kit according to the manufacturer's instructions.

A 2.5 µl volume of the diluted ligation mixture was used to transform *E. coli* TOP10 chemically competent cells. Three colonies were selected on LB agar plates containing 100 µg of ampicillin per ml and cultivated overnight in 3 ml of LB medium supplemented with 100 µg of ampicillin per ml. Plasmid DNA was purified using an E.Z.N.A.® Plasmid Mini Kit

according to the manufacturer's instructions. The *Penicillium oxalicum* beta-glucosidase gene sequence was verified by Sanger sequencing before heterologous expression. One plasmid designated pIF113#1 was selected for expressing the *P. oxalicum* beta-glucosidase in an *Aspergillus oryzae* host cell.

The coding sequence is 2793 bp including the stop codon with 2 predicted introns of 82 bp (nucleotides 85 to 116) and 59 bp (nucleotides 346 to 404). The encoded predicted protein is 883 amino acids. Using the SignalP program (Nielsen *et al.*, 1997, *Protein Engineering* 10: 1-6), a signal peptide of 21 residues was predicted. The predicted mature protein contains 862 amino acids with a predicted molecular mass of 94.7 kDa and an isoelectric point of 5.04.

Protoplasts of *Aspergillus oryzae* BECh2 (WO 2000/39322) were prepared according to WO 95/002043. One hundred µl of protoplasts were mixed with 2.5-15 µg of the *Aspergillus* expression vector and 250 µl of 60% PEG 4000 (Applichem, Darmstadt, Germany) (polyethylene glycol, molecular weight 4,000), 10 mM $CaCl_2$, and 10 mM Tris-HCl pH 7.5 and gently mixed. The mixture was incubated at 37°C for 30 minutes and the protoplasts were spread onto COVE plates for selection. After incubation for 4-7 days at 37°C spores of sixteen transformants were inoculated into 0.5 ml of YP medium supplemented with 2% maltodextrin in 96 deep-well plates. After 4 days cultivation at 30°C, the culture broths were analyzed to identify the best transformants producing large amounts of active *P. oxalicum* beta-glucosidase. The analysis was based on SDS-PAGE and activity of the enzyme on 4-nitrophenyl-beta-D-glucopyranoside (pNPG) as described in Example 16.

Spores of the best transformant were spread on COVE plates containing 0.01% TRITON® X-100 in order to isolate single colonies. The spreading was repeated twice in total on COVE plates containing 10 mM sodium nitrate. Spores were then inoculated into 100 ml of YP medium supplemented with 2% maltodextrin in 250 ml shake flasks and incubated for 4 days at 30°C with shaking at 100 rpm.

Combined supernatants of the shake flask cultures were first filtered using a glass micro fiber filter with a 0.7 µm pore size, and then sterile filtered using a filtration unit equipped with a PES (Polyether sulfone) with a 0.22 µm pore size (Nalge Nunc International, New York, NY USA). The sterile filtered supernatant was then adjusted to a concentration of 2 M ammonium sulfate by slowly adding solid ammonium sulfate, dissolving by gentle stirring, and then filtering using a glass micro fiber filter with a 0.7 µm pore size. If there was any precipitate, it was discarded.

The filtered supernatant was applied to a 50 ml Toyopearl Phenyl-650M column (TOSOH Bioscience GmbH, Germany) equilibrated with 2 M ammonium sulfate in water and unbound material was eluted with 2 M ammonium sulfate until the UV absorbance at 280 nm

was below 0.05. Bound protein was eluted with 50% ethanol as solution B using a step gradient. Ten ml fractions were collected and moniterd by UV absorbance at 280 nm. The fractions were analyzed by SDS-PAGE and pooled the fractions containing protein with theexpected molecular weight. The pooled fractions were dialyzed using 50 mM HEPES pH 7.5 buffer, so the ionic strength was below 4 MSi and the pH was 7.5.

The pooled protein was applied to a 50 ml Q Sepharose® Fast Flow column equilibrated with 50 mM HEPES pH 7.5 buffer and unbound material was eluted with 50 mM HEPES buffer pH 7.5. The bound protein was then eluted with a linear 20 column volume salt gradient in 50 mM HEPES buffer pH 7.5 containing 1 M NaCl as buffer B. Ten ml fractions of the eluate were collected and each were analyzed for purity of the protein by SDS-PAGE. The fractions with highest purity were pooled. Identity of the beta-glucosidase was confirmed by mass spectroscopy and protein identification was carried out by in gel digestion. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 78: Preparation of *Penicillium oxalicum* strain IBT5387 GH3 beta-glucosidase

The *Penicillium oxalicum* strain IBT5387 (Technical University of Denmark; NN005786) GH3 beta-glucosidase (SEQ ID NO: 187 [DNA sequence] and SEQ ID NO: 188 [deduced amino acid sequence]) was obtained according to the procedure described below.

Genomic DNA was isolated according to the procedure described in Example 73.

A set of degenerate primers (shown below) was hand-designed according to the strategy described by Rose *et al.*, 1998, *Nucleic Acids Res.* 26: 1628–1635, for targetting beta-glucosidases (EC 3.2.1.21) belonging to Family GH3.

GH3scree.f1 atgaccctggccgaaaaagtcaacytnacnacngg (SEQ ID NO: 258)
GH3scree.f2 ggtggccggaactgggaaggcttctsnccngaycc (SEQ ID NO: 259)
GH3scree.f5 gagctgggcttccagggctttgtnatgwsngaytgg (SEQ ID NO: 260)
GH3scree.f6 agcgctttggccggcctcgayatgwsnatgcc (SEQ ID NO: 261)
GH3scree.r1 atcccagttgctcaggtcccknckngt (SEQ ID NO: 262)
GH3scree.r2 aaaggttgtgtagctcagnccrtgnccraaytc (SEQ ID NO: 263)
GH3scree.r3 gtcaaagtggcggtagtcgatraanacnccytc (SEQ ID NO: 264)
GH3scree.r4 ggtgggcgagttgccgacggggttgactctgccrtanar (SEQ ID NO: 265)
GH3scree.r5 gccgggcagaccggcccagaggatggcngtnacrttngg (SEQ ID NO: 266)
GH3scree.r6 caggacggggccaaccgagtgaatgacnacdatngtrtt (SEQ ID NO: 267)

PCR screening of *Penicillium oxalicum* strain IBT5387 genomic DNA was performed with two successive PCRs. Each forward primers (f1, f2, f5, and f6) (0.33 µl of a 10 mM stock) was combined with each reverse primers (r1, r2, r3, r4, r5, and r6) (0.33 µl of a 10 mM stock) in a 10 µl mix containing 0.33 µl genomic DNA, 5 µl of Reddy Mix Extensor PCR

Master Mix 1 (Cat# AB-0794/A, ABgene UK, commercialized by Thermo Fisher Scientific). A hot-start PCR reaction was carried out in a DYAD® PCR machine for 2 minutes at 94°C followed by 9 cycles each at 94°C for 15 seconds, 63°C for 30 seconds with a 1°C decrease for each cycle, 68°C for 1 minute 45 seconds, and followed by 24 cycles each at 94°C for 15 seconds, 55°C for 30 seconds, 68°C for 1 minute 45 seconds, supplemented by a 7 minutes extension at 68°C. PCR-products produced during this first PCR were re-amplified with their corresponding primers by transferring 0.5µl of the first PCR reaction to a second 20 µl mix containing the same concentration of primers, dNTPs, polymerase, and buffer than in the first PCR reaction. The second PCR was carried out on the same PCR block at 94°C for 2 minutes followed by 24 cycles each at 94°C for 15 seconds, 58°C for 30 seconds, 68°C for 1 minute 45 seconds and completed by a 7 minutes extension at 68°C. PCR products produced during the second amplification were analyzed on 1% agarose gel electrophoresis in TAE buffer. Single bands ranging from 2000 to 800 nts in size were collected and eluted using the GFX® PCR DNA and Gel Band Purification Kit according to manufacturer's instructions. Purified DNA samples were directly sequenced with primers used for amplification.

Sequences were assembled in SeqMan v7.2.1 (DNA star, Madison, WI, USA) into a contig that was used for nblast searches (Altschul *et al.*, 1997, *Nucleic Acids Res.* 25: 3389-3402) on publicly available sequences. Results of the search indicated that the sequence GenBank EU700488.1 was nearly identical to the contig identified. Therefore, the cloning primers 5786GH3-50f and 5786GH3-50r were designed based on the sequence information of the cds from GenBank EU700488.1 for IN-FUSION™ cloning according to the manufacturer instructions for cloning in the expression vector pDAu109 (WO 2005/042735).

5786GH3-50f1:

5'-acacaactggggatccaccATGAAGCTCGAGTGGCTGGAAGC-3' (SEQ ID NO: 268)

5786GH3-50r1

5'-agatctcgagaagcttaCTGCACCTTGGGCAGATCGGCTG-3' (SEQ ID NO: 269)

The *Penicillium oxalicum* beta-glucosidase gene was amplified by PCR using the two cloning primers described previously in a PCR reaction that was performed with genomic DNA prepared from the strain IBT5387 (from DTU received in 1992). The PCR reaction was composed of 1 µl of genomic DNA, 0.75 µl of primer 5786GH3-51f1 (10 µM); 0.75 µl of primer 5786GH3-51r1 (10 µM); 3 µl of 5X HF buffer, 0.25 µl of 50 mM $MgCl_2$, 0.3 µl of 10mM dNTP; 0.15 µl of PHUSION® DNA polymerase, and PCR-grade water up to 15 µl. The PCR reaction was performed using a DYAD® PCR machine programmed for 2 minutes at 98°C followed by 10 touchdown cycles at 98°C for 15 seconds, 70°C (-1°C/cycle) for 30 seconds, and 72°C for 2 minutes 30 seconds; and 25 cycles each at 98°C for 15 seconds, 60°C for 30 seconds, 72°C for 2 minutes 30 seconds, and 5 minutes at 72°C.

The reaction product was isolated by 1.0% agarose gel electrophoresis using TAE buffer where an approximately 2.8 kb PCR product band was excised from the gel and purified using a GFX® PCR DNA and Gel Band Purification Kit according to manufacturer's instructions. DNA corresponding to the *Penicillium oxalicum* beta-glucosidase gene was cloned into the expression vector pDAu109 (WO 2005042735) linearized with *Bam* HI and *Hind* III, using an IN-FUSION™ Dry-Down PCR Cloning Kit according to the manufacturer's instructions.

A 2.5 µl volume of the diluted ligation mixture was used to transform *E. coli* TOP10 chemically competent cells. Three colonies were selected on LB agar plates containing 100 µg of ampicillin per ml and cultivated overnight in 3 ml of LB medium supplemented with 100 µg of ampicillin per ml. Plasmid DNA was purified using an E.Z.N.A.® Plasmid Mini Kit according to the manufacturer's instructions. The *Penicillium oxalicum* beta-glucosidase gene sequence was verified by Sanger sequencing before heterologous expression.

The coding sequence is 2964 bp including the stop codon and is interrupted by 5 introns of 101 bp (nucleotides 61 to 161), 64 bp (nucleotides 302 to 365), 79 bp (nucleotides 411 to 489), 63 bp (nucleotides 543 to 605), and 71 bp (nucleotides 2660 to 2730). The encoded predicted protein is 861 amino acids. Using the SignalP program (Nielsen *et al.*, 1997, *Protein Engineering* 10: 1-6), a signal peptide of 19 residues was predicted. The predicted mature protein contains 842 amino acids.

Protoplasts of *Aspergillus oryzae* BECh2 (WO 2000/39322) were prepared as described in WO 95/02043. One hundred microliters of protoplast suspension were mixed with 2.5-15 µg of the *Aspergillus* expression vector and 250 µl of 60% PEG 4000, 10 mM $CaCl_2$, and 10 mM Tris-HCl pH 7.5 were added and gently mixed. The mixture was incubated at 37°C for 30 minutes and the protoplasts were spread on COVE sucrose (1 M) plates supplemented with 10 mM acetamide and 15 mM CsCl for transformant selection. After incubation for 4-7 days at 37°C spores of several transformants were seeded on YP-2% maltodextrin medium. After 4 days cultivation at 30°C culture broth was analyzed in order to identify the best transformants based on their ability to produce a large amount of active *Penicillium oxalicum* beta-glucosidase. The screening was based on intensity of the band corresponding to the heterologous expressed protein determined by SDS-PAGE and activity of the enzyme on 4-nitrophenyl-beta-D-glucopyranoside (pNPG) as described in Example 16.

Spores of the best transformant were spread on COVE plates containing 0.01% TRITON® X-100 in order to isolate single colonies. The spreading was repeated twice in total on COVE sucrose medium (Cove, 1996, *Biochim. Biophys. Acta* 133: 51-56) containing 1 M sucrose and 10 mM sodium nitrate, supplemented with 10 mM acetamide and 15 mM

CsCl. Fermentation was then carried out in 250 ml shake flasks using DAP-2C-1 medium for 4 days at 30°C with shaking at 100 rpm.

Filtered broth was concentrated and washed with deionized water using a tangential flow concentrator equipped with a Sartocon® Slice cassette with a 10 kDa MW-CO polyethersulfone membrane (Sartorius Stedim Biotech GmbH, Goettingen, Germany). The concentrate was added to M ammonium sulphate and loaded onto a Phenyl Toyopearl (650M) column (Tosoh Corporation, 3-8-2 Shiba, Minato-ku, Tokyo, Japan) equilibrated in 2 M ammonium sulphate, and bound proteins were eluted with 1 M ammonium sulphate. The eluted protein was buffer exchanged with 25 mM HEPES pH 7.5 with sodium chloride by ultrafiltration with a 10 kDa polyethersulfone membrane using Vivaspin 20 (Sartorius Stedim Biotech GmbH, Goettingen, Germany). Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 79: Preparation of *Talaromyces emersonii* strain CBS 549.92 GH3 beta-glucosidase

The *Talaromyces emersonii* strain CBS 549.92 GH3 beta-glucosidase (SEQ ID NO: 189 [DNA sequence] and SEQ ID NO: 190 [deduced amino acid sequence]) was obtained according to the procedure described below.

Genomic DNA was isolated according to the procedure described in Example 73.

The *Talaromyces emersonii* beta-glucosidase gene was isolated by PCR using two cloning primers GH3-11f and GH3-11r shown below, which were designed based on the publicly available *Talaromyces emersonii* full-length sequence (Genbank AY072918.4) for direct cloning using the IN-FUSION™ strategy.

Primer GH3-11f:

acacaactggggatccaccatgaggaacgggttgctcaaggtcg (SEQ ID NO: 270)

Primer GH3-11r:

agatctcgagaagcttaaattccagggtatggcttaaggggc (SEQ ID NO: 271)

A PCR reaction was performed with genomic DNA prepared from *Talaromyces emersonii* strain CBS 549.92 in order to amplify the full-length gene. The PCR reaction was composed of 1 µl of genomic DNA, 0.75 µl of primer GH3-11f (10 µM); 0.75 µl of primer GH3-11r (10 µM); 3 µl of 5X HF buffer, 0.25 µl of 50 mM $MgCl_2$, 0.3 µl of 10 mM dNTP; 0.15 µl of PHUSION® DNA polymerase, and PCR-grade water up to 15 µl. The PCR reaction was performed using a DYAD® PCR machine programmed for 2 minutes at 98°C followed by 10 touchdown cycles at 98°C for 15 seconds, 70°C (-1°C/cycle) for 30 seconds, and 72°C for 2 minutes 30 seconds; and 25 cycles each at 98°C for 15 seconds, 60°C for 30 seconds, 72°C for 2 minutes 30 seconds, and 5 minutes at 72°C.

The reaction product was isolated by 1.0% agarose gel electrophoresis using TAE buffer where an approximately 2.9 kb PCR product band was excised from the gel and purified using a GFX® PCR DNA and Gel Band Purification Kit according to manufacturer's instructions. DNA corresponding to the *Talaromyces emersonii* beta-glucosidase gene was cloned into the expression vector pDAu109 (WO 2005042735) linearized with *Bam* HI and *Hind* III, using an IN-FUSION™ Dry-Down PCR Cloning Kit according to the manufacturer's instructions.

A 2.5 µl volume of the diluted ligation mixture was used to transform *E. coli* TOP10 chemically competent cells. Three colonies were selected on LB agar plates containing 100 µg of ampicillin per ml and cultivated overnight in 3 ml of LB medium supplemented with 100 µg of ampicillin per ml. Plasmid DNA was purified using an E.Z.N.A.® Plasmid Mini Kit according to the manufacturer's instructions. The *Talaromyces emersonii* beta-glucosidase gene sequence was verified by Sanger sequencing before heterologous expression.

The coding sequence is 2925 bp including the stop codon and is interrupted by 6 introns of 60 bp (nucleotides 61 to 120), 61 bp (nucleotides 261 to 321), 60 bp (nucleotides 367 to 426), 57 bp (nucleotides 480 to 536), 56 bp (nucleotides 1717 to 1772), and 54 bp (nucleotides 2632 to 2685). The encoded predicted protein is 858 amino acids. Using the SignalP program (Nielsen *et al.*, 1997, *Protein Engineering* 10: 1-6), a signal peptide of 19 residues was predicted. The predicted mature protein contains 839 amino acids.

Protoplasts of *Aspergillus oryzae* BECh2 (WO 2000/39322) were prepared as described in WO 95/02043. One hundred microliters of protoplast suspension were mixed with 2.5-15 µg of the *Aspergillus* expression vector and 250 µl of 60% PEG 4000, 10 mM $CaCl_2$, and 10 mM Tris-HCl pH 7.5 were added and gently mixed. The mixture was incubated at 37°C for 30 minutes and the protoplasts were spread on COVE sucrose (1 M) plates supplemented with 10 mM acetamide and 15 mM CsCl for transformant selection. After incubation for 4-7 days at 37°C spores of several transformants were seeded on YP-2% maltodextrin medium. After 4 days cultivation at 30°C culture broth was analyzed in order to identify the best transformants based on their ability to produce a large amount of active *Talaromyces emersonii* beta-glucosidase. The screening was based on intensity of the band corresponding to the heterologous expressed protein determined by SDS-PAGE and activity of the enzyme on 4-nitrophenyl-beta-D-glucopyranoside (pNPG) as described in Example 16.

Spores of the best transformant were spread on COVE plates containing 0.01% TRITON® X-100 in order to isolate single colonies. The spreading was repeated twice in total on COVE sucrose medium (Cove, 1996, *Biochim. Biophys. Acta* 133: 51-56) containing 1 M sucrose and 10 mM sodium nitrate, supplemented with 10 mM acetamide and 15 mM CsCl. Fermentation was then carried out in 250 ml shake flasks using YP-2% maltodextrin

medium for 4 days at 30°C with shaking at 100 rpm. The broth was filtered using standard methods. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 80: Preparation of *Thermoascus crustaceus* strain CBS 181.67 GH61A polypeptide

The *Thermoascus crustaceus* strain CBS 181.67 GH61A polypeptide having cellulolytic enhancing activity (SEQ ID NO: 191 [DNA sequence] and SEQ ID NO: 192 [deduced amino acid sequence]) was obtained according to the procedure described below.

Aspergillus oryzae strain HowB101 (WO 95/35385) was used as a host for recombinantly expressing the *Thermoascus crustaceus* GH61 polypeptides having cellulolytic enhancing activity.

Thermoascus crustaceus strain CBS 181.67 was inoculated onto a PDA plate and incubated for 3-4 days at 45ºC in the darkness. Several mycelia-PDA plugs were inoculated into 500 ml shake flasks containing 100 ml of NNCYP-PCS medium. The flasks were incubated for 6 days at 45ºC with shaking at 160 rpm. The mycelia were collected at day 3, day 4, day 5, and day 6. Then the mycelia from each day were combined and frozen in liquid nitrogen, and then stored in a -80°C freezer until use.

Genomic DNA was extracted using a DNEASY® Plant Mini Kit (QIAGEN Inc., Valencia, CA, USA). Total RNA was isolated by using a RNEASY® Plant Mini Kit. cDNA was synthesized by following the instructions of the 3' Rapid Amplification of cDNA End System (3' RACE) (Invitrogen Corporation, Carlsbad, CA, USA).

Four degenerate primers shown below were designed based on conserved regions of known GH61 sequences.

GH61A scF1:

5'-GCNACNGAYCTNGGNTTTG-3' (SEQ ID NO: 272)

GH61A scF2:

5'-GCNACNGAYCTNGGNTTCG-3' (SEQ ID NO: 273)

GH61A scF3:

5'-GCNACNGAYTTRGGNTTYG-3' (SEQ ID NO: 274)

GH61A scR1:

5'-CAYTGNGGRTARTTYTGNGC-3' (SEQ ID NO: 275)

PCR was performed by using a combination of forward primers GH61A scF1, GH61 scF2, and GH61A scF3, and reverse primer GH61A scR1 and cDNA as template. The amplification reaction was composed of 5 µl of 10X PCR buffer (Invitrogen Corporation, Carlsbad, CA, USA), 2 µl of 25 mM $MgCl_2$, 1 µl of 10 mM dNTP, 1 µl of 100 µM forward primer, 1 µl of 100 µM reverse primer, 2 µl of cDNA, 0.5 µl of *Taq* DNA polymerase High

Fidelity (Invitrogen Corporation, Carlsbad, CA, USA), and 37.5 µl of H_2O. The amplification was performed using an Peltier Thermal Cycler programmed for denaturing at 94°C for 2 minutes; 30 cycles each at 94°C for 40 seconds, 50°C for 40 seconds, and 72°C for 1 minute; and a final extension at 72°C for 10 minutes.

A PCR product of approximately 500 base pairs was detected by 1% agarose gel electrophoresis using TBE buffer. The PCR fragment was excised from the gel and purified using an ILLUSTRA® GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions, directly sequenced, and confirmed to be a GH61A partial gene by blast. Based on this partial sequence, new primers shown below were designed for 5' and 3' end cloning using a Genome Walking Kit (Takara Bio Inc., Otsu, Shiga, Japan):

61ASPR1:

5'-TGCAAGGAGCAAGGTAGTTGA-3' (SEQ ID NO: 276)

61ASPR2:

5'-GAGTCCATTCCAGCTTGACGGT-3' (SEQ ID NO: 277)

61ASPF1:

5'-TCAGACAATCTGATAGCGGC-3' (SEQ ID NO: 278)

61ASPF2:

5'-ATCCCAACCACAACTGCACCT-3' (SEQ ID NO: 279)

For 5' end and 3' end cloning, the primary amplifications were composed of 2 µl of genomic DNA as template, 2.5 mM each of dATP, dTTP, dGTP, and dCTP, 100 pmol of AP2 (provided by the Genome Walking Kit) and 10 pmol of primer 61ASPR1 for 5' end cloning or 100 pmol of AP3 (provided by the Genome Walking Kit), and 10 pmol of primer 61ASPF1 for 3' end cloning, 5 µl of 10X LA PCR Buffer II (provided by the Genome Walking Kit), and 2.5 units of TakaRa LA *Taq* DNA polymerase (provided by the Genome Walking Kit) in a final volume of 50 µl. The amplifications were performed using an Peltier Thermal Cycler programmed for pre-denaturing at 94°C for 1 minute and 98°C for 1 minute; five cycles each at a denaturing temperature of 94°C for 30 seconds; annealing at 60°C for 1 minute and elongation at 72°C for 2 minutes; 1 cycle of denaturing at 94°C for 30 seconds; annealing at 25°C for 3 minutes and elongation at 72°C for 2 minutes; fifteen repeats of 2 cycles at 94°C for 30 seconds, 62°C for 1 minutes, and 72°C for 2 minutes; followed by 1 cycle at 94°C for 30 seconds, 44°C for 1 minutes, and 72°C for 2 minutes; and a final extension at 72°C for 10 minutes. The heat block then went to a 4°C soak cycle.

The secondary ampliifications were composed of 2 µl of 20X diluted primary PCR product as templates, 2.5 mM each of dATP, dTTP, dGTP, and dCTP, 100 pmol of AP2, and 10 pmol of primer 61ASPR2 for 5' end cloning or 100 pmol of AP3 and 10 pmol of primer 61ASPF2 for 3' end cloning, 5 µl of 10X LA PCR Buffer II, and 2.5 units of TakaRa LA *Taq* DNA polymerase in a final volume of 50 µl. The amplifications were performed using an

Peltier Thermal Cycler programmed for fifteen repeats of 2 cycles of 94°C for 30 seconds; 62°C for 1 minutes; 72°C for 2 minutes; followed by 1 cycle at 94°C for 30 seconds, 44°C for 1 minutes, and 72°C for 2 minutes; and a final extension at 72°C for 10 minutes. The heat block then went to a 4°C soak cycle.

The PCR products from the 5' and 3' end PCR were recovered and sequenced. They were identified as the 5' end and 3' end of the GH61A polypeptide gene. Then the three sequences including the partial gene, 5' end, and 3' end were assembled to generate the full-length GH61A.

The obtained full-length gene showed that the sequence contains a coding region of 871 nucleotides including 1 intron and stop codon, and encodes 251 amino acids with a predicted signal peptide of 22 amino acids.

Based on the full-length *Thermoascus crustaceus* GH61A gene sequence, oligonucleotide primers, shown below, were designed to amplify the GH61A gene from genomic DNA of *Thermoascus crustaceus* CBS 181.67. An IN-FUSION® CF Dry-Down Cloning Kit (Clontech Laboratories, Inc., Mountain View, CA, USA) was used to clone the fragment directly into the expression vector pPFJO355, without the need for restriction digestion and ligation.

Sense primer:

5'-ACACAACTGGGGATCCACC**ATGGCCTTTTCCCAGATAATGGCTA**-3' (SEQ ID NO: 280)

Antisense primer:

5'-GTCACCCTCTAGATCT**GGATCGCAGGAGCGTTCAGA**-3' (SEQ ID NO: 281)

Bold letters represent the coding sequence for the sense primer and the downstream sequence of the stop codon for the antisense primer. The remaining sequence is homologous to the insertion sites of pPFJO355.

Twenty picomoles of each of the primers above were used in a PCR reaction composed of *Thermoascus crustaceus* genomic DNA, 10 µl of 5X GC Buffer, 1.5 µl of DMSO, 2 µl of 2.5 mM each of dATP, dTTP, dGTP, and dCTP, and 1 unit of PHUSION™ High-Fidelity DNA Polymerase in a final volume of 50 µl. The amplification was performed using a Peltier Thermal Cycler programmed for denaturing at 98°C for 1 minute; 5 cycles of denaturing at 98°C for 15 seconds, annealing at 70°C for 30 seconds, with a 1°C decrease per cycle, and elongation at 72°C for 30 seconds; 25 cycles each at 98°C for 15 seconds and 72°C for 90 seconds; and a final extension at 72°C for 10 minutes. The heat block then went to a 4°C soak cycle.

The reaction products were isolated by 1.0% agarose gel electrophoresis using TBE buffer where an approximately 1.0 kb product band was excised from the gel, and purified using an ILLUSTRA® GFX® PCR DNA and Gel Band Purification Kit according to the

manufacturer's instructions.

Plasmid pPFJO355 was digested with *Bam* HI and *Bgl* II, isolated by 1.0% agarose gel electrophoresis using TBE buffer, and purified using an ILLUSTRA® GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

The gene fragment and the digested vector were ligated together using an IN-FUSION® CF Dry-Down PCR Cloning Kit resulting in pGH61a51486 in which transcription of the *Thermoascus crustaceus* GH61A gene was under the control of the *Aspergillus oryzae* TAKA-alpha-amylase promoter. In brief, 30 ng of pPFJO355 digested with *Bam* HI and *Bgl* II and 50 ng of the *Thermoascus crustaceus* GH61A gene purified PCR product were added to a reaction vial and resuspended in a final volume of 10 µl with deionized water. The reaction was incubated at 37°C for 15 minutes and then 50°C for 15 minutes. Three µl of the reaction were used to transform *E. coli* TOP10 competent cells according to the manufacturer's instructions. The transformation was spread on LB plates supplemented with 100 µg of ampicillin per ml and incubated at at 37°C for 1 day. An *E. coli* transformant containing a plasmid designated pGH61a51486 was detected by colony PCR and plasmid DNA was prepared using a QIAprep Spin Miniprep Kit (QIAGEN Inc., Valencia, CA, USA). The *Thermoascus crustaceus* GH61A gene insert in pGH61a51486 was confirmed by DNA sequencing using a 3730XL DNA Analyzer.

The same PCR fragment was cloned into pGEM-T vector using a pGEM-T Vector System to generate pGEM-T-GH61a51486. The *Thermoascus crustaceus* GH61A gene insert in pGEM-T-GH61a51486 was confirmed by DNA sequencing using a 3730XL DNA Analyzer. *E. coli* strain T-51486A, designated NN059126, containing pGEM-T-GH61a51486, was deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM), Mascheroder Braunschweig, Germany, on June 10, 2009, and assigned accession number DSM 22656.

Nucleotide sequence data were scrutinized for quality and all sequences were compared to each other with assistance of PHRED/PHRAP software (University of Washington, Seattle, WA, USA).

The nucleotide sequence and deduced amino acid sequence of the *Thermoascus crustaceus gh61a* gene are shown in SEQ ID NO: 191 and SEQ ID NO: 192, respectively. The coding sequence is 871 bp including the stop codon and is interrupted by one intron of 115 base pairs (nucleotides 105-219). The encoded predicted protein is 251 amino acids. The% G+C content of the full-length coding sequence and the mature coding sequence are 50.23% and 52.55%, respectively. Using the SignalP software program (Nielsen *et al.*, 1997, *Protein Engineering* 10: 1-6), a signal peptide of 22 residues was predicted. The predicted mature protein contains 229 amino acids with a predicted molecular mass of 26.35 kDa.

Aspergillus oryzae HowB101 protoplasts were prepared according to the method of

Christensen *et al.*, 1988, *Bio/Technology* 6: 1419-1422 and transformed with 3 µg of pGH61a51486. The transformation yielded about 50 transformants. Twelve transformants were isolated to individual Minimal medium plates.

Four transformants were inoculated separately into 3 ml of YPM medium in a 24-well plate and incubated at 30°C with shaking at 150 rpm. After 3 days incubation, 20 µl of supernatant from each culture were analyzed by SDS-PAGE using a NUPAGE® NOVEX® 4-12% Bis-Tris Gel with MES buffer according to the manufacturer's instructions. The resulting gel was stained with INSTANT BLUE™ (Expedeon Ltd., Babraham Cambridge, UK). SDS-PAGE profiles of the cultures showed that the majority of the transformants had a major band of approximately 30 kDa. The expression strain was designated *Aspergillus oryzae* EXP03151.

A slant of *Aspergillus oryzae* EXP03151 was washed with 10 ml of YPM medium and inoculated into a 2 liter flask containing 400 ml of YPM medium to generate broth for characterization of the enzyme. The culture was harvested on day 3 and filtered using a 0.45 µm DURAPORE® Membrane (Millipore, Bedford, MA, USA).

The filtered broth was concentrated and buffer exchanged using a tangential flow concentrator equipped with a Sartocon® Slice cassette with a 10kDa cut-off polyethersulfone membrane (Sartorius Stedim Biotech GmbH, Goettingen, Germany) with 25 mM HEPES, pH 7.0. The protein was applied to a Q Sepharose™ Fast Flow column (GE Healthcare, Piscataway, NJ, USA) equilibrated in 25 mM HEPES pH 7.0. The protein was recovered in the eluate. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 81: Preparation of *Talaromyces emersonii* strain NN05002 GH10 xylanase

The *Talaromyces emersonii* strain NN05002 GH10 xylanase (SEQ ID NO: 193 [DNA sequence] and SEQ ID NO: 194 [deduced amino acid sequence]) was obtained according to the procedure described below.

Talaromyces emersonii was grown on a PDA agar plate at 45°C for 3 days. Mycelia were collected directly from the agar plate into a sterilized mortar and frozen under liquid nitrogen. Frozen mycelia were ground, by mortar and pestle, to a fine powder, and genomic DNA was isolated using a DNeasy® Plant Mini Kit.

Oligonucleotide primers, shown below, were designed to amplify the GH10 xylanase gene from genomic DNA of *Talaromyces emersonii*. An IN-FUSION™ CF Dry-down Cloning Kit was used to clone the fragment directly into the expression vector pPFJO355, without the need for restriction digestion and ligation.

Sense primer:

5'-ACACAACTGGGGATCCACC**ATGGTTCGCCTCAGTCCAG**-3' (SEQ ID NO: 282)

Antisense primer:

5'-GTCACCCTCTAGATCT**TTACAGACACTGCGAGTAATACTCATTG**-3' (SEQ ID NO: 283)

Bold letters represented the coding sequence. The remaining sequence was homologous to the insertion sites of pPFJO355.

Twenty picomoles of each of the primers above were used in a PCR reaction composed of *Talaromyces emersonii* genomic DNA, 10 µl of 5X GC Buffer, 1.5 µl of DMSO, 5 mM each of dATP, dTTP, dGTP, and dCTP, and 0.6 unit of Phusion™ High-Fidelity DNA Polymerase in a final volume of 50 µl. The amplification was performed using a Peltier Thermal Cycler programmed for denaturing at 98°C for 40 seconds; 8 cycles of denaturing at 98°C for 15 seconds, annealing at 65°C for 30 seconds, with a 1°C decrease per cycle and elongation at 72°C for 80 seconds; and another 23 cycles each at 98°C for 15 seconds, 58°C for 30 seconds and 72°C for 80 seconds; final extension at 72°C for 7 minutes. The heat block then went to a 10°C soak cycle.

The reaction products were isolated by 1.0% agarose gel electrophoresis using TBE buffer where an approximately 1.4 kb product band was excised from the gel, and purified using an ILLUSTRA® GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

Plasmid pPFJO355 was digested with *Bam* I and *Bgl* II, isolated by 1.0% agarose gel electrophoresis using TBE buffer, and purified using an ILLUSTRA® GFX® PCR DNA and Gel Band Purification Kit according to the manufacturer's instructions.

The gene fragment and the digested vector were ligated together using an IN-FUSION™ CF Dry-down PCR Cloning resulting in pxynTe50022 in which transcription of the *Talaromyces emersonii* GH10 xylanase gene was under the control of the *Aspergillus oryzae* TAKA amylase promoter. In brief, 20 ng of pPFJO355 digested with *Bam* I and *Bgl* II, and 60 ng of the *Talaromyces emersonii* GH10 xylanase gene purified PCR product were added to a reaction vial and resuspended in a final volume of 10 µl with addition of deionized water. The reaction was incubated at 37°C for 15 minutes and then 50°C for 15 minutes. Three µl of the reaction were used to transform *E. coli* TOP10 competent cells. An *E. coli* transformant containing pxynTe50022 was detected by colony PCR and plasmid DNA was prepared using a QIAprep Spin Miniprep Kit. The *Talaromyces emersonii* GH10 xylanase gene insert in pxynTe50022 was confirmed by DNA sequencing using a 3730XL DNA Analyzer.

Aspergillus oryzae HowB101 (WO 95/35385) protoplasts were prepared according to the method of Christensen *et al.*, 1988, *Bio/Technology* 6: 1419-1422 and transformed with 3 µg of pxynTe50022. The transformation yielded about 50 transformants. Twelve transformants were isolated to individual Minimal medium plates.

Four transformants were inoculated separately into 3 ml of YPM medium in a 24-well plate and incubated at 30°C, 150 rpm. After 3 days incubation, 20 µl of supernatant from each culture were analyzed by SDS-PAGE using a NUPAGE® NOVEX® 4-12% Bis-Tris Gel with MES buffer according to the manufacturer's instructions. The resulting gel was stained with INSTANT® Blue. SDS-PAGE profiles of the cultures showed that the majority of the transformants had a major smeary band of approximately 55kDa. The expression strain was designated as *A. oryzae* EXP03373.

A slant of *A. oryzae* EXP03373 was washed with 10 ml of YPM medium and inoculated into six 2 liter flasks, each containing 400 ml of YPM medium, to generate broth for characterization of the enzyme. The culture was harvested on day 3 by filtering the culture through MIRACLOTH® (CALBIOCHEM, Inc. La Jolla, CA, USA). The filtered culture broth was then again filtered using a 0.45 µm DURAPORE Membrane. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

A 1600 ml volume of filtered broth supernatant of *A. oryzae* EXP03373 was precipitated with ammonium sulfate (80% saturation), re-dissolved in 100 ml of 25mM Bis-Tris pH 6.0, dialyzed against the same buffer, and filtered through a 0.45 µm filter; the final volume was 200 ml. The solution was applied to a 40 ml Q Sepharose® Fast Flow column equilibrated with 25 mM Bis-Tris pH 6.0, and the proteins were eluted with a linear NaCl gradient (0–0.4 M). Fractions with activity against AZCL-xylan were analyzed by SDS-PAGE using a NUPAGE® NOVEX® 4-12% Bis-Tris Gel with MES buffer. Fractions with the correct molecular weight were pooled and concentrated by ultra filtration.

The supernatants were tested for endocellulase activity by microtiter plate assay as described below. A solution of 0.2% of the blue substrate AZCL-Xylan (Megazyme) was suspended in a 0.1 M sodium acetate buffer (pH 5.5) under stirring. The solution was distributed under stirring to a microtiter plate (200 µl to each well). Twenty µl of enzyme sample was added and incubated in an EPPENDORF® Thermomixer for 20 minutes at 50°C and 650 rpm. A denatured enzyme sample (100°C boiling for 20 minutes) was used as blank. After incubation the colored solution was separated from the solid by centrifugation at 3000 rpm for 5 minutes at 4°C. Then 150 µl of supernatant was transferred to a microtiter plate and the absorbance was measured using a Spectra Max M2 at 595 nm.

Example 82: Preparation of *Penicillium* sp. Strain NN51602 GH10 xylanase

The *Penicillium* sp. strain NN51602 GH10 xylanase (SEQ ID NO: 195 [DNA sequence] and SEQ ID NO: 196 [deduced amino acid sequence]) was obtained according to PCT/US10/032034. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 83: Preparation of *Meripilus giganteus* strain CBS 521.95 GH10 xylanase

The *Meripilus giganteus* strain CBS 521.95 GH10 xylanase (SEQ ID NO: 197 [DNA sequence] and SEQ ID NO: 198 [deduced amino acid sequence]) was recombinantly prepared according to WO 97/27290 except *Aspergillus oryzae* Bech2 (WO 2000/39322) was used as a host and pDAu75 as the expression vector. Vector pDAu75 is a derivative of pJal721 (WO 03/008575). Plasmid pDAu75 mainly differs from pJal721 in that the selection marker URA3 of *E. coli* has been dirupted by the insertion of the ampicillin resistance gene *E. coli* beta lactamase, which allows for rapid selection of positive recombinant *E. coli* clones using commercially available and highly competent strains on commonly used LB ampicillin plates. The ampicillin resistance gene is entirely removable using the two flanking *Not* I sites restoring a functional selection marker URA3. The techniques used for making pDAu75 from pJal721 are common molecular biology techniques for DNA cloning. Cloning of the cDNA sequence of the *Meripilus giganteus* GH10 xylanase gene into pDAu75 was performed by a restriction/ligation cloning procedure from the xylanase producing yeast colony as described in WO 97/27290.

The broth was filtered using Whatmann glass filter GF/D, GF/A, GF/C, GF/F (2.7 µm, 1.6 µm, 1.2 µm and 0.7 µm, respectively) followed by filtration through a 0.45 µm filter.

Ammonia sulfate was added to the filtered broth to a final concentration of 3 M and the precipitate was collected after centrifugation at 10,000 x *g* for 30 minutes. The precipitate was dissolved in 10 mM Tris/HCl pH 8.0 and dialyzed against 10 mM Tris/HCl pH 8.0 overnight. The dialyzed preparation was applied to a 150 ml Q SEPHAROSE® Fast Flow column equilibrated with 10 mM Tris/HCl pH 8.0 and the enzyme was eluted with a 1050 ml (7 column volumes) linear salt gradient from 0 to 1 M NaCl in 10 mM Tris/HCl pH 8.0. Elution was followed with A280 nm detection and fractions were collected and assayed for xylanase activity using 0.2% AZCL-Arabinoxylan from wheat (Megazyme) in 0.2 M sodium phosphate pH 6.0 plus 0.01% TRITON® X100. Fractions containing xylanase activity were pooled and stored at -20°C. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 84: Preparation of *Dictyoglomus thermophilum* strain ATCC 35947 GH11 xylanase

The *Dictyoglomus thermophilum* GH11 xylanase (SEQ ID NO: 199 [DNA sequence] and SEQ ID NO: 200 [deduced amino acid sequence]) was recombinantly prepared according to the following procedure.

Bacillus subtilis strains were made competent using the method described by Anagnostopoulos and Spizizen, 1961, *Journal of Bacteriology* 81: 741-746. DNA sequencing

was conducted with an ABI 3700 Sequencing (Applied Biosystems, Inc., Foster City, CA, USA).

Bacillus subtilis strain SMO25 was constructed as described below to delete an intracellular serine protease (*isp*A) gene in *Bacillus subtilis* strain A164Δ10 (Bindel-Connelly *et al.*, 2004, *J. Bacteriol.* 186: 4159–4167).

A deletion plasmid, pNNB194-*isp*AΔ, was constructed by splicing by overlap extension (SOE) (Horton *et al.*, 1989, *Gene* 77: 61-8). Flanking DNA sequences 5' and 3' of the *isp*A gene were obtained by PCR amplification from chromosomal DNA derived from *Bacillus subtilis* strain 164Δ5 (U.S. Patent No. 5,891,701) using primer pairs 994525/994526 and 994527/994528, respectively, shown below. Chromosomal DNA was obtained according to the procedure of Pitcher *et al.*, 1989, *Lett. Appl. Microbiol.* 8: 151-156.

Primer 994525:

5'-GGATCCATTATGTAGGGCGTAAAGC-3' (SEQ ID NO: 284)

Primer 994526:

5'-TTAGCAAGCTTAATCACTTTAATGCCCTCAG-3' (SEQ ID NO: 285)

Primer 994527:

5'-TGATTAAGCTTGCTAATCCGCAGGACACTTC-3' (SEQ ID NO: 286)

Primer 994528:

5'-GGTACCAACACTGCCTCTCTCATCTC-3' (SEQ ID NO: 287)

PCR amplifications were conducted in 50 µl reactions composed of 10 ng of *Bacillus subtilis* strain 164Δ5 chromosomal DNA, 0.4 µM of each primer, 200 µM each of dATP, dCTP, dGTP, and dTTP, 1X PCR Buffer II (Applied Biosystems, Inc., Foster City, CA, USA) with 2.5 mM $MgCl_2$, and 2.5 units of AmpliTaq GOLD® DNA Polymerase (Applied Biosystems, Inc., Foster City, CA, USA). The reactions were performed in a ROBOCYCLER® 40 Temperature Cycler (Stratagene, Corp., La Jolla, CA, USA) programmed for 1 cycle at 95°C for 10 minutes; 25 cycles each at 95°C for 1 minute, 50°C for 1 minute, and 72°C for 1 minute; and 1 cycle at 72°C for 7 minutes.

The PCR products were resolved by 0.8% agarose gel electrophoresis using 0.5X TBE buffer (50 mM Tris base-50 mM boric acid-1 mM disodium EDTA). A band of approximately 400 bp obtained using the primer pair 994525/994526 for the 5' flanking DNA sequence of the *isp*A gene was excised from the gel and extracted using a QIAQUICK® Gel Extraction Kit (QIAGEN Inc., Valencia, CA, USA). A band of approximately 400 bp obtained using the primer pair 994527/994528 for the 3' flanking DNA sequence of the *isp*A gene was excised from the gel and extracted using a QIAQUICK® Gel Extraction Kit.

The final SOE fragment was amplified using the same procedure above with the 400 bp fragments as templates and primers 994525 and 994528, shown above, to produce an *isp*A deletion fragment. The PCR product of approximately 800 kb was resolved by 0.8%

agarose gel electrophoresis using 0.5X TBE buffer.

The final 800 kb SOE fragment was cloned into pCR®2.1 (Invitrogen, Carlsbad, CA, USA) using a TA-TOPO® Cloning Kit (Invitrogen, Carlsbad, CA, USA) and transformed into ONE SHOT® TOP10 Chemically Competent *E. coli* cells (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Transformants were selected on 2X YT agar plates supplemented with 100 µg of ampicillin per ml and incubated at 37°C for 16 hours. The DNA sequence of the cloned fragment was verified by DNA sequencing with M13 forward and reverse primers (Invitrogen, Inc, Carlsbad, CA, USA). The plasmid was designated pCR®2.1-*isp*AΔ.

Plasmid pCR2.1-*isp*AΔ was digested with *Bam* HI and *Asp*718 and subjected to 0.8% agarose gel electrophoresis using 0.5X TBE buffer to isolate the *isp*A deletion fragment. A 800 bp fragment corresponding to the *isp*A deletion fragment was excised from the gel and extracted using a QIAQUICK® Gel Extraction Kit.

The temperature sensitive plasmid pNNB194 (pSK^+/pE194; U.S. Patent No. 5,958,728) was digested with *Bam* HI and *Asp*718 and resolved by 0.8% agarose gel electrophoresis using 0.5X TBE buffer to isolate the vector fragment. A 6600 bp vector fragment of pNNB194 was excised from the gel and extracted using a QIAQUICK® Gel Extraction Kit.

The *isp*A deletion fragment and the pNNB194 fragment were ligated together using a Rapid DNA Ligation Kit (Roche Applied Science, Indianapolis, IN, USA) and the ligation mix was transformed into *E. coli* SURE® cells (Stratagene Corp., La Jolla, CA, USA) selecting for ampicillin resistance according to the manufacturer's instructions. Plasmid DNA was isolated from eight transformants using a BIOROBOT® 9600, digested with *Bam* HI and *Asp*718, and analyzed by agarose electrophoresis as described above to identify plasmids which harbored the *isp*AΔ fragment. One transformant was identified and designated pNNB194-*isp*AΔ.

Plasmid pNNB194-*isp*AΔ was introduced into *Bacillus subtilis* A164Δ10 (Bindel-Connelly *et al.*, 2004, *J. Bacteriol.* 186: 4159–4167) and integrated at the *ispA* locus by selective growth at 45°C on Tryptose blood agar base (TBAB) plates supplemented with 1 µg of erythromycin and 25 µg of lincomycin per ml. The integrated plasmid was then excised by non-selective growth on LB medium at 34°C. Chromosomal DNA was isolated from several erythromycin sensitive clones according to the method of Pitcher *et al.*, 1989, *supra*, and analyzed by PCR using primers 994525 and 994528 using the same method above to confirm the presence of the *isp*A deletion. One such clone was designated *Bacillus subtilis* SMO25.

A linear integration vector-system was used for the expression cloning of a synthetic *Dictyoglomus thermophilum* Family 11 xylanase gene without a binding domain and without

a signal peptide. The synthetic gene sequence was based on the public gene sequence UNIPROT: P77853. The synthetic gene was codon optimized for expression in *Bacillus subtilis* following recommendations by Gustafsson *et al.*, 2004, *Trends in Biotechnology* 22: 346-353. The synthetic gene was generated by DNA2.0 (Menlo Park, CA, USA) and delivered as a cloned fragment in their standard cloning vector (kanamycin resistant). The xylanase gene was cloned as a truncated gene without binding domain and with the signal peptide from *Bacillus clausii* serine protease gene (*apr*H, SAVINASE™, Novo Nordisk A/S, Bagsværd, Denmark) (included in the flanking region). The gene was designed to contain a C-terminal HQHQHQHQP tag to ease purification. The forward primer was designed so the gene was amplified from the signal peptide cleavage site and it had 26 bases overhang (shown in italic in the table below). This overhang was complementary to part of one of the two linear vector fragments and was used when the PCR fragment and the vector fragments were assembled (described below). The reverse primer was designed to amplify the truncated version of the gene and contained an overhang consisting of 30 bp encoding a HQHQHQHQP-tag and a stop codon (the overhang is shown in italic in the table below). This overhang was complementary to part of one of the two linear vector fragments and was used when the PCR fragment and the vector fragments were assembled (described below).

The linear integration construct was a PCR fusion product made by fusion of each gene between two *Bacillus subtils* homologous chromosomal regions along with a strong promoter and a chloramphenicol resistance marker. The fusion was made by splicing by overlap extension (SOE) (Horton *et al.*, 1989, *supra*). The SOE PCR method is also described in WO 2003/095658. Each gene was expressed under the control of a triple promoter system (described in WO 99/43835), consisting of the promoters from *Bacillus licheniformis* alpha-amylase gene (*amyL*), *Bacillus amyloliquefaciens* alpha-amylase gene (*amyQ*), and the *Bacillus thuringiensis cryIIIA* promoter including the mRNA stabilizing sequence. The gene coding for chloramphenicol acetyl-transferase was used as marker (described, for example, by Diderichsen *et al.*, 1993, *Plasmid* 30: 312). The final gene construct was integrated by homologous recombination into the pectate lyase locus of the *Bacillus* chromosome.

The GH11 xylanase gene was amplified from plasmid 7587 by PCR using the primers shown in the Table below 1 below. The plasmid 7587 contains the synthetic *Dictyoglomus thermophilum* GH11 xylanase gene (SEQ ID NO: 304 for the DNA sequence and SEQ ID NO: 305 for the deduced amino acid sequence) without a binding domain and without a signal peptide.

Three fragments were PCR amplified to make the construct: the gene fragment containing the truncated xylanase gene and the 26 bp and 30 bp flanking DNA sequences included in the primers as overhang, the upstream flanking fragment (including a signal

peptide from Savinase and amplified with the primers 260558 and iMB1361Uni2) and the downstream flanking fragment (amplified with the primers 260559 and HQHQHQHQP-f). The flanking fragments were amplified from genomic DNA of the strain iMB1361 (described in patent application WO 2003/095658). All primers used are listed in the Table 1 below.

The gene fragment was amplified using a proofreading polymerase PHUSION™ DNA Polymerase according to the manufacturer's instructions. The two flanking DNA fragments were amplified with "Expand High Fidelity PCR System" (Roche-Applied-Science) according to standard procedures (following the manufacturer's recommendations). The PCR conditions were as follows: 94°C for 2 minutes followed by 10 cycles of (94°C for 15 seconds, 50°C for 45 seconds, 68°C for 4 minutes) followed by 20 cycles of (94°C for 15 seconds, 50°C for 45 seconds, 68°C for 4 minutes (+20 seconds extension per cycle)) and ending with one cycle at 68°C for 10 min. The 3 PCR fragments were subjected to a subsequent Splicing by Overlap Extension (SOE) PCR reaction to assemble the 3 fragments into one linear vector construct. This was pereformed by mixing the 3 fragments in equal molar ratios and a new PCR reaction were run under the following conditions: initial 2 minutes. at 94°C, followed by 10 cycles of (94°C for 15 seconds, 50°C for 45 seconds, 68°C for 5 minutes), 10 cycles of (94°C for 15 seconds, 50°C for 45 seconds, 68°C for 8 minutes), 15 cycles of (94°C for 15 seconds, 50°C for 45 seconds, 68°C for 8 minutes in addition 20 seconds extra per cycle). After the 1st cycle the two end primers 260558 and 260559 were added (20 pMol of each). Two µl of the PCR product were transformed into *Bacillus subtilis.* Transformants were selected on LB plates supplemented with 6 µg of chloramphenicol per ml. The truncated xylanase construct was integrated by homologous recombination into the genome of the *Bacillus subtilis* host PL4250 (AprE-, NprE-, SrfC-, SpoIIAC-, AmyE-, comS+). One transformant, EXP01955, was selected for further work. The xylanase coding region was sequenced in this transformant. It contained one mutation leading to a change of the HQHQHQHQP-tag to a HQHQHQHQQ-tag) but no other mutations were observed.

Table 1. Primers used

Amplification of	**SPECIFIC PRIMER FORWARD**	**SPECIFIC PRIMER REVERSE**
Truncated gene	FORWARD (SEQ ID NO: 288) 5'-*CTTTTAGTTCATCGATCGC ATCGGCT*GCTCAGACATCAA TCACACTTA-3'	REVERSE (SEQ ID NO: 289) 5'-*CTAGGGTTGATGCTGGTG TTGGTGCTGATG*GCTGCCC TGAGAGAAAGTG-3'

Upstream flanking fragment	260558: (SEQ ID NO: 290) 5'-GAGTATCGCCAGTAAGG GGCG-3'	iMB1361Uni2 (SEQ ID NO: 291) 5' AGCCGATGCGATCGATGAA CTA 3'
Downstream flanking fragment	HQHQHQHQP-f (SEQ ID NO: 292) 5'-CATCAGCACCAACACCAG CACCAGCCATAATCGCATGT TCAATCCGCTCCATA-3'	260559: (SEQ ID NO: 293) 5'-GCAGCCCTAAAATCGCAT AAAGC-3'

Chromosomal DNA from *Bacillus subtilis* strain EXP01955 was used as a template to PCR clone the *Bacillus clausii* serine protease gene (*apr*H, SAVINASE™, Novo Nordisk A/S, Bagsværd, Denmark) signal sequence/mature *D. thermophilum* xylanase gene (CBM-deleted) into pCR2.1-TOPO using the following primers which introduce a *Sac* I site at the 5' end (just upstream of the *apr*H ribosome binding site) and a *Mlu* I site at the 3' end (just after the translation stop codon which was introduced after the Ser codon at position 691-693, thereby avoiding the incorporation of the HQHQHQHQQ-tag). Chromosomal DNA was obtained according to the procedure of Pitcher *et al.*, 1989, *supra*.

Primer 062405:

5'-GAGCTCTATAAAAATGAGGAGGGAACCGAATGAAGAAACC-3' (SEQ ID NO: 294)

Primer 062406:

5'-ACGCGTTTAGCTGCCCTGAGAGAAAGTG-3' (SEQ ID NO: 295)

The PCR amplifications were conducted in 50 µl reactions composed of 10 ng of *B. subtilis* EXP01955 chromosomal DNA, 0.4 µM of each primer, 200 µM each of dATP, dCTP, dGTP, and dTTP, 1X PCR Buffer II with 2.5 mM $MgCl_2$, and 2.5 units of AmpliTaq GOLD® DNA Polymerase. The reactions were performed in a ROBOCYCLER® 40 Temperature Cycler programmed for 1 cycle at 95°C for 10 minutes; 25 cycles each at 95°C for 1 minute, 50°C for 1 minute, and 72°C for 1 minute; and 1 cycle at 72°C for 7 minutes. A PCR product of approximately 740 kb of the truncated xylanase gene was resolved by 0.8% agarose gel electrophoresis using 0.5X TBE buffer, excised from the gel, and extracted using a QIAQUICK® Gel Extraction Kit.

The 740 kb fragment was cloned into pCR®2.1 using a TA-TOPO® Cloning Kit according to the manufacturer's instructions and transformed into ONE SHOT® TOP10 Chemically Competent *E. coli* cells according to the manufacturer's instructions. Transformants were selected on 2X YT agar plates supplemented with 100 µg of ampicillin

per ml and incubated at 37°C for 16 hours. The DNA sequence of the cloned fragment was verified by DNA sequencing with M13 forward and reverse primers. The plasmid was designated pCR2.1-Dt xyl.

DNA sequencing revealed that there was an extra G at position 19 of the sequence encoding the *apr*H signal sequence. A QUIKCHANGE® XL Site-Directed Mutagenesis Kit (Stratagene Corp., La Jolla, CA, USA) was utilized to correct the mistake in plasmid pCR2.1-Dt xyl using the following primers to delete the extra G residue:

Primer 062535:

5'-CCGTTGGGGAAAATTGTCGC-3' (SEQ ID NO: 296)

Primer 062536:

5'-GCGACAATTTTCCCCAACGG-3' (SEQ ID NO: 297)

The kit was used according to the manufacturer's instructions and the change was successfully made resulting in plasmid pCR2.1-Dt xyl2.

Plasmid pCR2.1-Dt xyl2 and pMDT100 WO 2008/140615 were digested with *Sac* I and *Mlu* I. The digestions were each resolved by 0.8% agarose gel electrophoresis using 0.5X TBE buffer. A vector fragment of approximately 8.0 kb from pMDT100 and a xylanase gene fragment of approximately 700 bp from pCR2.1-Dt xyl2 were excised from the gels and extracted using a QIAQUICK® Gel Extraction Kit. The two purified fragments were ligated together using a Rapid DNA Ligation Kit.

Competent cells of *Bacillus subtilis* 168Δ4 were transformed with the ligation products according to the method of Young and Spizizen, 1961, *Journal of Bacteriology* 81: 823-829, or Dubnau and Davidoff-Abelson, 1971, *Journal of Molecular Biology* 56: 209-221. *Bacillus subtilis* 168Δ4 is derived from the *Bacillus subtilis* type strain 168 (BGSC 1A1, Bacillus Genetic Stock Center, Columbus, OH, USA) and has deletions in the *spo*IIAC, *apr*E, *npr*E, and *amy*E genes. The deletion of the four genes was performed essentially as described for *Bacillus subtilis* A164Δ5 (U.S. Patent No. 5,891,701).

Bacillus subtilis transformants were selected at 37°C after 16 hours of growth on TBAB plates supplemented with 5 µg of chloramphenicol per ml. To screen for integration of the plasmid by double cross-over at the *amy*E locus, *Bacillus subtilis* primary transformants were patched onto TBAB plates supplemented with 6 µg of neomycin per ml and onto TBAB plates supplemented with 5 µg of chloramphenicol per ml. Integration of the plasmid by double cross-over at the *amy*E locus does not incorporate the neomycin resistance gene and therefore renders the strain neomycin sensitive. A chloramphenicol resistant, neomycin sensitive transformant was identified, which harbored the *Dictyoglomus thermophilum* xylanase expression cassette in the *amy*E locus, and designated *Bacillus subtilis* 168 with pSMO271.

Genomic DNA was isolated from *Bacillus subtilis* 168 with pSMO271(Pitcher *et al.*,

1989, *supra*) and 0.1 µg was transformed into competent *Bacillus subtilis* SMO25. Transformants were selected on TBAB plates supplemented with 5 µg of chloramphenicol per ml at 37°C. A chloramphenicol resistant transformant was single colony purified and designated *Bacillus subtilis* SMO47.

The *Bacillus subtilis* strain designated SMO47 was streaked on agar slants and incubated for about 24 hours at 37°C. The agar medium was composed per liter of 10 g of soy peptone, 10 g of sucrose, 2 g of trisodium citrate dihydrate, 4 g of KH_2PO_4, 5 g of Na_2HPO_4, 15 g of Bacto agar, 0.15 mg of biotin, 2 ml of trace metals, and deionized water to 1 liter. The trace metals solution was composed of 1.59 g of $ZnSO_4 \cdot 7\ H_2O$, 0.76 g of $CuSO_4 \cdot 5\ H_2O$, 7.52 g of $FeSO_4 \cdot 7\ H_2O$, 1.88 g of $MnSO_4 \cdot H_2O$, 20 g of citric acid, and deionized water to 1 liter. Approximately 15 ml of sterile buffer (7.0 g of Na_2HPO_4, 3.0 g of KH_2PO_4, 4.0 g of NaCl, 0.2 g of $MgSO_4 \cdot 7\ H_2O$, and deionized water to 1 liter) was used to gently wash off some of the cells from the agar surface. The bacterial suspension was then used for inoculation of baffled shake flasks containing 100 ml of growth medium composed of 11 g of soy bean meal, 0.4 g of Na_2HPO_4, 5 drops of antifoam, and deionized water to 100 ml. The inoculated shake flasks were incubated at 37°C for about 20 hours with shaking at 300 rpm, after which 100 ml (obtained by combining the media from two independent shake flasks with the same strain) were used for inoculation of a 3 liter fermentor with 900 ml of medium composed of 40 g of hydrolyzed potato protein, 6 g of K_2SO_4, 4 g of Na_2HPO_4, 12 g of K_2HPO_4, 4 g of $(NH_4)_2SO_4$, 0.5 g of $CaCO_3$, 2 g of citric acid; 4 g of $MgSO_4$, 40 ml of trace metals solution (described above), 1 mg of biotin (biotin was added as 1 ml of a 1 g per liter biotin solution in the buffer described above), 1.3 ml of antifoam, and deionized water to 1 liter. The medium was adjusted to pH 5.25 with phosphoric acid prior to being autoclaved.

The fermentation was carried out as a fed-batch fermentation with sucrose solution being the feed. The fermentation temperature was held constant at 37°C. The tanks were aerated with 3 liter air per minute, and the agitation rate was held in the range of 1,500-1,800 rpm. The fermentation time was around 60-70 hours. The pH was maintained in the range of pH 6.5-7.3.

The fermentation was assayed for xylanase activity according to the following procedure. Culture supernatants were diluted appropriately in 0.1 M sodium acetate pH 5.0. A purified *Dictyoglomus thermophilum* xylanase was diluted using 2-fold steps starting with a 1.71 µg/ml concentration and ending with a 0.03 µg /ml concentration in the sample buffer. A total of 40 µl of each dilution including standard was transferred to a 96-well flat bottom plate. Using a Biomek NX (Beckman Coulter, Fullerton CA, USA), a 96-well pippetting workstation, 40 µl an Azo-Wheat arabinoxylan (Megazyme International, Ireland) substrate solution (1% w/v) was added to each well then incubated at 50°C for 30 minutes. Upon completion of the incubation the reaction was stopped with 200 µl of ethanol (95% v/v). The

samples were then incubated at ambient temperatures for 5 minutes followed by centrifugation at 3,000 rpm for 10 minutes. One hundred-fifty microliters of the supernatant was removed and dispensed into a new 96-well flat bottom plate. An optical density of 590 nm was obtained for the 96-well plate using a SPECTRAMAX® 250 plate reader (Molecular Devices, Sunnvale CA, USA). Sample concentrations were determined by extrapolation from the generated standard curve.

Filtrated broth was added to 2% v/v GC-850 (Gulbrandsen, SC, USA) followed by centrifugation at 20,000 x *g* for 20 minutes. The supernatant was again added 2% v/v GC-850 followed by centrifugation at 20,000 x *g* for 20 minutes. The supernatant was concentrated using a tangential flow concentrator equipped with a Sartocon® Slice cassette with a 10 kDa MW-CO polyethersulfone membrane. The concentrate was 80°C treated for 30 minutes and filtrated through a 1.2 µm glass microfibre filter (Whatman, International Ltd, Maidstone, England). The pH was adjusted to pH 8.0 and loaded onto a MEP HyperCel™ (Pall Corporation, East Hills, NY USA) column. The bound proteins were eluted with 50 mM acetic acid pH 4.5. The eluted proteins were stirred with activated carbon 1% w/v (Picatif FGV 120, Pica, France) for 15 minutes and filtrated on 0.2 µm PES filter (Nalge Nunc International, New York, NY USA). The pH was adjusted to pH 5.0 using 3 M Tris. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 85: Preparation of *Aspergillus aculeatus* strain CBS 172.66 GH3 beta-xylosidase

The *Aspergillus aculeatus* strain CBS 172.66 GH beta-xylosidase (SEQ ID NO: 201 [DNA sequence] and SEQ ID NO: 202 [deduced amino acid sequence]) was recombinantly prepared according to the following procedure.

Aspergillus aculeatus CBS 172.66 was used as the source of the polypeptide having beta-xylosidase activity.

Genomic sequence information was generated by the U.S. Department of Energy Joint Genome Institute (JGI). A preliminary assembly of the genome was downloaded from JGI and analyzed using the Pedant-Pro™ Sequence Analysis Suite (Biomax Informatics AG, Martinsried, Germany). Gene models constructed by the software were used as a starting point for detecting GH3 homologues in the genome. More precise gene models were constructed manually using multiple known GH3 protein sequences as a guide.

To generate genomic DNA for PCR amplification, *Aspergillus aculeatus* CBS 172.66 was propagated on PDA agar plates by growing at 26°C for 7 days. Spores harvested from the PDA plates were used to inoculate 25 ml of YP+2% glucose medium in a baffled shake flask and incubated at 26°C for 48 hours with agitation at 200 rpm.

Genomic DNA was isolated according to a modified FastDNA® SPIN protocol (Qbiogene, Inc., Carlsbad, CA, USA). Briefly a FastDNA® SPIN Kit for Soil (Qbiogene, Inc., Carlsbad, CA, USA) was used in a FastPrep® 24 Homogenization System (MP Biosciences, Santa Ana, CA, USA). Two ml of fungal material from the above cultures were harvested by centrifugation at 14,000 x *g* for 2 minutes. The supernatant was removed and the pellet resuspended in 500 µl of deionized water. The suspension was transferred to a Lysing Matrix E FastPrep® tube (Qbiogene, Inc., Carlsbad, CA, USA) and 790 µl of sodium phosphate buffer and 100 µl of MT buffer from the FastDNA® SPIN Kit were added to the tube. The sample was then secured in the FastPrep® Instrument (Qbiogene, Inc., Carlsbad, CA, USA) and processed for 60 seconds at a speed of 5.5 m/sec. The sample was then centrifuged at 14,000 x *g* for two minutes and the supernatant transferred to a clean EPPENDORF® tube. A 250 µl volume of PPS reagent from the FastDNA® SPIN Kit was added and then the sample was mixed gently by inversion. The sample was again centrifuged at 14,000 x *g* for 5 minutes. The supernatant was transferred to a 15 ml tube followed by 1 ml of Binding Matrix suspension from the FastDNA® SPIN Kit and then mixed by inversion for two minutes. The sample was placed in a stationary tube rack and the silica matrix was allowed to settle for 3 minutes. A 500 µl volume of the supernatant was removed and discarded and then the remaining sample was resuspended in the matrix. The sample was then transferred to a SPIN filter tube from the FastDNA® SPIN Kit and centrifuged at 14,000 x *g* for 1 minute. The catch tube was emptied and the remaining matrix suspension added to the SPIN filter tube. The sample was again centrifuged (14,000 x *g*, 1 minute). A 500 µl volume of SEWS-M solution from the FastDNA® SPIN Kit was added to the SPIN filter tube and the sample was centrifuged at the same speed for 1 minute. The catch tube was emptied and the SPIN filter replaced in the catch tube. The unit was centrifuged at 14,000 x *g* for 2 minutes to "dry" the matrix of residual SEWS-M wash solution. The SPIN filter was placed in a fresh catch tube and allowed to air dry for 5 minutes at room temperature. The matrix was gently resuspended in 100 µl of DES (DNase/Pyrogen free water) with a pipette tip. The unit was centrifuged (14,000 x *g*, 1 minute) to elute the genomic DNA followed by elution with 100 µl of 10 mM Tris, 0.1 mM EDTA, pH 8.0 by renewed centrifugation at 14,000 x *g* for 1 minute and the eluates were combined. The concentration of the DNA harvested from the catch tube was measured by a UV spectrophotometer at 260 nm.

Synthetic oligonucleotide primers shown below are designed to PCR amplify *Aspergillus aculeatus* CBS 172.66 GH3 genes from the genomic DNA prepared in Example 2. An IN-FUSION™ Cloning Kit (Clontech, Mountain View, CA, USA) is used to clone the fragments directly into the expression vector pDau109 (WO 2005/042735).

Primer GH3-114f:

5'-ACACAACTGGGGATCCACCATGGCTGTGGCGGCTCTT-3' (SEQ ID NO: 298)
Primer GH3-114r:
5'-AGATCTCGAGAAGCTTACTACTCATCCCCCTGCAC-3' (SEQ ID NO: 299)

PCR reactions are carried out with genomic DNA prepared from Example 2 for amplification of the genes identified in Example 1. The PCR reaction is composed of 1 µl of genomic DNA, 1 µl of primer forward (f) (50 µM); 1 µl of primer reverse (r) (50 µM); 10 µl of 5X HF buffer, 2 µl of 10 mM dNTP; 1 µl of PHUSION® DNA polymerase, and PCR-grade water up to 50 µl. Primers GH3-114f and GH3-114r are used simultaneously.

The PCR reactions are performed using a DYAD® PCR machine programmed for 2 minutes at 98°C followed by 20 touchdown cycles at 98°C for 15 seconds, 70°C (-1°C/cycle) for 30 seconds, and 72°C for 2 minutes 30 seconds; and 25 cycles each at 98°C for 15 seconds, 60°C for 30 seconds, 72°C for 2 minutes 30 seconds; and 5 minutes at 72°C.

The reaction products are isolated by 1.0% agarose gel electrophoresis using 40 mM Tris base, 20 mM sodium acetate, 1 mM disodium EDTA (TAE) buffer where approximately 2.5 to 3.0 kb PCR product bands are excised from the gels and purified using a GFX® PCR DNA and Gel Band Purification Kit according to manufacturer's instructions. DNA corresponding to the *A. aculeatus* GH3 genes are cloned into the expression vector pDAu109 (WO 2005042735) linearized with *Bam* HI and *Hind* III, using an IN-FUSION™ Dry-Down PCR Cloning Kit according to the manufacturer's instructions.

A 2.5 µl volume of the five times diluted ligation mixture is used to transform *E. coli* TOP10 chemically competent cells. Five colonies are selected on LB agar plates containing 100 µg of ampicillin per ml and cultivated overnight in 3 ml of LB medium supplemented with 100 µg of ampicillin per ml. Plasmid DNA is purified using an E.Z.N.A.® Plasmid Mini Kit according to the manufacturer's instructions. The *Aspergillus aculeatus* GH3 gene sequences were verified by Sanger sequencing with an Applied Biosystems Model 3700 Automated DNA Sequencer using version 3.1 BIG-DYE™ terminator chemistry (Applied Biosystems, Inc., Foster City, CA, USA) (Applied Biosystems, Inc., Foster City, CA, USA). Nucleotide sequence data are scrutinized for quality and all sequences are compared to each other with assistance of PHRED/PHRAP software (University of Washington, Seattle, WA, USA).

The coding sequence is 2412 bp including the stop codon and contains no introns. The encoded predicted protein is 803 amino acids. Using the SignalP program (Nielsen *et al.*, 1997, *supra*), a signal peptide of 17 residues was predicted. The predicted mature protein contains 786 amino acids.

Spores of the best transformant were spread on COVE plates containing 0.01% TRITON® X-100 in order to isolate single colonies. The spreading was repeated twice in total on COVE sucrose medium (Cove, 1996, *Biochim. Biophys. Acta* 133: 51-56) containing

1 M sucrose and 10 mM sodium nitrate, supplemented with 10 mM acetamide and 15 mM CsCl. Fermentation was then carried out in 250 ml shake flasks using DAP-2C-1 medium for 4 days at 30°C with shaking at 100 rpm. The fermentation broth was filtered using standard methods.

Ammonium sulphate was added to the filtrated broth to a consentration of 2 M. After filtration using a 0.2 µm PES filter (Thermo Fisher Scientific, Roskilde, Denmark), the filtrate was loaded onto a Phenyl Sepharose™ 6 Fast Flow column (high sub) (GE Healthcare, Piscataway, NJ, USA) equilibrated in 25 mM HEPES pH 7.0 with 2 M ammonium sulphate, and bound proteins were eluted with 25 mM HEPES pH 7.0 with no ammonium sulphate. The fractions were pooled and applied to a Sephadex™ G-25 (medium) (GE Healthcare, Piscataway, NJ, USA) column equilibrated in 25 mM HEPES pH 7.0. The fractions were pooled and then applied to a SOURCE™ 15Q (GE Healthcare, Piscataway, NJ, USA) column equilibrated in 25 mM HEPES pH 7.0, and bound proteins were eluted with a linear gradient from 0-1000 mM sodium chloride. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 86: Preparation of *Aspergillus aculeatus* strain CBS 186.67 GH3 beta-xylosidase

The *Aspergillus aculeatus* strain CBS 186.67 GH3 beta-xylosidase (SEQ ID NO: 203 [DNA sequence] and SEQ ID NO: 204 [deduced amino acid sequence]) was recombinantly prepared according to the following procedure.

Genomic DNA was isolated according to the procedure described in Example 73.

The *Aspergillus aculeatus* beta-xylosidase gene was isolated by PCR using two cloning primers GH3-101f and GH3-101r, shown below, which were designed based on the publicly available *Aspergillus aculeatus* xyl2 full-length sequence (GenBank AB462375.1) for direct cloning using the IN-FUSION™ strategy.

Primer GH3-101f:

5'-acacaactggggatccaccatggctgtggcggctcttgctctgctgg-3' (SEQ ID NO: 300)

Primer GH3-101r:

5'-agatctcgagaagcttaCTCATCCCCCGCCACCCCCTGCACCTCC-3' (SEQ ID NO: 301)

A PCR reaction was performed with genomic DNA prepared from *Aspergillus aculeatus* CBS 186.67 in order to amplify the full-length gene. The PCR reaction was composed of 1 µl of genomic DNA, 0.75 µl of primer GH3-101.1f (10 µM), 0.75 µl of primer GH3-101.1r (10 µM), 3 µl of 5X HF buffer, 0.25 µl of 50 mM $MgCl_2$, 0.3 µl of 10 mM dNTP, 0.15 µl of PHUSION® DNA polymerase, and PCR-grade water up to 15 µl. The PCR reaction was performed using a DYAD® PCR machine programmed for 2 minutes at 98°C

followed by 10 touchdown cycles at 98°C for 15 seconds, 70°C (-1°C/cycle) for 30 seconds, and 72°C for 2 minutes 30 seconds; and 25 cycles each at 98°C for 15 seconds, 60°C for 30 seconds, 72°C for 2 minutes 30 seconds, and 5 minutes at 72°C.

The reaction products were isolated by 1.0% agarose gel electrophoresis using TAE buffer where an approximately 2.4 kb PCR product band was excised from the gel and purified using a GFX® PCR DNA and Gel Band Purification Kit according to manufacturer's instructions. DNA corresponding to the *Aapergillus aculeatus* beta-xylosidase gene was cloned into the expression vector pDAu109 (WO 2005042735) linearized with *Bam* HI and *Hind* III, using an IN-FUSION™ Dry-Down PCR Cloning Kit according to the manufacturer's instructions.

A 2.5 µl volume of the diluted ligation mixture was used to transform *E. coli* TOP10 chemically competent cells. Three colonies were selected on LB agar plates containing 100 µg of ampicillin per ml and cultivated overnight in 3 ml of LB medium supplemented with 100 µg of ampicillin per ml. Plasmid DNA was purified using an E.Z.N.A.® Plasmid Mini Kit according to the manufacturer's instructions. The *Aspergillus aculeatus* beta-xylosidase gene sequence was verified by Sanger sequencing before heterologous expression.

The coding sequence is 2454 bp including the stop codon. The gene does not contain introns. The encoded predicted protein is 817 amino acids. Using the SignalP program (Nielsen *et al.*, 1997, *Protein Engineering* 10: 1-6), a signal peptide of 17 residues was predicted. The predicted mature protein contains 800 amino acids.

Protoplasts of *Aspergillus oryzae* MT3568 were prepared as described in WO 95/02043. *A. oryzae* MT3568 is an *amdS* (acetamidase) disrupted gene derivative of *Aspergillus oryzae* JaL355 (WO 2002/40694) in which *pyrG* auxotrophy was restored by disrupting the *A. oryzae* acetamidase (*amdS*) gene with the *pyrG* gene. One hundred microliters of protoplast suspension were mixed with 2.5-15 µg of the *Aspergillus* expression vector and 250 µl of 60% PEG 4000, 10 mM $CaCl_2$, and 10 mM Tris-HCl pH 7.5 were added and gently mixed. The mixture was incubated at 37°C for 30 minutes and the protoplasts were spread on COVE sucrose (1 M) plates supplemented with 10 mM acetamide and 15 mM CsCl for transformant selection. After incubation for 4-7 days at 37°C spores of several transformants were seeded on YP-2% maltodextrin medium. After 4 days cultivation at 30°C culture broth was analyzed in order to identify the best transformants based on their ability to produce a large amount of active *Aculeatus aculeatus* beta-xylosidase. The screening was based on intensity of the band corresponding to the heterologous expressed protein determined by SDS-PAGE and activity of the enzyme on 4-nitrophenyl-beta-D-xylopyranoside (pNPX) as follows. Ten µl of culture broth was mixed with 90 µl of assay reagent containing 10 µl of 0.1% TWEEN® 20, 10 µl of 1 M sodium citrate pH 5, 4 µl of 100 mM of pNPX substrate (Sigma Aldrich) solubilized in DMSO (0.4% final volume in stock

solution), and filtered water. The assay was performed for 30 minutes at 37°C and absorbance dertermined at 405 nm before and after addition of 100 µl of 1 M sodium carbonate pH 10. The highest absorbance values at 405 nm were correlated to the SDS-PAGE data for selection of the best transformant.

Spores of the best transformant designated *A. oryzae* EXP3611 were spread on COVE plates containing 0.01% TRITON® X-100 in order to isolate single colonies. The spreading was repeated twice in total on COVE sucrose medium (Cove, 1996, *Biochim. Biophys. Acta* 133: 51-56) containing 1 M sucrose and 10 mM sodium nitrate, supplemented with 10 mM acetamide and 15 mM CsCl. Fermentation was then carried out in 250 ml shake flasks using DAP-4C-1 medium for 4 days at 30°C with shaking at 100 rpm. The fermentation broth was filtered using standard methods. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 87: Preparation of *Aspergillus fumigatus* strain NN051616 GH3 beta-xylosidase Q0H905

The *Aspergillus fumigatus* strain NN051616 GH3 beta-xylosidase (SEQ ID NO: 205 [DNA sequence] and SEQ ID NO: 206 [deduced amino acid sequence]) was recombinantly prepared according to the following procedure.

Two synthetic oligonucleotide primers shown below were designed to PCR amplify the *Aspergillus fumigatus* beta xylosidase gene from the genomic DNA. An InFusion Cloning Kit (Clontech, Mountain View, CA) was used to clone the fragment directly into the expression vector, pAILo2 (WO 2005/074647), without the need for restriction digests and ligation.

Forward primer:

5'-ACTGGATTTACC**ATGGCGGTTGCCAAATCTATTGCT** -3' (SEQ ID NO: 302)

Reverse primer:

5'-TCACCTCTAGTTAATTAA**TCACGCAGACGAAATCTGCT**-3' (SEQ ID NO: 303)

Bold letters represent coding sequence. The remaining sequence is homologous to the insertion sites of pAILo2.

Fifteen picomoles of each of the primers above were used in a PCR reaction containing 250 ng of *Aspergillus fumigatus* genomic DNA, 1X Expand High Fidelity Buffer with $MgCl_2$ (Roche Applied Science, Indianapolis, IN), 1 µl of 10 mM blend of dATP, dTTP, dGTP, and dCTP, 0.75 units of Expand High fidelity Enzyme Mix (Roche Applied Science, Indianapolis, IN), in a final volume of 50 µl. The amplification conditions were one cycle at 94°C for 2 minutes; 10 cycles each at 94°C for 15 seconds, 56.5°C for 30 seconds, and

72°C for 2 minutes; and 20 cycles each at 94°C for 15 seconds, 56.5°C for 30 seconds, and 72°C for 2 minutes plus 5 seconds per successive cycle. The heat block was then held at 72°C for 7 minutes followed by a 4°C soak cycle.

The reaction products were isolated on a 1.0% agarose gel using TAE buffer and a 2.4 kb product band was excised from the gel and purified using a MinElute® Gel Extraction Kit (QIAGEN Inc., Valencia, CA) according to the manufacturer's instructions.

The fragment was then cloned into pAlLo2 using an InFusion Cloning Kit. The vector was digested with *Nco* I and *Pac* I (using conditions specified by the manufacturer). The fragment was purified by gel electrophoresis and QIAquick kit (QIAGEN Inc., Valencia, CA) gel purification. The gene fragment and the digested vector were combined together in a reaction resulting in the expression plasmid pAG57, in which transcription of the *Aspergillus fumigatus* beta-xylosidase gene was under the control of the NA2-tpi promoter (a hybrid of the promoters from the genes for *Aspergillus niger* neutral alpha-amylase and *Aspergillus oryzae* triose phosphate isomerase). The recombination reaction (20 µl) was composed of 1X InFusion Buffer (Clontech, Mountain View, CA) , 1X BSA (Clontech, Mountain View, CA) , 1 µl of InFusion enzyme (diluted 1:10) (Clontech, Mountain View, CA) , 182 ng of pAlLo2 digested with *Nco* I and *Pac* I, and 97.7 ng of the *Aspergillus fumigatus* beta-xylosidase purified PCR product. The reaction was incubated at 37°C for 15 minutes followed by 15 minutes at 50°C. The reaction was diluted with 40 µl of TE buffer and 2.5 µl of the diluted reaction was used to transform *E. coli* Top10 Competent cells. An *E. coli* transformant containing pAG57 (*Aspergillus fumigatus* beta-xylosidase gene) was identified by restriction enzyme digestion and plasmid DNA was prepared using a BIOROBOT® 9600. The pAG57 plasmid construct was sequenced using an Applied Biosystems 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) to verify the sequence.

Aspergillus oryzae JaL355 protoplasts were prepared according to the method of Christensen *et al.*, 1988, *Bio/Technology* 6: 1419-1422 and tansformed with 5 µg of pAG57. Twenty-four transformants were isolated to individual PDA plates.

Plugs taken from the original transformation plate of each of the twenty-four transformants were added to 1ml of M410 separately in 24 well plates, which were incubated at 34°C. After three days of incubation, 7.5 µl of supernatant from each culture was analyzed using Criterion stain-free, 8-16% gradient SDS-PAGE, (BioRad, Hercules, CA) according to the manufacturer's instructions. SDS-PAGE profiles of the cultures showed that several transformants had a new major band of approximately 130 kDa.

Confluent PDA plate of the highest expressing transformant was washed with 5 ml of 0.01% TWEEN® 20 and inoculated into a 500 ml Erlenmeyer flask containing 100 ml of M410 medium. Inoculated flask was incubated with shaking for 3 days at 34°C. The broth

was filtered through a 0.22 µm stericup suction filter (Millipore, Bedford, MA).

Filtered broth was concentrated and buffer exchanged using a tangential flow concentrator (Pall Filtron, Northborough, MA, USA) equipped with a 10 kDa polyethersulfone membrane (Pall Filtron, Northborough, MA, USA) with 50 mM sodium acetate pH 5.0. Protein concentration was determined using a Microplate BCA™ Protein Assay Kit in which bovine serum albumin was used as a protein standard.

Example 88: Evaluation of two cellobiohydrolases I replacing a CBHI component in a high-temperature enzyme composition in saccharification of milled unwashed PCS at 50-65°C

The ability of two cellobiohydrolase I proteins to replace a CBHI component in a high-temperature enzyme composition (3 mg total protein per g cellulose) was tested at 50°C, 55°C, 60°C, and 65°C using milled unwashed PCS as a substrate. The high-temperature enzyme composition included 37% CBHI, 25% *Aspergillus fumigatus* Cel6A CBHII, 10% *Myceliophthora thermophila* Cel5A EGII, 15% *Penicillium* sp. GH61A polypeptide having cellulolytic enhancing activity, 5% *Aspergillus fumigatus* Cel3A beta-glucosidase, 5% *Aspergillus fumigatus* GH10 xyn3 xylanase, and 3% *Trichoderma reesei* GH3 beta-xylosidase.

The following CBHIs were each tested in the high-temperature enzyme composition: *Aspergillus fumigatus* Cel7A CBHI and *Penicillium emersonii* Cel7A CBHI.

The assay was performed as described in Example 34. The 1 ml reactions with milled unwashed PCS (5% insoluble solids) were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

As shown in Figure 29, *Penicillium emersonii* Cel7A CBHI performed the same as *Aspergillus fumigatus* Cel7A CBHI at 50°C and performed better than *Aspergillus fumigatus* Cel7A CBHI at 55-65°C (as the degree of cellulose conversion to glucose was higher for *Penicillium emersonii* Cel7A CBHI than *Aspergillus fumigatus* Cel7A CBHI at 55-65°C).

Example 89: Evaluation of two cellobiohydrolases I replacing a CBHI component in a high-temperature enzyme composition in saccharification of milled unwashed PCS at 50-65°C

The ability of two cellobiohydrolase I proteins to replace a CBHI component in a high-temperature enzyme composition (3 mg total protein per g cellulose) was tested at 50°C, 55°C, 60°C, and 65°C using milled unwashed PCS as a substrate. The high-temperature enzyme composition included 40% CBHI, 25% *Aspergillus fumigatus* Cel6A CBHII, 10% *Myceliophthora thermophila* Cel5A EGII, 15% *Thermoascus aurantiacus* GH61A polypeptide

having cellulolytic enhancing activity, 5% *Aspergillus fumigatus* GH10 xylanase, and 5% *Aspergillus fumigatus* Cel3A beta-glucosidase.

The following CBHIs were each tested in the high-temperature enzyme composition: *Aspergillus fumigatus* Cel7A CBHI and *Penicillium pinophilum* Cel7A CBHI. The high-temperature composition including *Aspergillus fumigatus* Cel7A CBHI was loaded at 3.3 mg total protein per gram cellulose instead of 3 mg total protein per gram cellulose, in which *Penicillium pinophilum* Cel7A CBHI was loaded.

The assay was performed as described in Example 34. The 1 ml reactions with 5% milled unwashed PCS were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

As shown in Figure 30, *Penicillium pinophilum* Cel7A CBHI performed well in the entire range of temperatures. *Penicillium pinophilum* Cel7A CBHI performed about the same as *Aspergillus fumigatus* Cel7A CBHI at 50°C and 55°C, but the performance was slightly lower compared to *Aspergillus fumigatus* Cel7A CBHI at 60°C and 65°C.

Example 90: Evaluation of two cellobiohydrolases I replacing a CBHI component in a high-temperature enzyme composition in saccharification of milled unwashed PCS at 50-65°C

The ability of two cellobiohydrolase I proteins to replace a CBHI component in a high-temperature enzyme composition (3 mg total protein per g cellulose) was tested at 50°C, 55°C, 60°C, and 65°C using milled unwashed PCS as a substrate. The high-temperature enzyme composition included 37% CBHI, 25% *Aspergillus fumigatus* Cel6A CBHII, 10% *Myceliophthora thermophila* Cel5A EGII, 15% *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, 5% *Aspergillus fumigatus* Cel3A beta-glucosidase, 5% *Aspergillus fumigatus* GH10 xyn3 xylanase, and 3% *Trichoderma reesei* GH3 beta-xylosidase.

The following CBHIs were each tested in the high-temperature enzyme composition: *Aspergillus fumigatus* Cel7A CBHI and *Aspergillus terreus* Cel7A CBHI.

The assay was performed as described in Example 34. The 1 ml reactions with milled unwashed PCS (5% insoluble solids) were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

As shown in Figure 31, the performance of *Aspergillus terreus* Cel7A CBHI was the same as the performance of *Aspergillus fumigatus* Cel7A CBHI at 55°C and lower at 60-65°C; however, at 50°C, the degree of cellulose conversion to glucose was much higher for *Aspergillus terreus* Cel7A CBHI than *Aspergillus fumigatus* Cel7A CBHI at 50°C.

Example 91: Evaluation of three cellobiohydrolases I replacing a CBHI component in a high-temperature enzyme composition in saccharification of milled unwashed PCS at 50-60°C

The ability of three cellobiohydrolase I proteins to replace a CBHI component in a high-temperature enzyme composition (3 mg total protein per g cellulose) was tested at 50°C, 55°C, and 60°C using milled unwashed PCS as a substrate. The high-temperature enzyme composition included 45% CBHI, 25% *Thielavia terrestris* Cel6A CBHII, 5% *Trichoderma reesei* Cel7B EGI, 5% *Thermoascus aurantiacus* Cel5A EGII, 5% *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, 5% *Thielavia terrestris* GH61E polypeptide having cellulolytic enhancing activity, 5% *Aspergillus fumigatus* Cel3A beta-glucosidase, and 5% *Aspergillus fumigatus* GH10 xyn3 xylanase.

The following CBHIs were each tested in the high-temperature enzyme composition: *Aspergillus fumigatus* Cel7A CBHI, *Neosartorya fischeri* Cel7A CBHI, and *Aspergillus nidulans* Cel7A CBHI.

The assay was performed as described in Example 34. The 1 ml reactions with milled unwashed PCS (5% insoluble solids) were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

As shown in Figure 32, the performance of *Neosartorya fischeri* Cel7A CBHI was lower than the performance of *Aspergillus fumigatus* Cel7A CBHI at 50°C and 55°C, but performance was the same at 60°C. *Aspergillus nidulans* Cel7A CBHI performed almost the same as *Aspergillus fumigatus* Cel7A CBHI at 50°C, but showed lower hydrolysis at 55°C and 60°C.

Example 92: Evaluation of two cellobiohydrolases II replacing a CBHII component in a high-temperature enzyme composition in saccharification of milled unwashed PCS at 50-65°C

The ability of two cellobiohydrolase II proteins to replace a CBHII component in a high-temperature enzyme composition (3 mg total protein per g cellulose) was tested at 50°C, 55°C, 60°C, and 65°C using milled unwashed PCS as a substrate. The high-temperature enzyme composition included 37% *Aspergillus fumigatus* Cel7A CBHI, 25% CBHII, 10% *Myceliophthora thermophila* Cel5A EGII, 15% *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, 5% *Aspergillus fumigatus* Cel3A beta-glucosidase, 5% *Aspergillus fumigatus* GH10 xyn3 xylanase, and 3% *Trichoderma reesei* GH3 beta-xylosidase.

The following CBHIIs were each tested in the high-temperature enzyme composition:

Aspergillus fumigatus Cel6A CBHII and *Finnellia nivea* Cel6A CBHII.

The assay was performed as described in Example 34. The 1 ml reactions with milled unwashed PCS (5% insoluble solids) were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

As shown in Figure 33, *Finnellia nivea* Cel6A CBHII performed well at 50-55°C as it showed hydrolysis levels similar to *Aspergillus fumigatus* Cel6A CBHII at 50-55°C, but the performance declined at 60-65°C.

Example 93: Evaluation of three cellobiohydrolases II replacing a CBHII component in a high-temperature enzyme composition in saccharification of milled unwashed PCS at 50-65°C

The ability of three cellobiohydrolase II proteins to replace a CBHII component in a high-temperature enzyme composition (3 mg total protein per g cellulose) was tested at 50°C, 55°C, 60°C, and 65°C using milled unwashed PCS as a substrate. The high-temperature enzyme composition included 37% *Aspergillus fumigatus* Cel7A CBHI, 25% CBHII, 10% *Trichoderma reesei* Cel5A EGII, 15% *Penicillium* sp. GH61A polypeptide having cellulolytic enhancing activity, 5% *Aspergillus fumigatus* Cel3A beta-glucosidase, 5% *Aspergillus fumigatus* GH10 xyn3 xylanase, and 3% *Trichoderma reesei* GH3 beta-xylosidase.

The following CBHIIs were each tested in the high-temperature enzyme composition: *Aspergillus fumigatus* Cel6A CBHII, *Penicillium emersonii* Cel6A CBHII, and *Penicillium pinophilum* Cel6A CBHII.

The assay was performed as described in Example 34. The 1 ml reactions with milled unwashed PCS (5% insoluble solids) were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

As shown in Figure 34, *Penicillium emersonii* Cel6A CBHII performed well in the entire range of temperatures. *Penicillium emersonii* Cel6A CBHII performed almost as well as *Aspergillus fumigatus* Cel6A CBHII at 50°C, but performed slightly lower than *Aspergillus fumigatus* Cel6A CBHII at 55-65°C. The performance of *Penicillium pinophilum* Cel6A CBHII was comparable to that of *Aspergillus fumigatus* Cel6A CBHII at 50°C and 55°C; however, performance declined at 60°C and 65°C.

Example 94: Evaluation of three endoglucanases II replacing an endoglucanase component in a high-temperature enzyme composition in saccharification of milled unwashed PCS at 50-65°C

The ability of three endoglucanase II proteins to replace an endoglucanase component in a high-temperature enzyme composition (3 mg total protein per g cellulose) was tested at 50°C, 55°C, 60°C, and 65°C using milled unwashed PCS as a substrate. The high-temperature enzyme composition included 40% *Aspergillus fumigatus* Cel7A CBHI, 25% *Aspergillus fumigatus* Cel6A CBHII, 10% EG cellulase, 15% *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, 5% *Aspergillus fumigatus* Cel3A beta-glucosidase, and 5% *Aspergillus fumigatus* GH10 xyn3 xylanase.

The following EGIIs were each tested in the high-temperature enzyme composition: *Aspergillus fumigatus* Cel5A EGII, *Neosartorya fischeri* Cel5A EGII, and *Myceliophthora thermophila* Cel5A EGII.

The assay was performed as described in Example 34. The 1 ml reactions with milled unwashed PCS (5% insoluble solids) were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

As shown in Figure 35, all endoglucanase II proteins performed similarly within this temperature range, with *Neosartorya fischeri* Cel5A EGII and *Myceliophthora thermophila* Cel5A EGII having similar activity at 50°C and 55°C and *Aspergillus fumigatus* Cel5A EGII having comparable activity to *Neosartorya fischeri* Cel5A EGII and *Myceliophthora thermophila* Cel5A EGII at 60°C and 65°C.

Example 95: Evaluation of two beta-glucosidases replacing a beta-glucosidase component in a high-temperature enzyme composition in saccharification of milled unwashed PCS at 50-65°C

The ability of three beta-glucosidase proteins to replace a beta-glucosidase component in a high-temperature enzyme composition (3 mg total protein per g cellulose) was tested at 50°C, 55°C, 60°C, and 65°C using milled unwashed PCS as a substrate. The high-temperature enzyme composition included 37% *Aspergillus fumigatus* Cel7A CBHI, 25% *Aspergillus fumigatus* Cel6A CBHII, 10% *Myceliophthora thermophila* Cel5A EGII, 15% *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, 5% beta-glucosidase, 5% *Aspergillus fumigatus* GH10 xyn3 xylanase, and 3% *Trichoderma reesei* GH3 beta-xylosidase.

The following beta-glucosidases were each tested in the high-temperature enzyme composition: *Aspergillus fumigatus* Cel3A beta-glucosidase and *Aspergillus aculeatus* beta-glucosidases.

The assay was performed as described in Example 34, with the exception of glucose background, in which 40 g per liter of glucose was included in the reactions. The 1 ml reactions with milled unwashed PCS (5% insoluble solids) were conducted for 72 hours in 50

mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

As shown in Figure 36, *Aspergillus aculeatus* Cel3A beta-glucosidase had slightly higher or similar performance as *Aspergillus fumigatus* Cel3A beta-glucosidase at all temperatures

Example 96: Evaluation of four beta-glucosidases replacing a beta-glucosidase component in a high-temperature enzyme composition in saccharification of milled unwashed PCS at 50-60°C

Four beta-glucosidases, including *Aspergillus fumigatus* Cel3A beta-glucosidase, *Aspergillus kawashii* Cel3A beta-glucosidase, *Aspergillus clavatus* Cel3 beta-glucosidase, and *Talaromyces emersonii* Cel3A beta-glucosidase were each evaluated in a high-temperature enzyme composition at 50°C, 55°C, and 60°C using milled unwashed PCS as a substrate. The high-temperature enzyme composition included 45% *Aspergillus fumigatus* Cel7A CBHI, 25% *Thielavia terrestris* Cel6A CBHII, 5% *Trichoderma reesei* Cel7B EGI, 5% *Thermoascus aurantiacus* Cel5A EGII, 5% *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, 5% *Thielavia terrestris* GH61E polypeptide having cellulolytic enhancing activity, 5% *Aspergillus fumigatus* GH10 xyn3 xylanase, and 5% beta-glucosidase. The high-temperature enzyme composition was used at 3.0 mg total protein per g cellulose.

The assay was performed as described in Example 34. The 1 ml reactions with milled unwashed PCS (5% insoluble solids) were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

The results shown in Figure 37 demonstrated that all beta-glucosidases except *Talaromyces emersonii* Cel3A beta-glucosidase had similar performance all three temperatures. *Talaromyces emersonii* Cel3A beta-glucosidase had lower activity at 50°C and 55°C but had equivalent activity at 60°C.

Example 97: Evaluation of three beta-glucosidases replacing a beta-glucosidase component in a high-temperature enzyme composition in saccharification of milled unwashed PCS at 50-65°C

Three beta-glucosidases, including *Aspergillus fumigatus* Cel3A beta-glucosidase, *Penicillium oxalicum* Cel3A beta-glucosidase (Example 77), and *Penicillium oxalicum* Cel3A beta-glucosidase (Example 78) were each evaluated in a high-temperature enzyme composition at 50°C, 55°C, 60°C, and 65°C using milled unwashed PCS as a substrate. The high-temperature enzyme composition included 40% *Aspergillus fumigatus* Cel7A CBHI,

25% *Aspergillus fumigatus* Cel6A CBHII, 10% *Myceliophthora thermophila* Cel5A EGII, 15% *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, 5% *Aspergillus fumigatus* GH10 xyn3 xylanase, and 5% beta-glucosidase. The high-temperature enzyme composition was used at 3.0 mg total protein per g cellulose.

The assay was performed as described in Example 34. The 1 ml reactions with milled unwashed PCS (5% insoluble solids) were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

The results shown in Figure 38 demonstrated that the beta-glucosidases had similar activity at all temperatures.

Example 98: Evaluation of the ability of three GH61 polypeptides having cellulolytic enhancing activity to enhance PCS-hydrolyzing activity of a high-temperature enzyme composition at 50-65°C using milled washed PCS

The ability of three GH61 polypeptides having cellulolytic enhancing activity, *Thermoascus aurantiacus* GH61A, *Penicllium sp* GH61A, and *Thermoascus crustaceus* GH61A, were each evaluated for their ability to enhance the PCS-hydrolyzing activity of a high-temperature enzyme composition using milled washed PCS at 50°C, 55°C, 60°C, and 65°C. Each GH61 polypeptide was separately added at 11.6% enzyme to a high temperature enzyme mixture. The high-temperature enzyme composition included 43.5% *Aspergillus fumigatus* Cel7A CBHI, 29% *Aspergillus fumigatus* Cel6A CBHII, 12% *Myceliophthora thermophila* Cel5A EGII, 6% *Aspergillus fumigatus* Cel3A beta-glucosidase, 6% *Aspergillus fumigatus* GH10 xyn3 xylanase, and 4% *Trichoderma reesei* GH3 beta-xylosidase. The results for the enzyme compositions containing GH61 polypeptides (2.3725 mg total protein per g cellulose) were compared with the results for a similar enzyme composition to which no GH61 polypeptide was added (2.125 mg total protein per g cellulose).

The assay was performed as described in Example 34. The 1 ml reactions with milled washed PCS (5% insoluble solids) were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

As shown in Figure 39, all three GH61 polypeptides showed significant cellulase-enhancing activity, with *Thermoascus aurantiacus* GH61A polypeptide, *Penicllium sp* GH61A polypeptide, and *Thermoascus crustaceus* GH61A polypeptide having similar enhancement at 50°C and 55°C while *Thermoascus aurantiacus* GH61A polypeptide had higher activity than *Penicllium sp* GH61A polypeptide and *Thermoascus crustaceus* GH61A polypeptide at 60°C and 65°C.

Example 99: Evaluation of three xylanases replacing a xylanase component in a high-temperature enzyme composition in saccharification of milled unwashed PCS at 50-65°C

The ability of three xylanases to replace an xylanase component in a high-temperature enzyme composition (3 mg total protein per g cellulose) was tested at 50°C, 55°C, 60°C, and 65°C using milled unwashed PCS as a substrate. The high-temperature enzyme composition included 37% *Aspergillus fumigatus* Cel7A CBHI, 25% *Aspergillus fumigatus* Cel6A CBHII, 10% *Myceliophthora thermophila* Cel5A EGII, 15% *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, 5% *Aspergillus fumigatus* Cel3A beta-glucosidase, 3% *Trichoderma reesei* GH3 beta-xylosidase and 5% xylanase.

The following xylanases were each tested in the high-temperature enzyme composition: *Aspergillus fumigatus* GH10 xylanase 3, *Talaromyces emersonii* GH10 xylanase, and *Penicillium emersonii* GH10 xylanase.

The assay was performed as described in Example 34. The 1 ml reactions with milled unwashed PCS (5% insoluble solids) were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

As shown in Figure 40, *Talaromyces emersonii* GH10 xylanase and *Penicillium emersonii* GH10 xylanase had similar activity as *Aspergillus fumigatus* GH10 xylanase 3 at all three temperatures.

Example 100: Evaluation of three xylanases by adding a xylanase component to a high-temperature enzyme composition in saccharification of milled unwashed PCS at 50-60°C

Three xylanases were each evaluated as a 10% addition to a high-temperature enzyme composition (3.5 mg total protein per g cellulose) at 50°C, 55°C, and 60°C using milled unwashed PCS as a substrate. The high-temperature enzyme composition included 45% *Aspergillus fumigatus* Cel7A CBHI, 25% *Myceliophthora thermophila* Cel6A CBHII, 10% *Myceliophthora thermophila* Cel5A EGII, 5% *Thermoascus aurantiacus* GH61A polypeptide having cellulolytic enhancing activity, 5% *Thielavia terrestris* GH61E polypeptide having cellulolytic enhancing activity, and 5% *Penicillium brasilianum* Cel3A beta-glucosidase.

The following xylanases were each tested in the high-temperature enzyme composition: *Aspergillus fumigatus* GH10 xylanase (xyl3), *Meripilus giganteus* GH10 xylanase, and *Dictyoglomus thermophilum* GH11 xylanase.

The assay was performed as described in Example 34. The 1 ml reactions with milled unwashed PCS (5% insoluble solids) were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

As shown in Figure 41, all three xylanase increased hydrolysis of the high-temperature enzyme composition. *Aspergillus fumigatus* GH10 xylanase and *Meripilus giganteus* GH10 xylanase had the same activity at 50°C and 55°C but *Aspergillus fumigatus* GH10 xylanase 3 had significantly higher activity at 60°C than *Meripilus giganteus* GH10 xylanase. *Dictyoglomus thermophilum* GH11 xylanase had lower activity than *Aspergillus fumigatus* GH10 xylanase 3 at all three temperatures but *Dictyoglomus thermophilum* GH11 xylanase had increasing activity as temperature increases to 60°C.

Example 101: Comparison of XCL-602-based enzyme compositions containing different cellobiohydrolases and xylanases in hydrolysis of milled unwashed PCS at 50-60°C

Four XCL-602 based enzyme compositions containing a different cellobiohydrolase and xylanase were tested at 50°C, 55°C, and 60°C using milled unwashed PCS as a substrate. The cellobiohydrolases tested in the XCL-602 based enzyme compositions were *Aspergillus fumigatus* Cel7A CBHI and *Penicillium emersonii* Cel7 CBHI. The xylanases tested were *Aspergillus fumigatus* GH10 xylanase 3 and *Trichophaea saccata* GH10 xylanase. The XCL-602 based enzyme compositions included 40% XCL-602, 20% CBHI, 20% *Aspergillus fumigatus* Cel6A CBHII, 12.5% *Penicillium* species GH61A, 5% xylanase, and 2.5% *Talaromyces emersonii* GH3 beta-xylosidase. XCL-602 based enzyme compositions containing *Aspergillus fumigatus* Cel7A CBHI were tested at 3.0, 5.0, and 7.0 mg protein per g cellulose while XCL-602 based enzyme compositions containing *Penicillium emersonii* Cel7 CBHI were tested at 3.0 mg protein per g cellulose. For comparison, *Trichoderma reesei*-based XCL-602 cellulase was tested at 3.0, 5.0, and 7.0 mg protein per g cellulose.

The assay was performed as described in Example 34. The 1 ml reactions with milled unwashed PCS (5% insoluble solids) were conducted for 72 hours in 50 mM sodium acetate pH 5.0 buffer containing 1 mM manganese sulfate. All reactions were performed in triplicate and involved single mixing at the beginning of hydrolysis.

The results for 3 mg protein per g cellulose are shown in Figure 42. The two xylanases, *Aspergillus fumigatus* GH10 xylanase 3 and *Trichophaea saccata* GH10 xylanase showed similar performance in the XCL-602 based enzyme compositions with either *Aspergillus fumigatus* Cel7A CBHI or *Penicillium emersonii* Cel7 CBHI. For the CBHIs in the XCL-602 based enzyme compositions with either xylanase, *Penicillium emersonii* Cel7

CBHI had slightly higher performance than *Aspergillus fumigatus* Cel7A CBHI at all three temperatures. Finally, all XCL-602 based enzyme compositions replaced with CBHI, CBHII, GH61, xylanase, and beta-xylosidase had significantly higher hydrolysis over the non-replaced Trichoderma reesei-based XCL-602 cellulase at all three temperatures. At 80% glucose conversion, the XCL-602 based enzyme compositions containing *Aspergillus fumigatus* Cel7A CBHI and either xylanase at 55°C required 4.3 mg protein per g cellulose while XCL-602 at 50°C required 6.5 mg protein per g cellulose, a 1.5-fold reduction in protein loading.

The present invention is further described by the following numbered paragraphs:

[1] A method for degrading or converting a cellulosic material, comprising: treating the cellulosic material with an enzyme composition of the present invention.

[2] The method of paragraph 1, wherein the cellulosic material is pretreated.

[3] The method of paragraph 1 and 2, further comprising recovering the degraded cellulosic material.

[4] The method of paragraph 19, wherein the degraded cellulosic material is a sugar.

[5] The method of paragraph 20, wherein the sugar is selected from the group consisting of glucose, xylose, mannose, galactose, and arabinose.

[6] A method for producing a fermentation product, comprising: (a) saccharifying a cellulosic material with an enzyme composition of the present invention 4; (b) fermenting the saccharified cellulosic material with one or more (several) fermenting microorganisms to produce the fermentation product; and (c) recovering the fermentation product from the fermentation.

[7] The method of paragraph 6, wherein the cellulosic material is pretreated.

[8] The method of paragraph 6 or 7, wherein steps (a) and (b) are performed simultaneously in a simultaneous saccharification and fermentation.

[9] The method of any of paragraphs 6-8, wherein the fermentation product is an alcohol, an organic acid, a ketone, an amino acid, or a gas.

[10] A method of fermenting a cellulosic material, comprising: fermenting the cellulosic material with one or more (several) fermenting microorganisms, wherein the cellulosic material is saccharified with an enzyme composition of the present invention.

[11] The method of paragraph 10, wherein the cellulosic material is pretreated before saccharification.

[12] The method of paragraph 10 or 11, wherein the fermenting of the cellulosic material produces a fermentation product.

[13] The method of paragraph 12, further comprising recovering the fermentation product from the fermentation.

[14] The method of paragraph 12 or 13, wherein the fermentation product is an alcohol, an organic acid, a ketone, an amino acid, or a gas.

The invention described and claimed herein is not to be limited in scope by the specific aspects herein disclosed, since these aspects are intended as illustrations of several aspects of the invention. Any equivalent aspects are intended to be within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims. In the case of conflict, the present disclosure including definitions will control.

Claims

What is claimed is:

1. An enzyme composition, comprising two or more (several) components selected from the group consisting of:

(I) a polypeptide having cellobiohydrolase I activity selected from the group consisting of:

(A) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 2; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 1, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 1, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 1;

(B) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 4; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 3, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 3, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 3;

(C) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 6; (b) a polypeptide encoded by a polynucleotide that hybridizes

under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 5, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 5, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 5;

(D) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 8; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 7, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 7, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 7;

(E) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 158; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 157, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 157, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 157;

(F) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 160; (b) a polypeptide encoded by a polynucleotide that

hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 159, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 159, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 159;

(G) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 162; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 161, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 161, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 161;

(H) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 164; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 163, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 163, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 163; and

(I) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more

preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 166; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 165, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 165, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 165;

(II) a polypeptide having cellobiohydrolase II activity selected from the group consisting of:

(A) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 10; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 9, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 9, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 9;

(B) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 12; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 11, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 11, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 11;

(C) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 14; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 13, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 13, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 13;

(D) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 16; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 15, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 15, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 15;

(E) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 18; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 17, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 17, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at

least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 17;

(F) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 168; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 167, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 167, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 167;

(G) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 170; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 169, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 169, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 169; and

(H) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 172; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 172, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 172, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even

more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 172;

(III) a polypeptide having endoglucanase I activity selected from the group consisting of: (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 20; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 19, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 19, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 19;

(IV) a polypeptide having endoglucanase II activity selected from the group consisting of:

(A) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 22; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 21, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 21, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 21;

(B) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 24; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature

polypeptide coding sequence of SEQ ID NO: 23, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 23, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 23;

(C) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 26; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 25, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 25, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 25;

(D) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 174; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 173, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 173, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 173; and

(E) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 176; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at

least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 175, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 175, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 175; and

(V) a polypeptide having beta-glucosidase activity selected from the group consisting of:

(A) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 28; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 27, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 27, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 27;

(B) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 30; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 29, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 29, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 29;

(C) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature

polypeptide of SEQ ID NO: 32; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 31, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 31, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 31;

(D) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 178; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 177, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 177, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 177;

(E) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 180; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 179, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 179, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 179;

(F) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more

preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 182; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 181, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 181, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 181;

(G) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 184; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 183, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 183, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 183;

(H) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 186; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 185, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 185, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 185;

(l) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 188; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 187, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 187, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 187; and

(J) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 190; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 189, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 189, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 189.

2. The enzyme composition of claim 1, further comprising a polypeptide having cellulolytic enhancing activity selected from the group consisting of:

(l) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 34; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 33, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 33, or (iii) a full-length complementary strand of

(i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 33;

(II) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 36; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 35, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 35, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 35;

(III) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 38; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 37, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 37, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 37;

(IV) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 40; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 39, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 39, or (iii) a full-length complementary strand of

(i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 39;

(V) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 42; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 41, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 41, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 41;

(VI) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 44; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 43, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 43, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 43;

(VII) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 192; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 191, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 191, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a

nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 191; and

(VIII) a combination of any of I, II, III, IV, V, VI, and VII.

3. The enzyme composition of claim 1 or 2, further comprising a polypeptide having xylanase activity.

4. The enzyme composition of claim 3, wherein the polypeptide having xylanase activity is a Family 10 polypeptide having xylanase activity.

5. The enzyme composition of claim 4, wherein the Family 10 polypeptide having xylanase activity is selected from the group consisting of:

(I) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 46; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 45, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 45, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 45;

(II) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 48; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 47, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 47, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more

preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 47;

(III) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 50; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 49, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 49, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 49;

(IV) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 52; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 51, (ii) the cDNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 51, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 51;

(V) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 54; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 53, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 53, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even

more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 53;

(VI) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 194; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 193, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 193, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 193;

(VII) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 196; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 195, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 195, or (iii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 195; and

(VIII) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 198; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 197, (ii) the genomic DNA sequence of the mature polypeptide coding sequence of SEQ ID NO: 197, or (iii) a full-length

complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 197.

6. The enzyme composition of claim 3, wherein the polypeptide having xylanase activity is a Family 11 polypeptide having xylanase activity.

7. The enzyme composition of claim 6, wherein the Family 11 polypeptide having xylanase activity is selected from the group consisting of:

(I) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 56; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with (i) the mature polypeptide coding sequence of SEQ ID NO: 55 or (ii) a full-length complementary strand of (i) or (ii); and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 55; and

(II) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 200 or SEQ ID NO: 305; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with the mature polypeptide coding sequence of SEQ ID NO: 199 or SEQ ID NO: 304 or its full-length complementary strand; and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 199 or SEQ ID NO: 304.

8. The enzyme composition of any of claims 1-7, further comprising a beta-xylosidase.

9. The enzyme composition of claim 8, wherein the beta-xylosidase is selected from the group consisting of:

(I) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 58; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with the mature polypeptide coding sequence of SEQ ID NO: 57 or its full-length complementary strand; and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 57;

(II) a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 60; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with the mature polypeptide coding sequence of SEQ ID NO: 59 or its full-length complementary strand; and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 59;

(III) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 202; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with the mature polypeptide coding sequence of SEQ ID NO: 201 or its full-length complementary strand; and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 201;

(IV) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more

preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 204; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with the mature polypeptide coding sequence of SEQ ID NO: 203 or its full-length complementary strand; and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 203; and

(V) (a) a polypeptide comprising an amino acid sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide of SEQ ID NO: 206; (b) a polypeptide encoded by a polynucleotide that hybridizes under preferably at least medium-high stringency conditions, more preferably at least high stringency conditions, and most preferably very high stringency conditions with the mature polypeptide coding sequence of SEQ ID NO: 205 or its full-length complementary strand; and (c) a polypeptide encoded by a polynucleotide comprising a nucleotide sequence having preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, even more preferably at least 95% identity, and most preferably at least 97% identity to the mature polypeptide coding sequence of SEQ ID NO: 205.

10. The enzyme composition of any of claims 1-9, further comprising one or more (several) enzymes selected from the group consisting of a hemicellulase, a carbohydrate-esterase, a pectinase, a protease, a catalase, a laccase, a peroxidase, an H_2O_2-producing enzyme, an oxidoreductase, an expansin, a swollenin, or a mixture thereof.

11. The enzyme composition of any of claims 1-10, which is employed at a temperature in the range of about 40°C to about 70°C, *e.g.*, about 50°C to about 65°C, about 55°C to about 63°C, or about 55°C to about 60°C.

12. The enzyme composition of any of claims 1-11, which reduces the amount of protein required to achieve the same degree of cellulose hydrolysis of PCS at 55°C, pH 3.5 to pH 6.0 at least 1.1-fold, *e.g.*, at least 1.25-fold, at least 1.5-fold, at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 7.5-fold, or at least 10-fold compared to a *Trichoderma reesei*-based enzyme composition SaMe-MF268 at 50°C, pH 5.0.

13. The enzyme composition of any of claims 1-11, which reduces the amount of protein

required to achieve the same degree of cellulose hydrolysis of PCS at 60°C, pH 3.5 to pH 6.0 at least 1.1-fold, *e.g.*, at least 1.25-fold, at least 1.5-fold, at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 7.5-fold, or at least 10-fold compared to a *Trichoderma reesei*-based enzyme composition SaMe-MF268 at 50°C, pH 5.0.

14. The enzyme composition of any of claims 1-11, which reduces the amount of protein required to achieve the same degree of cellulose hydrolysis of PCS at 65°C, pH 3.5 to pH 6.0 at least 1.1-fold, *e.g.*, at least 1.25-fold, at least 1.5-fold, at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 7.5-fold, or at least 10-fold compared to a *Trichoderma reesei*-based enzyme composition SaMe-MF268 at 50°C, pH 5.0.

15. The enzyme composition of any of claims 1-11, which reduces the amount of protein required to achieve the same degree of cellulose hydrolysis of PCS at 70°C, pH 3.5 to pH 6.0 at least 1.1-fold, *e.g.*, at least 1.25-fold, at least 1.5-fold, at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 7.5-fold, or at least 10-fold compared to a *Trichoderma reesei*-based enzyme composition SaMe-MF268 at 50°C, pH 5.0.

16. A recombinant host cell encoding the enzyme composition of any of claims 1-15.

17. A method of producing an enzyme composition, comprising: (a) cultivating the recombinant host cell of claim 16 under conditions conducive for production of the enzyme composition; and (b) recovering the enzyme composition.

18. A method for degrading or converting a cellulosic material, comprising: treating the cellulosic material with the enzyme composition of any of claims 1-15.

19. The method of claim 18, further comprising recovering the degraded cellulosic material.

20. A method for producing a fermentation product, comprising:

(a) saccharifying a cellulosic material with the enzyme composition of any of claims 1-15;

(b) fermenting the saccharified cellulosic material with one or more (several) fermenting microorganisms to produce the fermentation product; and

(c) recovering the fermentation product from the fermentation.

21. A method of fermenting a cellulosic material, comprising: fermenting the cellulosic

material with one or more (several) fermenting microorganisms, wherein the cellulosic material is saccharified with the enzyme composition of any of claims 1-15.

22. The method of claim 21, wherein the fermenting of the cellulosic material produces a fermentation product.

23. The method of claim 22, further comprising recovering the fermentation product from the fermentation.

24. The method of claim 22 or 23, wherein the fermentation product is an alcohol, an organic acid, a ketone, an amino acid, or a gas.

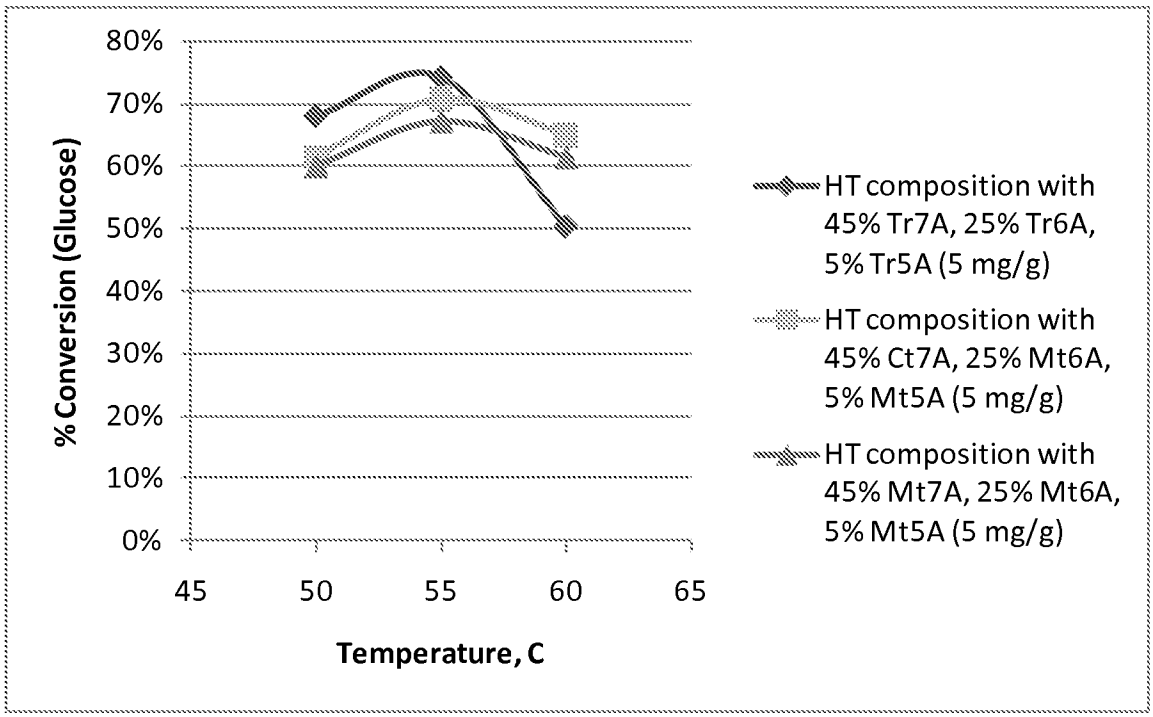

80%
70%
60%
50%
40%
30%
20%
10%
0%
% Conversion (Glucose)
45
50
55
60
65
Temperature, C
HT composition with 45% Tr7A, 25% Tr6A, 5% Tr5A (5 mg/g)
HT composition with 45% Ct7A, 25% Mt6A, 5% Mt5A (5 mg/g)
HT composition with 45% Mt7A, 25% Mt6A, 5% Mt5A (5 mg/g)

Fig. 1

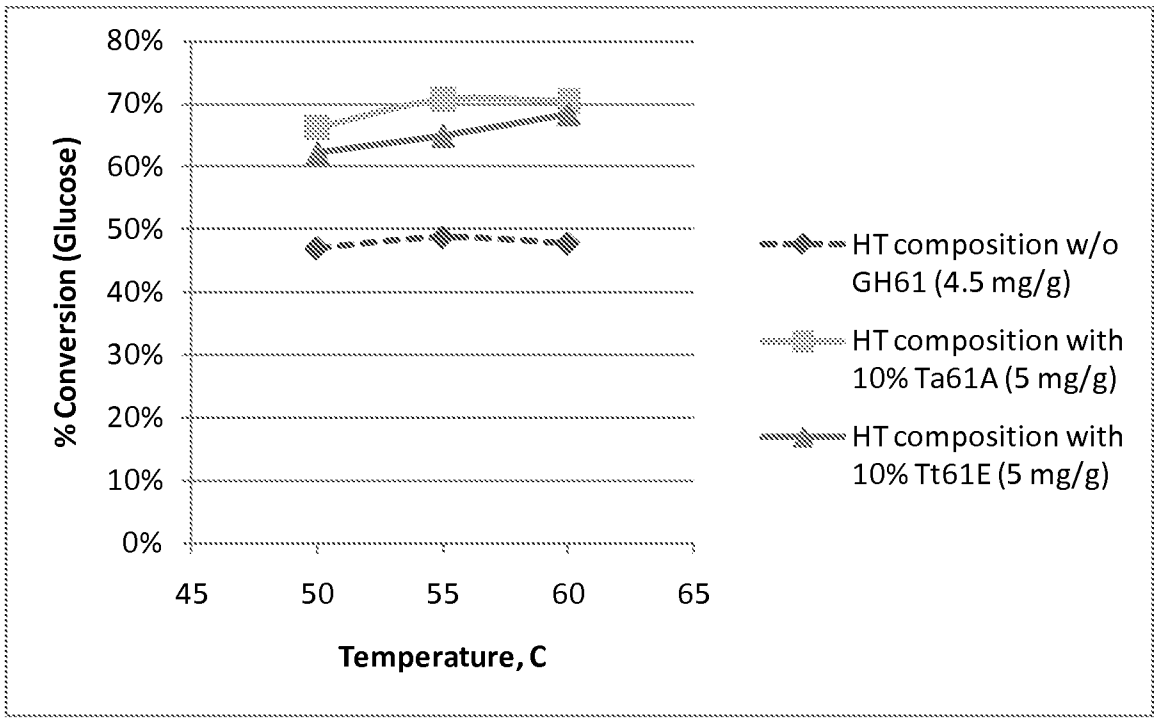

80%
70%
60%
50%
40%
30%
20%
10%
0%
% Conversion (Glucose)
45
50
55
60
65
Temperature, C
HT composition w/o GH61 (4.5 mg/g)
HT composition with 10% Ta61A (5 mg/g)
HT composition with 10% Tt61E (5 mg/g)

Fig. 2

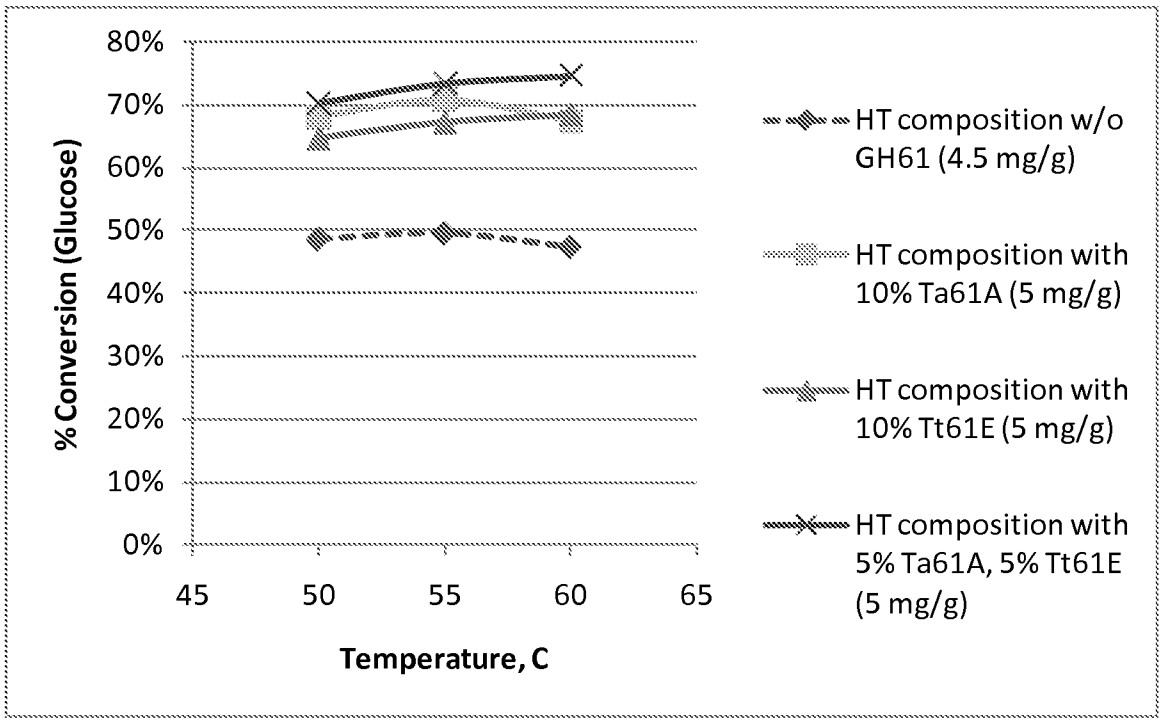

80%
70%
60%
50%
40%
30%
20%
10%
0%
% Conversion (Glucose)
45
50
55
60
65
Temperature, C
HT composition w/o GH61 (4.5 mg/g)
HT composition with 10% Ta61A (5 mg/g)
HT composition with 10% Tt61E (5 mg/g)
HT composition with 5% Ta61A, 5% Tt61E (5 mg/g)

Fig. 3

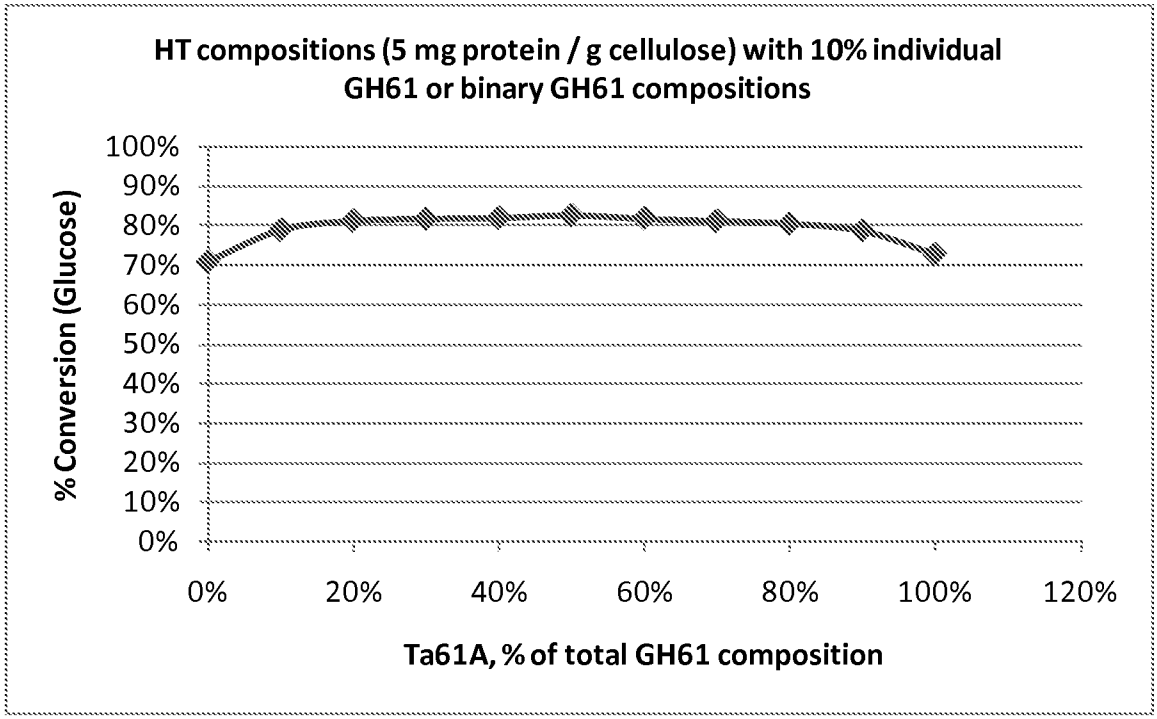

HT compositions (5 mg protein / g cellulose) with 10% individual GH61 or binary GH61 compositions
% Conversion (Glucose)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0%
20%
40%
60%
80%
100%
120%
Ta61A, % of total GH61 composition

Fig. 4

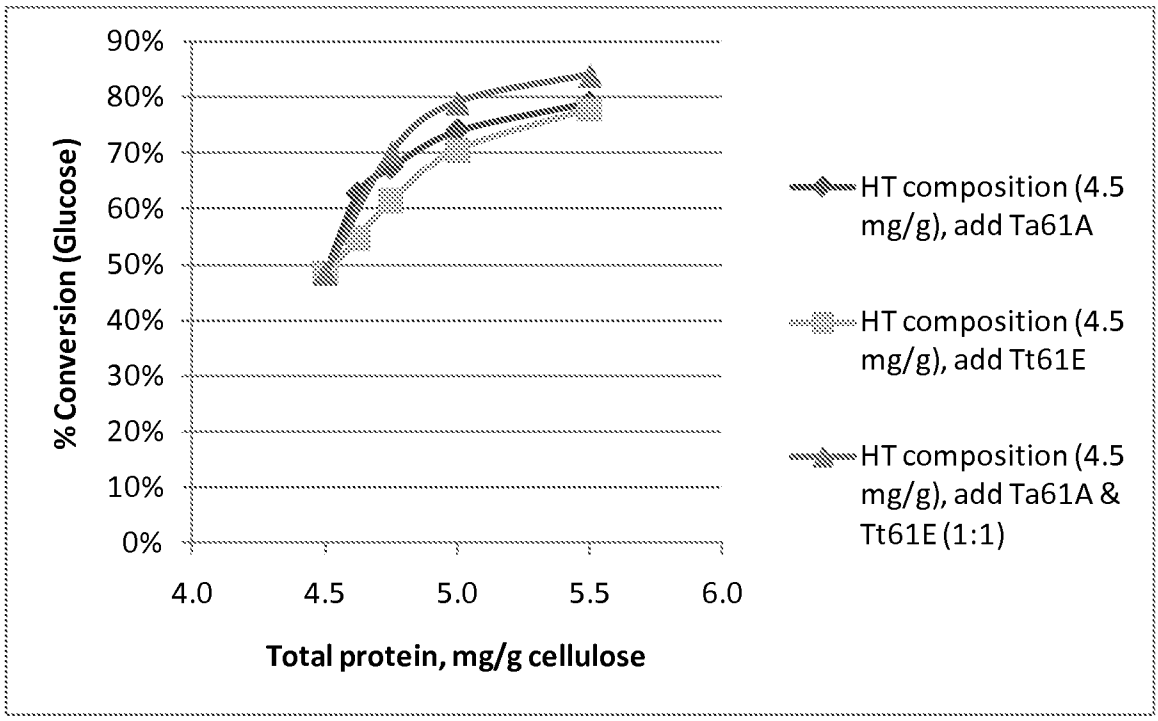

90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% Conversion (Glucose)
4.0
4.5
5.0
5.5
6.0
Total protein, mg/g cellulose
HT composition (4.5 mg/g), add Ta61A
HT composition (4.5 mg/g), add Tt61E
HT composition (4.5 mg/g), add Ta61A & Tt61E (1:1)

Fig. 5

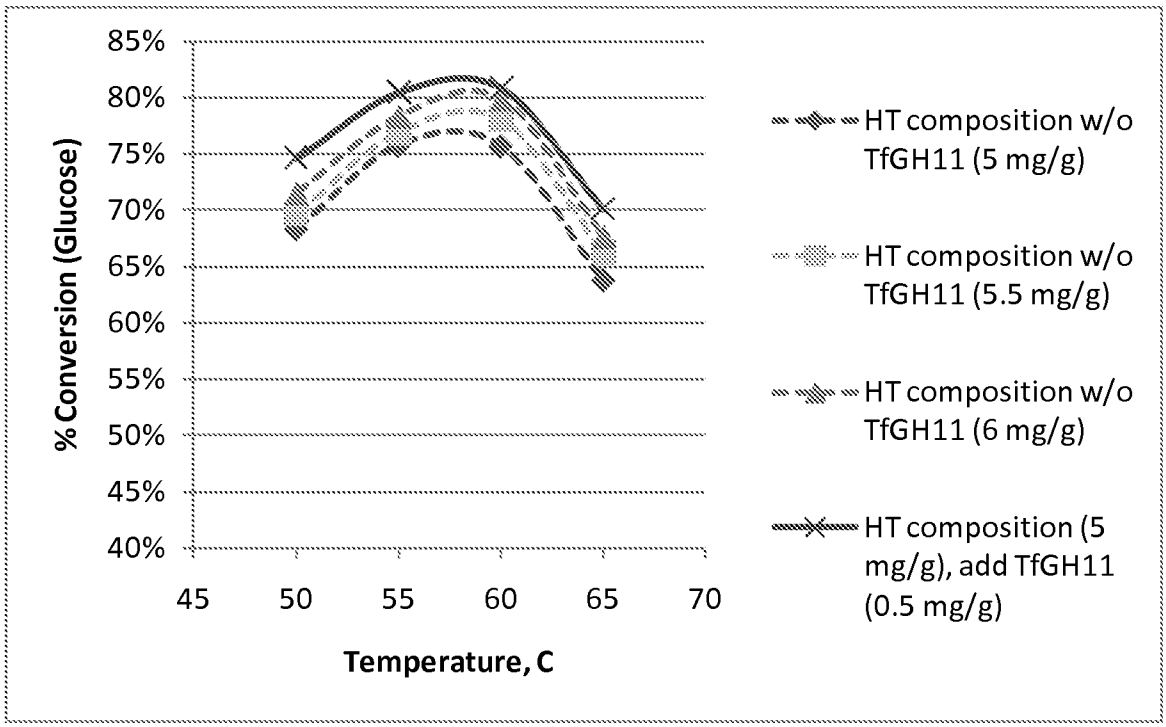

85%
80%
75%
70%
65%
60%
55%
50%
45%
40%
% Conversion (Glucose)
45
50
55
60
65
70
Temperature, C
HT composition w/o TfGH11 (5 mg/g)
HT composition w/o TfGH11 (5.5 mg/g)
HT composition w/o TfGH11 (6 mg/g)
HT composition (5 mg/g), add TfGH11 (0.5 mg/g)

Fig. 6

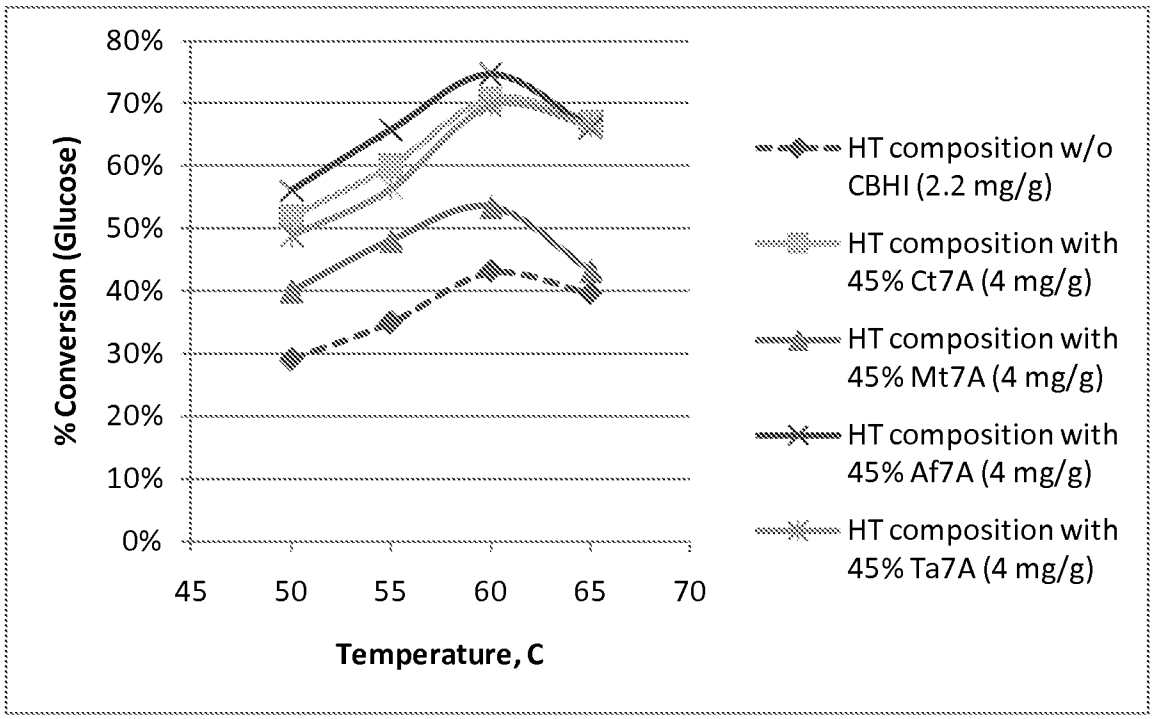

80%
70%
60%
50%
40%
30%
20%
10%
0%
% Conversion (Glucose)
45
50
55
60
65
70
Temperature, C
HT composition w/o CBHI (2.2 mg/g)
HT composition with 45% Ct7A (4 mg/g)
HT composition with 45% Mt7A (4 mg/g)
HT composition with 45% Af7A (4 mg/g)
HT composition with 45% Ta7A (4 mg/g)

Fig. 7

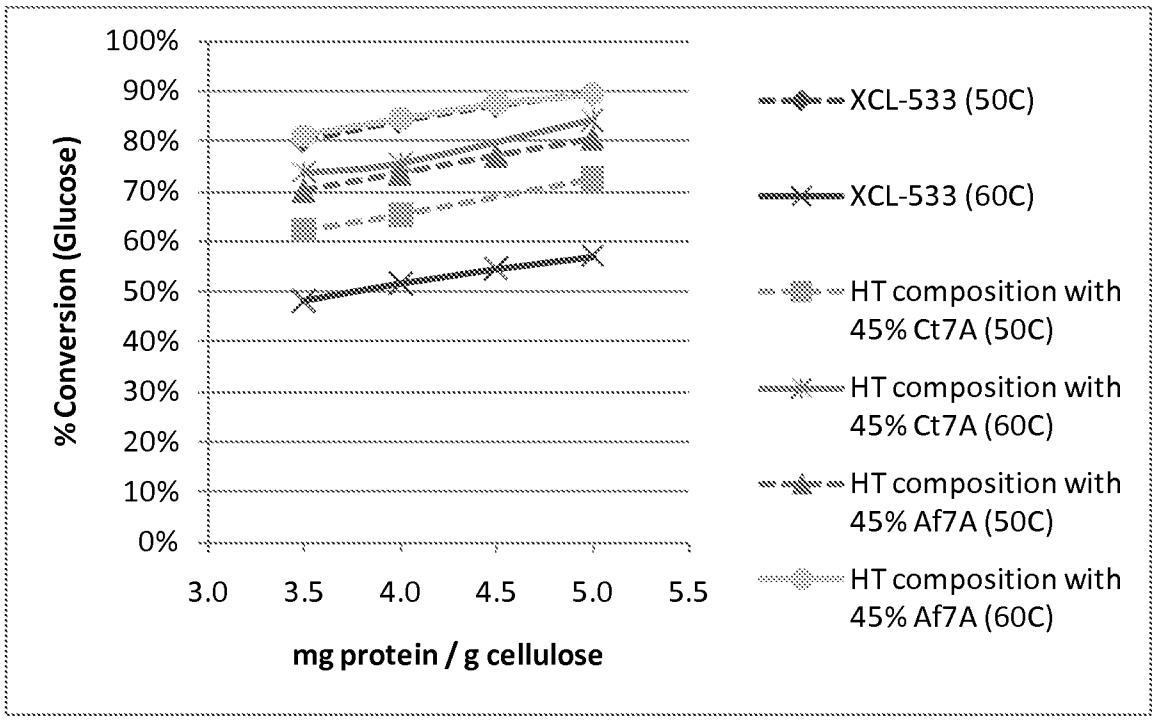

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% Conversion (Glucose)
3.0
3.5
4.0
4.5
5.0
5.5
mg protein / g cellulose
XCL-533 (50C)
XCL-533 (60C)
HT composition with 45% Ct7A (50C)
HT composition with 45% Ct7A (60C)
HT composition with 45% Af7A (50C)
HT composition with 45% Af7A (60C)

Fig. 8

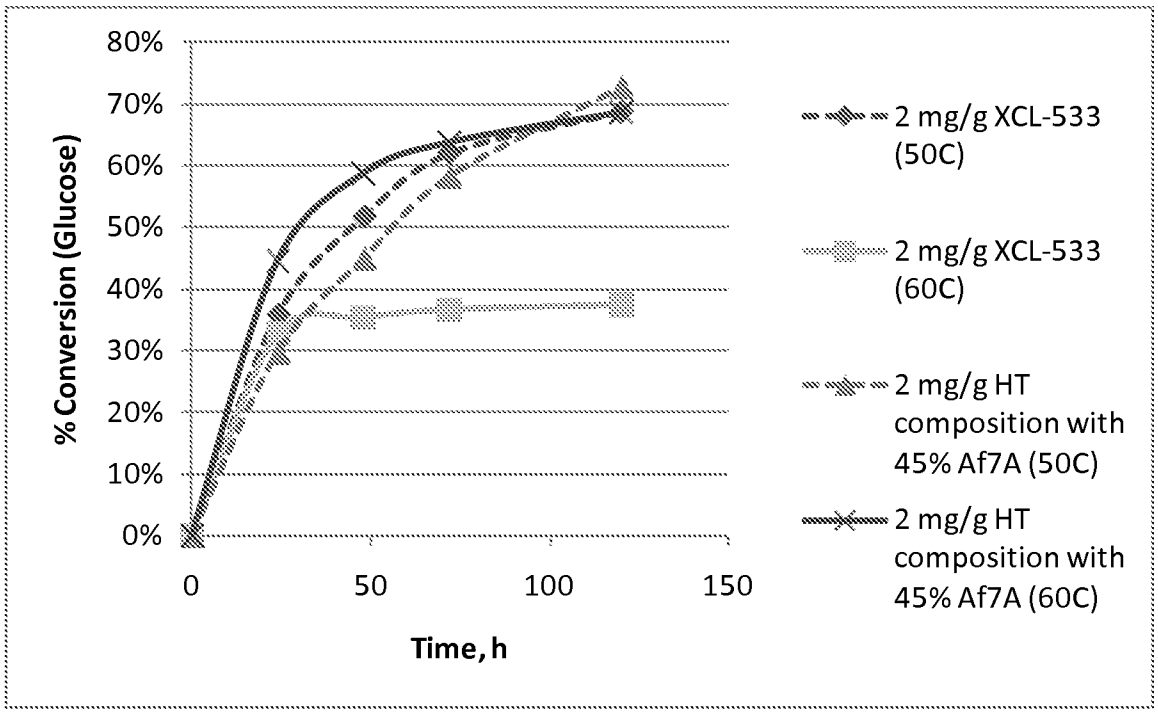

80%
70%
60%
50%
40%
30%
20%
10%
0%
% Conversion (Glucose)
0
50
100
150
Time, h
2 mg/g XCL-533 (50C)
2 mg/g XCL-533 (60C)
2 mg/g HT composition with 45% Af7A (50C)
2 mg/g HT composition with 45% Af7A (60C)

Fig. 9

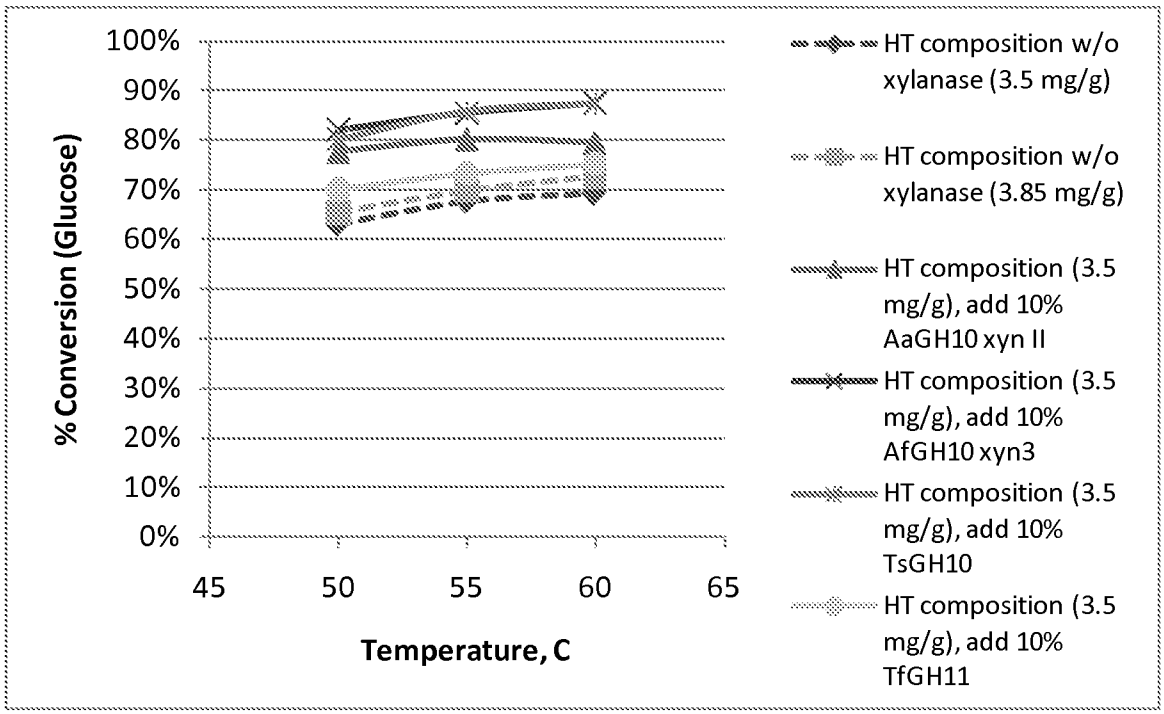

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% Conversion (Glucose)
45
50
55
60
65
Temperature, C
HT composition w/o xylanase (3.5 mg/g)
HT composition w/o xylanase (3.85 mg/g)
HT composition (3.5 mg/g), add 10% AaGH10 xyn II
HT composition (3.5 mg/g), add 10% AfGH10 xyn3
HT composition (3.5 mg/g), add 10% TsGH10
HT composition (3.5 mg/g), add 10% TfGH11

Fig. 10

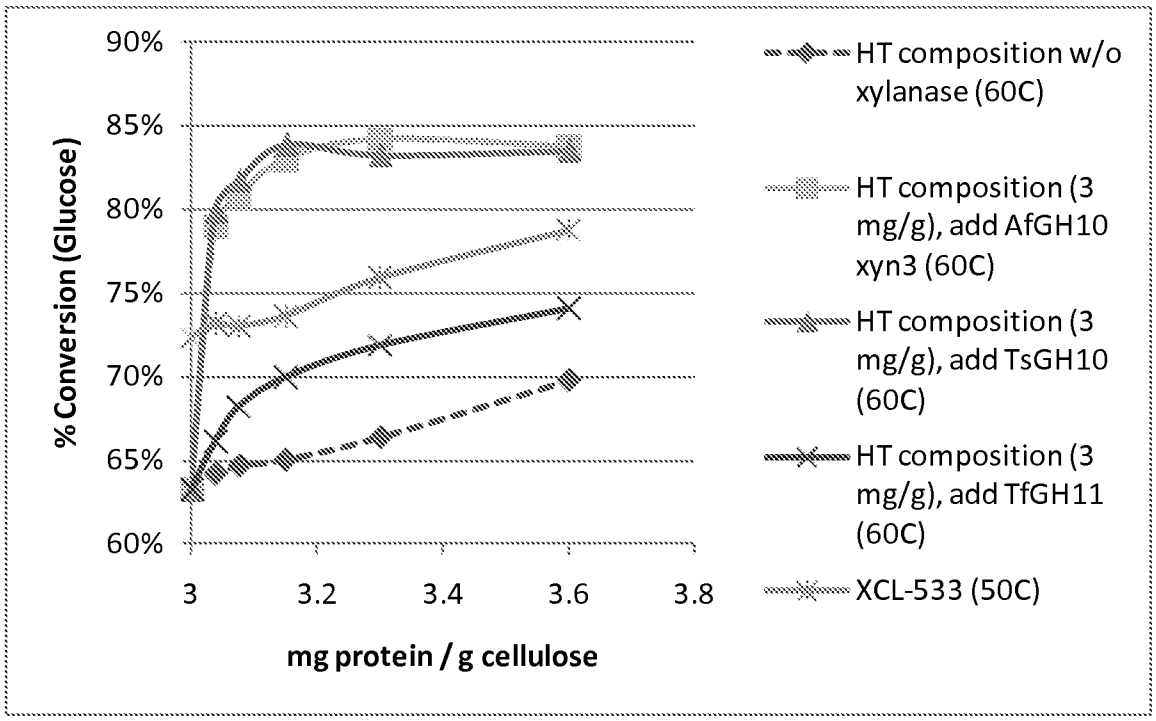

90%
85%
80%
75%
70%
65%
60%
% Conversion (Glucose)
3
3.2
3.4
3.6
3.8
mg protein / g cellulose
HT composition w/o xylanase (60C)
HT composition (3 mg/g), add AfGH10 xyn3 (60C)
HT composition (3 mg/g), add TsGH10 (60C)
HT composition (3 mg/g), add TfGH11 (60C)
XCL-533 (50C)

Fig. 11

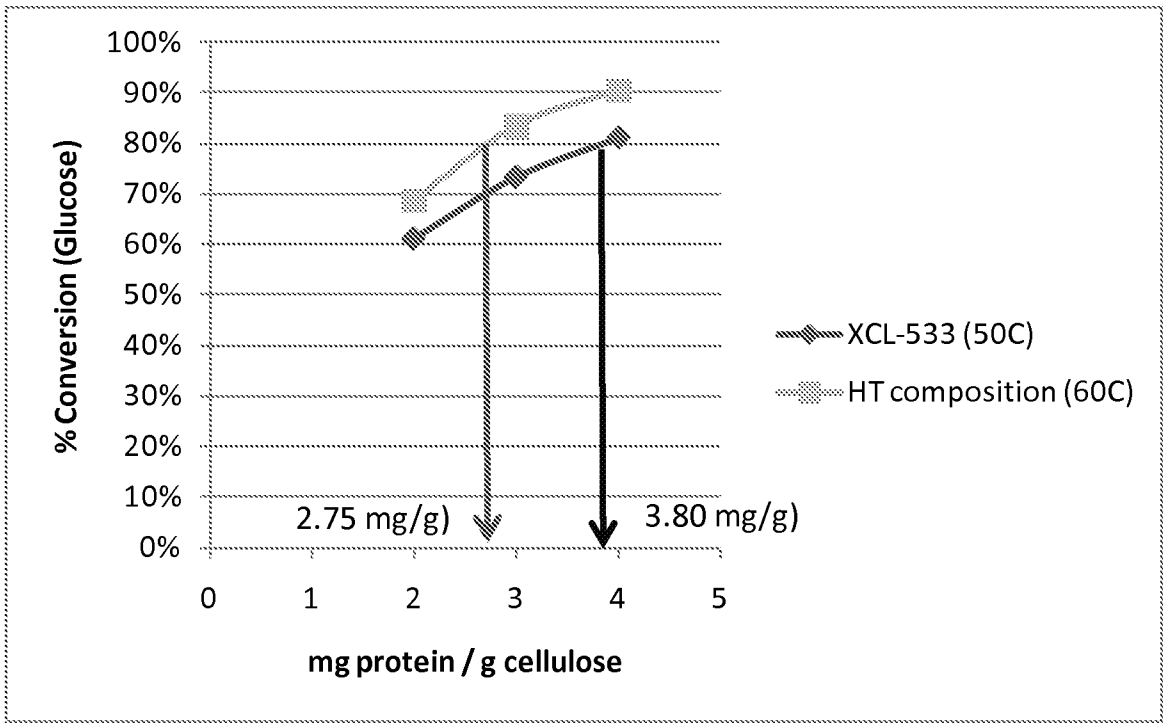

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% Conversion (Glucose)
0
1
2
3
4
5
mg protein / g cellulose
2.75 mg/g)
3.80 mg/g)
XCL-533 (50C)
HT composition (60C)

Fig. 12

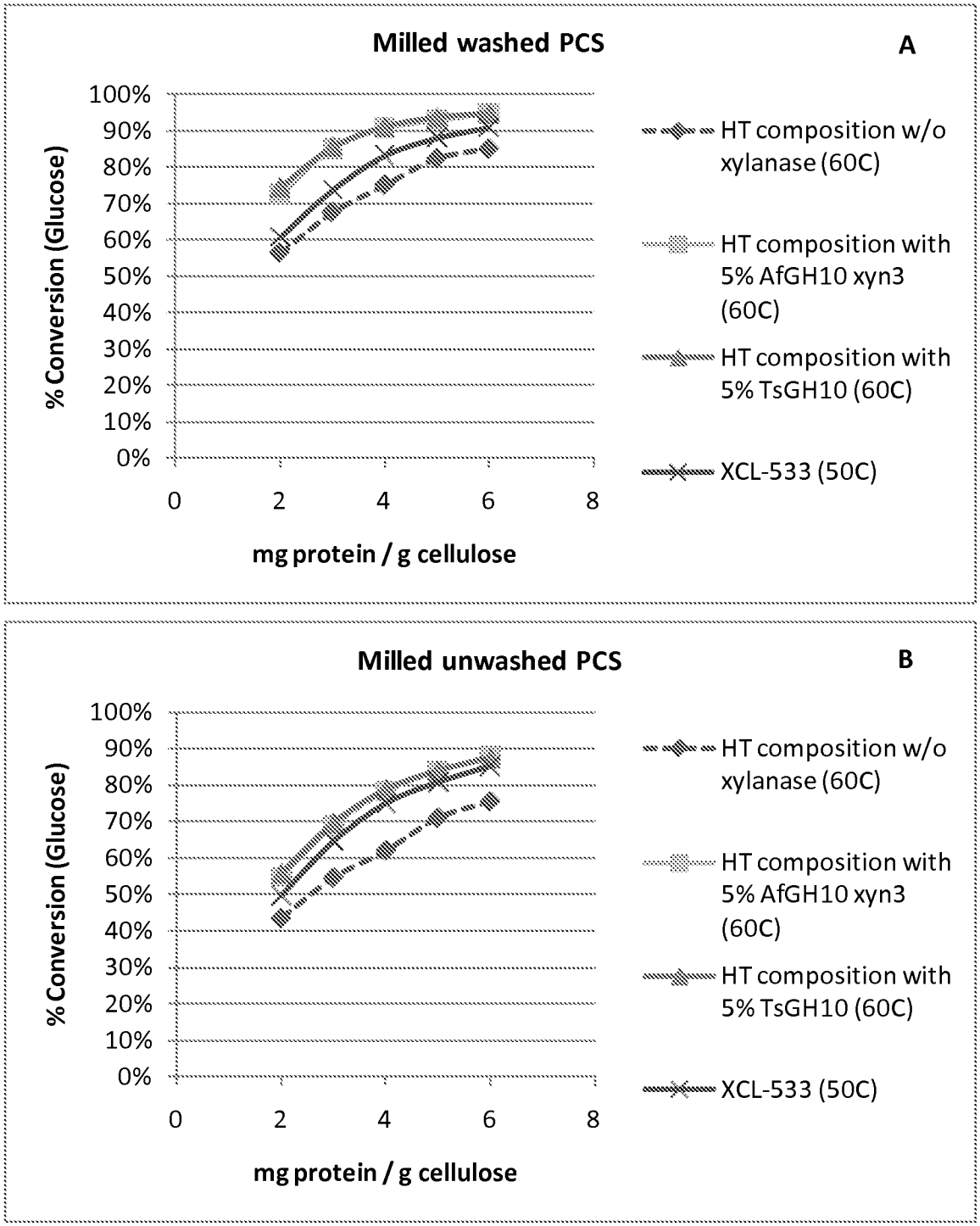
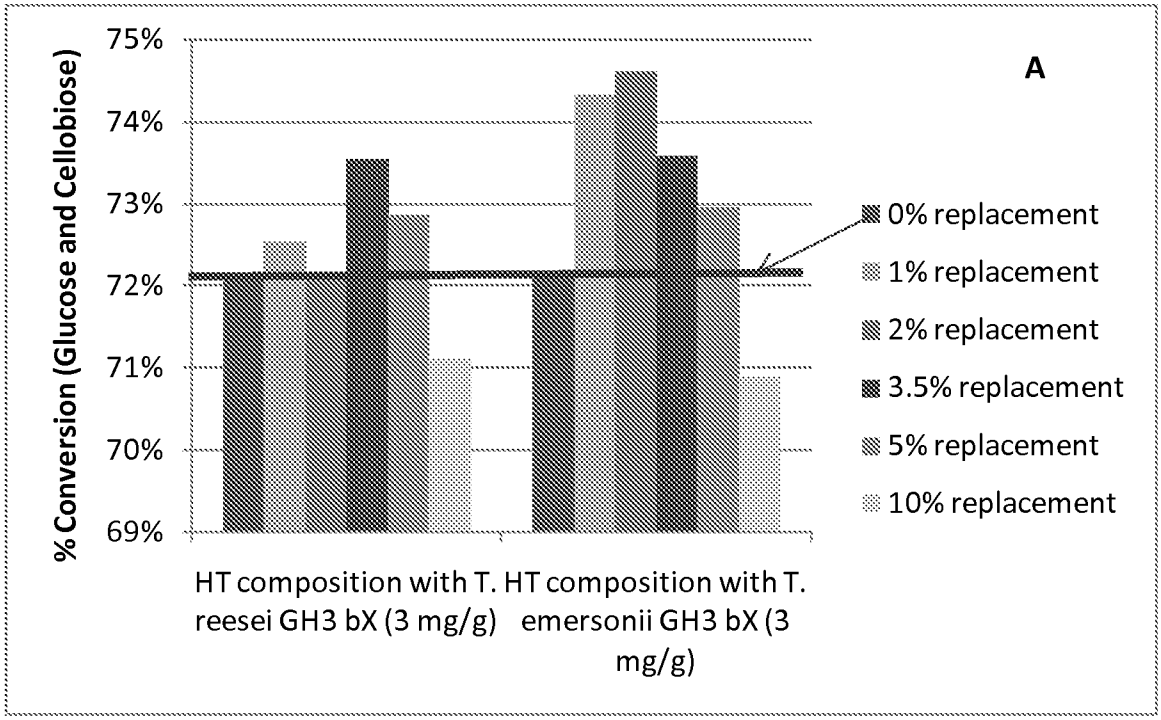

Milled washed PCS
A
% Conversion (Glucose)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
2
4
6
8
mg protein / g cellulose
HT composition w/o xylanase (60C)
HT composition with 5% AfGH10 xyn3 (60C)
HT composition with 5% TsGH10 (60C)
XCL-533 (50C)
Milled unwashed PCS
B
% Conversion (Glucose)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
2
4
6
8
mg protein / g cellulose
HT composition w/o xylanase (60C)
HT composition with 5% AfGH10 xyn3 (60C)
HT composition with 5% TsGH10 (60C)
XCL-533 (50C)

Fig. 13


A
% Conversion (Glucose and Cellobiose)
75%
74%
73%
72%
71%
70%
69%
0% replacement
1% replacement
2% replacement
3.5% replacement
5% replacement
10% replacement
HT composition with T. reesei GH3 bX (3 mg/g)
HT composition with T. emersonii GH3 bX (3 mg/g)

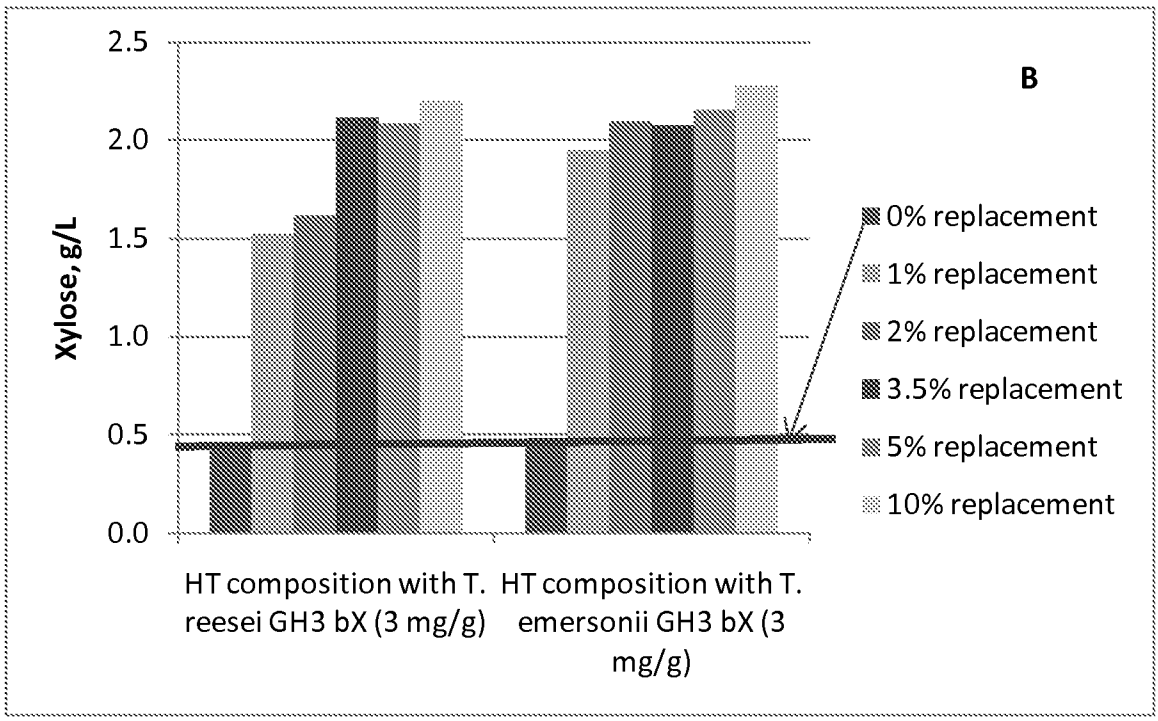

B
Xylose, g/L
2.5
2.0
1.5
1.0
0.5
0.0
0% replacement
1% replacement
2% replacement
3.5% replacement
5% replacement
10% replacement
HT composition with T. reesei GH3 bX (3 mg/g)
HT composition with T. emersonii GH3 bX (3 mg/g)

Fig. 14

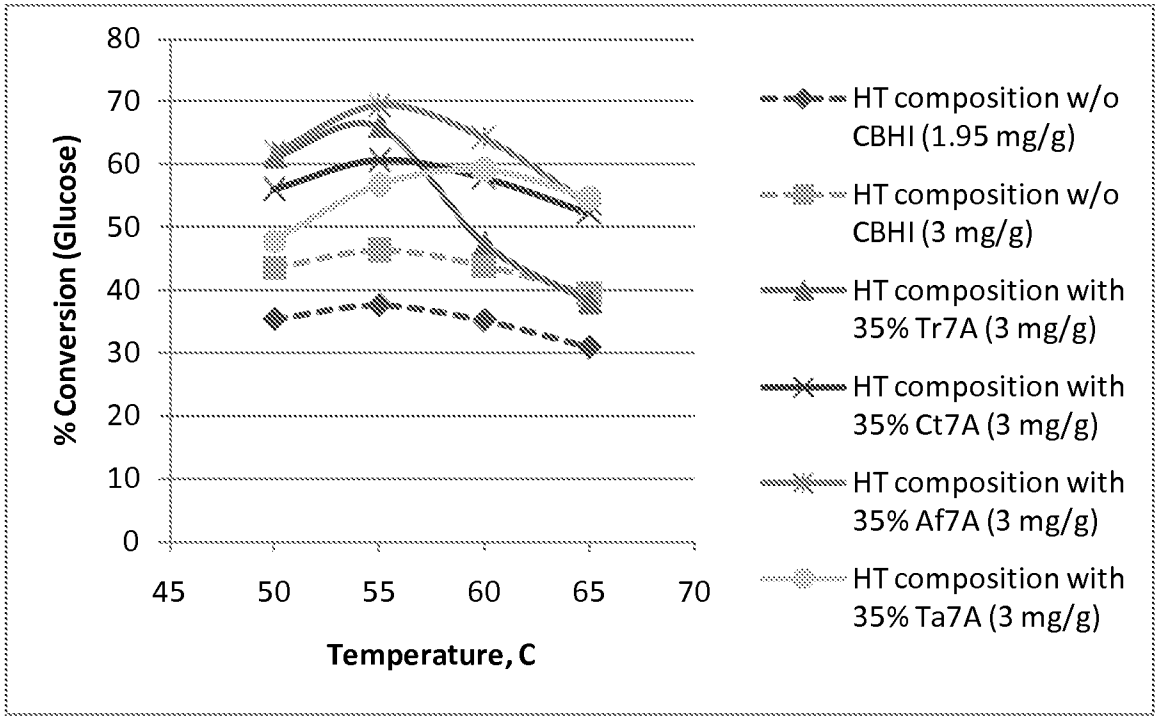

80
70
60
50
40
30
20
10
0
% Conversion (Glucose)
45
50
55
60
65
70
Temperature, C
HT composition w/o CBHI (1.95 mg/g)
HT composition w/o CBHI (3 mg/g)
HT composition with 35% Tr7A (3 mg/g)
HT composition with 35% Ct7A (3 mg/g)
HT composition with 35% Af7A (3 mg/g)
HT composition with 35% Ta7A (3 mg/g)

Fig. 15

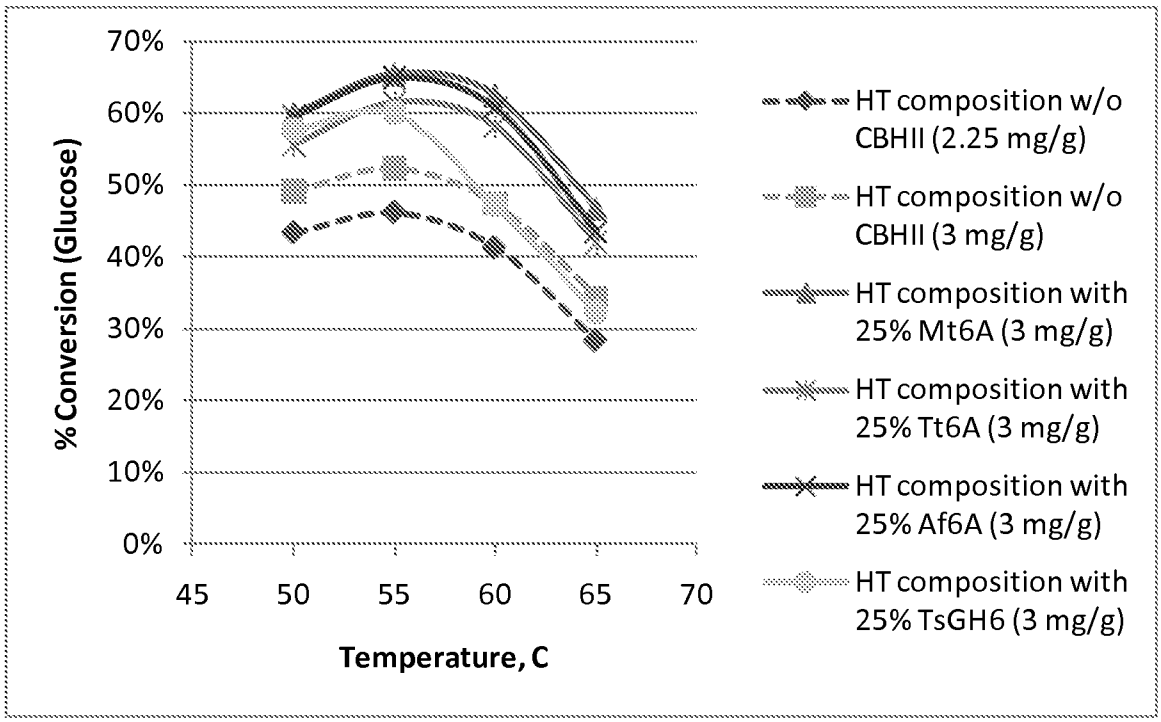

70%
60%
50%
40%
30%
20%
10%
0%
% Conversion (Glucose)
45
50
55
60
65
70
Temperature, C
HT composition w/o CBHII (2.25 mg/g)
HT composition w/o CBHII (3 mg/g)
HT composition with 25% Mt6A (3 mg/g)
HT composition with 25% Tt6A (3 mg/g)
HT composition with 25% Af6A (3 mg/g)
HT composition with 25% TsGH6 (3 mg/g)

Fig. 16

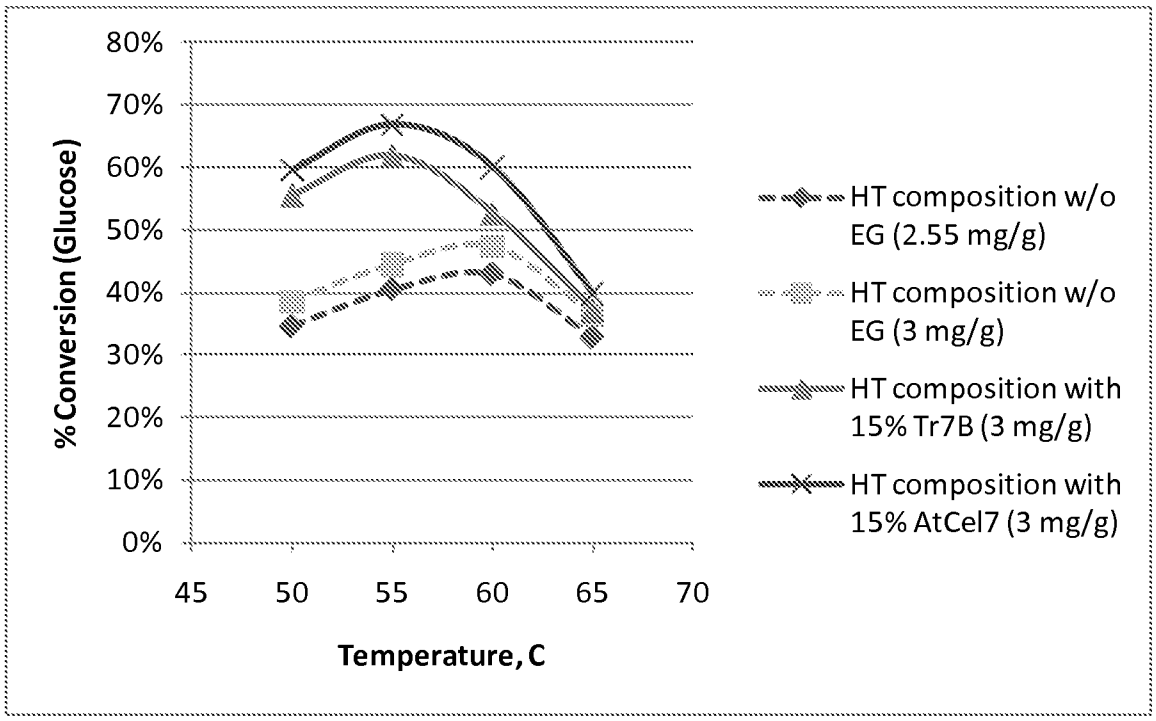

80%
70%
60%
50%
40%
30%
20%
10%
0%
% Conversion (Glucose)
45
50
55
60
65
70
Temperature, C
HT composition w/o EG (2.55 mg/g)
HT composition w/o EG (3 mg/g)
HT composition with 15% Tr7B (3 mg/g)
HT composition with 15% AtCel7 (3 mg/g)

Fig. 17

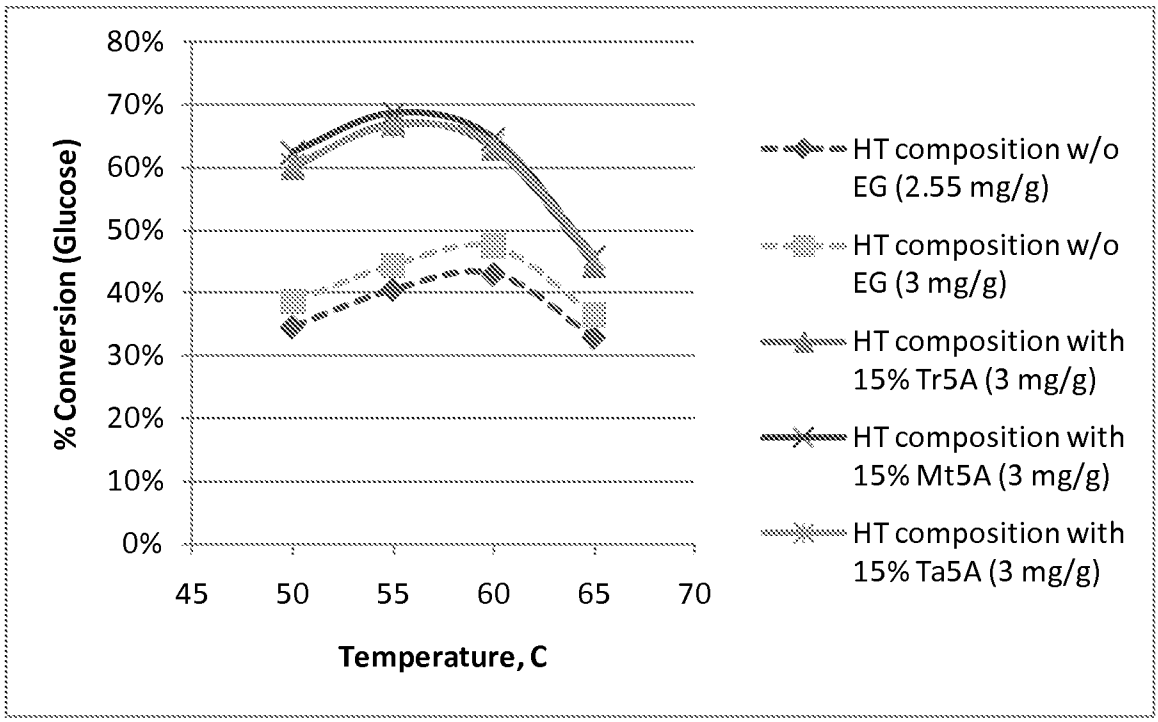

80%
70%
60%
50%
40%
30%
20%
10%
0%
% Conversion (Glucose)
45
50
55
60
65
70
Temperature, C
HT composition w/o EG (2.55 mg/g)
HT composition w/o EG (3 mg/g)
HT composition with 15% Tr5A (3 mg/g)
HT composition with 15% Mt5A (3 mg/g)
HT composition with 15% Ta5A (3 mg/g)

Fig. 18

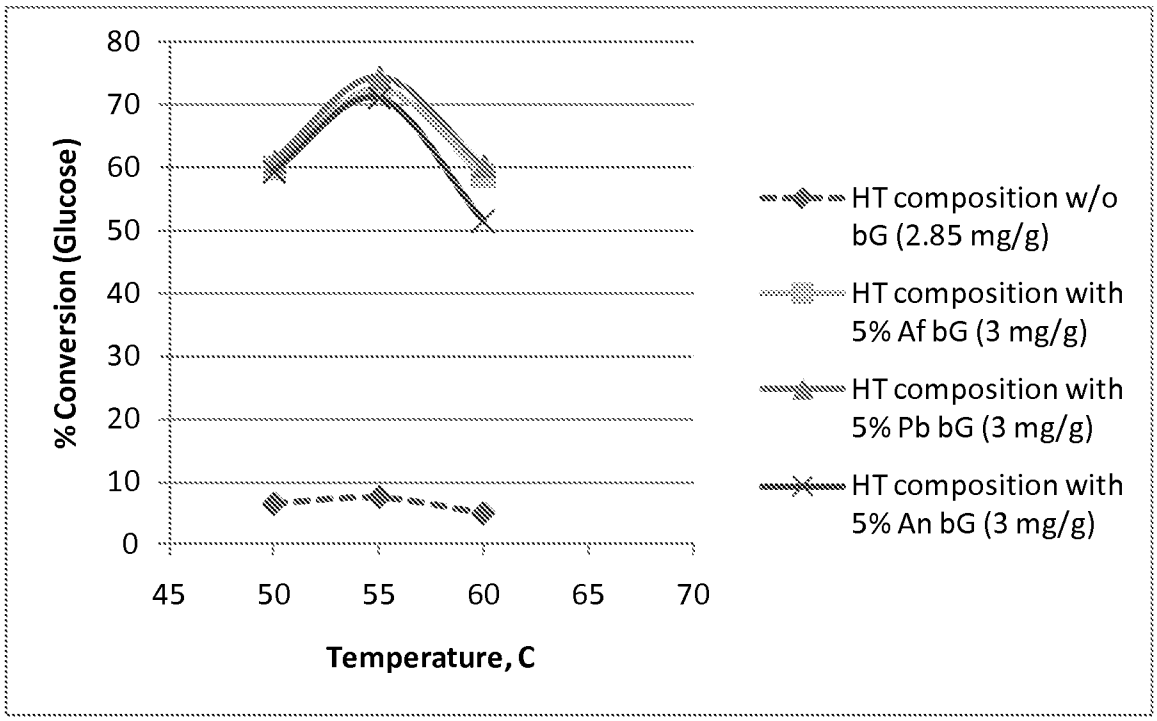

80
70
60
50
40
30
20
10
0
% Conversion (Glucose)
45
50
55
60
65
70
Temperature, C
HT composition w/o bG (2.85 mg/g)
HT composition with 5% Af bG (3 mg/g)
HT composition with 5% Pb bG (3 mg/g)
HT composition with 5% An bG (3 mg/g)

Fig. 19

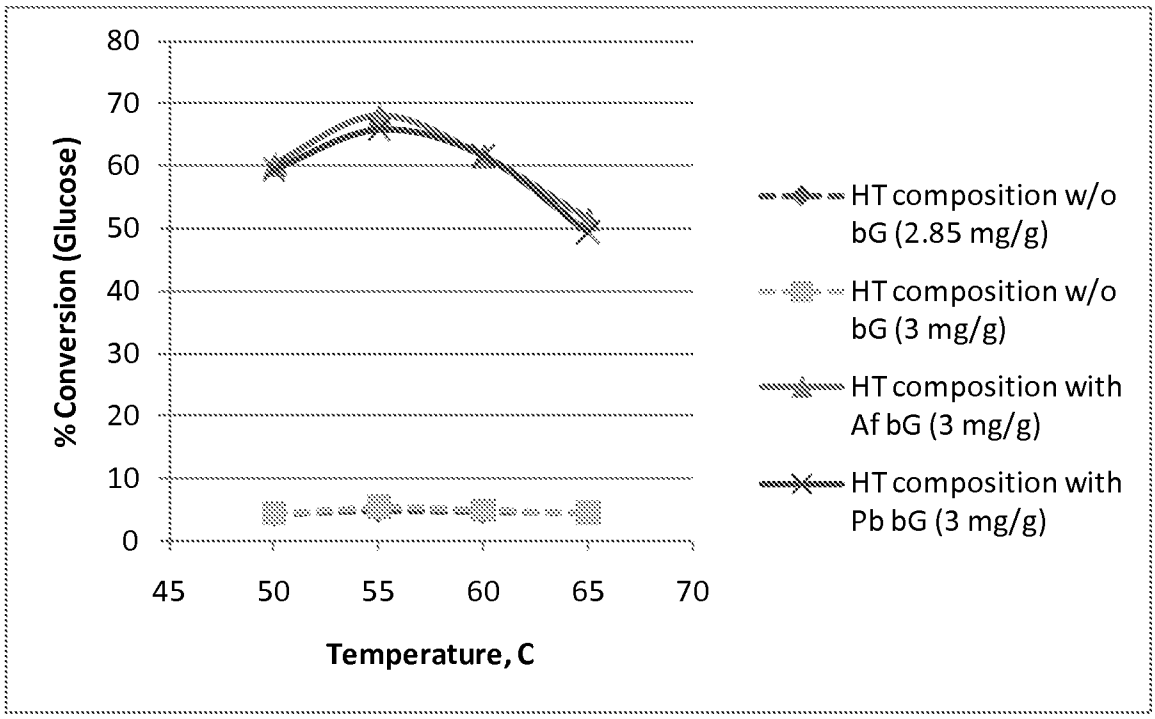

80
70
60
50
40
30
20
10
0
% Conversion (Glucose)
45
50
55
60
65
70
Temperature, C
HT composition w/o bG (2.85 mg/g)
HT composition w/o bG (3 mg/g)
HT composition with Af bG (3 mg/g)
HT composition with Pb bG (3 mg/g)

Fig. 20

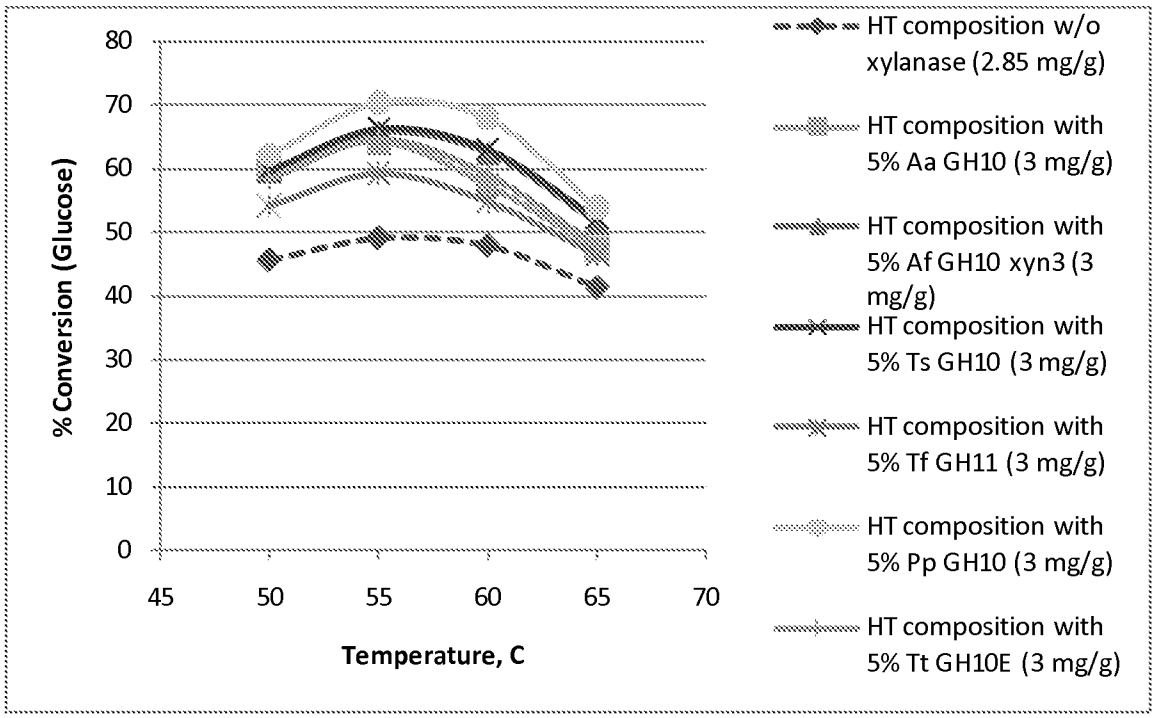

80
70
60
50
40
30
20
10
0
% Conversion (Glucose)
45
50
55
60
65
70
Temperature, C
HT composition w/o xylanase (2.85 mg/g)
HT composition with 5% Aa GH10 (3 mg/g)
HT composition with 5% Af GH10 xyn3 (3 mg/g)
HT composition with 5% Ts GH10 (3 mg/g)
HT composition with 5% Tf GH11 (3 mg/g)
HT composition with 5% Pp GH10 (3 mg/g)
HT composition with 5% Tt GH10E (3 mg/g)

Fig. 21

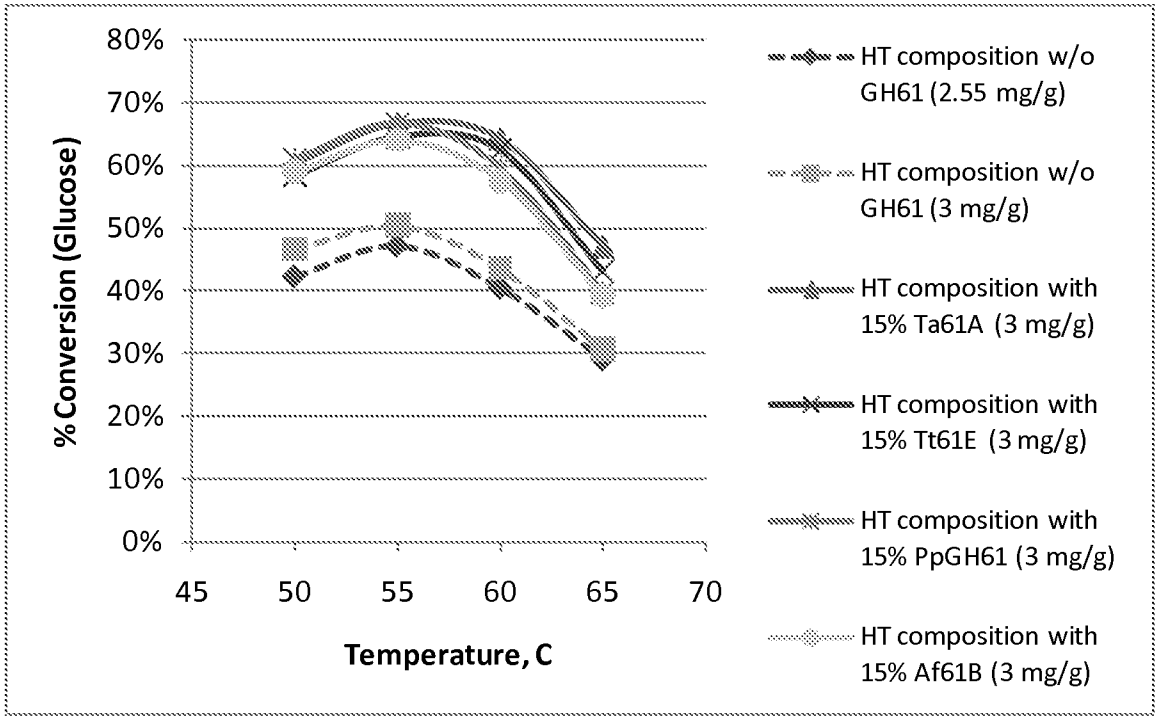

80%
70%
60%
50%
40%
30%
20%
10%
0%
% Conversion (Glucose)
45
50
55
60
65
70
Temperature, C
HT composition w/o GH61 (2.55 mg/g)
HT composition w/o GH61 (3 mg/g)
HT composition with 15% Ta61A (3 mg/g)
HT composition with 15% Tt61E (3 mg/g)
HT composition with 15% PpGH61 (3 mg/g)
HT composition with 15% Af61B (3 mg/g)

Fig. 22

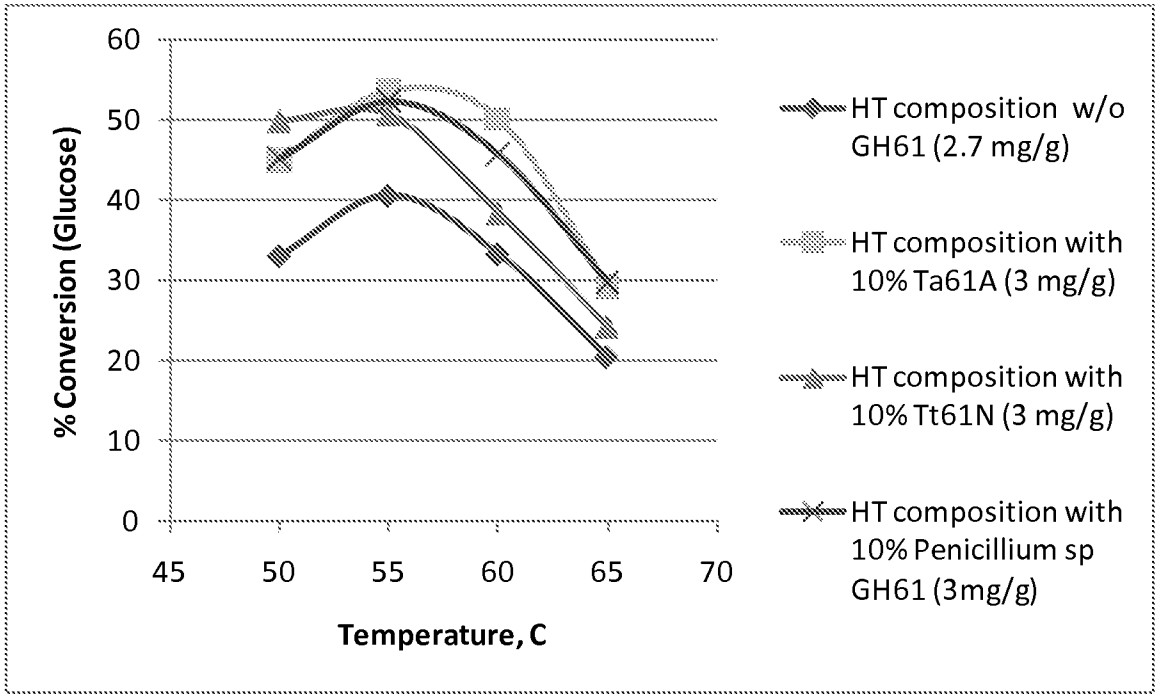

60
50
40
30
20
10
0
% Conversion (Glucose)
45
50
55
60
65
70
Temperature, C
HT composition w/o GH61 (2.7 mg/g)
HT composition with 10% Ta61A (3 mg/g)
HT composition with 10% Tt61N (3 mg/g)
HT composition with 10% Penicillium sp GH61 (3mg/g)

Fig. 23

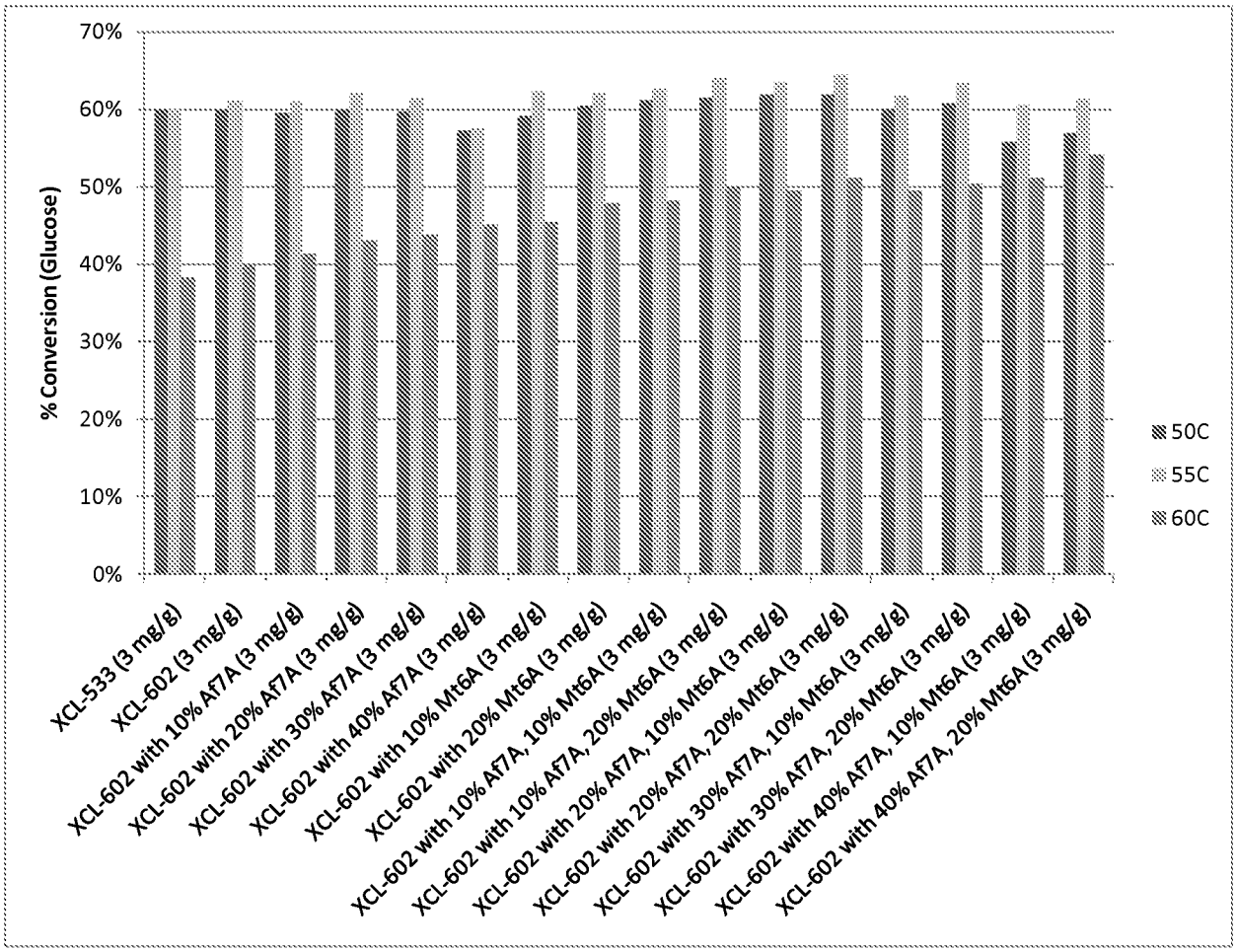

70%
60%
50%
40%
30%
20%
10%
0%
% Conversion (Glucose)
50C
55C
60C
XCL-533 (3 mg/g)
XCL-602 (3 mg/g)
XCL-602 with 10% Af7A (3 mg/g)
XCL-602 with 20% Af7A (3 mg/g)
XCL-602 with 30% Af7A (3 mg/g)
XCL-602 with 40% Af7A (3 mg/g)
XCL-602 with 10% Mt6A (3 mg/g)
XCL-602 with 20% Mt6A (3 mg/g)
XCL-602 with 10% Af7A, 10% Mt6A (3 mg/g)
XCL-602 with 10% Af7A, 20% Mt6A (3 mg/g)
XCL-602 with 20% Af7A, 10% Mt6A (3 mg/g)
XCL-602 with 20% Af7A, 20% Mt6A (3 mg/g)
XCL-602 with 30% Af7A, 10% Mt6A (3 mg/g)
XCL-602 with 30% Af7A, 20% Mt6A (3 mg/g)
XCL-602 with 40% Af7A, 10% Mt6A (3 mg/g)
XCL-602 with 40% Af7A, 20% Mt6A (3 mg/g)

Fig. 24

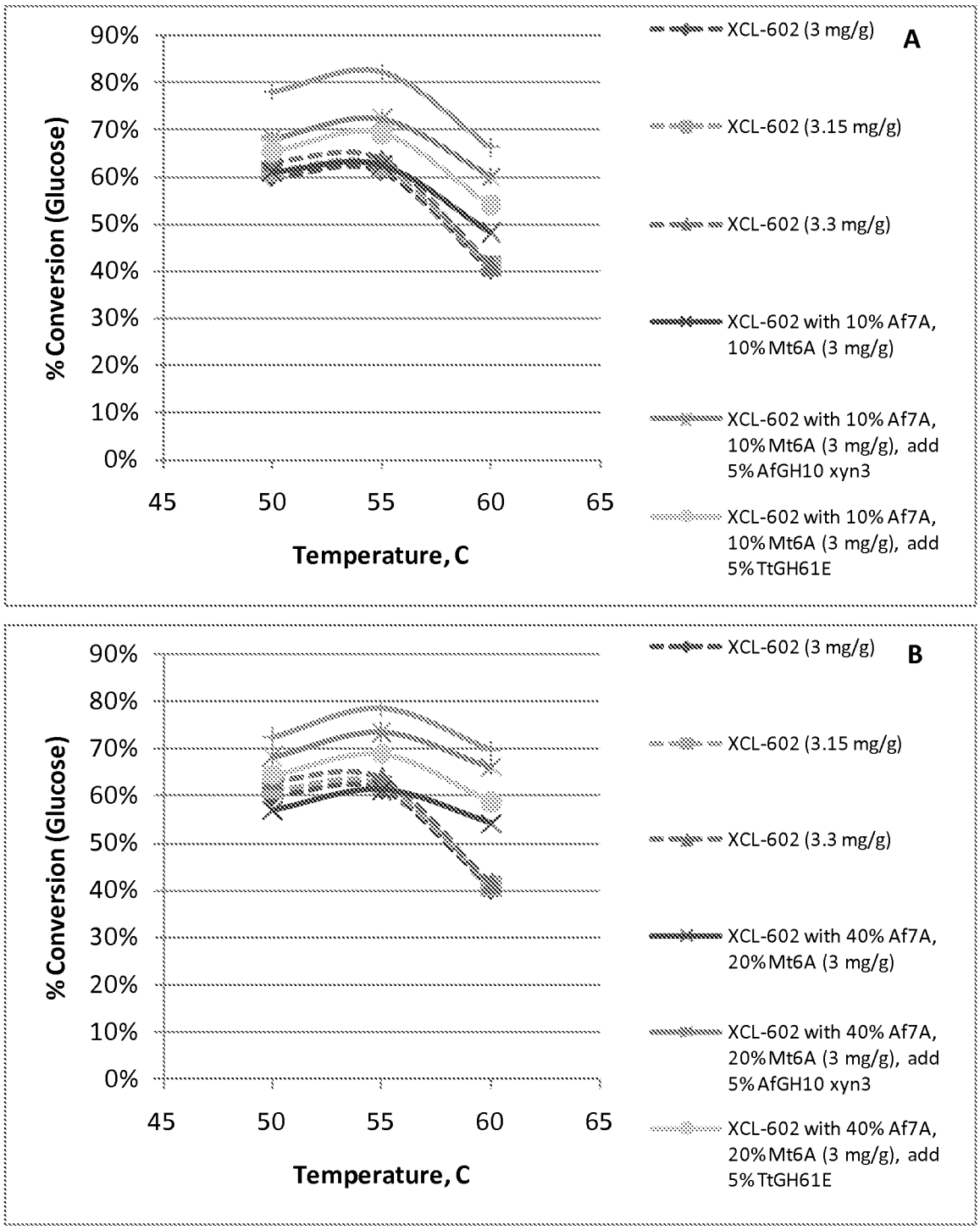

A
% Conversion (Glucose)
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
45
50
55
60
65
Temperature, C
XCL-602 (3 mg/g)
XCL-602 (3.15 mg/g)
XCL-602 (3.3 mg/g)
XCL-602 with 10% Af7A, 10% Mt6A (3 mg/g)
XCL-602 with 10% Af7A, 10% Mt6A (3 mg/g), add 5% AfGH10 xyn3
XCL-602 with 10% Af7A, 10% Mt6A (3 mg/g), add 5% TtGH61E
B
% Conversion (Glucose)
Temperature, C
XCL-602 (3 mg/g)
XCL-602 (3.15 mg/g)
XCL-602 (3.3 mg/g)
XCL-602 with 40% Af7A, 20% Mt6A (3 mg/g)
XCL-602 with 40% Af7A, 20% Mt6A (3 mg/g), add 5% AfGH10 xyn3
XCL-602 with 40% Af7A, 20% Mt6A (3 mg/g), add 5% TtGH61E

Fig. 25

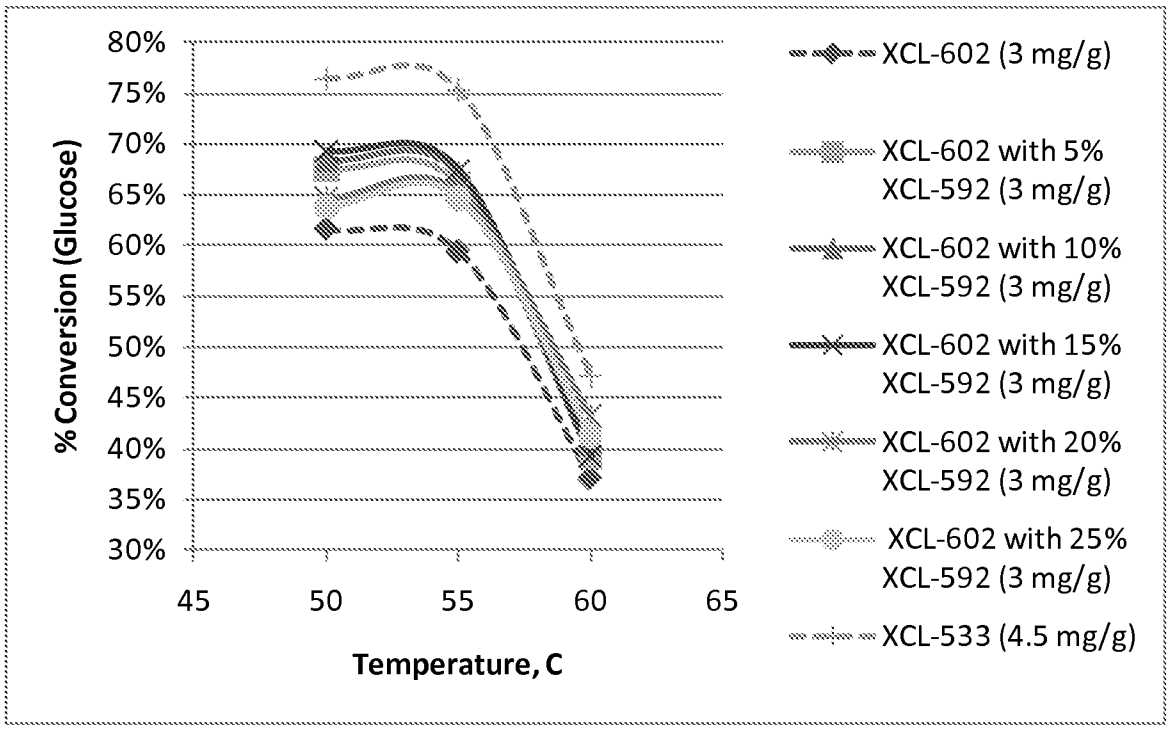
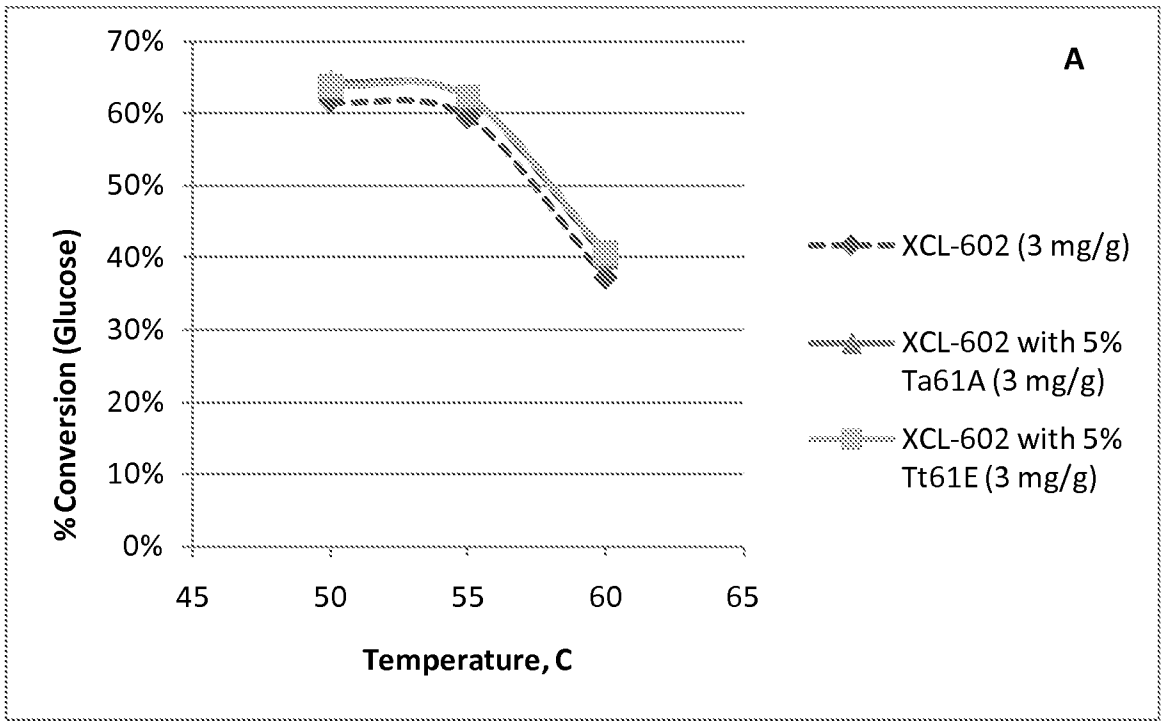

80%
75%
70%
65%
60%
55%
50%
45%
40%
35%
30%
% Conversion (Glucose)
45
50
55
60
65
Temperature, C
XCL-602 (3 mg/g)
XCL-602 with 5% XCL-592 (3 mg/g)
XCL-602 with 10% XCL-592 (3 mg/g)
XCL-602 with 15% XCL-592 (3 mg/g)
XCL-602 with 20% XCL-592 (3 mg/g)
XCL-602 with 25% XCL-592 (3 mg/g)
XCL-533 (4.5 mg/g)

Fig. 26


A
70%
60%
50%
40%
30%
20%
10%
0%
% Conversion (Glucose)
45
50
55
60
65
Temperature, C
XCL-602 (3 mg/g)
XCL-602 with 5% Ta61A (3 mg/g)
XCL-602 with 5% Tt61E (3 mg/g)

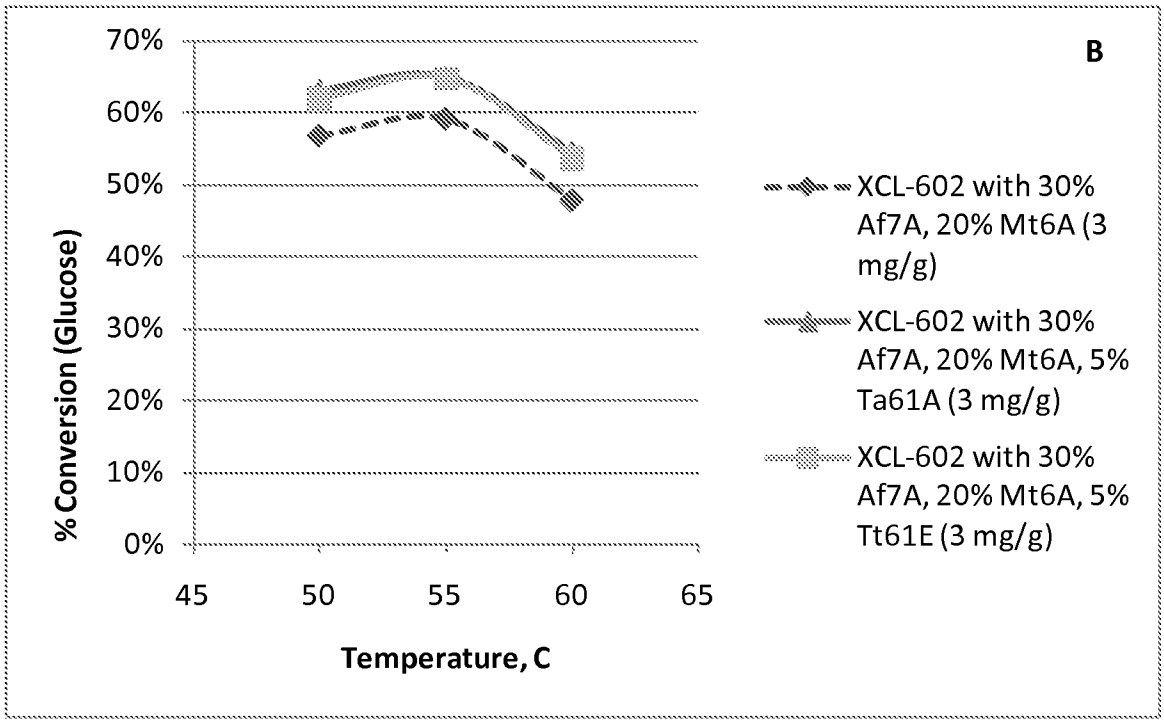

B
70%
60%
50%
40%
30%
20%
10%
0%
% Conversion (Glucose)
45
50
55
60
65
Temperature, C
XCL-602 with 30% Af7A, 20% Mt6A (3 mg/g)
XCL-602 with 30% Af7A, 20% Mt6A, 5% Ta61A (3 mg/g)
XCL-602 with 30% Af7A, 20% Mt6A, 5% Tt61E (3 mg/g)

Fig. 27

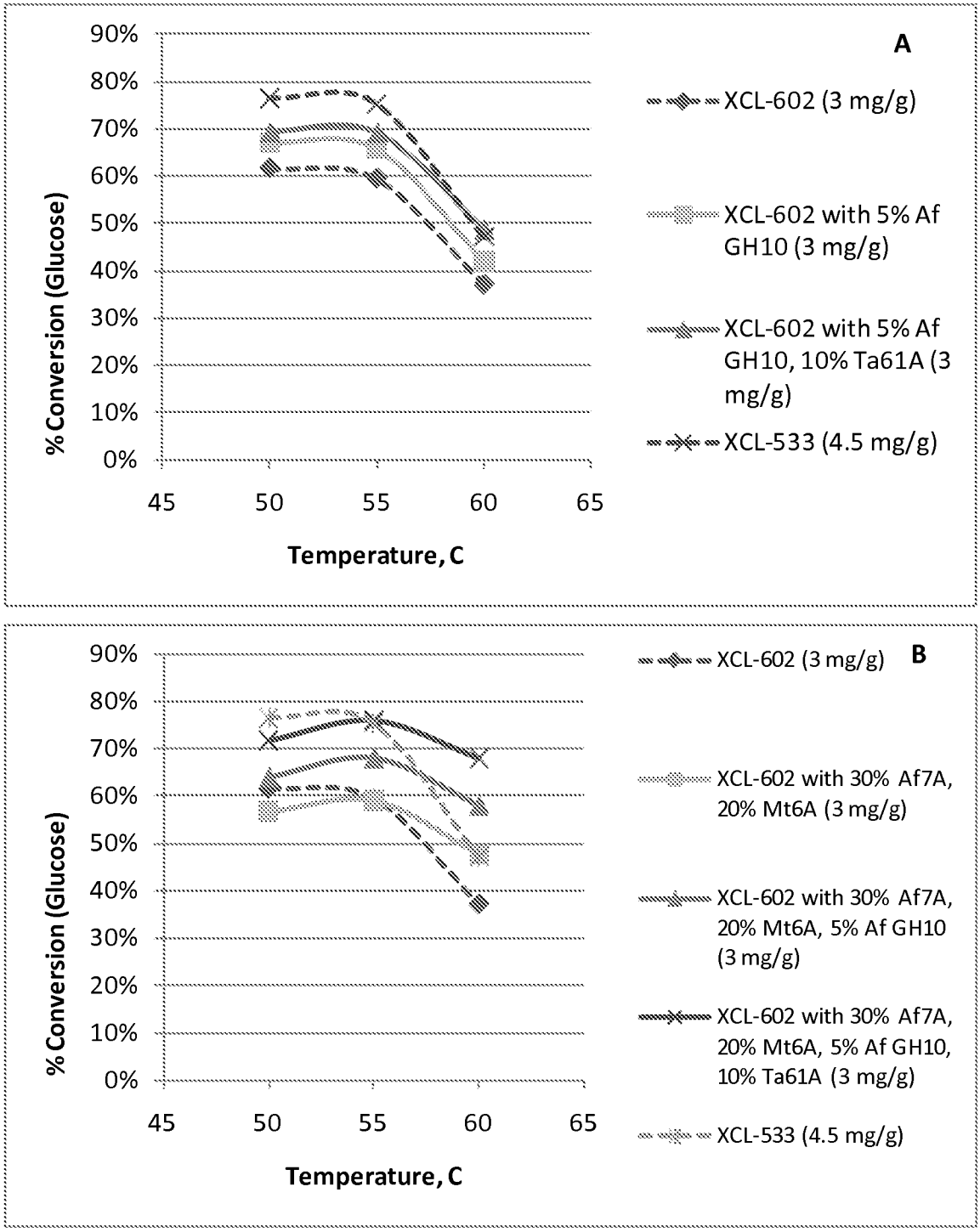

A
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% Conversion (Glucose)
45
50
55
60
65
Temperature, C
XCL-602 (3 mg/g)
XCL-602 with 5% Af GH10 (3 mg/g)
XCL-602 with 5% Af GH10, 10% Ta61A (3 mg/g)
XCL-533 (4.5 mg/g)
B
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% Conversion (Glucose)
45
50
55
60
65
Temperature, C
XCL-602 (3 mg/g)
XCL-602 with 30% Af7A, 20% Mt6A (3 mg/g)
XCL-602 with 30% Af7A, 20% Mt6A, 5% Af GH10 (3 mg/g)
XCL-602 with 30% Af7A, 20% Mt6A, 5% Af GH10, 10% Ta61A (3 mg/g)
XCL-533 (4.5 mg/g)

Fig. 28

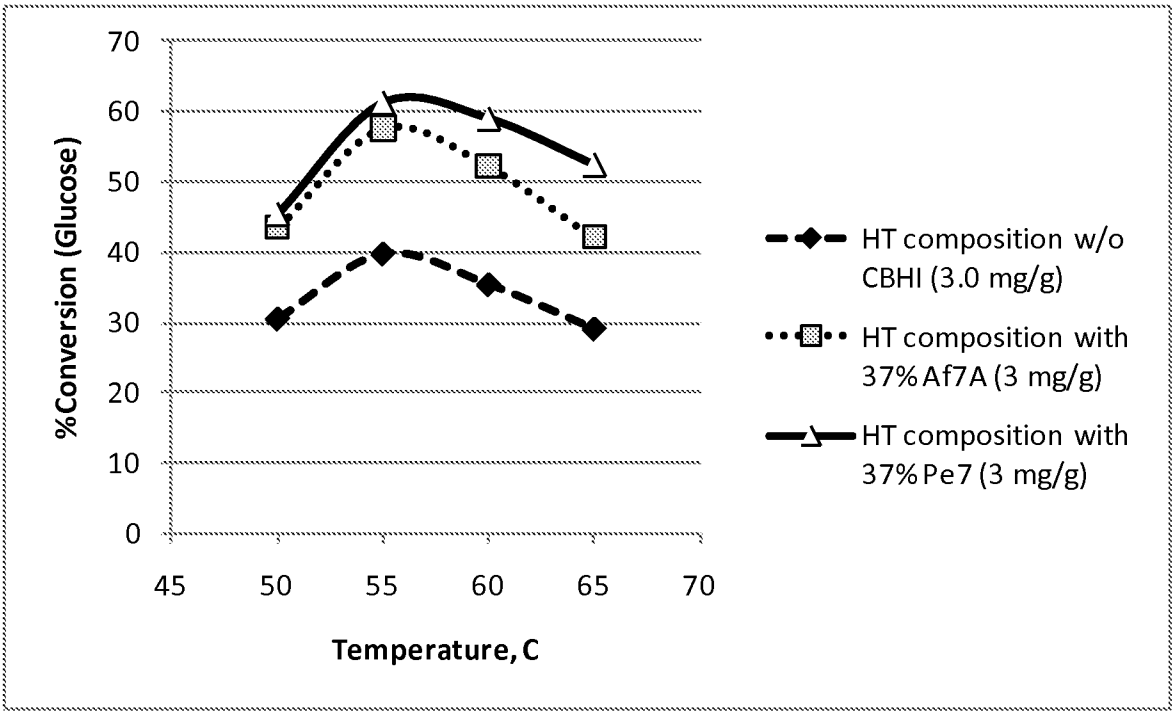

70
60
50
40
30
20
10
0
%Conversion (Glucose)
45
50
55
60
65
70
Temperature, C
HT composition w/o CBHI (3.0 mg/g)
HT composition with 37% Af7A (3 mg/g)
HT composition with 37% Pe7 (3 mg/g)

Fig. 29

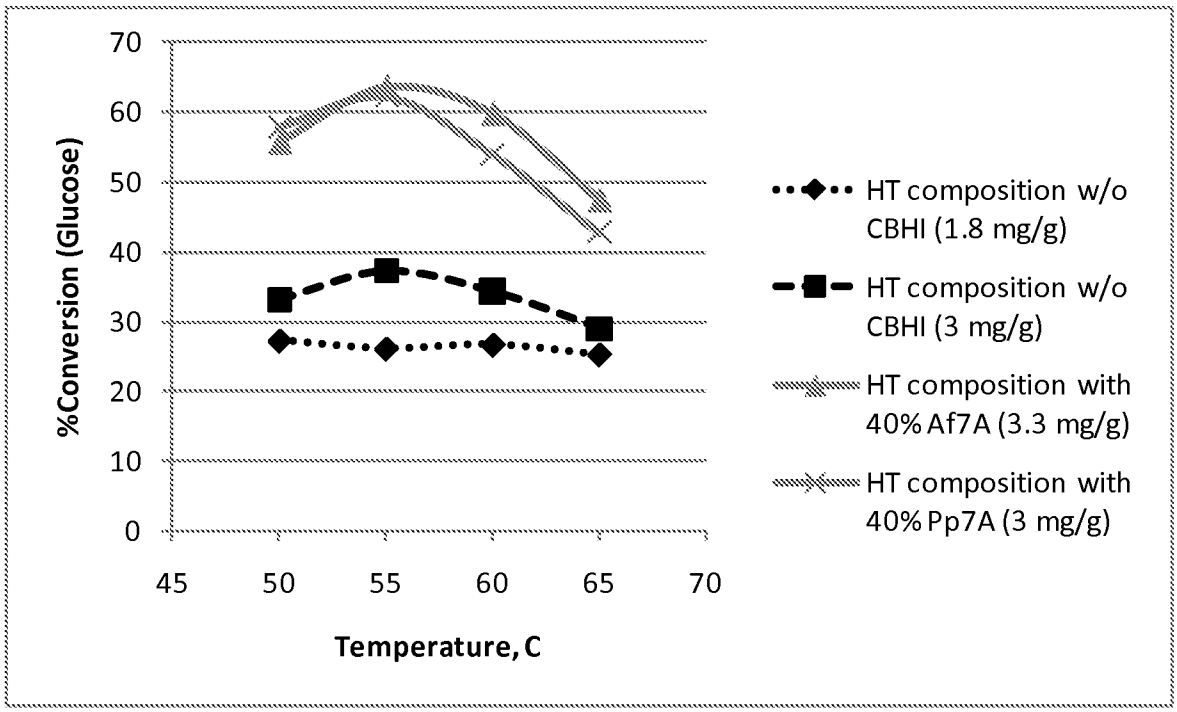

70
60
50
40
30
20
10
0
%Conversion (Glucose)
45
50
55
60
65
70
Temperature, C
HT composition w/o CBHI (1.8 mg/g)
HT composition w/o CBHI (3 mg/g)
HT composition with 40% Af7A (3.3 mg/g)
HT composition with 40% Pp7A (3 mg/g)

Fig. 30

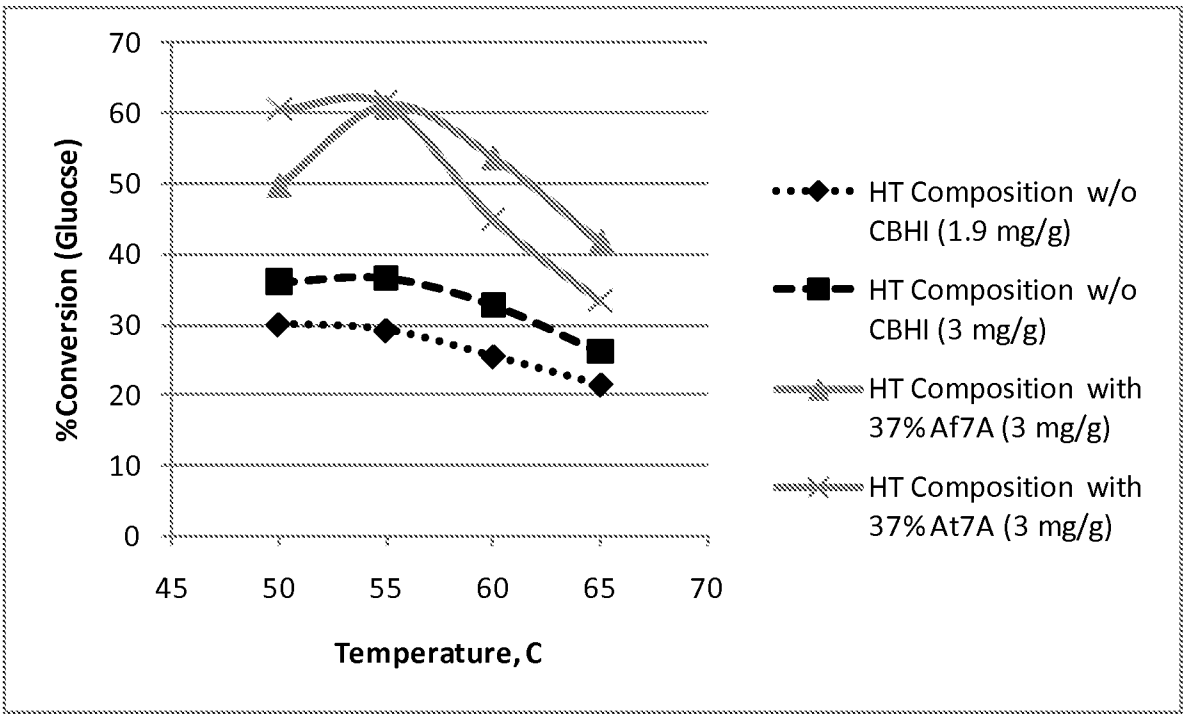

70
60
50
40
30
20
10
0
%Conversion (Gluocse)
45
50
55
60
65
70
Temperature, C
HT Composition w/o CBHI (1.9 mg/g)
HT Composition w/o CBHI (3 mg/g)
HT Composition with 37% Af7A (3 mg/g)
HT Composition with 37% At7A (3 mg/g)

Fig. 31

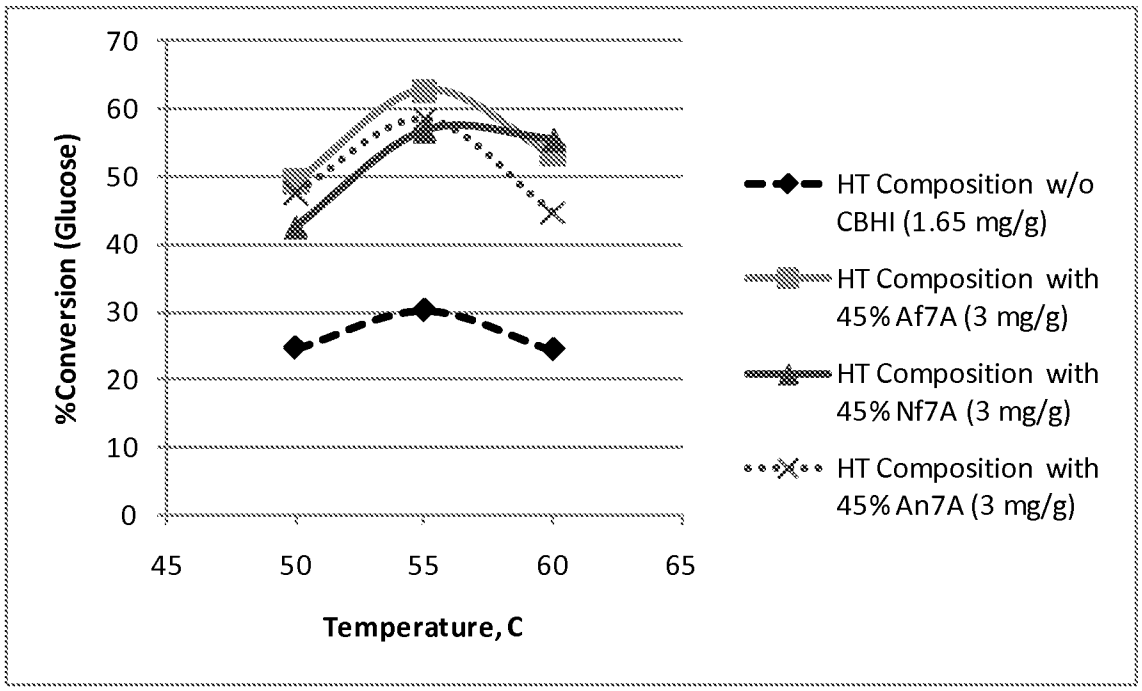

70
60
50
40
30
20
10
0
%Conversion (Glucose)
45
50
55
60
65
Temperature, C
HT Composition w/o CBHI (1.65 mg/g)
HT Composition with 45% Af7A (3 mg/g)
HT Composition with 45% Nf7A (3 mg/g)
HT Composition with 45% An7A (3 mg/g)

Fig. 32

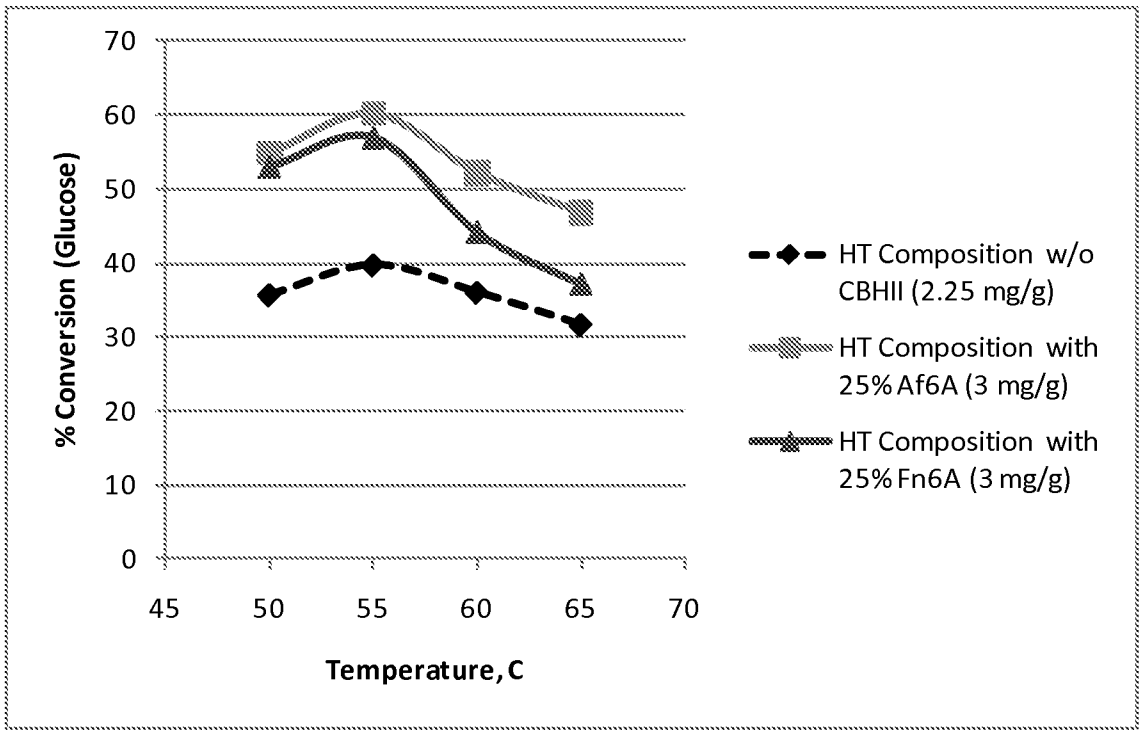

70
60
50
40
30
20
10
0
% Conversion (Glucose)
45
50
55
60
65
70
Temperature, C
HT Composition w/o CBHII (2.25 mg/g)
HT Composition with 25% Af6A (3 mg/g)
HT Composition with 25% Fn6A (3 mg/g)

Fig. 33

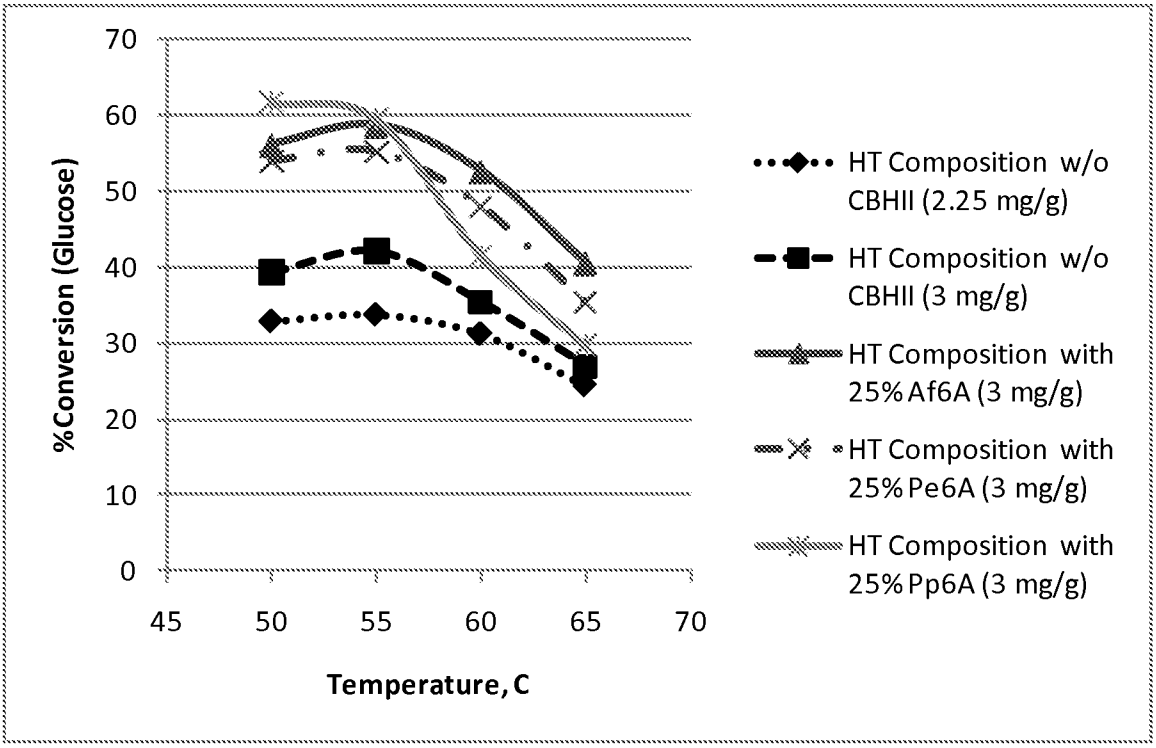

70
60
50
40
30
20
10
0
%Conversion (Glucose)
45
50
55
60
65
70
Temperature, C
HT Composition w/o CBHII (2.25 mg/g)
HT Composition w/o CBHII (3 mg/g)
HT Composition with 25% Af6A (3 mg/g)
HT Composition with 25% Pe6A (3 mg/g)
HT Composition with 25% Pp6A (3 mg/g)

Fig. 34

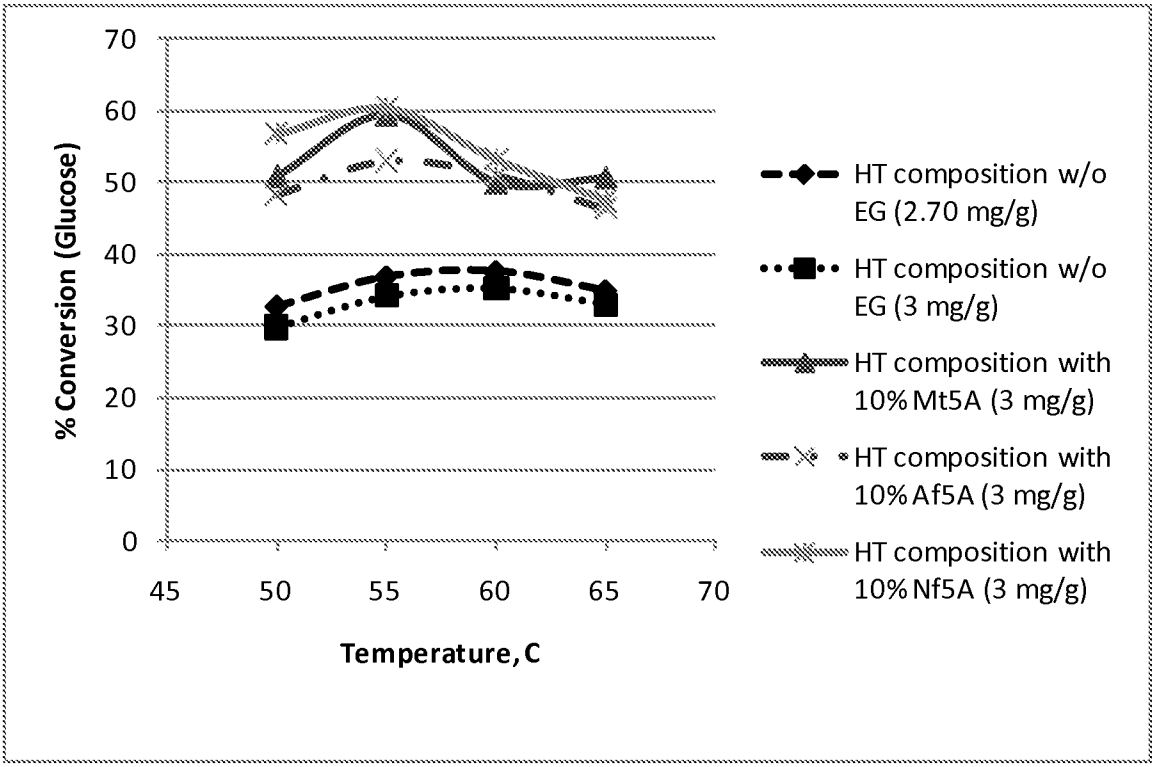

70
60
50
40
30
20
10
0
% Conversion (Glucose)
45
50
55
60
65
70
Temperature, C
HT composition w/o EG (2.70 mg/g)
HT composition w/o EG (3 mg/g)
HT composition with 10% Mt5A (3 mg/g)
HT composition with 10% Af5A (3 mg/g)
HT composition with 10% Nf5A (3 mg/g)

Fig. 35

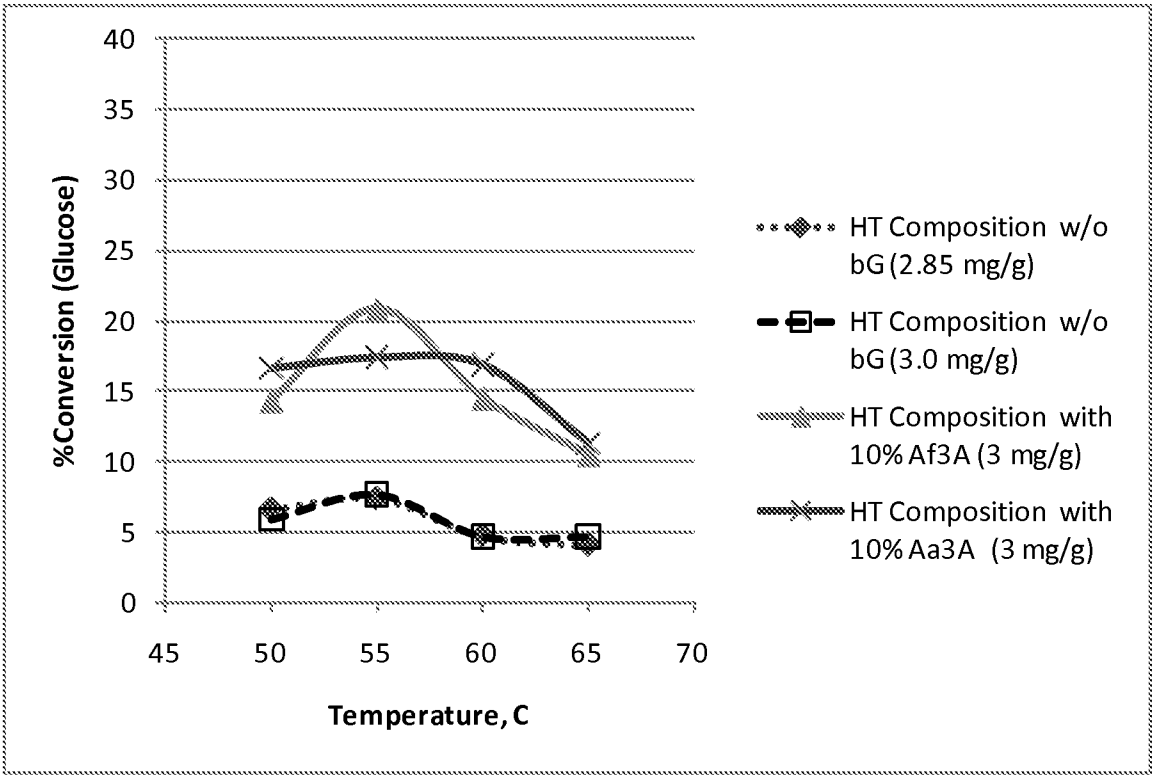

40
35
30
25
20
15
10
5
0
%Conversion (Glucose)
45
50
55
60
65
70
Temperature, C
HT Composition w/o bG (2.85 mg/g)
HT Composition w/o bG (3.0 mg/g)
HT Composition with 10% Af3A (3 mg/g)
HT Composition with 10% Aa3A (3 mg/g)

Fig. 36

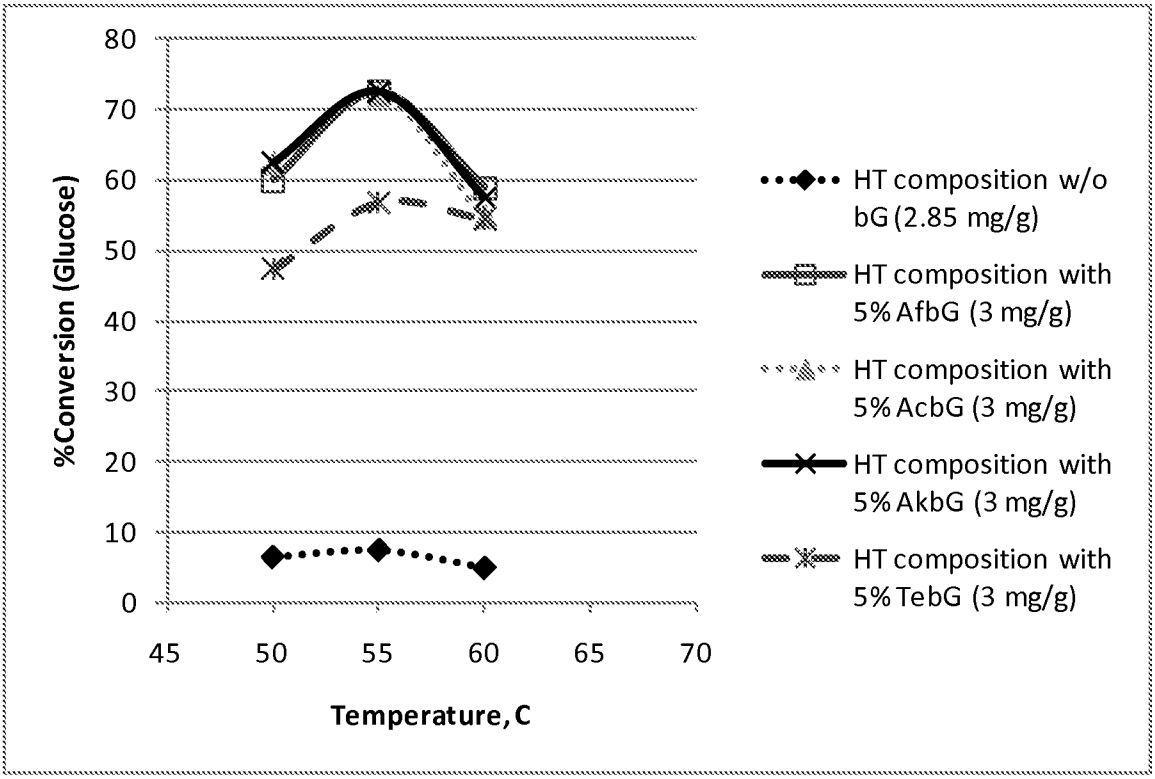

80
70
60
50
40
30
20
10
0
%Conversion (Glucose)
45
50
55
60
65
70
Temperature, C
HT composition w/o bG (2.85 mg/g)
HT composition with 5% AfbG (3 mg/g)
HT composition with 5% AcbG (3 mg/g)
HT composition with 5% AkbG (3 mg/g)
HT composition with 5% TebG (3 mg/g)

Fig. 37

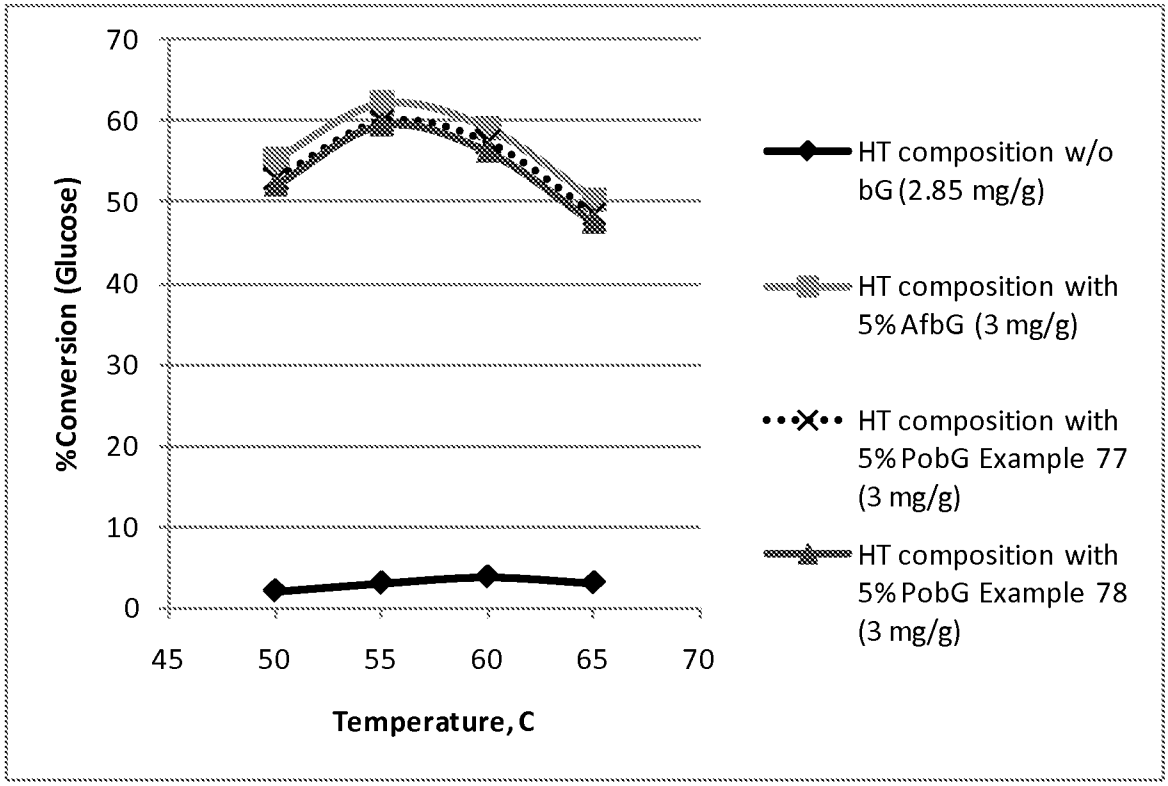

70
60
50
40
30
20
10
0
%Conversion (Glucose)
45
50
55
60
65
70
Temperature, C
HT composition w/o bG (2.85 mg/g)
HT composition with 5% AfbG (3 mg/g)
HT composition with 5% PobG Example 77 (3 mg/g)
HT composition with 5% PobG Example 78 (3 mg/g)

Fig. 38

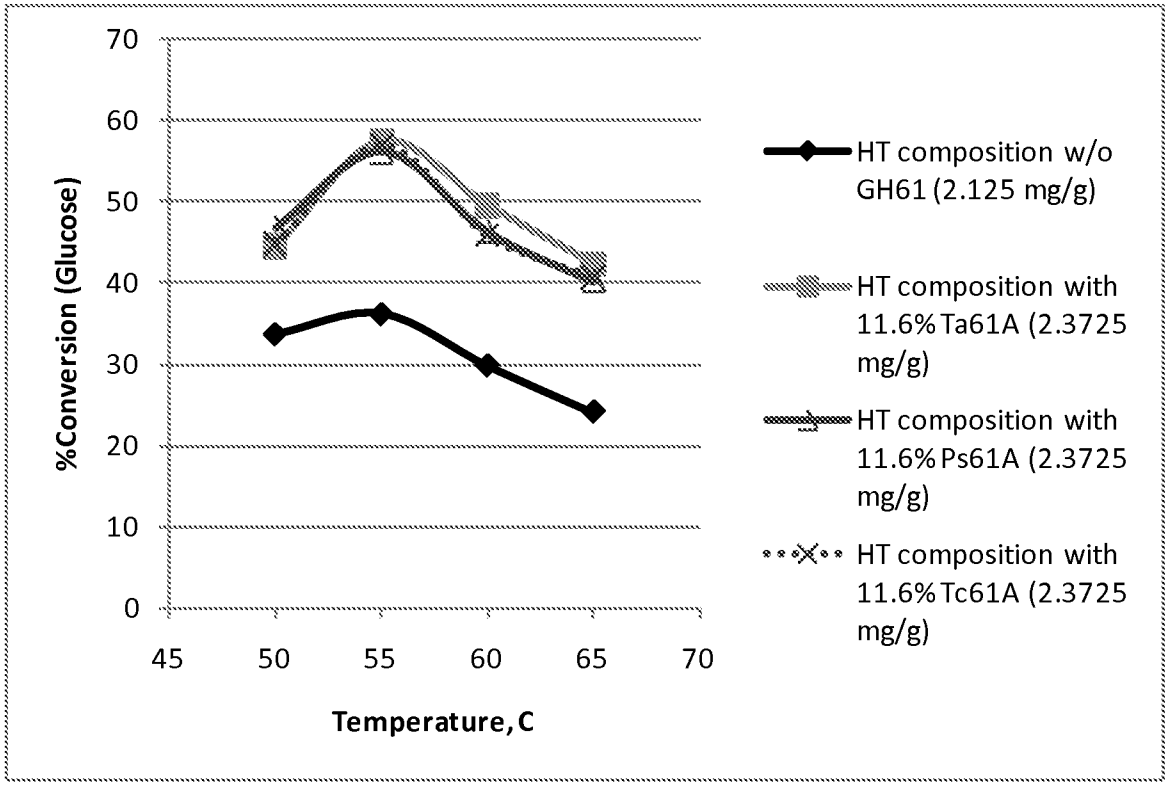

70
60
50
40
30
20
10
0
%Conversion (Glucose)
45
50
55
60
65
70
Temperature, C
HT composition w/o GH61 (2.125 mg/g)
HT composition with 11.6% Ta61A (2.3725 mg/g)
HT composition with 11.6% Ps61A (2.3725 mg/g)
HT composition with 11.6% Tc61A (2.3725 mg/g)

Fig. 39

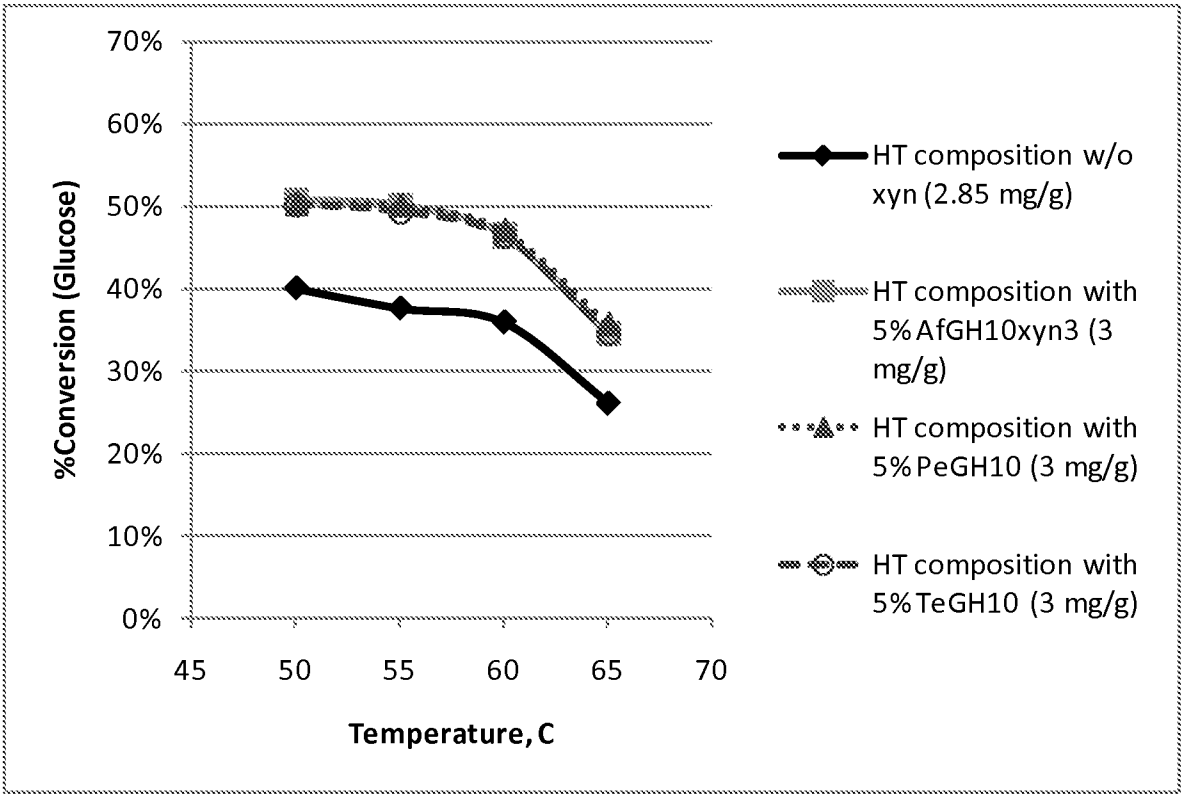

70%
60%
50%
40%
30%
20%
10%
0%
%Conversion (Glucose)
45
50
55
60
65
70
Temperature, C
HT composition w/o xyn (2.85 mg/g)
HT composition with 5% AfGH10xyn3 (3 mg/g)
HT composition with 5% PeGH10 (3 mg/g)
HT composition with 5% TeGH10 (3 mg/g)

Fig. 40

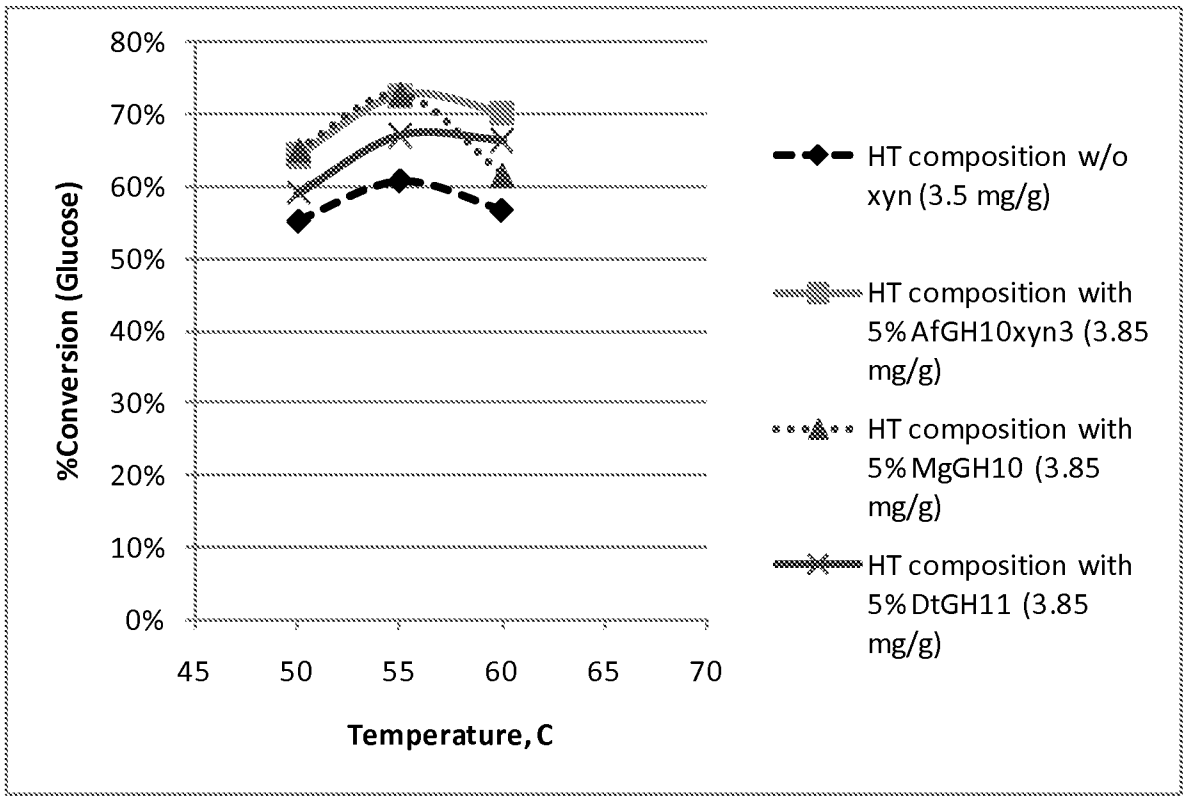

80%
70%
60%
50%
40%
30%
20%
10%
0%
%Conversion (Glucose)
45
50
55
60
65
70
Temperature, C
HT composition w/o xyn (3.5 mg/g)
HT composition with 5% AfGH10xyn3 (3.85 mg/g)
HT composition with 5% MgGH10 (3.85 mg/g)
HT composition with 5% DtGH11 (3.85 mg/g)

Fig. 41

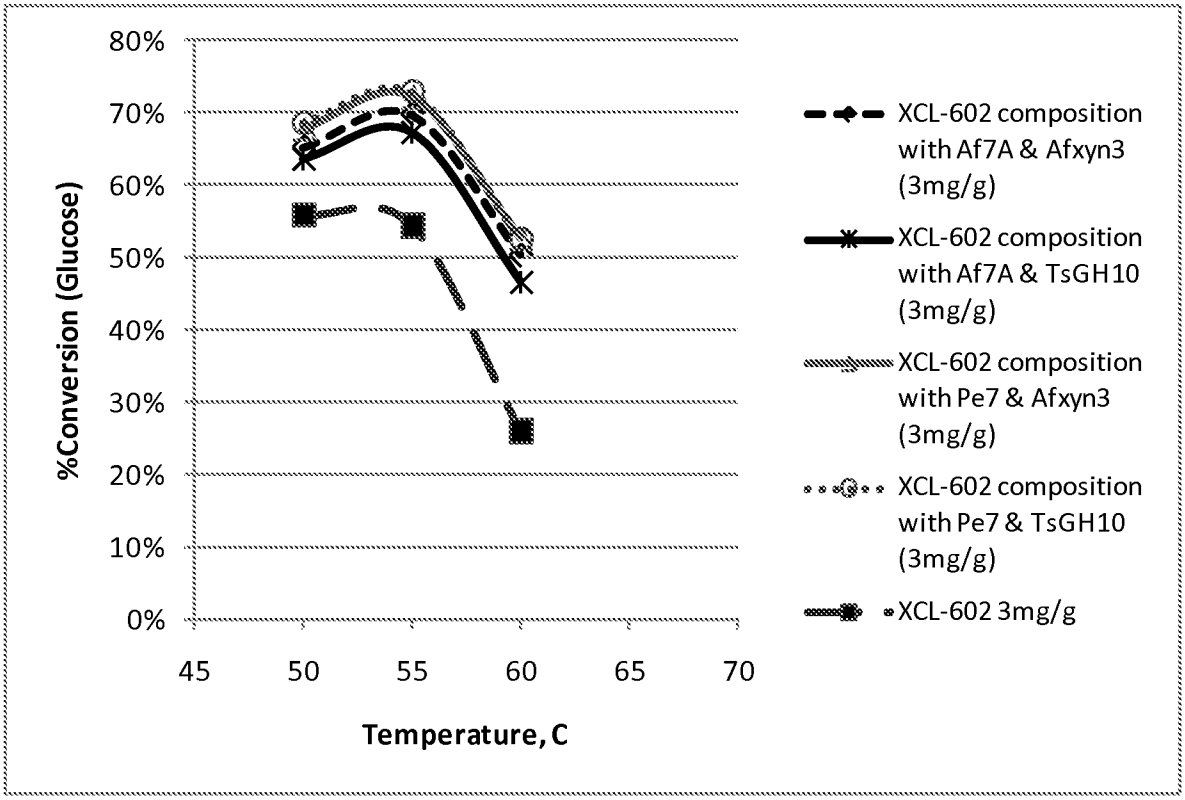

80%
70%
60%
50%
40%
30%
20%
10%
0%
%Conversion (Glucose)
45
50
55
60
65
70
Temperature, C
XCL-602 composition with Af7A & Afxyn3 (3mg/g)
XCL-602 composition with Af7A & TsGH10 (3mg/g)
XCL-602 composition with Pe7 & Afxyn3 (3mg/g)
XCL-602 composition with Pe7 & TsGH10 (3mg/g)
XCL-602 3mg/g

Fig. 42

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/055723

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N9/42 C12P19/02 D06M16/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N C12P D06M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, Sequence Search, EMBASE, FSTA, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/071818 A1 (ROAL OY [FI]; VEHMAANPERAE JARI [FI]; ALAPURANEN MARIKA [FI]; PURANEN) 28 June 2007 (2007-06-28) page 5, lines 24-32 page 18 - page 20 examples 1-32 sequence 8 table 30 -----	1,3,5-24
X	WO 2009/059175 A2 (NOVOZYMES INC [US]; XU FENG [US]) 7 May 2009 (2009-05-07) pages 35-38 examples 26, 29-30 claims 27-29 sequence 44 ----- -/--	1-4,6, 10-24

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 January 2011	13/04/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Behrens, Joyce

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/055723

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/085935 A2 (NOVOZYMES AS [DK]; HARRIS PAUL [US]; MAIYURAN SUCHINDRA [US]; BROWN KI) 9 July 2009 (2009-07-09) pages 62-65 pages 86-87 sequence 59	1,11-24
X	WO 03/000941 A2 (NOVOZYMES AS [DK]; LANGE LENE [DK]; WU WENPING [CN]; AUBERT DOMINIQUE) 3 January 2003 (2003-01-03) page 1 pages 53-55 claims 1, 26, 29 sequence 4	1,3, 10-24
X	VIIKARI L ET AL: "Thermostable enzymes in lignocellulose hydrolysis", ADVANCES IN BIOCHEMICAL ENGINEERING, BIOTECHNOLOGY, SPRINGER, BERLIN, DE, vol. 108, 23 June 2007 (2007-06-23), pages 121-145, XP002510178, ISSN: 0724-6145, DOI: DOI:10.1007/10_2007_065 [retrieved on 2007-06-23] pages 123-139	5,7-9
A	WO 2008/025165 A1 (IOGEN ENERGY CORP [CA]; HILL CHRISTOPHER [CA]; SCOTT BRIAN R [CA]; TOM) 6 March 2008 (2008-03-06) pages 4-7 example 6	1-24
A	WOOD T M ET AL: "THE MECHANISM OF FUNGAL CELLULASE ACTION SYNERGISM BETWEEN ENZYME COMPONENTS OF PENICILLIUM PINOPHILUM CELLULASE IN SOLUBILIZING HYDROGEN BOND-ORDERED CELLULOSE", BIOCHEMICAL JOURNAL, THE BIOCHEMICAL SOCIETY, LONDON, GB, vol. 260, no. 1, 1 January 1989 (1989-01-01), pages 37-43, XP008028526, ISSN: 0264-6021 abstract tables 2,3	1-24

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ZHANG YI-HENG PERCIVAL ET AL: "Toward an aggregated understanding of enzymatic hydrolysis of cellulose: Noncomplexed cellulase systems", BIOTECHNOLOGY AND BIOENGINEERING, WILEY & SONS, HOBOKEN, NJ, US, vol. 88, no. 7, 30 December 2004 (2004-12-30), pages 797-824, XP002572377, ISSN: 0006-3592, DOI: DOI:10.1002/BIT.20282 [retrieved on 2004-11-10] page 808, column 2 - page 811, column 1 table 5 -----	1-24
A	ZHOU JIN ET AL: "Optimization of cellulase mixture for efficient hydrolysis of steam-exploded corn stover by statistically designed experiments", BIORESOURCE TECHNOLOGY, ELSEVIER BV, GB, vol. 100, no. 2, 1 January 2009 (2009-01-01), pages 819-825, XP002596453, ISSN: 0960-8524, DOI: DOI:10.1016/J.BIORTECH.2008.06.068 [retrieved on 2008-09-03] page 822, column 2 - page 825, column 1 -----	1-24
A	MERINO SANDRA T ET AL: "Progress and challenges in enzyme development for biomass utilization", ADVANCES IN BIOCHEMICAL ENGINEERING, BIOTECHNOLOGY, SPRINGER, BERLIN, DE, vol. 108, 1 January 2007 (2007-01-01), pages 95-120, XP009132055, ISSN: 0724-6145 [retrieved on 2007-06-27] figure 103 pages 108-111 -----	1-24
A	DASHTBAN MEHDI ET AL: "Fungal Bioconversion of Lignocellulosic Residues; Opportunities & Perspectives", INTERNATIONAL JOURNAL OF BIOLOGICAL SCIENCES, IVYSPRING INTERNATIONAL PUBLISHER, AU, vol. 5, no. 6, 4 September 2009 (2009-09-04), pages 578-595, XP002570268, ISSN: 1449-2288 the whole document ----- -/--	1-24

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/055723

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ROSGAARD LISA ET AL: "Efficiency of new fungal cellulase systems in boosting enzymatic degradation of barley straw lignocellulose", BIOTECHNOLOGY PROGRESS, AMERICAN INSTITUTE OF CHEMICAL ENGINEERS, US, vol. 22, no. 2, 1 March 2006 (2006-03-01), pages 493-498, XP002565690, ISSN: 8756-7938, DOI: DOI:10.1021/BP0503610 [retrieved on 2006-03-24] abstract page 496 -----	1-24

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2010/055723

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-24(partially)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

see additional sheet(s)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 2

---

2. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 4

---

3. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 6

---

4. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 8

---

5. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 158

---

6. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 160

---

7. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 162

---

8. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 164

---

9. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 166

---

10. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 10

---

11. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 12
---

12. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 14
---

13. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 16
---

14. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 18
---

15. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 168
---

16. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 170
---

17. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 172
---

18. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 20
---

19. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 22
---

20. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 24
---

21. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 26
---

22. claims: 1-24(partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Enzyme composition comprising SEQ ID NO: 174
---

23. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 176
---

24. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 28
---

25. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 30
---

26. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 32
---

27. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 178
---

28. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 180
---

29. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 182
---

30. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 184
---

31. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 186
---

32. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 188
---

33. claims: 1-24(partially)

Enzyme composition comprising SEQ ID NO: 190

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 1-24(partially)

Present claim 1 relates to an extremely large number of possible compositions. Support and disclosure in the sense of Article 6 and 5 PCT is to be found however for only a very small proportion of the compositions claimed, see examples 35-62, 88-101. The non-compliance with the substantive provisions is to such an extent, that the search was performed taking into consideration the non-compliance in determining the extent of the search of claim 1 (PCT Guidelines 9.19 and 9.23). The search of claim 1 was restricted to those claimed compositions which appear to be supported and a generalisation of the individual components (i.e. genetic variants).

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.2), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2010/055723

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007071818 A1	28-06-2007	AR 058721 A1	20-02-2008
		AU 2006326963 A1	28-06-2007
		CA 2632502 A1	28-06-2007
		CN 101384713 A	11-03-2009
		EP 1963498 A1	03-09-2008
		FI 120045 B1	15-06-2009
		US 2009042266 A1	12-02-2009
		US 2011045544 A1	24-02-2011
WO 2009059175 A2	07-05-2009	CN 101910405 A	08-12-2010
		EP 2215242 A2	11-08-2010
		US 2009123979 A1	14-05-2009
WO 2009085935 A2	09-07-2009	AU 2008343105 A1	09-07-2009
		CA 2709490 A1	09-07-2009
		CN 101952420 A	19-01-2011
		EA 201070764 A1	30-12-2010
		EP 2235173 A2	06-10-2010
		KR 20100105849 A	30-09-2010
		US 2010304434 A1	02-12-2010
WO 03000941 A2	03-01-2003	AU 2002316809 A1	08-01-2003
		CN 1620501 A	25-05-2005
		EP 1421224 A2	26-05-2004
		EP 2298868 A2	23-03-2011
		EP 2277997 A2	26-01-2011
		EP 2295544 A2	16-03-2011
		US 2004197890 A1	07-10-2004
		US 2010256037 A1	07-10-2010
WO 2008025165 A1	06-03-2008	AU 2007291865 A1	06-03-2008
		CA 2661568 A1	06-03-2008
		CN 101517073 A	26-08-2009
		EP 2076594 A1	08-07-2009
		JP 2010501190 T	21-01-2010
		US 2008057541 A1	06-03-2008