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CN 101492686 A,2009.07.29,
CN 104844703 A,2015.08.19,
CN 1480533 A,2004.03.10,
NCBI.Bacillus thuringiensis strain MEB4 insecticidal crystal protein Cry2Aa (cry2Aa) gene,complete cds.《GenBank database》.2016,
NCBI.Bacillus thuringiensis strain MEB4 insecticidal crystal protein Cry2Aa (cry2Aa) gene,complete cds.《GenBank database》.2016,
NCBI.Bacillus thuringiensis strain HST-S-38 insecticidal crystal protein B2 (cry2Ab) gene,complete cds.《GenBank database》.2014,
NCBI.Bacillus thuringiensis strain HST-S-38 insecticidal crystal protein B2 (cry2Ab) gene,complete cds.《GenBank database》.2014,
NCBI.Bacillus thuringiensis strain lyL cry2Ab-type insecticidal crystal protein gene,complete cds.《GenBank database》.2008,
NCBI.Bacillus thuringiensis strain lyL cry2Ab-type insecticidal crystal protein gene,complete cds.《GenBank database》.2008,
NCBI.Bacillus thuringiensis strain BTSC6H8 cry2-like protein gene, complete cds.《GenBank database》.2011,
NCBI.Bacillus thuringiensis strain BTSC6H8 cry2-like protein gene, complete cds.《GenBank database》.2011,
NCBI.Bacillus thuringiensis strain HD395 Cry2Ba2(cryaba2)gene,complete cds.《GenBank database》.2014,
NCBI.Bacillus thuringiensis strain HD395 Cry2Ba2(cryaba2)gene,complete cds.《GenBank database》.2014, (续)

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(54)发明名称

杀虫晶体蛋白、核酸、杀虫晶体蛋白的制备方法及其应用

(57)摘要

本发明涉及杀虫晶体蛋白领域,特别涉及对甜菜夜蛾、水稻二化螟、亚洲玉米螟和小菜蛾有杀虫活性的Cry2类杀虫晶体蛋白。

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(56)对比文件

Masataka Ohsawa等.A 50-Kilodalton Cry2A Peptide Is Lethal to Bombyx mori and Lymantria dispar.《Applied and Environment Microbiology》.2012,第78卷(第13期),

余宗兰等.苏云金芽胞杆菌晶体蛋白2Ab32(Cry2Ab32)基因的克隆、表达与杀虫活性分析.《农业生物技术学报》.2015,第23卷(第6期),

NCBI.Bacillus thuringiensis strain Rpp39 insecticidal crystal protein Cry2Aa gene,complete cds.《GenBank database》.2006,

NCBI.Bacillus thuringiensis strain Rpp39 insecticidal crystal protein Cry2Aa gene,complete cds.《GenBank database》.2006,

1.一种杀虫晶体蛋白，其为Cry2类蛋白，所述Cry2类蛋白包括Cry2Aa17、Cry2Ab29、mCry2Aa17、Cry2Ab4和Cry2Ah1中的至少一种；

其中，所述Cry2Aa17的氨基酸序列如SEQ ID No.1所示，所述Cry2Ab29的氨基酸序列如SEQ ID No.2所示，所述Cry2Ab4的氨基酸序列如SEQ ID No.5所示，所述Cry2Ah1的氨基酸序列如SEQ ID No.6所示，所述mCry2Aa17的氨基酸序列为 经如SEQ ID No.14所示核酸序列翻译后得到的氨基酸序列。

2.一种能够翻译为如权利要求1所述的杀虫晶体蛋白的核酸。

3.根据权利要求2所述的核酸，其特征在于，能够翻译为所述Cry2Aa17的氨基酸序列的核酸的序列如SEQ ID No.8所示，能够翻译为所述Cry2Ab29的氨基酸序列的核酸的序列如SEQ ID No.9所示，能够翻译为所述Cry2Ab4的氨基酸序列的核酸序列如SEQ ID No.12所示，和能够翻译为所述Cry2Ah1的氨基酸序列的核酸序列如SEQ ID No.13所示，能够翻译为所述mCry2Aa17的氨基酸序列的核酸的序列如SEQ ID No.14所示。

4.根据权利要求1所述的杀虫晶体蛋白在防治有害生物中的应用，其中，

Cry2Aa17对甜菜夜蛾、亚洲玉米螟和小菜蛾中的一种有活性；

mCry2Aa17对甜菜夜蛾、亚洲玉米螟和小菜蛾中的一种有活性；

Cry2Ab29对甜菜夜蛾、亚洲玉米螟和小菜蛾中的一种有活性；

Cry2Ah1对甜菜夜蛾、亚洲玉米螟和水稻二化螟中的一种有活性；

Cry2Ab4对甜菜夜蛾、亚洲玉米螟、小菜蛾和水稻二化螟中的一种有活性。

5.根据权利要求4所述的应用，其特征在于，所述杀虫晶体蛋白为在16-20℃下在活体生物中表达的杀虫晶体蛋白。

6.根据权利要求5所述的应用，其特征在于，所述杀虫晶体蛋白为在含有如权利要求2或3所述的核酸序列的活体生物中表达所述杀虫晶体蛋白。

7.根据权利要求5所述的应用，其特征在于，所述活体生物为微生物。

8.根据权利要求7所述的应用，其特征在于，所述活体生物为芽孢杆菌(Bacillus)、假单胞菌(Pseudomonas)、肠杆菌(Escherichia)和酵母(Saccharomyces)中的至少一种。

9.根据权利要求8所述的应用，其特征在于，所述活体生物为苏云金芽孢杆菌(Bacillus thuringiensis)、枯草芽孢杆菌(Bacilllus subtilis)、萎缩芽孢杆菌(Bacillus atrophaeus)和蜡样芽孢杆菌(Bacillus cereus)中的至少一种；所述假单胞菌包括荧光假单胞杆菌(Pseudomonas fluorescens)；所述肠杆菌包括大肠杆菌(Escherichia coli)。

10.根据权利要求7所述的应用，其特征在于，所述微生物为野生微生物和/或遗传工程微生物。

杀虫晶体蛋白、核酸、杀虫晶体蛋白的制备方法及其应用

技术领域

[0001] 本发明涉及杀虫晶体蛋白领域，特别涉及对鳞翅目有杀虫活性的Cry2类杀虫晶体蛋白。

背景技术

[0002] 到目前为止，已经发现了约600种的杀虫晶体蛋白，这些杀虫晶体蛋白主要来源于苏云金芽孢杆(Bacillus thuringiensis)。其对9个目500多种昆虫以及线虫、螨类和多种原生动物具有杀虫活性。由于Bt杀虫活性广泛并且与化学农药相比专一性高、安全性好、防治效果好、不污染环境等优点，已经成为目前世界上研究最多，应用最广、最成功的昆虫病原微生物，Bt杀虫剂占生物农药的70％以上，并已经应用于转基因植物，为人类带来很好的经济和生态效益，对其研究还在不断扩展和深入。

[0003] 但是杀虫晶体蛋白的专一性强的特点是一把双刃剑。一方面其专一性强，意味着其对除目标害虫以外的生物，特别是人畜安全；但是另一方面也使得其杀虫谱过窄，对于多种害虫的综合防治来说，需要开发出针对不同害虫有效的杀虫晶体蛋白才能达到防治的目的。

[0004] 然而，现有技术中仅发现苏云金芽孢杆菌的营养期杀虫蛋白(Vip蛋白，一种不同于杀虫晶体蛋白的蛋白)对甜菜夜蛾有较好的活性，而在杀虫晶体蛋白中至今还未发现能够对甜菜夜蛾等具有较好活性的蛋白。

[0005] 因此，有必要从已知或新分离的苏云金芽孢杆菌菌株中寻找能够对甜菜夜蛾等有较好的活性杀虫晶体蛋白。

发明内容

[0006] 本发明之一提供了一种杀虫晶体蛋白，其为Cry2类蛋白，所述Cry2类蛋白包括Cry2Aa17、Cry2Ab29、Cry2Ba2、Cry2Aa9、Cry2Ab4和Cry2Ah1中的至少一种；其中，所述Cry2Aa17的氨基酸序列如SEQ ID No.1所示，所述 Cry2Ab29的氨基酸序列如SEQ ID No.2所示，所述Cry2Ba2的氨基酸序列如 SEQ ID No.3所示；所述Cry2Aa9的氨基酸序列如SEQ ID No.4所示，所述 Cry2Ab4的氨基酸序列如SEQ ID No.5所示，所述Cry2Ah1的氨基酸序列如 SEQ ID No.6所示。

[0007] 其中，由于氨基酸序列与杀虫活性之间并不是简单的一一对应关系，有的氨基酸序列高度同源但杀虫活性却有很大的不同。随着新基因的不断发现，一种基因具有两种或多种活性的情况不断被发现，为了避免杀虫谱和氨基酸序列的不一致性，Crickmore等提出了一种根据氨基酸序列同源性进行分类，形成了一个开放式分类系统，共分为四级，杀虫晶体蛋白氨基酸序列的同源性小于 45％为第一等级；在45％-78％之间为第二等级；在78％-95％之间为第三等级；同源性在95％以上为第四等级。其中第一、第四等级用阿拉伯数表示；第二、第三等级分别用大、小写英文字母表示。只要是来自Bt的伴胞晶体蛋白 (又称为杀虫晶体蛋白)，对目标生物具有毒性，或与已知的Cry或Cyt蛋白具有很高同源性，就可以

纳入这一分类系统。按照Crickmore等提出的基因分类系统，Cry蛋白约70类，本发明的Cry2Aa17、Cry2Ab29、Cry2Ba2、 Cry2Aa9、Cry2Ab4和Cry2Ah1之间的同源性在45％-78％之间。

[0008] 现有研究证实，杀虫晶体蛋白原毒素大小约130kDa，但其在130kDa大小的状态下并没有杀虫活性，而是必须经过蛋白酶等作用的切割，切割得到约52- 68kDa的蛋白后才能够发挥其活性。其中，所述的切割可以一般发生在有害生物的肠道之内，但也可以人为地有目的地改造为具有相同功能的52-68kDa的蛋白。

[0009] 因此，在一个具体实施例中，所述杀虫晶体蛋白为利用胰凝乳蛋白酶和/或胰蛋白酶消化所述Cry2Aa17、Cry2Ab29、Cry2Ba2、Cry2Aa9、Cry2Ab4和 Cry2Ah1中的至少一种之后获得的52-68kDa的蛋白；优选所述杀虫晶体蛋白为利用胰凝乳蛋白酶和/或胰蛋白酶消化所述Cry2Aa17、Cry2Ab29、Cry2Ba2、 Cry2Aa9、Cry2Ab4和Cry2Ah1中的至少一种之后获得的55-65kDa的蛋白；特别优选所述52-68kDa的蛋白或所述55-65kDa的蛋白与Cry2类蛋白具有相同的功能。在此所述的Cry2类蛋白包括Cry2Aa17、Cry2Ab29、Cry2Ba2、 Cry2Aa9、Cry2Ab4和Cry2Ah1中的至少一种；其中，所述Cry2Aa17的氨基酸序列如SEQ ID No.1所示，所述Cry2Ab29的氨基酸序列如SEQ ID No.2所示，所述Cry2Ba2的氨基酸序列如SEQ ID No.3所示；所述Cry2Aa9的氨基酸序列如SEQ ID No.4所示，所述Cry2Ab4的氨基酸序列如SEQ ID No.5所示，所述Cry2Ah1的氨基酸序列如SEQ ID No.6所示。其中，例如与杀虫蛋白Cry2Aa9、Cry2Ab4或Cry2Ah1的相同的功能可以为在防治甜菜夜蛾和/或水稻二化螟中的应用；与杀虫蛋白Cry2Aa17、Cry2Ab29、或Cry2Ba2的相同的功能可以为在防治甜菜夜蛾、亚洲玉米螟和小菜蛾中的应用。

[0010] 在一个具体实施例中，所述杀虫晶体蛋白为所述Cry2Aa17、Cry2Ab29、 Cry2Ba2、Cry2Aa9、Cry2Ab4和Cry2Ah1中的至少一种的氨基酸序列经过取代和/或缺失和/或添加一个或多个氨基酸的与所述Cry2Aa17、Cry2Ab29、 Cry2Ba2、Cry2Aa9、Cry2Ab4和Cry2Ah1中的至少一种具有相同功能的蛋白；

[0011] 优选所述杀虫晶体蛋白为mCry2Aa17，所述mCry2Aa17的氨基酸序列为经如SEQ ID No.14所示核酸序列翻译后得到的氨基酸序列。

[0012] 本发明之二提供了一种能够翻译为如上的本发明所述的杀虫晶体蛋白的核酸。

[0013] 在一个具体实施例中，能够翻译为所述Cry2Aa17的氨基酸序列的核酸的序列如SEQ ID No.8所示，能够翻译为所述Cry2Ab29的氨基酸序列的核酸的序列如SEQ ID No.9所示，能够翻译为所述Cry2Ba2的氨基酸序列的核酸的序列如SEQ ID No.10所示，能够翻译为所述Cry2Aa9的氨基酸序列的核酸序列如SEQ ID No.11所示，能够翻译为所述Cry2Ab4的氨基酸序列的核酸序列如 SEQ ID No.12所示，和能够翻译为所述Cry2Ah1的氨基酸序列的核酸序列如 SEQ ID No.13所示，能够翻译为所述mCry2Aa17的氨基酸序列的核酸的序列如SEQ ID No.14所示。这些基因的序列可以从Bt菌株中克隆出来，也可以人工合成。

[0014] 本发明之三提供了一种制备如上的本发明所述的杀虫晶体蛋白的方法，其包括如下步骤：在活体生物中表达上述杀虫晶体蛋白；优选在含有如上的本发明所述的核酸序列的活体生物中表达所述杀虫晶体蛋白。

[0015] 本发明之四提供了一种制备杀虫晶体蛋白的方法，其包括如下步骤：在16- 20℃下表达杀虫晶体蛋白。

[0016] 在一个具体实施例中，所述杀虫晶体蛋白为Cry1-70类蛋白中的至少一种，例如表1中的蛋白中的至少一种；优选为Cry1类蛋白、Cry2类蛋白、Cry3 类蛋白和Cry8类蛋白中的至少一种，更优选的为在含有能够翻译为如上的本发明所述的杀虫晶体蛋白的核酸，或更具体的讲在含有SEQ ID No.8、如SEQ ID No.9所示、如SEQ ID No.10所示、如SEQ ID No.11所示、如SEQ ID No. 12、如SEQ ID No.13所示和如SEQ ID No.14所示所示核酸的活体生物中表达所述杀虫蛋白。其中Cry1-70类蛋白包括如表1所示的杀虫蛋白模式基因。

[0017] 表1杀虫蛋白的模式基因

名称	登录号	来源菌株	发现时间
Cry1Aa1	AAA22353	Bt kurstaki HD1	1985
Cry1Ab1	AAA22330	Bt berliner 1715	1986
Cry1Ac1	AAA22331	Bt kurstaki HD73	1985
Cry1Ad1	AAA22340	Bt aizawai PS81I	1993
Cry1Ae1	AAA22410	Bt alesti	1991
Cry1Af1	AAB82749	Bt NT0423	1997
Cry1Ag1	AAD46137		1999
Cry1Ah1	AAQ14326		2000
Cry1Ai1	AAO39719		2002
Cry1Ba1	CAA29898	Bt thuringiensis HD2	1988
Cry1Bb1	AAA22344	Bt EG5847	1994
Cry1Bc1	CAA86568	Bt morrisoni	1994
Cry1Bd1	AAD10292	Bt wuhanensis HD525	2000
Cry1Be1	AAC32850	Bt PS158C2	1998
Cry1Bf1	CAC50778		2001
Cry1Bg1	AAO39720		2002
Cry1Bh1	HQ589331	Bt PS46L	2010
Cry1Ca1	CAA30396	Bt entomocidus 60.5	1988
Cry1Cb1	M97880	Bt galleriae HD29	1993
Cry1Da1	CAA38099	Bt aizawai HD68	1990
Cry1Db1	CAA80234	Bt BTS00349A	1993
Cry1Dc1	ABK35074	Bt JC291	2006
Cry1Ea1	CAA37933	Bt kenyae 4F1	1990
Cry1Eb1	AAA22346	Bt aizawai PS81A2	1993
Cry1Fa1	AAA22348	Bt aizawai EG6346	1991
Cry1Fb1	CAA80235	Bt BTS00349A	1993
Cry1Ga1	CAA80233	Bt BTS0349A	1993
Cry1Gb1	AAD10291	Bt wuhanensis HD525	1999
Cry1Gc1	AAQ52381		2003
Cry1Ha1	CAA80236	Bt BTS02069AA	1993
Cry1Hb1	AAA79694	Bt morrisoni BF190	1995
Cry1Ia1	CAA44633	Bt kurstaki	1992
Cry1Ib1	AAA82114	Bt entomocidus BP465	1995
Cry1Ic1	AAC62933	Bt C18	1998
Cry1Id1	AAD44366		2000
Cry1Ie1	AAG43526	Bt BTC007	2000

[0018]

[0019]

Cry1If1	AAQ52382		2003
Cry1Ja1	AAA22341	Bt EG5847	1994
Cry1Jb1	AAA98959	Bt EG5092	1994
Cry1Jc1	AAC31092		1998
Cry1Jd1	CAC50779	Bt	2001
Cry1Ka1	AAB00376	Bt morrisoni BF190	1995
Cry1La1	AAS60191	Bt kurstaki K1	2004
Cry1Ma1	FJ884067		2009
Cry2Aa1	AAA22335	Bt kurstaki	1989
Cry2Ab1	AAA22342	Bt kurstaki HD1	1989
Cry2Ac1	CAA40536	Bt shanghaiensis S1	1991
Cry2Ad1	AAF09583	Bt BR30	1999
Cry2Ae1	AAQ52362		2003
Cry2Af1	ABO30519	Bt C81	2007
Cry2Ag1	ACH91610	Bt JF19-2	2008
Cry2Ah1	EU939453	Bt	2008
Cry2Ai1	FJ788388	Bt	2009
Cry3Aa1	AAA22336	Bt san diego	1987
Cry3Ba1	CAA34983	Bt tolworthi 43F	1990
Cry3Bb1	AAA22334	Bt EG4961	1992
Cry3Ca1	CAA42469	Bt kurstaki BtI109P	1992
Cry4Aa1	CAA68485	Bt israelensis	1987
Cry4Ba1	CAA30312	Bt israelensis 4Q2-72	1988
Cry4Ca1	EU646202		2008
Cry4Cb1	FJ403208	Bt HS18-1	2008
Cry4Cc1	FJ403207	Bt MC28	2008
Cry5Aa1	AAA67694	Bt darmstadiensis PS17	1994
Cry5Ab1	AAA67693	Bt darmstadiensis PS17	1991
Cry5Ac1	I34543		1997
Cry5Ad1	ABQ82087	Bt L366	2007
Cry5Ba1	AAA68598	Bt PS86Q3	1997
Cry5Ca1	HM461869	Sbt003	2010
Cry5Da1	HM461870	Sbt003	2010
Cry5Ea1	HM461880	Sbt003	2010
Cry6Aa1	AAA22357	Bt PS52A1	1993
Cry6Ba1	AAA22358	Bt PS69D1	1991
Cry7Aa1	AAA22351	Bt galleriae PGSI245	1992
Cry7Ab1	AAA21120	Bt dakota HD511	1994
Cry7Ba1	ABB70817	Bt huazhongensis	2006
Cry7Ca1	ABR67863	Bt BTH-13	2007
Cry7Da1	ACQ99547	Bt LH-2	2009
Cry7Ea1	HM035086	Sbt009	2010
Cry7Fa1	HM035088	Sbt009	2010
Cry7Fb1	HM572235	Bt	2010
Cry7Ga1	HM572237	Bt	2010

[0020]

Cry8Aa1	AAA21117	Bt kumamotoensis	1992
Cry8Ab1	EU044830	Bt B-JJX	2007
Cry8Ba1	AAA21118	Bt kumamotoensis	1993
Cry8Bb1	CAD57542		2002
Cry8Bc1	CAD57543		2002
Cry8Ca1	AAA21119	Bt japonensis Buibui	1995
Cry8Da1	BAC07226	Bt galleriae	2002
Cry8Db1	BAF93483	Bt BBT2-5	2007
Cry8Ea1	AAQ73470	Bt 185	2003
Cry8Fa1	AAT48690	Bt 185	2004
Cry8Ga1	AAT46073	Bt HBF-18	2004
Cry8Ha1	EF465532	Bt 185	2006
Cry8Ia1	EU381044	Bt su4	2008
Cry8Ib1	GU325772		2010
Cry8Ja1	EU625348	Bt FPT-2	2008
Cry8Ka1	FJ422558		2008
Cry8Kb1	HM123758	ST8	2010
Cry8La1	GU325771		2010
Cry8Ma1	HM044665	Sbt016	2010
Cry8Na1	HM640939	BtQ52-7	2010
Cry8Pa1	HQ388415	Bt ST8	2010
Cry8Qa1	HQ441166	Bt ST8	2010
Cry9Aa1	CAA41122	Bt galleriae	1991
Cry9Ba1	CAA52927	Bt galleriae	1993
Cry9Bb1	AAV28716	Bt japonensis	2004
Cry9Ca1	CAA85764	Bt tolworthi	1996
Cry9Da1	BAA19948	Bt japonensis N141	1997
Cry9Db1	AAX78439	Bt kurstaki DP1019	2005
Cry9Ea1	BAA34908	Bt aizawai SSK-10	1998
Cry9Eb1	CAC50780		2001
Cry9Ec1	AAC63366	Bt galleriae	2003
Cry9Ed1	AAX78440	Bt kurstaki DP1019	2005
Cry9Ee1	GQ249296	Bt T03B001	2009
Cry10Aa1	AAA22614	Bt israelensis	1986
Cry11Aa1	AAA22352	Bt israelensis	1988
Cry11Ba1	CAA60504	Bt jegathesan 367	1995
Cry11Bb1	AAC97162	Bt medellin	1998
Cry12Aa1	AAA22355	Bt PS33F2	1991
Cry13Aa1	AAA22356	Bt PS63B	1992
Cry14Aa1	AAA21516	Bt sotto PS80JJ1	1994
Cry15Aa1	AAA22333	Bt thompsoni	1992
Cry16Aa1	CAA63860	Cb malaysia CH18	1996
Cry17Aa1	CAA67841	Cb malaysia CH18	1998
Cry18Aa1	CAA67506	Paenibacillus popilliae	1997
Cry18Ba1	AAF89667	Paenibacillus popilliae	1999
Cry18Ca1	AAF89668	Paenibacillus	1999

			popilliae	
	Cry19Aa1	CAA68875	Bt jegathesan 367	1996
	Cry19Ba1	BAA32397	Bt higo	1998
	Cry20Aa1	AAB93476	Bt fukuokaensis	1997
	Cry20Ba1	ACS93601	Bt higo LBIT-976	2009
	Cry21Aa1	I32932		1996
	Cry21Ba1	BAC06484	Bt roskildiensis	2002
	Cry22Aa1	I34547		1997
	Cry22Ab1	AAK50456	Bt EG4140	2000
	Cry22Ba1	CAD43578	Bt	2002
	Cry23Aa1	AAF76375	Bt	2000
	Cry24Aa1	AAC61891	Bt jegathesan	1998
	Cry24Ba1	BAD32657	Bt sotto	2004
	Cry24Ca1	CAJ43600	Bt FCC-41	2005
	Cry25Aa1	AAC61892	Bt jegathesan	1998
	Cry26Aa1	AAD25075	Bt finitimus B-1166	1999
	Cry27Aa1	BAA82796	Bt higo	1999
	Cry28Aa1	AAD24189	Bt finitimus B-1161	1999
	Cry29Aa1	CAC80985	Bt medellin	2000
	Cry30Aa1	CAC80986	Bt medellin	2000
	Cry30Ba1	BAD00052	Bt entomocidus	2003
	Cry30Ca1	BAD67157	Bt sotto	2004
	Cry30Da1	EF095955	Bt Y41	2006
[0021]	Cry30Db1	BAE80088	Bt aizawai BUN1-14	2006
	Cry30Ea1	ACC95445	Bt S2160-1	2007
	Cry30Fa1	ACI22625	Bt MC28	2008
	Cry30Ga1	ACG60020	Bt HS18-1	2008
	Cry31Aa1	BAB11757	Bt 84-HS-1-11	2000
	Cry31Ab1	BAE79809	Bt B0195	2006
	Cry31Ac1	BAF34368	Bt 87-29	2006
	Cry31Ad1	BAI44022	Bt MO19	2010
	Cry32Aa1	AAG36711	Bt yunnanensis	2001
	Cry32Ab1	GU063850		2010
	Cry32Ba1	BAB78601	Bt	2001
	Cry32Ca1	BAB78602	Bt	2001
	Cry32Da1	BAB78603	Bt	2001
	Cry32Ea1	GU324274	Bt	2010
	Cry33Aa1	AAL26871	Bt dakota	2001
	Cry34Aa1	AAG50341	Bt PS80JJ1	2001
	Cry34Ab1	AAG41671	Bt PS149B1	2001
	Cry34Ac1	AAG50118	Bt PS167H2	2001
	Cry34Ba1	AAK64565	Bt EG4851	2001
	Cry35Aa1	AAG50342	Bt PS80JJ1	2001
	Cry35Ab1	AAG41672	Bt PS149B1	2001
	Cry35Ac1	AAG50117	Bt PS167H2	2001
	Cry35Ba1	AAK64566	Bt EG4851	2001
	Cry36Aa1	AAK64558	Bt	2001
	Cry37Aa1	AAF76376	Bt	2000

[0022]

Cry38Aa1	AAK64559	Bt	2000
Cry39Aa1	BAB72016	Bt aizawai	2001
Cry40Aa1	BAB72018	Bt aizawai	2001
Cry40Ba1	BAC77648	Bun1-14	2003
Cry40Ca1	EU381045	Bt Y41	2008
Cry40Da1	ACF15199	Bt S2096-2	2008
Cry41Aa1	BAD35157	Bt A1462	2003
Cry41Ab1	BAD35163	Bt A1462	2003
Cry41Ba1	HM461871	Sbt021	2010
Cry42Aa1	BAD35166	Bt A1462	2003
Cry43Aa1	BAD15301	P. lentimorbus semadara	2003
Cry43Ba1	BAD15303	P. lentimorbus semadara	2003
Cry44Aa1	BAD08532	Btentomocidus INA288	2004
Cry45Aa1	BAD22577	Bt 89-T-34-22	2004
Cry46Aa1	BAC79010	Bt dakota	2004
Cry46Ab1	BAD35170	Bt	2004
Cry47Aa1	AAY24695	Bt CAA890	2005
Cry48Aa1	CAJ18351	Bs IAB59	2005
Cry48Ab1	CAJ86548	Bs LP1G	2006
Cry49Aa1	CAH56541	Bs IAB59	2005
Cry49Ab1	CAJ86542	Bs LP1G	2006
Cry50Aa1	BAE86999	Bt sotto	2006
Cry50Ba1	GU446675	Bt S2160-1	2010
Cry51Aa1	ABI14444	Bt F14-1	2006
Cry52Aa1	EF613489	Bt Y41	2007
Cry52Ba1	FJ361760	Bt BM59-2	2008
Cry53Aa1	EF633476	Bt Y41	2007
Cry53Ab1	FJ361759	Bt MC28	2008
Cry54Aa1	ACA52194	Bt MC28	2009
Cry54Ba1	GU446677	Bt S2160-1	2010
Cry55Aa1	ABW88932	YBT 1518	2008
Cry56Aa1	FJ597621	Bt Ywc2-8	2008
Cry57Aa1	ANC87261	Bt kim	2009
Cry58Aa1	ANC87260	Bt entomocidus	2009
Cry59Aa1	ACR43758	Bt kim LBIT-980	2009
Cry60Aa1	ACU24782	Bt jegathesan	2009
Cry60Ba1	GU810818	Bt malayensis	2009
Cry61Aa1	HM035087	Sbt009	2010
Cry62Aa1	HM054509	ST7	2010
Cry63Aa1	BAI44028	MO19	2010
Cry64Aa1	BAJ05397	Bt tohokuensis	2010
Cry65Aa1	HM461868	SBt003	2010
Cry66Aa1	HM485581	SBt021	2010
Cry67Aa1	HM485582	SBt009	2010
Cry68Aa1	HQ113114	Bt MC28	2010
Cry69Aa1	HQ401006	Bt MC28	2011
Cry69Ab1	JN209957	Bt hs18-1	2011
Cry70Aa	JN646781	Bt hs18-1	2011

[0023]

Cry70Ba	ADO51070	Bt MC28	2011
Cry70Bb	EEL67276	Bc AH603	2011

[0024] 在一个具体实施例中,所述活体生物包括微生物和/或植物。

[0025] 在一个具体实施例中,所述微生物选自芽孢杆菌(Bacillus)、假单胞菌(Pseudomonas)、肠杆菌(Escherichia)和酵母(Saccharomyces)中的至少一种。

[0026] 在一个具体实施例中,所述芽孢杆菌包括苏云金芽孢杆菌(Bacillus thuringiensis)、枯草芽孢杆菌(Bacilllus subtilis)、萎缩芽孢杆菌(Bacillus atrophaeus)和蜡样芽孢杆菌(Bacillus cereus)中的至少一种;所述假单胞菌包括荧光假单胞杆菌(Pseudomonas fluorescens);所述肠杆菌包括大肠杆菌 (Escherichia coli)。

[0027] 在一个具体实施例中,所述微生物为野生微生物和/或遗传工程微生物。

[0028] 其中,野生微生物是指从自然界中分离出的未经过人工改造的微生物。

[0029] 遗传工程微生物是指将野生微生物人工改造的微生物。

[0030] 在一个具体实施例中,含有如上的本发明所述的核酸序列被连接在表达载体上,例如能够在大肠杆菌和苏云金芽孢杆菌中穿梭的pSTK表达载体。

[0031] 在一个具体实施例中,所述植物选自大葱、甘蓝、大白菜、芹菜、菜花、胡萝卜、芦笋、莴笋、蕹菜、苋菜、辣椒、豇豆、花椰菜、茄子、芥兰、番茄、菜心、小白菜、青花菜、菠菜、萝卜和四季豆中的至少一种。

[0032] 本发明之五提供了一种如上的本发明所述的杀虫晶体蛋白在防治有害生物中的应用,优选所述有害生物为昆虫,更优选所述有害生物为鳞翅目昆虫,最优选所述有害生物为甜菜夜蛾。其中所述的鳞翅目昆虫包括甜菜夜蛾、亚洲玉米螟、小菜蛾和水稻二化螟等。例如杀虫蛋白Cry2Aa17、Cry2Ab29、Cry2Ba2 中的至少一种在防治甜菜夜蛾、亚洲玉米螟和小菜蛾中的应用;杀虫蛋白 Cry2Aa9、Cry2Ab4和Cry2Ah1中的至少一种在防治甜菜夜蛾和/或水稻二化螟中的应用。

具体实施方式

[0033] 以下通过优选的实施例的形式对本发明的上述内容再作进一步的详细说明,但不构成对本发明的限制。

[0034] 1)液体LB:胰蛋白胨1%,酵母粉0.5%,NaCl 1%,pH 7.0,15磅灭菌 15min。用于培养大肠杆菌。

[0035] 2)固体LB:在液体LB培养基中加1.3%琼脂,15磅灭菌15min。用于培养大肠杆菌。

[0036] 3)牛肉膏蛋白胨培养基:0.3%牛肉膏,0.5%蛋白胨,pH 7.2,15磅灭菌 15min。用于培养含有杀虫晶体蛋白基因的Bt菌株。

[0037] 4)BH培养基:脑心浸液肉汤,3.7%,15磅灭菌20min。用于制备Bt感受态。

[0038] 5)抗生素:氨苄青霉素水溶液100mg/ml,用时稀释500倍-20℃保存。其中pEB质粒载体具有氨苄青霉素抗性。

[0039] 蛋白电泳检测及定量分析

[0040] 120V预电泳约10-20min,取蛋白样品40μL,加入10μL 5×加样缓冲液,混匀,再将上清和沉淀分别梯度稀释,煮沸5-10min,12000rpm离心5 min,取10L上清点样。80V恒压电泳至样品浓缩呈直线,150V恒压电泳至指示的溴酚蓝到达凝胶底部。

[0041] 脱色:电泳后取出凝胶,电泳后取出凝胶并用蒸馏水冲洗,加入50mL溶液I,微波炉中加热30s,60rpm振荡10min。

[0042] 染色:倒掉溶液I后加入溶液II(每50mL溶液II加200L溶液III)微波炉加热30s,60rpm振荡15min以上。

[0043] 倒掉溶液II,加入无菌水,凝胶成像系统照相保存。

[0044] 观察结果,根据蛋白条带着色程度比较目的蛋白表达情况,用凝胶成像系统照相保存。目标蛋白大小与预期蛋白大小基本一致。

[0045] 蛋白定量分析:

[0046] 制备以下5种不同浓度梯度的BSA:0.8μg/μL、0.4μg/μL、0.2μg/μL、0.1 μg/μL、0.05μg/μL,分别将目标蛋白进行梯度稀释,使其终浓度介于0.8-0.05 μg/μL,目标蛋白和5种不同浓度的BSA上样量均为10μL,进行蛋白电泳检测。表2为SDS聚丙烯酰氨凝胶的制备表。

[0047] 表2 SDS聚丙烯酰氨凝胶的制备表

[0048]

	分离胶 8%（mL）	浓缩胶 4%（mL）
蒸馏水	4.6	3.1
30%脱气的丙烯酰胺	2.7	0.65
凝胶缓冲液（pH 8.8）	2.5	—
凝胶缓冲液（pH 6.8）	—	1.25
10%过硫酸铵（APS）	0.1	0.05
四甲基乙二胺（TEMED）	0.006	0.006

[0049] 实施例1

[0050] 1.1cry2Aa17、cry2Ab29、cry2Ba2以及mcry2Aa17的克隆

[0051] 通过本领域的常规方法,从苏云金芽孢杆菌的野生菌株DS415克隆出 cry2Aa17基因,如SEQ ID No.8所示;野生菌株LS5115-3克隆出cry2Ab29基因,如SEQ ID No.9所示;野生菌株HD395克隆出cry2Ba2基因,如SEQ ID No.10所示;从苏云金芽孢杆菌的野生菌株克隆出或人工合成cry2Aa9基因,如SEQ ID No.11所示;野生菌株Bt B-Pr-88克隆出cry2Ab4基因,如SEQ ID No.12所示;野生菌株Bt SC6H8克隆出cry2Ah1基因,如SEQ ID No.13所示。

[0052] 对克隆的cry2Aa17基因进行定点突变,获得mcry2Aa17基因,如SEQ ID No.14所示。

[0053] 将8种cry2类基因:cry2Aa17、mcry2Aa17、cry2Ab29、cry2Ba2、 cry2Aa9、cry2Ab4和cry2Ah1分别插入到pEB载体Ecl 136II位置,连接反应按照以下连接体系进行,充分混匀后,16℃连接4h。

[0054] 目的DNA 4μl

[0055] 载体DNA 1μl

[0056] Solution I 5μl

[0057] 1.2大肠杆菌热激转化

[0058] 将连接产物10μL加入到100μL大肠杆菌DH5α感受态细胞中,轻轻混匀,冰浴30min,42℃热激90s,立即置于冰上,冰浴3min,加入800μL LB 液体培养基,150rpm/min、37℃培养1h,取200μL涂布于含有氨苄抗生素的 LB固体平板,加入X-gal溶液40μL,IPTG溶液4μL,37℃培养过夜,进行蓝白斑筛选。以载体正向引物pEB_F和克隆基因反向引物cry2_R进行PCR鉴定,筛选出连接方向正确的阳性克隆。

[0059] 1.3大肠杆菌质粒DNA提取

[0060] 提取阳性克隆菌株的质粒，转入大肠杆菌Rosetta感受态细胞中，经PCR 鉴定及序列测定，鉴定出正确重组的转化子进行目的蛋白诱导表达。

[0061] 质粒提取方法具体如下：

[0062] (1)挑取阳性转化子于5mL LB液体培养基中培养过夜，取1-4ml菌液于12000r/min离心1min，弃去上清；

[0063] (2)加入250μL含有50mg/ml RNAase的S1溶液悬浮沉淀；

[0064] (3)加入250μL细菌裂解液S2并充分缓慢温和地上下翻转4-6次，直到溶液透明清亮为止，这一步骤不应该超过5分钟；

[0065] (4)加入350μL S3中和液并充分温和地上下翻转6-8次，12000r/min离心10min；

[0066] (5)随后吸取上清，然后转移到制备管中，12000r/min离心1min并弃掉滤液；

[0067] (6)随后加入500μL洗涤液W1，12000r/min离心1min并弃掉滤液；

[0068] (7)随后加入700μLW2，12000r/min离心1min并弃掉滤液，该步骤重复一次；

[0069] (8)将收集柱转移到新1.5ml离心管上，收集柱中央滴加60-80μL预热至65℃的灭菌水，静止1min，12000r/min离心1min，-20℃保存备用。

[0070] 1.4cry2基因诱导表达

[0071] 将重组质粒转化通过热激转化的方式导入到大肠杆菌表达宿主Rosetta中，获得含有cry2类杀虫基因的重组菌株V14-F4(cry2Aa17)、V15-A10 (mcry2Aa17)、V14-F7(cry2Ab29)、V14-F10(cry2Ba2)、V15-A1 (cry2Aa9)、V15-A4(cry2Ab4)和V15-A6(cry2Ah1)。

[0072] 将上述E.coli的单克隆分别转接于5mL液体LB培养基中(每个样品接种于3管5mL液体LB培养基中)，220rpm、37℃培养12h；按1％的接种量分别转接至含有400mL LB的三角瓶中(每个样品转接至3瓶含有400mL LB的三角瓶中)，220rpm、37℃培养约2.5小时，至OD_{600}为0.6；加入IPTG 200μL，220rpm，16℃、20℃和30℃(将相同样品的3瓶培养液分别置于此三种温度下)分别培养12小时，诱导蛋白表达。

[0073] 样品处理：菌液8000rmp离心10min收集菌体；菌体沉淀中加入20 mmol/L Tris-HCl(pH 8.0)，超声波破碎细胞5min(Ampl 70％，超声时间3 s，超声停止5s)，12000rpm离心15min，将上清转移至一个新的EP管中，沉淀加入20mmol/L Tris-HCl(pH 8.0)重新悬浮，分别进行蛋白电泳检测定量。

[0074] 实施例2

[0075] 杀虫蛋白对亚洲甜菜夜蛾、亚洲玉米螟、小菜蛾和的活性分析

[0076] 甜菜夜蛾的人工饲料由中国农业科学院植物保护研究所迁飞害虫组提供。

[0077] 甜菜夜蛾的生测方法：(1)取30g人工饲料置于培养皿中，加入3mL待测样品溶液，充分搅拌均匀，室温放置，根据饲料干湿程度适当室温晾置，直至饲料不能挤出水分；

[0078] (2)将全部饲料分装于3个已消毒的24孔细胞培养板中；

[0079] (3)用毛笔轻轻挑取幼虫接于24孔板中，每孔一头小于等于12小时的初孵幼虫；接完后，加盖(内附一层吹塑纸)，并用橡皮筋固定；

[0080] (4)放置25℃光照培养箱中培养，光周期为16L:8D，湿度50-75％，每天观察饲料湿度，并做出适当的调整，保持培养箱湿度；

[0081] (5)7天后分别调查死、活虫数及活虫的体重，计算抑制率、校正抑制率、死亡率和校正死亡率。

[0082] 亚洲玉米螟选用初孵幼虫，生测方法参照He et al进行(HE K L,WANG Z Y,WEN L P,et al.Determination of baseline susceptibility to Cry1Ab protein for Asian corn borer(Lep.,Crambidae)[J].Applied Entomology,2005,129(8):407- 412)，并在培养后的调查期间称量活虫的体重。

[0083] 小菜蛾的人工饲料购于美国Southland Products Incorporated公司，配制方法见说明。

[0084] 小菜蛾的生测方法：采用人工饲料的方法，人工饲料购于Southland Products Incorporated公司，配制方法见产品说明。称取饲料10g与1mL待测样品稀释液混匀，平均分到3个无菌培养皿中。每个培养皿接2龄幼虫20头， 25℃生化培养箱中保温，光周期为16L:8D，湿度50-75％，观察饲料湿度，并做出适当的调整，保持培养箱湿度。培养48h后调查死、活虫数以及活虫的体重，并观察幼虫取食情况。计算抑制率、校正抑制率、死亡率和校正死亡率。水稻二化螟的人工饲料由中国农业科学院植物保护研究所水稻害虫组提供。水稻二化螟的生测方法：

[0085] (1)称取15g人工饲料放置于灭菌的培养皿中。

[0086] (2)加入待测样品的溶液1.5mL，充分搅拌混匀平均分装于三个培养皿中。

[0087] (3)根据饲料的干湿程度室温放置一段时间，直到饲料表面没有水滴。每皿接入20头初孵幼虫，用医用胶带封口，每个浓度重复3次，共处理60头试虫。

[0088] (4)将样品置于25℃光照培养箱培养，用黑布遮盖住所有生测样品，不能有光线的进入(水稻二化螟趋光性很强，会咬穿平皿逃逸)，光周期为 16L:8D，湿度60％-70％左右，每天观察饲料干湿程度，适当做出微调。

[0089] (5)培养6天调查死虫和活虫数以及活虫的体重，计算抑制率、校正抑制率、死亡率和校正死亡率等数据。

[0090] 其中，校正抑制率(％)＝1-处理组平均体重/对照组平均体重；

[0091] 校正死亡率(％)＝(对照组存活率-处理组存活率)/对照组存活率。

[0092] 表3杀虫蛋白对甜菜夜蛾(Spodoptera exigua)的杀虫活性

[0093]

蛋白名称	表达温度（℃）	蛋白含量（μg/ml）	校正抑制率（％）
Cry2Aa17	30	100	-3.08
mCry2Aa17	30	100	-0.14
Cry2Ab29	30	100	44.22
Cry2Ah1	30	100	65.55
Cry2Ab4	30	100	47.74
Cry2Aa17	30	33	-0.89
mCry2Aa17	30	33	5.45
Cry2Ab29	30	33	43.28

[0094]

Cry2Ah1	30	33	55.55
Cry2Ab4	30	33	47.74
Cry2Aa17	30	11	-5.01
mCry2Aa17	30	11	-2.10
Cry2Ab29	30	11	31.25
Cry2Ah1	30	11	49.01
Cry2Ab4	30	11	34.38
Cry2Aa17	30	3.7	-4.70
mCry2Aa17	30	3.7	-3.61
Cry2Ab29	30	3.7	17.29
Cry2Ah1	30	3.7	43.75
Cry2Ab4	30	3.7	29.65
Cry2Aa17	30	1.23	-3.37
mCry2Aa17	30	1.23	-1.80
Cry2Ab29	30	1.23	7.61
Cry2Ah1	30	1.23	35.24
Cry2Ab4	30	1.23	0.00
Cry2Aa17	20	100	42.76
mCry2Aa17	20	100	64.43
Cry2Ab29	20	100	89.19
Cry2Ah1	20	100	85.01
Cry2Ab4	20	100	87.03
Cry2Aa17	20	33	26.22
mCry2Aa17	20	33	40.60
Cry2Ab29	20	33	79.23
Cry2Ah1	20	33	72.98
Cry2Ab4	20	33	79.28
Cry2Aa17	20	11	12.92
mCry2Aa17	20	11	23.87
Cry2Ab29	20	11	69.69
Cry2Ah1	20	11	64.85
Cry2Ab4	20	11	73.03
Cry2Aa17	20	3.7	6.46
mCry2Aa17	20	3.7	21.95
Cry2Ab29	20	3.7	56.91
Cry2Ah1	20	3.7	47.65
Cry2Ab4	20	3.7	65.18
Cry2Aa17	20	1.23	3.15
mCry2Aa17	20	1.23	6.39
Cry2Ab29	20	1.23	38.63

[0095]

Cry2Ah1	20	1.23	40.32
Cry2Ab4	20	1.23	39.71
Cry2Aa17	16	100	48.53
mCry2Aa17	16	100	70.21
Cry2Ab29	16	100	88.58
Cry2Ah1	16	100	87.82
Cry2Ab4	16	100	96.91
Cry2Aa17	16	33	30.52
mCry2Aa17	16	33	46.03
Cry2Ab29	16	33	79.68
Cry2Ah1	16	33	71.65
Cry2Ab4	16	33	83.57
Cry2Aa17	16	11	11.54
mCry2Aa17	16	11	28.49
Cry2Ab29	16	11	76.83
Cry2Ah1	16	11	71.16
Cry2Ab4	16	11	72.93
Cry2Aa17	16	3.7	7.54
mCry2Aa17	16	3.7	25.19
Cry2Ab29	16	3.7	62.19
Cry2Ah1	16	3.7	48.35
Cry2Ab4	16	3.7	68.58
Cry2Aa17	16	1.23	6.63
mCry2Aa17	16	1.23	6.53
Cry2Ab29	16	1.23	46.21
Cry2Ah1	16	1.23	42.19
Cry2Ab4	16	1.23	46.27

[0096] 表4杀虫蛋白在64μg/ml时对亚洲玉米螟(Ostrinia nubilalis)的杀虫活性

[0097]

蛋白名称	表达温度(℃)	校正抑制率	校正死亡率
Cry2Aa17	20	40.9%	22.5%
mCry2Aa17	20	60.8%	50.7%
Cry2Ab29	20	100.0%	35.8%
Cry2Ba2	20	28.7%	36.6%
Cry2Ah1	20	87.3%	20.0%
Cry2Aa9	20	45.8%	35.3%
Cry2Ab4	20	90.2%	45.0%

[0098] 表5杀虫蛋白在64μg/ml时对小菜蛾(Plutella xylostella)的杀虫活性

[0099]

蛋白名称	表达温度(℃)	校正抑制率	校正死亡率

Cry2Aa17	20	93.3%	62.8%
mCry2Aa17	20	98.9%	90.5%
Cry2Ab29	20	73.3%	50.3%
Cry2Ba2	20	27.9%	26.1%
Cry2Ab4	20	83.1%	57.5%

[0100] 表6杀虫蛋白在64μg/ml时对水稻二化螟(Chilo supperssalis)的杀虫活性

[0101]

蛋白名称	表达温度(℃)	校正抑制率	校正死亡率
Cry2Ah1	20	21.5%	18.5%
Cry2Aa9	20	25.1%	18.5%
Cry2Ab4	20	85.4%	58.5%

<110> 中国农业科学院植物保护研究所

<120> 杀虫晶体蛋白、核酸、杀虫晶体蛋白的制备方法及其应用

<130> P1605IPP-1

<140> 2016102187810

<141> 2016-04-11

<160> 14

<170> SIPOSequenceListing 1.0

<210> 1

[0001] <211> 633

<212> PRT

<213> 苏云金芽孢杆菌(Bacillus thuringiensis)

<400> 1

Met Asn Ser Val Leu Asn Ser Gly Arg Thr Thr Ile Cys Asp Ala Tyr
1 5 10 15
Asn Val Ala Ala His Asp Pro Phe Ser Phe Gln His Lys Ser Leu Asp
20 25 30
Thr Val Gln Lys Glu Trp Thr Glu Trp Lys Lys Asn Asn His Ser Leu
35 40 45
Tyr Leu Asp Pro Ile Val Gly Thr Val Ala Ser Phe Leu Leu Lys Lys
50 55 60
Val Gly Ser Leu Val Gly Lys Arg Ile Leu Ser Glu Leu Arg Asn Leu
65 70 75 80

Ile Phe Pro Ser Gly Ser Thr Asn Leu Met Gln Asp Ile Leu Arg Glu
85 90 95

Thr Glu Lys Phe Leu Asn Gln Arg Leu Asn Thr Asp Thr Leu Ala Arg
100 105 110

Val Asn Ala Glu Leu Thr Gly Leu Gln Ala Asn Val Glu Glu Phe Asn
115 120 125

Arg Gln Val Asp Asn Phe Leu Asn Pro Asn Arg Asn Ala Val Pro Leu
130 135 140

Ser Ile Thr Ser Ser Val Asn Thr Met Gln Gln Leu Phe Leu Asn Arg
145 150 155 160

Leu Pro Gln Phe Gln Met Gln Gly Tyr Gln Leu Leu Leu Leu Pro Leu
165 170 175

Phe Ala Gln Ala Ala Asn Leu His Leu Ser Phe Ile Arg Asp Val Ile
180 185 190

Leu Asn Ala Asp Glu Trp Gly Ile Ser Ala Ala Thr Leu Arg Thr Tyr
[0002] 195 200 205

Arg Asp Tyr Leu Lys Asn Tyr Thr Arg Asp Tyr Ser Asn Tyr Cys Ile
210 215 220

Asn Thr Tyr Gln Ser Ala Phe Lys Gly Leu Asn Thr Arg Leu His Asp
225 230 235 240

Met Leu Glu Phe Arg Thr Tyr Met Phe Leu Asn Val Phe Glu Tyr Val
245 250 255

Ser Ile Trp Ser Leu Phe Lys Tyr Gln Ser Leu Leu Val Ser Ser Gly
260 265 270

Ala Asn Leu Tyr Ala Ser Gly Ser Gly Pro Gln Gln Thr Gln Ser Phe
275 280 285

Thr Ala Gln Asn Trp Pro Phe Leu Tyr Ser Leu Phe Gln Val Asn Ser
290 295 300

Asn Tyr Ile Leu Ser Gly Ile Ser Gly Thr Arg Leu Ser Ile Thr Phe
305 310 315 320

Pro Asn Ile Gly Gly Leu Pro Gly Ser Thr Thr Thr His Ser Leu Asn

325 330 335

Ser Ala Arg Val Asn Tyr Ser Gly Gly Val Ser Ser Gly Leu Ile Gly

340 345 350

Ala Thr Asn Leu Asn His Asn Phe Asn Cys Ser Thr Val Leu Pro Pro

355 360 365

Leu Ser Thr Pro Phe Val Arg Ser Trp Leu Asp Ser Gly Thr Asp Arg

370 375 380

Glu Gly Val Ala Thr Ser Thr Asn Trp Gln Thr Glu Ser Phe Gln Thr

385 390 395 400

Thr Leu Ser Leu Arg Cys Gly Ala Phe Ser Ala Arg Gly Asn Ser Asn

405 410 415

Tyr Phe Pro Asp Tyr Phe Ile Arg Asn Ile Ser Gly Val Pro Leu Val

420 425 430

Ile Arg Asn Glu Asp Leu Thr Arg Pro Leu His Tyr Asn Gln Ile Arg

435 440 445

[0003] Asn Ile Ala Ser Pro Ser Gly Thr Pro Gly Gly Ala Arg Ala Tyr Leu

450 455 460

Val Ser Val His Asn Arg Lys Asn Asn Ile Tyr Ala Ala Asn Glu Asn

465 470 475 480

Gly Thr Met Ile His Leu Ala Pro Glu Asp Tyr Thr Gly Phe Thr Ile

485 490 495

Ser Pro Ile His Ala Thr Gln Val Asn Asn Gln Thr Arg Thr Phe Ile

500 505 510

Ser Glu Lys Phe Gly Asn Gln Gly Asp Ser Leu Arg Phe Glu Gln Ser

515 520 525

Asn Thr Thr Ala Arg Tyr Thr Leu Arg Gly Asn Gly Asn Ser Tyr Asn

530 535 540

Leu Tyr Leu Arg Val Ser Ser Ile Gly Asn Ser Thr Ile Arg Val Thr

545 550 555 560

Ile Asn Gly Arg Val Tyr Thr Val Ser Asn Val Asn Thr Thr Thr Asn

565 570 575

Asn Asp Gly Val Asn Asp Asn Gly Ala Arg Phe Ser Asp Ile Asn Ile
580 585 590
Gly Asn Ile Val Ala Ser Asp Asn Thr Asn Val Thr Leu Asp Ile Asn
595 600 605
Val Thr Leu Asn Ser Gly Thr Pro Phe Asp Leu Met Asn Ile Met Phe
610 615 620
Val Pro Thr Asn Leu Ser Pro Leu Tyr
625 630

<210> 2
<211> 633
<212> PRT
<213> 苏云金芽孢杆菌(Bacillus thuringiensis)

<400> 2

[0004] Met Asn Ser Val Leu Asn Ser Gly Arg Thr Thr Ile Cys Asp Ala Tyr
1 5 10 15
Asn Val Ala Ala His Asp Pro Phe Ser Phe Gln His Lys Ser Leu Asp
20 25 30
Thr Val Gln Lys Glu Trp Thr Glu Trp Lys Lys Asn Asn His Ser Leu
35 40 45
Tyr Leu Asp Pro Ile Val Gly Thr Val Ala Ser Phe Leu Leu Lys Lys
50 55 60
Val Gly Ser Leu Val Gly Lys Arg Ile Leu Ser Glu Leu Arg Asn Leu
65 70 75 80
Ile Phe Pro Ser Gly Ser Thr Asn Leu Met Gln Asp Ile Leu Arg Glu
85 90 95
Thr Glu Lys Phe Leu Asn Gln Arg Leu Asn Thr Asp Thr Leu Ala Arg
100 105 110
Val Asn Ala Glu Leu Thr Gly Leu Gln Ala Asn Val Glu Glu Phe Asn
115 120 125

Arg Gln Val Asp Asn Phe Leu Asn Pro Asn Arg Asn Ala Val Pro Leu
130 135 140
Ser Ile Thr Ser Ser Val Asn Thr Met Gln Gln Leu Phe Leu Asn Arg
145 150 155 160
Leu Pro Gln Phe Gln Met Gln Gly Tyr Gln Leu Leu Leu Leu Pro Leu
165 170 175
Phe Ala Gln Ala Ala Asn Leu His Leu Ser Phe Ile Arg Asp Val Ile
180 185 190
Leu Asn Ala Asp Glu Trp Gly Ile Ser Ala Ala Thr Leu Arg Thr Tyr
195 200 205
Arg Asp Tyr Leu Lys Asn Tyr Thr Arg Asp Tyr Ser Asn Tyr Cys Ile
210 215 220
Asn Thr Tyr Gln Ser Ala Phe Lys Gly Leu Asn Thr Arg Leu His Asp
225 230 235 240
Met Leu Glu Phe Arg Thr Tyr Met Phe Leu Asn Val Phe Glu Tyr Val

[0005]

245 250 255
Ser Ile Trp Ser Leu Phe Lys Tyr Gln Ser Leu Leu Val Ser Ser Gly
260 265 270
Ala Asn Leu Tyr Ala Ser Gly Ser Gly Pro Gln Gln Thr Gln Ser Phe
275 280 285
Thr Ser Gln Asp Trp Pro Phe Leu Tyr Ser Leu Phe Gln Val Asn Ser
290 295 300
Asn Tyr Val Leu Asn Gly Phe Ser Gly Ala Arg Leu Ser Asn Thr Phe
305 310 315 320
Pro Asn Ile Val Gly Leu Pro Gly Ser Thr Thr Thr His Ala Leu Leu
325 330 335
Ala Ala Arg Val Asn Tyr Ser Gly Gly Ile Ser Ser Gly Asp Ile Gly
340 345 350
Ala Ser Pro Phe Asn Gln Asn Phe Asn Cys Ser Thr Phe Leu Pro Pro
355 360 365
Leu Leu Thr Pro Phe Val Arg Ser Trp Leu Asp Ser Gly Ser Asp Arg

370 375 380

Glu Gly Val Ala Thr Val Thr Asn Trp Gln Thr Glu Ser Phe Glu Thr

385 390 395 400

Thr Leu Gly Leu Arg Ser Gly Ala Phe Thr Ala Arg Gly Asn Ser Asn

405 410 415

Tyr Phe Pro Asp Tyr Phe Ile Arg Asn Ile Ser Gly Val Pro Leu Val

420 425 430

Val Arg Asn Glu Asp Leu Arg Arg Pro Leu His Tyr Asn Glu Ile Arg

435 440 445

Asn Ile Ala Ser Pro Ser Gly Thr Pro Gly Gly Ala Arg Ala Tyr Met

450 455 460

Val Ser Val His Asn Arg Lys Asn Asn Ile His Ala Val His Glu Asn

465 470 475 480

Gly Ser Met Ile His Leu Ala Pro Asn Asp Tyr Thr Gly Phe Thr Ile

485 490 495

[0006] Ser Pro Ile His Ala Thr Gln Val Asn Asn Gln Thr Arg Thr Phe Ile

500 505 510

Ser Glu Lys Phe Gly Asn Gln Gly Asp Ser Leu Arg Phe Glu Gln Asn

515 520 525

Asn Thr Thr Ala Arg Tyr Thr Leu Arg Gly Asn Gly Asn Ser Tyr Asn

530 535 540

Leu Tyr Leu Arg Val Ser Ser Ile Gly Asn Ser Thr Ile Arg Val Thr

545 550 555 560

Ile Asn Gly Arg Val Tyr Thr Val Ser Asn Val Asn Thr Thr Thr Asn

565 570 575

Asn Asp Gly Val Asn Asp Asn Gly Ala Arg Phe Ser Asp Ile Asn Ile

580 585 590

Gly Asn Ile Val Ala Ser Asp Asn Thr Asn Val Thr Leu Asp Ile Asn

595 600 605

Val Thr Leu Asn Ser Gly Thr Pro Phe Asp Leu Met Asn Ile Met Phe

610 615 620

Val Pro Thr Asn Leu Ser Pro Leu Tyr
625 630

<210> 3
<211> 624
<212> PRT
<213> 苏云金芽孢杆菌(Bacillus thuringiensis)

<400> 3
Met Asn Ser Val Leu Asn Ser Gly Arg Thr Asn Lys Cys Asp Ala Tyr
1 5 10 15
Asn Val Val Ala His Asp Pro Phe Ser Phe Glu His Lys Ser Leu Asp
20 25 30
Thr Ile Gln Gln Glu Trp Met Glu Trp Lys Arg Thr Asp His Ser Leu
35 40 45

[0007]

Tyr Val Ser Pro Ile Val Gly Thr Ile Ala Ser Phe Pro Leu Lys Lys
50 55 60
Val Ala Gly Leu Ile Gly Lys Arg Ile Leu Ser Glu Leu Lys Asn Leu
65 70 75 80
Ile Phe Pro Ser Gly Ser Ile Glu Ser Met Gln Asp Ile Leu Arg Gly
85 90 95
Ala Glu Gln Phe Leu Asn Gln Arg Leu Asp Ala Asp Thr Phe Ala Arg
100 105 110
Val Glu Ala Glu Leu Ile Gly Leu Gln Ala Asn Val Glu Glu Phe Asn
115 120 125
Gln Gln Val Asp Asn Phe Leu Asn Pro Asn Gln Asn Pro Val Pro Leu
130 135 140
Ala Ile Ile Asp Ser Val Asn Thr Met Gln Gln Leu Phe Leu Ser Arg
145 150 155 160
Leu Pro Gln Phe Gln Ile Gln Arg Tyr Gln Leu Leu Leu Leu Pro Leu
165 170 175

Phe Ala Gln Ala Ala Asn Leu His Leu Thr Phe Ile Arg Asp Val Ile
180 185 190
Leu Asn Ala Asp Glu Trp Gly Ile Pro Ala Ala Thr Val Arg Thr Tyr
195 200 205
Arg Glu His Leu Lys Arg Tyr Thr Arg Asp Tyr Ser Asn Tyr Cys Ile
210 215 220
Asn Thr Tyr Gln Thr Ala Phe Arg Gly Leu Asn Thr Arg Leu His Asp
225 230 235 240
Met Leu Glu Phe Arg Thr Phe Met Phe Leu Asn Val Leu Asp Tyr Val
245 250 255
Ser Ile Trp Ser Leu Phe Lys Tyr Gln Ser Leu Met Val Thr Ser Ser
260 265 270
Ala Asn Leu Tyr Ala Ser Gly Ser Gly Ser Asn Gln Pro Phe Thr Ala
275 280 285
Gln Asp Trp Pro Phe Leu Tyr Ser Leu Phe Gln Val Asn Ser Asn Tyr

[0008]

290 295 300
Ile Met Ser Asn Phe Gly Gly Asn Arg Glu Thr Ala Ser Phe Gly Val
305 310 315 320
Pro Ile Leu Gly Gly Phe Ile Ile Asn Phe Leu Leu Ser Phe Arg Val
325 330 335
Asn Tyr Thr Gly Gly Val Ser Ser Gly Leu Leu Gly Val Glu Gly Ile
340 345 350
Ser Asn Asn Phe Asn Cys Asn Ser Ser Leu Ser Thr Pro Val Val Arg
355 360 365
Ser Trp Leu Asp Ser Gly Val Tyr Arg Gly Asp Leu Gln His Asn Trp
370 375 380
Arg Thr Asp Ile Phe Met Arg Thr Asn Ile Val Pro Cys Gly Ala Phe
385 390 395 400
Leu Leu Ser Leu Ala Met Phe Pro Asp Val Lys Ser Asn Tyr Phe Pro
405 410 415
Asp Tyr Phe Ile Arg Asn Ile Ser Gly Ile Ile Arg Asn Ile Asp Asn

420 425 430

Met Asn Leu Ser Arg Pro Leu His Phe Asn Glu Val Arg Asp Leu Arg

435 440 445

Asp Thr Glu Val Ala Thr Leu Val Ser Val His Asn Arg Lys Asn Asn

450 455 460

Ile Tyr Ala Ala His Glu Asn Gly Thr Met Ile His Phe Ala Pro Glu

465 470 475 480

Gly Tyr Ile Gly Phe Thr Ile Ser Pro Ile Tyr Ala Thr Gln Val Asn

485 490 495

Asn Gln Thr Arg Thr Phe Ile Ser Glu Lys Phe Gly Asn Gln Gly Asp

500 505 510

Ser Leu Arg Phe Glu Gln Thr Asn Thr Thr Ala Arg Tyr Thr Phe Arg

515 520 525

Gly Asn Gly Asn Asn Tyr Asn Leu Tyr Leu Arg Val Ser Ser Gln Gly

530 535 540

[0009]

Asn Ser Thr Phe Arg Val Thr Ile Asn Gly Arg Val Tyr Thr Val Ser

545 550 555 560

Asn Val Asn Thr Thr Thr Asn Asn Asp Gly Val Ile Asp Asn Gly Ala

565 570 575

Arg Phe Ser Asp Ile His Ile Gly Asn Ile Val Ala Ser Asn Asn Thr

580 585 590

Asn Val Pro Leu Asp Ile Asn Val Ile Leu Asn Ser Gly Thr Gln Phe

595 600 605

Glu Leu Met Asn Ile Ile Phe Val Pro Thr Asn Leu Ser Pro Leu Tyr

610 615 620

<210> 4

<211> 633

<212> PRT

<213> 苏云金芽孢杆菌(Bacillus thuringiensis)

<400> 4

Met Asn Ser Val Leu Asn Ser Gly Arg Thr Thr Ile Cys Asp Ala Tyr
1 5 10 15

Asn Val Val Ala His Asp Pro Phe Ser Phe Glu His Lys Ser Leu Asp
20 25 30

Thr Ile Gln Lys Glu Trp Met Glu Trp Lys Arg Thr Asp His Ser Leu
35 40 45

Tyr Val Ala Pro Val Val Gly Thr Val Ser Ser Phe Leu Leu Lys Lys
50 55 60

Val Gly Ser Leu Ile Gly Lys Arg Ile Leu Ser Glu Leu Trp Gly Ile
65 70 75 80

Ile Phe Pro Ser Gly Ser Thr Asn Leu Met Gln Asp Ile Leu Arg Glu
85 90 95

Thr Glu Gln Phe Leu Asn Gln Arg Leu Asn Thr Asp Thr Leu Ala Arg
100 105 110

[0010] Val Asn Ala Glu Leu Ile Gly Leu Gln Ala Asn Ile Arg Glu Phe Asn
115 120 125

Gln Gln Val Asp Asn Phe Leu Asn Pro Thr Gln Asn Pro Val Pro Leu
130 135 140

Ser Ile Thr Ser Ser Val Asn Thr Met Gln Gln Leu Phe Leu Asn Arg
145 150 155 160

Leu Pro Gln Phe Gln Ile Gln Gly Tyr Gln Leu Leu Leu Leu Pro Leu
165 170 175

Phe Ala Gln Ala Ala Asn Met His Leu Ser Phe Ile Arg Asp Val Ile
180 185 190

Leu Asn Ala Asp Glu Trp Gly Ile Ser Ala Ala Thr Leu Arg Thr Tyr
195 200 205

Arg Asp Tyr Leu Arg Asn Tyr Thr Arg Asp Tyr Ser Asn Tyr Cys Ile
210 215 220

Asn Thr Tyr Gln Thr Ala Phe Arg Gly Leu Asn Thr Arg Leu His Asp
225 230 235 240

Met Leu Glu Phe Arg Thr Tyr Met Phe Leu Asn Val Phe Glu Tyr Val
245 250 255
Ser Ile Trp Ser Leu Phe Lys Tyr Gln Ser Leu Met Val Ser Ser Gly
260 265 270
Ala Asn Leu Tyr Ala Ser Gly Ser Gly Pro Gln Gln Thr Gln Ser Phe
275 280 285
Thr Ala Gln Asn Trp Pro Phe Leu Tyr Ser Leu Phe Gln Val Asn Ser
290 295 300
Asn Tyr Ile Leu Ser Gly Ile Ser Gly Thr Arg Leu Ser Ile Thr Phe
305 310 315 320
Pro Asn Ile Gly Gly Leu Pro Gly Ser Thr Thr Thr His Ser Leu Asn
325 330 335
Ser Ala Arg Val Asn Tyr Ser Gly Gly Val Ser Ser Gly Leu Ile Gly
340 345 350
Ala Thr Asn Leu Asn His Asn Phe Asn Cys Ser Thr Val Leu Pro Pro
[0011] 355 360 365
Leu Ser Thr Pro Phe Val Arg Ser Trp Leu Asp Ser Gly Thr Asp Arg
370 375 380
Glu Gly Val Ala Thr Ser Thr Asn Trp Gln Thr Glu Ser Phe Gln Thr
385 390 395 400
Thr Leu Ser Leu Arg Cys Gly Ala Phe Ser Ala Arg Gly Asn Ser Asn
405 410 415
Tyr Phe Pro Asp Tyr Phe Ile Arg Asn Ile Ser Gly Val Pro Leu Val
420 425 430
Ile Arg Asn Glu Asp Leu Thr Arg Pro Leu His Tyr Asn Gln Ile Arg
435 440 445
Asn Ile Glu Ser Pro Ser Gly Thr Pro Gly Gly Ala Arg Ala Tyr Leu
450 455 460
Val Ser Val His Asn Arg Lys Asn Asn Ile Tyr Ala Ala Asn Glu Asn
465 470 475 480
Gly Thr Met Ile His Leu Ala Pro Glu Asp Tyr Thr Gly Phe Thr Ile

485 490 495

Ser Pro Ile His Ala Thr Gln Val Asn Asn Gln Thr Arg Thr Phe Ile

500 505 510

Ser Glu Lys Phe Gly Asn Gln Gly Asp Ser Leu Arg Phe Glu Gln Ser

515 520 525

Asn Thr Thr Ala Arg Tyr Thr Leu Arg Val Asn Gly Asn Ser Tyr Asn

530 535 540

Leu Tyr Leu Arg Val Ser Ser Ile Gly Asn Ser Thr Ile Arg Val Thr

545 550 555 560

Ile Asn Gly Arg Val Tyr Thr Val Ser Asn Val Asn Thr Thr Thr Asn

565 570 575

Asn Asp Gly Val Asn Asp Asn Gly Ala Arg Phe Ser Asp Ile Asn Ile

580 585 590

Gly Asn Ile Val Ala Ser Asp Asn Thr Asn Val Thr Leu Asp Ile Asn

595 600 605

[0012] Val Thr Leu Asn Ser Gly Thr Pro Phe Asp Leu Met Asn Ile Met Phe

610 615 620

Val Pro Thr Asn Leu Ser Pro Leu Tyr

625 630

<210> 5

<211> 633

<212> PRT

<213> 苏云金芽孢杆菌(Bacillus thuringiensis)

<400> 5

Met Asn Ser Val Leu Asn Ser Gly Arg Thr Thr Ile Cys Asp Ala Tyr

1 5 10 15

Asn Val Ala Ala His Asp Pro Phe Ser Phe Gln His Lys Ser Leu Asp

20 25 30

Thr Val Gln Lys Glu Trp Thr Glu Trp Lys Lys Asn Asn His Ser Leu

35 40 45

Tyr Leu Asp Pro Ile Val Gly Thr Val Ala Ser Phe Leu Leu Lys Lys

50 55 60

Val Gly Ser Leu Val Gly Lys Arg Ile Leu Ser Glu Leu Arg Asn Leu

65 70 75 80

Ile Phe Pro Ser Gly Ser Thr Asn Leu Met Gln Asp Ile Leu Arg Glu

85 90 95

Thr Glu Lys Phe Leu Asn Gln Arg Leu Asn Thr Asp Thr Val Ala Arg

100 105 110

Val Asn Ala Glu Leu Thr Gly Leu Gln Ala Asn Val Glu Glu Phe Asn

115 120 125

Arg Gln Val Asp Asn Phe Leu Asn Pro Asn Arg Asn Ala Val Pro Leu

130 135 140

Ser Ile Thr Ser Ser Val Asn Thr Met Gln Gln Leu Phe Leu Asn Arg

145 150 155 160

[0013] Leu Pro Gln Phe Gln Met Gln Gly Tyr Gln Leu Leu Leu Leu Pro Leu

165 170 175

Phe Ala Gln Ala Ala Asn Leu His Leu Ser Phe Ile Arg Asp Val Ile

180 185 190

Leu Asn Ala Asp Glu Trp Gly Ile Ser Ala Ala Thr Leu Arg Thr Tyr

195 200 205

Arg Asp Tyr Leu Lys Asn Tyr Thr Arg Asp Tyr Ser Asn Tyr Cys Ile

210 215 220

Asn Thr Tyr Gln Ser Ala Phe Lys Gly Leu Asn Thr Arg Leu His Asp

225 230 235 240

Met Leu Glu Phe Arg Thr Tyr Met Phe Leu Asn Val Phe Glu Tyr Val

245 250 255

Ser Ile Trp Ser Leu Phe Lys Tyr Gln Ser Leu Leu Val Ser Ser Gly

260 265 270

Ala Asn Leu Tyr Ala Ser Gly Ser Gly Pro Gln Gln Thr Gln Ser Phe

275 280 285

Thr Ser Gln Asp Trp Pro Phe Leu Tyr Ser Leu Phe Gln Val Asn Ser
290 295 300
Asn Tyr Val Leu Asn Gly Phe Ser Gly Ala Arg Leu Ser Asn Thr Phe
305 310 315 320
Pro Asn Ile Val Gly Leu Pro Gly Ser Thr Thr Thr His Ala Leu Leu
325 330 335
Ala Ala Arg Val Asn Tyr Ser Gly Gly Ile Ser Ser Gly Asp Ile Gly
340 345 350
Ala Ser Pro Phe Asn Gln Asn Phe Asn Cys Ser Thr Phe Leu Pro Pro
355 360 365
Leu Leu Thr Pro Phe Val Arg Ser Trp Leu Asp Ser Gly Ser Asp Arg
370 375 380
Glu Gly Val Ala Thr Val Thr Asn Trp Gln Thr Glu Ser Phe Glu Thr
385 390 395 400
Thr Leu Gly Leu Arg Ser Gly Ala Phe Thr Ala Arg Gly Ile Ser Asn

[0014]

405 410 415
Tyr Phe Pro Asp Tyr Phe Ile Arg Asn Ile Ser Gly Val Pro Leu Val
420 425 430
Val Arg Asn Glu Asp Leu Arg Arg Pro Leu His Tyr Asn Glu Ile Arg
435 440 445
Asn Ile Ala Ser Pro Ser Gly Thr Pro Gly Gly Ala Arg Ala Tyr Met
450 455 460
Val Ser Val His Asn Arg Lys Asn Asn Ile His Ala Val His Glu Asn
465 470 475 480
Gly Ser Met Ile His Leu Ala Pro Asn Asp Tyr Thr Gly Phe Thr Ile
485 490 495
Ser Pro Ile His Ala Thr Gln Val Asn Asn Gln Thr Arg Thr Phe Ile
500 505 510
Ser Glu Lys Phe Gly Asn Gln Gly Asp Ser Leu Arg Phe Glu Gln Asn
515 520 525
Asn Thr Thr Ala Arg Tyr Thr Leu Arg Gly Asn Gly Asn Ser Tyr Asn

530 535 540

Leu Tyr Leu Arg Val Ser Ser Ile Gly Asn Ser Thr Ile Arg Val Thr
545 550 555 560

Ile Asn Gly Arg Val Tyr Thr Ala Thr Asn Val Asn Thr Thr Thr Asn
565 570 575

Asn Asp Gly Val Asn Asp Asn Gly Ala Arg Phe Ser Asp Ile Asn Ile
580 585 590

Gly Asn Val Val Ala Ser Ser Asn Ser Asp Val Pro Leu Asp Ile Asn
595 600 605

Val Thr Leu Asn Ser Gly Thr Gln Phe Asp Leu Met Asn Ile Met Leu
610 615 620

Val Pro Thr Asn Leu Ser Pro Leu Tyr
625 630

<210> 6

[0015] <211> 632

<212> PRT

<213> 苏云金芽孢杆菌(Bacillus thuringiensis)

<400> 6

Met Asn Ser Val Leu Asn Ser Gly Arg Ala Thr Asn Gly Asp Ala Tyr
1 5 10 15

Asn Val Val Ala His Asp Pro Phe Ser Phe Gln His Lys Ser Leu Asp
20 25 30

Thr Ile Gln Glu Glu Trp Met Glu Trp Lys Lys Asp Asn His Ile Leu
35 40 45

Tyr Val Asp Pro Ile Val Gly Thr Val Ala Ser Phe Leu Leu Lys Lys
50 55 60

Val Gly Ser Leu Val Glu Lys Arg Ile Leu Ser Glu Leu Arg Asn Leu
65 70 75 80

Ile Phe Pro Ser Gly Ser Thr Asn Leu Met Gln Asp Ile Leu Arg Glu

85 90 95

Thr Glu Lys Phe Leu Asn Gln Arg Leu Asn Thr Asp Thr Leu Ala Arg
100 105 110

Val Asn Ala Glu Leu Thr Gly Leu Gln Ala Asn Val Glu Glu Phe Asn
115 120 125

Arg Gln Val Asp Asn Phe Leu Asn Pro Asn Arg Asn Ala Val Pro Leu
130 135 140

Ser Ile Thr Ser Ser Val Asn Thr Met Gln Gln Leu Phe Leu Asn Arg
145 150 155 160

Leu Pro Gln Phe Gln Met Gln Gly Tyr Gln Leu Leu Leu Leu Pro Leu
165 170 175

Phe Ala Gln Ala Ala Asn Leu His Leu Ser Phe Ile Arg Asp Val Ile
180 185 190

Leu Asn Ala Asp Glu Trp Gly Ile Ser Ala Ala Thr Leu Arg Thr Tyr
195 200 205

[0016] Gln Asn His Leu Arg Asn Tyr Thr Arg Glu Tyr Ser Asn Tyr Cys Ile
210 215 220

Thr Thr Tyr Gln Thr Ala Phe Arg Gly Leu Asn Thr Arg Leu His Asp
225 230 235 240

Met Leu Glu Phe Arg Thr Tyr Met Phe Leu Asn Val Phe Glu Tyr Val
245 250 255

Ser Ile Trp Ser Leu Phe Lys Tyr Gln Ser Leu Leu Val Ser Ser Gly
260 265 270

Ala Asn Leu Tyr Ala Ser Gly Ser Gly Pro Gln Gln Thr Gln Ser Phe
275 280 285

Thr Ser Gln Asp Trp Pro Phe Leu Tyr Ser Leu Phe Gln Val Asn Ser
290 295 300

Asn Tyr Val Leu Asn Gly Phe Ser Gly Ala Arg Leu Thr Gln Thr Phe
305 310 315 320

Pro Asn Ile Val Gly Leu Pro Gly Thr Thr Thr Thr His Ala Leu Leu
325 330 335

Ala Ala Arg Val Asn Tyr Ser Gly Gly Val Ser Ser Gly Asp Ile Gly
340 345 350
Ala Val Phe Asn Gln Asn Phe Ser Cys Ser Thr Phe Leu Pro Pro Leu
355 360 365
Leu Thr Pro Phe Val Arg Ser Trp Leu Asp Ser Gly Ser Asp Arg Gly
370 375 380
Gly Ile Asn Thr Val Thr Asn Trp Gln Thr Glu Ser Phe Glu Thr Thr
385 390 395 400
Leu Gly Leu Arg Ser Gly Ala Phe Thr Ala Arg Gly Asn Ser Asn Tyr
405 410 415
Phe Pro Asp Tyr Phe Ile Arg Asn Ile Ser Gly Val Pro Leu Val Val
420 425 430
Arg Asn Glu Asp Leu Arg Arg Pro Leu His Tyr Asn Gln Ile Arg Asn
435 440 445
Ile Glu Ser Pro Ser Gly Thr Pro Gly Gly Leu Arg Ala Tyr Met Val

[0017]

450 455 460
Ser Val His Asn Arg Lys Asn Asn Ile Tyr Ala Val His Glu Asn Gly
465 470 475 480
Thr Met Ile His Leu Ala Pro Glu Asp Tyr Thr Gly Phe Thr Ile Ser
485 490 495
Pro Ile His Ala Thr Gln Val Asn Asn Gln Thr Arg Thr Phe Ile Ser
500 505 510
Glu Lys Phe Gly Asn Gln Gly Asp Ser Leu Arg Phe Glu Gln Ser Asn
515 520 525
Thr Thr Ala Arg Tyr Thr Leu Arg Gly Asn Gly Asn Ser Tyr Asn Leu
530 535 540
Tyr Leu Arg Val Ser Ser Ile Gly Asn Ser Thr Ile Arg Val Thr Ile
545 550 555 560
Asn Gly Arg Val Tyr Thr Ala Ser Asn Val Asn Thr Thr Thr Asn Asn
565 570 575
Asp Gly Val Asn Asp Asn Gly Ala Arg Phe Ser Asp Ile Asn Ile Gly

580 585 590

Asn Val Val Ala Ser Asp Asn Thr Asn Val Pro Leu Asp Ile Asn Val

595 600 605

Thr Leu Asn Ser Gly Thr Gln Phe Glu Leu Met Asn Ile Met Phe Val

610 615 620

Pro Thr Asn Leu Ser Pro Leu Tyr

625 630

<210> 7

<211> 633

<212> PRT

<213> 苏云金芽孢杆菌(Bacillus thuringiensis)

<400> 7

Met Asn Ser Val Leu Asn Ser Gly Arg Thr Thr Ile Cys Asp Ala Tyr

[0018] 1 5 10 15

Asn Val Ala Ala His Asp Pro Phe Ser Phe Gln His Lys Ser Leu Asp

20 25 30

Thr Val Gln Lys Glu Trp Thr Glu Trp Lys Lys Asn Asn His Ser Leu

35 40 45

Tyr Leu Asp Pro Ile Val Gly Thr Val Ala Ser Phe Leu Leu Lys Lys

50 55 60

Val Gly Ser Leu Val Gly Lys Arg Ile Leu Ser Glu Leu Arg Asn Leu

65 70 75 80

Ile Phe Pro Ser Gly Ser Thr Asn Leu Met Gln Asp Ile Leu Arg Glu

85 90 95

Thr Glu Lys Phe Leu Asn Gln Arg Leu Asn Thr Asp Thr Leu Ala Arg

100 105 110

Val Asn Ala Glu Leu Thr Gly Leu Gln Ala Asn Val Glu Glu Phe Asn

115 120 125

Arg Gln Val Asp Asn Phe Leu Asn Pro Asn Arg Asn Ala Val Pro Leu

130 135 140

Ser Ile Thr Ser Ser Val Asn Thr Met Gln Gln Leu Phe Leu Asn Arg

145 150 155 160

Leu Pro Gln Phe Gln Met Gln Gly Tyr Gln Leu Leu Leu Leu Pro Leu

165 170 175

Phe Ala Gln Ala Ala Asn Leu His Leu Ser Phe Ile Arg Asp Val Ile

180 185 190

Leu Asn Ala Asp Glu Trp Gly Ile Ser Ala Ala Thr Leu Arg Thr Tyr

195 200 205

Arg Asp Tyr Leu Lys Asn Tyr Thr Arg Asp Tyr Ser Asn Tyr Cys Ile

210 215 220

Asn Thr Tyr Gln Ser Ala Phe Lys Gly Leu Asn Thr Arg Leu His Asp

225 230 235 240

Met Leu Glu Phe Arg Thr Tyr Met Phe Leu Asn Val Phe Glu Tyr Val

245 250 255

[0019] Ser Ile Trp Ser Leu Phe Lys Tyr Gln Ser Leu Leu Val Ser Ser Gly

260 265 270

Ala Asn Leu Tyr Ala Ser Gly Ser Gly Pro Gln Gln Thr Gln Ser Phe

275 280 285

Thr Ala Gln Asn Trp Pro Phe Leu Tyr Ser Leu Phe Gln Val Asn Ser

290 295 300

Asn Tyr Ile Leu Ser Gly Ile Ser Gly Thr Arg Leu Ser Ile Thr Phe

305 310 315 320

Pro Asn Ile Gly Gly Leu Pro Gly Ser Thr Thr Thr His Ser Leu Asn

325 330 335

Ser Ala Arg Val Asn Tyr Ser Gly Gly Val Ser Ser Gly Leu Ile Gly

340 345 350

Ala Thr Asn Leu Asn His Asn Phe Asn Cys Ser Thr Val Leu Pro Pro

355 360 365

Leu Ser Thr Pro Phe Val Arg Ser Trp Leu Asp Ser Gly Thr Asp Arg

370 375 380

Glu Gly Val Ala Thr Ser Thr Asn Trp Gln Thr Glu Ser Phe Gln Thr
385 390 395 400
Thr Leu Ser Leu Arg Cys Gly Ala Phe Ser Ala Arg Gly Asn Ser Asn
405 410 415
Tyr Phe Pro Asp Tyr Phe Ile Arg Asn Ile Ser Gly Val Pro Leu Val
420 425 430
Ile Arg Asn Glu Asp Leu Thr Arg Pro Leu His Tyr Asn Gln Ile Arg
435 440 445
Asn Ile Glu Ser Pro Ser Gly Thr Pro Gly Gly Ala Arg Ala Tyr Leu
450 455 460
Val Ser Val His Asn Arg Lys Asn Asn Ile Tyr Ala Ala Asn Glu Asn
465 470 475 480
Gly Thr Met Ile His Leu Ala Pro Glu Asp Tyr Thr Gly Phe Thr Ile
485 490 495
Ser Pro Ile His Ala Thr Gln Val Asn Asn Gln Thr Arg Thr Phe Ile

[0020]

500 505 510
Ser Glu Lys Phe Gly Asn Gln Gly Asp Ser Leu Arg Phe Glu Gln Ser
515 520 525
Asn Thr Thr Ala Arg Tyr Thr Leu Arg Gly Asn Gly Asn Ser Tyr Asn
530 535 540
Leu Tyr Leu Arg Val Ser Ser Ile Gly Asn Ser Thr Ile Arg Val Thr
545 550 555 560
Ile Asn Gly Arg Val Tyr Thr Val Ser Asn Val Asn Thr Thr Thr Asn
565 570 575
Asn Asp Gly Val Asn Asp Asn Gly Ala Arg Phe Ser Asp Ile Asn Ile
580 585 590
Gly Asn Ile Val Ala Ser Asp Asn Thr Asn Val Thr Leu Asp Ile Asn
595 600 605
Val Thr Leu Asn Ser Gly Thr Pro Phe Asp Leu Met Asn Ile Met Phe
610 615 620
Val Pro Thr Asn Leu Pro Pro Leu Tyr

625 630

<210> 8

<211> 1899

<212> DNA

<213> 苏云金芽孢杆菌(Bacillus thuringiensis)

<400> 8

[0021]

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catgatccat ttagttttca acacaaatca ttagataccg tacaaaagga atggacggag 120
tggaaaaaaa ataatcatag tttataccta gatcctattg ttggaactgt ggctagtttt 180
ctgttaaaga aagtggggag tcttgttgga aaaaggatac taagtgagtt acggaattta 240
atatttccta gtggtagtac aaatctaatg caagatattt taagagagac agaaaaattc 300
ctgaatcaaa gacttaatac agacactctt gcccgtgtaa atgcggaatt gacagggctg 360
caagcaaatg tagaagagtt taatcgacaa gtagataatt ttttgaaccc taaccgaaac 420
gctgttcctt tatcaataac ttcttcagtt aatacaatgc aacaattatt tctaaataga 480
ttaccccagt tccagatgca aggataccaa ctgttattat tacctttatt tgcacaggca 540
gccaatttac atctttcttt tattagagat gttattctaa atgcagatga atggggaatt 600
tcagcagcaa cattacgtac gtatcgagat tacttgaaaa attatacaag agattactct 660
aactattgta taaatacgta tcaaagtgcg tttaaaggtt taaacactcg tttacacgat 720
atgttagaat ttagaacata tatgttttta aatgtatttg agtatgtatc tatctggtcg 780
ttgtttaaat atcaaagtct tctagtatct tccggtgcta atttatatgc aagtggtagt 840
ggaccacagc agacacaatc atttacagca caaaactggc atttttata ttctcttttc 900
caagttaatt cgaattatat attatctggt attagtggta ctaggctttc tattaccttc 960
cctaatattg gtggtttacc gggtagtact acaactcatt cattgaatag tgccagggtt 1020
aattatagcg gaggagtttc atctggtctc ataggggcga ctaatctcaa tcacaacttt 1080
aattgcagca cggtcctccc tcctttatca acaccatttg ttagaagttg gctggattca 1140
ggtacagatc gagagggcgt tgctacctct acgaattggc agacagaatc ctttcaaaca 1200
actttaagtt taaggtgtgg tgctttttca gcccgtggaa attcaaacta tttcccagat 1260
tattttatcc gtaatatttc tggggttcct ttagttatta gaaacgaaga tctaacaaga 1320
ccgttacact ataaccaaat aagaaatata gcaagtcctt cgggaacacc tggtggagca 1380

cgggcctatt tggtatctgt gcataacaga aaaaataata tctatgccgc taatgaaaat 1440

ggtactatga tccatttggc gccagaagat tatacaggat ttactatatc gccaatacat 1500

gccactcaag tgaataatca aactcgaaca tttatttctg aaaaatttgg aaatcaaggt 1560

gattccttaa gatttgaaca aagcaacacg acagctcgtt atacgcttag agggaatgga 1620

aatagttaca atctttattt aagagtatct tcaataggaa attcaactat tcgagttact 1680

ataaacggta gagtttatac tgtttcaaat gttaatacca ctacaaataa cgatggagtt 1740

aatgataatg gagctcgttt ttcagatatt aatatcggta atatagtagc aagtgataat 1800

actaatgtaa cgctagatat aaatgtgaca ttaaactccg gtactccatt tgatctcatg 1860

aatattatgt ttgtgccaac taatctttca ccactttat 1961

<210> 9

<211> 1899

<212> DNA

<213> 苏云金芽孢杆菌(Bacillus thuringiensis)

[0022] <400> 9

atgaatagtg tattgaatag cggaagaact actatttgtg atgcgtataa tgtagcggct 60

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tggaaaaaaa ataatcatag tttataccta gatcctattg ttggaactgt ggctagtttt 180

ctgttaaaga aagtggggag tcttgttgga aaaaggatac taagtgagtt acggaattta 240

atatttccta gtggtagtac aaatctaatg caagatattt taagagagac agaaaaattc 300

ctgaatcaaa gacttaatac agacactctt gcccgtgtaa atgcggaatt gacagggctg 360

caagcaaatg tagaagagtt taatcgacaa gtagataatt ttttgaaccc taaccgaaac 420

gctgttcctt tatcaataac ttcttcagtt aatacaatgc aacaattatt tctaaataga 480

ttaccccagt tccagatgca aggataccaa ctgttattat tacctttatt tgcacaggca 540

gccaatttac atctttcttt tattagagat gttattctaa atgcagatga atggggaatt 600

tcagcagcaa cattacgtac gtatcgagat tacttgaaaa attatacaag agattactct 660

aactattgta taaatacgta tcaaagtgcg tttaaaggtt taaacactcg tttacacgat 720

atgttagaat ttagaacata tatgttttta aatgtatttg agtatgtatc tatctggtcg 780

ttgtttaaat atcaaagtct tctagtatct tccggtgcta atttatatgc aagtggtagt 840

ggaccacagc agacccaatc atttacttca caagactggc catttttata ttctcttttc 900

caagttaatt caaattatgt gttaaatgga tttagtggtg ctaggctttc taataccttc 960

cctaatatag ttggtttacc tggttctact acaactcacg cattgcttgc tgcaagggtt 1020

aattacagtg gaggaatttc gtctggtgat ataggtgcat ctccgtttaa tcaaaatttt 1080

aattgtagca catttctccc cccattgtta acgccatttg ttaggagttg gctagattca 1140

ggttcagatc gggagggcgt tgccaccgtt acaaattggc aaacagaatc ctttgagaca 1200

actttagggt taaggagtgg tgcttttaca gctcgcggta attcaaacta tttcccagat 1260

tattttattc gtaatatttc tggagttcct ttagttgtta gaaatgaaga tttaagaaga 1320

ccgttacact ataatgaaat aagaaatata gcaagtcctt caggaacacc tggtggagca 1380

cgagcttata tggtatctgt gcataacaga aaaaataata tccatgctgt tcatgaaaat 1440

ggttctatga ttcatttagc gccaaatgac tatacaggat ttactatttc gccgatacat 1500

gcaactcaag tgaataatca aacacgaaca tttatttctg aaaaatttgg aaatcaaggt 1560

gattctttaa ggtttgaaca aaacaacacg acagctcgtt atacgcttag agggaatgga 1620

aatagttaca atctttattt aagagtatct tcaataggaa attcaactat tcgagttact 1680

ataaacggta gagtttatac tgtttcaaat gttaatacca ctacaaataa cgatggagtt 1740

aatgataatg gagctcgttt ttcagatatt aatatcggta atatagtagc aagtgataat 1800

[0023] actaatgtaa cgctagatat aaatgtgaca ttaaactccg gtactccatt tgatctcatg 1860

aatattatgt ttgtgccaac taatctttca ccactttat 1961

<210> 10

<211> 1872

<212> DNA

<213> 苏云金芽孢杆菌(Bacillus thuringiensis)

<400> 10

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catgatccat ttagttttga gcataaatca ttagatacca tacagcaaga atggatggag 120

tggaaaagaa ccgatcatag tttatatgta tctcctattg tgggaactat agctagtttt 180

ccgctaaaga aagtagcggg gcttatagga aaaagaatat taagtgagtt aaagaattta 240

atttttccta gtggtagtat agaatcaatg caagatattt taagaggggc agaacaattc 300

ctaaatcaaa gacttgatgc agacaccttt gctcgggtag aggcagaatt gatagggctt 360

caagcaaatg tagaggaatt taatcaacaa gtggacaatt ttttaaaccc aaatcaaaac 420

cctgttcctt tagcaataat tgattcggtt aatacaatgc aacaattatt cctaagtaga 480

ttaccccagt tccagataca acgctatcag ctattattat tacctttatt tgcacaagca 540

gccaatttac accttacctt tattagagat gttattctta atgcagatga atggggaata 600

ccagcagcaa cagtgcgcac atatagagag cacctaaaaa gatatacacg cgattattcc 660

aattattgta taaacacgta ccaaactgct ttccgaggtt taaacactcg tttacatgat 720

atgttagagt ttagaacatt tatgttttta aatgtattag actatgtatc tatctggtcg 780

ttgtttaaat atcaaagtct gatggttact tcaagtgcta atttatatgc ttcgggaagt 840

ggtagtaatc aacctttac tgcacaagac tggccatttt tatattctct tttccaagtg 900

aattcaaatt atataatgtc taattttggt ggtaaccgag agactgctag ttttggtgtt 960

cctattctgg ggggattcat aataaatttt ttacttagtt ttagggttaa ttatactgga 1020

ggagtttcat ctggtctcct aggtgttgaa ggaatttcaa acaactttaa ttgcaactcc 1080

tctttatcaa caccagttgt aagaagttgg ctagattcag gtgtatatcg aggtgacctg 1140

caacacaatt ggcgaacaga catctttatg aggactaata ttgtaccttg tggtgctttt 1200

ctattatctc ttgctatgtt tccagatgtt aaaagtaatt attttcctga ttatttcatt 1260

[0024] cgtaacattt ccggaattat tcgaaatatt gataacatga atttgagtag accattacac 1320

tttaatgaag taagagattt aagagacact gaagttgcta ctttagtatc tgtgcataat 1380

agaaaaaata atatctatgc tgctcatgaa aatggtacta tgattcattt tgcgccggaa 1440

ggttatatag gtttcacaat atcaccaata tatgcaactc aagtaaataa tcaaacacga 1500

acgtttattt ctgaaaaatt cggaaatcaa ggtgattcct tgagatttga acaaactaac 1560

acaacggctc gttatacgtt tagagggaat ggtaataatt ataatcttta tttaagagta 1620

tcctcacaag gaaattctac ttttcgagtt actataaacg gtagggttta tactgtttca 1680

aatgttaata ccactacaaa taatgatggg gttattgata atggggctcg tttttcagat 1740

attcacatcg ggaatatagt ggcaagtaac aatactaatg taccattaga tataaatgtg 1800

atacttaact ccggtactca atttgagctt atgaatatta tttttgttcc aactaatctt 1860

tcaccacttt at 1934

<210> 11

<211> 1899

<212> DNA

<213> 苏云金芽孢杆菌(Bacillus thuringiensis)

<400> 11

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tggaaaagaa cagatcatag tttatatgta gctcctgtag tcggaactgt gtctagtttt 180

ttgctaaaga aagtggggag tcttattgga aaaaggatat tgagtgaatt atgggggata 240

atatttccta gtggtagtac aaatctaatg caagatattt taagggagac agaacaattc 300

ctaaatcaaa gacttaatac agataccctt gctcgtgtaa atgcagaatt gatagggctc 360

caagcgaata taagggagtt taatcaacaa gtagataatt ttttaaaccc tactcaaaac 420

cctgttcctt tatcaataac ttcttcggtt aatacaatgc agcaattatt tctaaataga 480

ttaccccagt tccagataca aggataccag ttgttattat tacctttatt tgcacaggca 540

gccaatatgc atctttcttt tattagagat gttattctta atgcagatga atggggtatt 600

tcagcagcaa cattacgtac gtatcgagat tacctgagaa attatacaag agattattct 660

aattattgta taaatacgta tcaaactgcg tttagagggt taaacacccg tttacacgat 720

atgttagaat ttagaacata tatgttttta aatgtatttg aatatgtatc catttggtca 780

ttgtttaaat atcagagtct tatggtatct tctggcgcta atttatatgc tagcggtagt 840

[0025] ggaccacagc agacacaatc atttacagca caaaactggc catttttata ttctcttttc 900

caagttaatt cgaattatat attatctggt attagtggta ctaggctttc tattaccttc 960

cctaatattg gtggtttacc gggtagtact acaactcatt cattgaatag tgccagggtt 1020

aattatagcg gaggagtttc atctggtctc ataggggcga ctaatctcaa tcacaacttt 1080

aattgcagca cggtcctccc tcctttatca acaccatttg ttagaagttg gctggattca 1140

ggtacagatc gagagggcgt tgctacctct acgaattggc agacagaatc ctttcaaaca 1200

actttaagtt taaggtgtgg tgctttttca gcccgtggaa attcaaacta tttcccagat 1260

tattttatcc gtaatatttc tggggttcct ttagttatta gaaacgaaga tctaacaaga 1320

ccgttacact ataaccaaat aagaaatata gaaagtcctt cgggaacacc tggtggagca 1380

cgggcctatt tggtatctgt gcataacaga aaaaataata tctatgccgc taatgaaaat 1440

ggtactatga tccatttggc gccagaagat tatacaggat ttactatatc gccaatacat 1500

gccactcaag tgaataatca aactcgaaca tttatttctg aaaaatttgg aaatcaaggt 1560

gattccttaa gatttgaaca aagcaacacg acagctcgtt atacgcttag agtgaatgga 1620

aatagttaca atctttattt aagagtatct tcaataggaa attcaactat tcgagttact 1680

ataaacggta gagtttatac tgtttcaaat gttaatacca ctacaaataa cgatggagtt 1740

aatgataatg gagctcgttt ttcagatatt aatatcggta atatagtagc aagtgataat 1800

actaatgtaa cgctagatat aaatgtgaca ttaaactccg gtactccatt tgatctcatg 1860

aatattatgt ttgtgccaac taatctttca ccactttat 1961

<210> 12

<211> 1899

<212> DNA

<213> 苏云金芽孢杆菌(Bacillus thuringiensis)

<400> 12

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<210> 13

<211> 1896

<212> DNA

<213> 苏云金芽孢杆菌(Bacillus thuringiensis)

[0027]

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<211> 1899

<212> DNA

<213> 苏云金芽孢杆菌(Bacillus thuringiensis)

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