(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 September 2008 (12.09.2008)

PCT

(10) International Publication Number
WO 2008/108805 A2

(51) International Patent Classification: Not classified

(21) International Application Number:
PCT/US2007/016357

(22) International Filing Date: 19 July 2007 (19.07.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/831,821 19 July 2006 (19.07.2006) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

(54) **Title:** ATTENUATED FNR DEFICIENT ENTEROBACTERIA

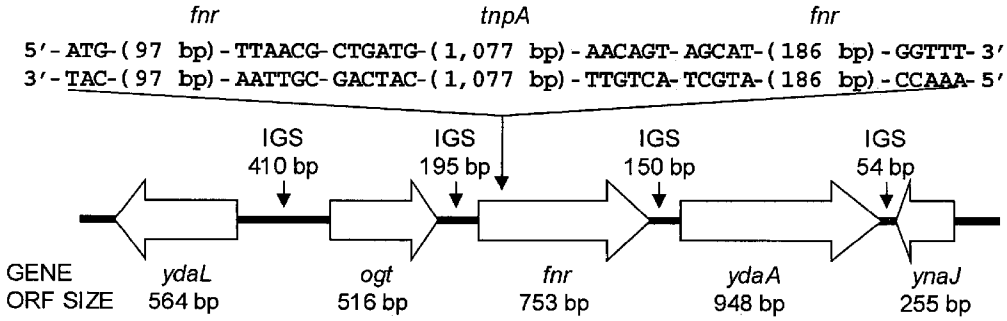

FIG. 1

(57) **Abstract:** The invention provides an attenuated enterobacterium comprising an attenuating mutation in the *fnr* gene, and optionally further comprising a heterologous nucleic acid encoding a foreign antigen. Also provided are pharmaceutical formulations comprising the attenuated enterobacteria of the invention. Further disclosed are methods of inducing an immune response in a subject by administration of an immunogenically effective amount of an attenuated enterobacterium or pharmaceutical formulation of the invention.

WO 2008/108805 A2

ATTENUATED FNR DEFICIENT ENTEROBACTERIA

CROSS REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application No. 60/831,821, filed July 19, 2006, the disclosure of which is incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

This invention relates to attenuated Fumarate-Nitrate Reductase (FNR) enterobacteria strains. In particular, this invention relates to attenuated FNR enterobacteria strains and methods of using the same to induce an immune response.

BACKGROUND OF THE INVENTION

Salmonella enterica serovar Typhimurium is a gram-negative facultative intracellular pathogen. Serovar Typhimurium infections usually result from ingestion of contaminated food or water. The organism generally targets and colonizes the intestinal epithelium of the host and causes gastroenteritis (*i.e.*, salmonellosis). During a *Salmonella* infection, the growth phase and growth conditions of the organism are important in attachment, invasion, and the regulation of many of the virulence genes. Cells grown under limited oxygen concentrations are more invasive and adhere better to mammalian cells than do aerobically grown or stationary-phase cells. *Salmonella* invasion genes have been identified and localized. During infection, serovar Typhimurium must adapt to changes in $[O_2]$ encountered in the gastrointestinal tract of the host. In *Escherichia coli*, transitions from aerobic to anaerobic environments or vice versa, involve changes in a large number of genes. However, upon sudden reappearance of oxygen, these cellular processes must be reversed in a precise and orderly fashion to ensure the safe transition to the oxygenated environment. This complex regulatory system has been extensively studied in *E. coli*, where the DNA-binding protein FNR encoded by *fnr*, senses changes in $[O_2]$ and controls the

expression of the different genes either alone or in cooperation with other regulators, *e.g.*, ArcA.

SUMMARY OF THE INVENTION

The present invention is based, in part, on the inventors' discovery that enterobacteria comprising an attenuating mutation in the *fnr* (Fumarate-Nitrate Reductase) gene have an avirulent (*i.e.*, highly attenuated) phenotype. Thus, the present invention provides attenuated enterobacteria and methods of using the same as attenuated immunogenic compositions, attenuated vaccines and/or as attenuated vaccine vectors to induce an immune response against a heterologous antigen in a subject.

Accordingly, a first aspect of the invention provides a pharmaceutical composition comprising an attenuated enterobacterium comprising an attenuating mutation (*e.g.*, deletion) in the *fnr* gene in a pharmaceutically acceptable carrier.

A further aspect of the invention provides an attenuated enterobacterium comprising an attenuating mutation (*e.g.*, a deletion) in the *fnr* gene and, optionally, further comprising a heterologous nucleic acid sequence encoding a foreign antigen. In particular embodiments, the attenuated enterobacterium is present in a pharmaceutical composition in a pharmaceutically acceptable carrier.

A further aspect of the present invention is a method of inducing an immune response in a subject comprising administering to the subject an immunogenically effective amount of an attenuated enterobacterium comprising an attenuating mutation (*e.g.*, a deletion) in the *fnr* gene. In embodiments of the invention, the attenuated enterobacterium is provided in a pharmaceutical composition further comprising a pharmaceutically acceptable carrier. In further embodiments of the invention, the attenuated enterobacterium comprises a heterologous nucleic acid encoding a foreign antigen.

The invention further provides for the use of an attenuated enterobacterium or pharmaceutical composition of the invention to induce an immune response in a subject.

These and other aspects of the invention are set forth in more detail in the following description of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the location of the *tnpA* insertion (between bp 106 and 107) in the *fnr* gene. WT *fnr* sequences are in bold, and the sequences of the beginning and ending junctions of the *tnpA* insert are in italics. Arrows indicate the direction of transcription. IGS, intergenic spacer region. (Complete DNA sequences [*i.e.*, *ogt*, *tnpA/fnr* junctions, and *ydaA*] are available at GenBank accession number AH015911.)

Figure 2 shows a logo graph of the information matrix obtained from the consensus alignment of FNR motif sequences for serovar Typhimurium (derived from the corresponding FNR-regulated genes in *E. coli*). The total height of each column of characters represents the amount of information for that specific position, and the height of each character represents the frequency of each nucleotide.

Figure 3 shows the correlation between the microarray and qRT-PCR data for 19 selected genes. The ratios of changes in gene expression, from the microarray and qRT-PCR experiments, for the FNR mutant relative to the WT were $\log_2$ transformed and linearly correlated.

Figure 4 shows a scheme representing the structural organization of the major genes involved in virulence/SPI-1 (A), ethanolamine utilization (B), and flagellar biosynthesis and motility/swarming (C to E). The names of genes are listed to the right of the arrows, an asterisk next to the gene indicates the presence of at least one FNR motif in the 5' region, and the numbers to the left of the arrows indicate the ratio of gene expression in the *fnr* mutant relative to that in the WT.

Figure 5 shows a comparison of the *fnr* mutant and the WT strain for virulence in 6- to 8-week-old C57BL/6 mice. (**A**) Groups of 10 mice were inoculated p.o. with 5×10^6 and 5×10^7 CFU/mouse. (**B**) Groups of five mice

were challenged i.p. with 250 CFU/mouse, as described. Percent survival is the number of mice surviving relative to the number of mice challenged at time zero.

Figure 6 shows a comparison of the WT, the *fnr* mutant, and the mutant strain harboring pfnr for survival inside peritoneal macrophages from C57BL/6 mice. The macrophages were harvested and treated as described. (**A**) Comparison between the *fnr* mutant and the WT strain. The number of viable cells found inside the macrophages, at time zero, following the removal of extracellular bacteria by washing/gentamicin treatment is defined as 100% survival. (**B**) Comparison between the WT, the *fnr* mutant, and the pfnr-complemented mutant. The number of viable cells found inside macrophages at 20 h is expressed as percent survival relative to that found inside macrophages at 2 h.

Figure 7 shows the virulence of the WT and the *fnr* mutant in C57BL/6 mice and congenic gp91*phox*$^{-/-}$ mice and survival of the bacteria inside peritoneal macrophages. The mice were challenged i.p. with 250 CFU/mouse, as described. (**A**) C57BL/6 and gp91*phox*$^{-/-}$ mice treated with the WT strain. (**B**) C57BL/6 and gp91*phox*$^{-/-}$ mice treated with the *fnr* mutant. (**C**) Survival of the WT and the *fnr* mutant inside macrophages from C57BL/6 and gp91*phox*$^{-/-}$ mice. The number of viable cells at 20 h is expressed as percent survival relative to that found inside the macrophages at time zero.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is explained in greater detail below. This description is not intended to be a detailed catalog of all the different ways in which the invention may be implemented, or all the features that may be added to the instant invention. For example, features illustrated with respect to one embodiment may be incorporated into other embodiments, and features illustrated with respect to a particular embodiment may be deleted from that embodiment. In addition, numerous variations and additions to the various embodiments suggested herein will be apparent to those skilled in the art in light of the instant disclosure, which do not depart from the instant

invention. Hence, the following specification is intended to illustrate some particular embodiments of the invention, and not to exhaustively specify all permutations, combinations, and variations thereof.

All publications, patents, and patent publications cited herein are incorporated by reference in their entireties for the teachings relevant to the sentence and/or paragraph in which the citation is presented.

As used herein, "a," "an" or "the" can mean one or more than one. For example, "a" mutation can mean a single mutation or a multiplicity of mutations.

As used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative ("or").

FNR (Fumarate-Nitrate Reductase) is a DNA-binding regulator protein expressed by all enterobacteria including *Salmonella* spp., *Escherichia* spp., and *Shigella* spp. The *fnr* gene was previously known as *oxrA* in *Salmonella* spp. The present inventors have identified FNR as a global regulatory protein for the expression of virulent genes in enterobacteria. An FNR deleted (Δ*fnr*) strain of a known virulent strain of *S. enterica* serovar Typhimurium was shown to be non-motile, lacking flagella, and having an avirulent phenotype. Thus, the present invention provides FNR deficient (*e.g.*, Δ*fnr* or *fnr* mutants) strains of enterobacteria that can be used to study the *fnr* gene, its role in virulence in these organisms, and can further be used as attenuated immunogenic compositions, attenuated vaccines (*e.g.*, live attenuated vaccines) and/or attenuated vaccine vectors.

Enterobacteria are known in the art and are generally pathogens that can infect the gastrointestinal tract of avians and/or mammals. The present invention can be practiced with any suitable enterobacterium in the order Enterobacteriales and optionally in the family Enterobacteriaceae that encodes FNR, including but not limited to bacteria classified in the following genera: *Alishewanella, Alterococcus, Aquamonas, Aranicola, Arsenophonus, Azotivirga, Blochmannia, Brenneria, Buchnera, Budvicia, Buttiauxella, Candidatus, Cedecea, Citrobacter, Dickeya, Edwardsiella, Enterobacter, Erwinia (e.g., E. amylovora), Escherichia, Ewingella, Grimontella, Hafnia, Klebsiella (e.g., K. pneumoniae), Kuyvera, Leclercia, Leminorella, Moellerella,*

Morganella, Obesumbacterium, Pantoea, Pectobacterium, Candidatus Phlomobacter, Photohabdus, Plesiomonas (e.g., P. shigelloides), Pragia, Proteus (e.g., P. vulgaris), Providencia, Rahnella, Raoultella, Salmonella, Samsonia, Serratia (e.g., S. marcenscens), Shigella, Sodalis, Tatumella, Travulsiella, Wigglesworthia, Xenorhabdus, Yersinia (e.g., Y. pestis), and Yokenella.

In particular embodiments, the enterobacterium is a *Salmonella* spp., an *Escherichia* spp., or a *Shigella* spp.

Further, the enterobacterium can optionally be a pathogenic enterobacterium. In particular embodiments, the enterobacterium from which the FNR deficient strain is derived is a pathogenic (*e.g.*, virulent) bacterium as that term is understood in the art, where the attenuating *fnr* mutation results in a reduction in the pathogenicity. In representative embodiments, the FNR deficient strain is highly attenuated so as to be avirulent (*e.g.*, induces no or insignificant levels of pathogenicity).

The term "pathogenic" is understood in the art, for example, as causing pathogenicity such as morbidity and/or mortality in a subject or population of subjects.

The term "attenuating" with respect to pathogenic microorganisms is understood in the art, for example, as a reduction in pathogenicity (including no detectable pathogenicity) produced in the subject as a result of administration of the FNR deficient enterobacterium strain as compared with the level of pathogenicity produced if an enterobacterium with a fully functional *fnr* gene (*e.g.*, the wild-type strain) were administered.

Methods of assessing pathogenicity of enterobacteria, and attenuation thereof, are known in the art (*e.g.*, morbidity and/or mortality following challenge in a suitable animal model such as mice or survival in cultured macrophages).

Suitable *Salmonella* species within the scope of the present invention include but are not limited to *S. bongori* and *S. enterica* as well as *S. enterica* subspecies (*e.g.*, *enterica, salamae, arizonae, diarizonae, houtenae* and *indica*). Numerous serovars of *S. bongori* and *S. enterica* are known and are within the scope of the present invention. Exemplary *S. enterica* serovars include Typhimurium, Typhi and Enteritidis.

The present invention can further be practiced with any species of *Escherichia* including but not limited to *E. adecarboxylata, E. albertii, E. blattae, E. coli* (including toxigenic strains such as *E. coli* O157:H7), *E. fergusonii, E. hermannii,* and *E. vulneris.*

Suitable species of *Shigella* include without limitation species in Serogroup A (*e.g., S. dysenteriae* and serotypes thereof), species in Serogroup B (*e.g., S. flexneri* and serotypes thereof), species in Serogroup C (*e.g., S. boydii* and serotypes thereof), and species in Serogroup D (*e.g., S. sonnei* and serotypes thereof).

The genomic sequences of numerous enterobacteria are known in the art. *See, e.g.*, NCBI Accession No. NC_004337 (*Shigella flexneri* 2a str. 301); NCBI Accession No. NC_007613 (*Shigella boydii* Sb227); NCBI Accession No. AP009048 (*E. coli* W3110); NCBI Accession No. BA000007 (*E. coli* O157:H7 str. Sakai); NCBI Accession No. AE009952 (*Y. pestis* KIM); NCBI Accession No. NC_003197 (*S. typhimurium* LT2); and NCBI Accession No. NC_003198 (*S. enterica* subsp. enterica serovar Typhi str. CT18).

Likewise, the nucleic acid and amino acid sequences of the *fnr* gene from various enterobacteria are known in the art.

The attenuated enterobacteria of the present invention comprise an attenuating mutation in the *fnr* gene. In representative embodiments, the mutation is an attenuating deletion mutation (including truncations) that results in attenuation of the pathogenicity of the bacterium. Other mutations include without limitation attenuating insertions, substitutions and/or frame-shift mutations that result in attenuation of the pathogenicity of the bacterium. In embodiments of the invention, the mutation is a non-polar alteration in the *fnr* gene.

Deletion and insertion mutations can be any deletion/insertion mutation in the *fnr* gene that results in attenuation of the pathogenicity of the bacterium. In representative embodiments, the alteration is a deletion or an insertion of at least about 9, 30, 50, 75, 90, 120, 150, 180, 240, 300, 450 or more consecutive nucleotides in the *fnr* gene that results in attenuation of the pathogenicity of the bacterium. Optionally, essentially all (*e.g.*, at least about 95%, 97%, 98% or more) or all of the *fnr* coding sequence is deleted. In other embodiments, essentially all or all of the *fnr* gene, including regulatory

elements, is deleted. In particular embodiments, the deletion can extend beyond the *fnr* gene. Generally, however, the deletion does not render genes essential for growth, multiplication and/or survival non-functional. In particular embodiments, the deletion does not extend into any genes essential for growth, multiplication and/or survival. In embodiments of the invention, the deletion does not extend to genes that are 5' and/or 3' of the *fnr* coding region or the *fnr* gene.

One FNR deficient strain of *S. enterica* serovar Typhimurium has been constructed by the inventors and is shown in the Examples.

The FNR deficient enterobacterium strains of the invention can further comprise other mutations, including other attenuating mutations.

Generally, the FNR deficient enterobacterium strains will retain other appropriate genomic sequences to be able to grow, multiply and survive (*e.g.*, in the gut of a host). Thus, the *fnr* mutations of the invention exclude lethal mutations that unduly inhibit the survival of the organism.

In embodiments of the invention, the FNR mutation results in at least about a 25%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 97%, 98% or more reduction in FNR mRNA, protein and/or activity. Methods of assessing levels of mRNA and proteins levels and FNR activity are known in the art.

The *fnr* mutation can be combined with any other mutation known in the art, including other attenuating mutations. For example, the *fnr* mutant enterobacterium can also comprise an *arcA* mutation. The ArcA protein cooperates with FNR for controlling the transitions from aerobic/anaerobic conditions and *vice versa*.

The attenuated enterobacteria of the present invention can be used as an attenuated immunogenic compositions or attenuated vaccine against enterobacteria (*e.g.*, a live attenuated vaccine). Enterobacteria are described above. In embodiments of the invention, the enterobacterium is a pathogenic enterobacterium. For example, in particular embodiments, the invention provides live immunogenic compositions or live attenuated vaccines against *Salmonella, Shigella* or *Escherichia*. The attenuated enterobacterium vaccine can be used to induce an immune response against one species or against multiple closely related species/genera of enterobacteria (*e.g.*, that cross-

react with antibodies produced in response to administration of the attenuated enterobacterium).

Further, the attenuated FNR deficient enterobacterium strains of the invention can be used as vectors, *e.g.*, to deliver an antigen(s) that is heterologous (*e.g.*, foreign) to the enterobacterium vector (including any plasmids carried by the enterobacterium) to induce an immune response against other organisms (*e.g.*, pathogenic organisms). In one embodiment, a heterologous nucleic acid sequence encoding the foreign antigen(s) is incorporated into the genomic DNA of the enterobacterium (*e.g.*, inserted into or in place of a deleted *fnr* gene). In other embodiments, the heterologous nucleic acid sequence encoding the foreign antigen is incorporated into a plasmid that is carried by an attenuated FNR deficient host (*e.g.*, a Δ*fnr* host). Plasmids that are compatible with the various enterobacteria are known in the art.

The attenuated FNR deficient strains can further be used as vectors to deliver therapeutic proteins and untranslated RNAs (*e.g.*, siRNA, shRNA, antisense RNA).

Methods of expressing foreign antigens in enterobacteria are known to those skilled in the art. For example, the foreign antigen can be expressed as part of a fusion with one of the structural proteins of the bacterial host (*e.g.*, expressed on the surface of the bacterium) such as a flagellin protein (*see, e.g.*, Chauhan et al., (2005) *Molecular and Cellular Biochemistry* 276:1-6) or a membrane protein as known in the art. *See also*, Chinchilla et al., (2007) *Infection Immun.* 75: 3769. In other embodiments, the foreign antigen is not expressed as a fusion with a host structural protein. According to this embodiment, the heterologous nucleic acid encoding the foreign antigen can optionally be operably associated with a leader sequence directing secretion of the foreign antigen from the bacterial cell.

The heterologous nucleic acid sequence encoding the foreign antigen can be operatively associated with any suitable promoter or other regulatory sequence. The promoter or regulatory sequence can be native or foreign to the host, can be native or foreign to the heterologous nucleic acid, and can further be partially or completely synthetic.

The codon usage of the heterologous nucleic acid sequence can be optimized for expression in the enterobacterium using methods known to those skilled in the art (*see, e.g.*, Chinchilla et al., (2007) *Infection Immun.* 75: 3769.

The foreign antigen can be any suitable antigen known in the art, and can further be from a bacterial, yeast, fungal, protozoan or viral source. Suitable antigens include, but are not limited to antigens from pathogenic infectious agents.

The antigen can be an antigen from a pathogenic microorganism, which includes but is not limited to, *Rickettsia, Chlamydia, Mycobacteria, Clostridia, Corynebacteria, Mycoplasma, Ureaplasma, Legionella, Shigella, Salmonella,* pathogenic *Escherichia coli* species, *Bordatella, Neisseria, Treponema, Bacillus, Haemophilus, Moraxella, Vibrio, Staphylococcus* spp., *Streptococcus* spp., *Campylobacter* spp., *Borrelia* spp., *Leptospira* spp., *Erlichia* spp., *Klebsiella* spp., *Pseudomonas* spp., *Helicobacter* spp., and any other pathogenic microorganism now known or later identified (see, *e.g.*, Microbiology, Davis et al, Eds., 4th ed., Lippincott, New York, 1990, the entire contents of which are incorporated herein by reference for the teachings of pathogenic microorganisms).

Specific examples of microorganisms from which the antigen can be obtained include, but are not limited to, *Helicobacter pylori, Chlamydia pneumoniae, Chlamydia trachomatis, Ureaplasma urealyticum, Mycoplasma pneumoniae, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, Streptococcus viridans, Enterococcus faecalis, Neisseria meningitidis, Neisseria gonorrhoeae, Treponema pallidum, Bacillus anthracis, Salmonella typhi, Vibrio cholera, Pasteurella pestis, Pseudomonas aeruginosa, Campylobacter jejuni, Clostridium difficile, Clostridium tetani, Clostridium botulinum, Mycobacterium tuberculosis, Borrelia burgdorferi, Haemophilus ducreyi, Corynebacterium diphtheria, Bordetella pertussis, Bordetella parapertussis, Bordetella bronchiseptica, Haemophilus influenza,* and enterotoxic *Escherichia coli.*

The antigen can further be an antigen from a pathogenic protozoa, including, but not limited to, *Plasmodium* spp. (*e.g.*, malaria antigens), *Babeosis* spp., *Schistosoma* spp., *Trypanosoma* spp., *Pneumocystis carnii,*

Toxoplasma spp., *Leishmania* spp., and any other protozoan pathogen now known or later identified.

The antigen can also be an antigen from pathogenic yeast and fungi, including, but not limited to, *Aspergillus* spp., *Candida* spp., *Cryptococcus* spp., *Histoplasma* spp., *Coccidioides* spp., and any other pathogenic fungus now known or later identified.

Suitable antigens can include, but are not limited to, viral antigens such as antigens including but not limited to human hepatitis C virus (HCV) antigens and influenza antigens.

Other specific examples of various antigens include, but are not limited to, the B1 protein of hepatitis C virus (Bruna-Romero et al. (1997) *Hepatology* 25: 470-477), amino acids 252-260 of the circumsporozoite protein of *Plasmodium berghei* [Allsopp et al. (1996) *Eur. J. Immunol.* 26: 1951-1958], the influenza A virus nucleoprotein [*e.g.*, residues 366-374; Nomura et al. (1996) *J. Immunol. Methods* 193: 4149], the listeriolysin O protein of *Listeria monocytogenes* [residues 91-99; An et al. (1996) *Infect. Immun.* 64: 1685-1693], *P. falciparum* antigens (causing malaria, *e.g.*, tCSP), hepatitis B surface antigen [Gilbert et al. (1997) *Nature Biotech.* 15: 1280-1283], and *E coli* O157.H1.

The term "antigen" as used herein includes toxins such as the neurotoxin tetanospasmin produced by *Clostridium tetani* and the toxin produced by *E. coli* O157:H7.

In particular embodiments, the attenuated enterobacteria of the invention express a foreign antigen(s) and can be used to induce an immune response against both the enterobacterium and the organism(s) from which the foreign antigen(s) is derived and, optionally, other species/genera closely related to either of the foregoing (*e.g.*, that cross-react with antibodies produced in response to administration of the attenuated enterobacterium).

There is no particular size limitation to the heterologous nucleic acid encoding the foreign antigen. When incorporated into the genomic DNA, the heterologous nucleic acid will generally be at least about 30, 50, 75, 100, 150 or 200 nucleotides in length and/or less than about 1, 1.5, 2, 2.5 or 3 kilobases in length. When carried by a plasmid, the heterologous nucleic acid can generally be longer, *e.g.*, at least about 30, 50, 75, 100, 150, 200, 500 or

1000 nucleotides in length and/or less than about 5, 10, 12, 14, 16, 18 or 20 kilobases in length.

In representative embodiments, the FNR deficient enterobacterium is a Δ*fnr* mutant, which advantageously reduces the probability of reversion to the wild-type pathogenic phenotype. For example, most current live attenuated vaccine strains against typhoid are auxotrophs for some nutrients, which are likely less stable than the deletion mutants.

The present invention can be used for therapeutic/prophylactic and non-therapeutic/prophylactic purposes. For example, the present invention provides FNR deficient (*e.g.*, Δ*fnr*) enterobacteria strains that can be used to study the *fnr* gene, its role in virulence in these organisms, and as attenuated immunogenic compositions, attenuated vaccines (*e.g.*, live attenuated vaccines) and attenuated vaccine vectors (*e.g.*, live attenuated vaccine vectors).

With respect to uses as an attenuated vaccine or vaccine vector, the present invention finds use in both veterinary and medical applications. Suitable subjects include avians, mammals and fish, with mammals being preferred. The term "avian" as used herein includes, but is not limited to, chickens, ducks, geese, quail, turkeys and pheasants. The term "mammal" as used herein includes, but is not limited to, primates (*e.g.*, simians and humans), bovines, ovines, caprines, porcines, equines, felines, canines, lagomorphs, rodents (*e.g.*, rats and mice), *etc.* Human subjects include fetal, neonatal, infant, juvenile and adult subjects.

The invention can be used in a therapeutic and/or prophylactic manner. For example, in one embodiment, to protect against an infectious disease, subjects may be vaccinated prior to exposure, *e.g.*, as neonates or adolescents. Adults that have not previously been exposed to the disease may also be vaccinated.

In particular embodiments, the present invention provides a pharmaceutical composition comprising a FNR deficient (*e.g.*, Δ*fnr*) strain enterobacterium (optionally, a live FNR deficient enterobacterium) in a pharmaceutically-acceptable carrier, which can also include other medicinal agents, pharmaceutical agents, carriers, adjuvants, diluents, *etc.* For injection, the carrier is typically a liquid. For other methods of administration,

the carrier may be either solid or liquid, such as sterile, pyrogen-free water or sterile pyrogen-free phosphate-buffered saline solution. For inhalation administration, the carrier will be respirable, and is optionally in solid or liquid particulate form. Formulation of pharmaceutical compositions is well known in the pharmaceutical arts [*see, e.g.*, Remington's Pharmaceutical Sciences, 15th Edition, Mack Publishing Company, Easton, Pa. (1975)].

By "pharmaceutically acceptable" it is meant a material that is not biologically or otherwise undesirable, *e.g.*, the material may be administered to a subject without causing undesirable biological effects.

The FNR deficient strains of the invention can be administered to elicit an immune response. Typically, immunological compositions of the present invention comprise an immunogenically effective amount of the FNR deficient strain enterobacterium as disclosed herein, optionally in combination with a pharmaceutically acceptable carrier.

An "immunogenically effective amount" is an amount that is sufficient to induce an immune response in the subject to which the composition is administered. Nonlimiting examples of dosages include about 10^4 to 10^9 colony forming units (cfu), about 10^5 to 10^8 cfu or about 10^6 to 10^7 cfu. Optionally, one or more booster dosages (*e.g.*, about 10^3 to 10^8 cfu or 10^4 to 10^5 cfu) can be administered.

The invention also encompasses methods of producing an immune response in a subject, the method comprising: administering a FNR deficient (*e.g.*, Δ*fnr*) enterobacterium strain of the invention or a pharmaceutical formulation containing the same to a subject in an immunogenically effective amount so that an immune response is produced in the subject.

The terms "vaccination" or "immunization" are well-understood in the art. For example, the terms vaccination or immunization can be understood to be a process that increases a subject's immune reaction to antigen and thereby enhance the ability to resist and/or overcome infection.

Any suitable method of producing an immune response (*e.g.*, immunization) known in the art can be employed in carrying out the present invention, as long as an active immune response (preferably, a protective immune response) is elicited.

In representative embodiments, less pathogenicity (including no detectable pathogenicity) is produced in the subject as a result of administration of the FNR deficient enterobacterium strain as compared with pathogenicity produced if an enterobacterium with a fully functional *fnr* gene (*e.g.*, the wild-type strain) were administered (*e.g.*, at least about a 25%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 97%, 98% or more reduction in pathogenicity).

Vaccines can be given as a single dose schedule or in a multiple dose schedule. A multiple dose schedule is one in which a primary course of administration may consist of about 1 to 10 separate doses, followed by other doses (*i.e.,* booster doses) given at subsequent time intervals to maintain and/or reinforce the immune response, for example, at about 1 to 4 months for a second dose, and if needed, a subsequent dose(s) after another several months or year. The dosage regimen will also, at least in part, be determined by the need of the individual and be dependent upon the judgment of the medical or veterinary practitioner.

An "active immune response" or "active immunity" is characterized by "participation of host tissues and cells after an encounter with the immunogen. It involves differentiation and proliferation of immunocompetent cells in lymphoreticular tissues, which lead to synthesis of antibody or the development of cell-mediated reactivity, or both." Herbert B. Herscowitz, *Immunophysiology: Cell Function and Cellular Interactions in Antibody Formation, in* IMMUNOLOGY: BASIC PROCESSES 117 (Joseph A. Bellanti ed., 1985). Alternatively stated, an active immune response is mounted by the host after exposure to immunogen by infection or by vaccination. Active immunity can be contrasted with passive immunity, which is acquired through the "transfer of preformed substances (antibody, transfer factor, thymic graft, interleukin-2) from an actively immunized host to a non-immune host." *Id.*

A "protective" immune response or "protective" immunity as used herein indicates that the immune response confers some benefit to the subject in that it prevents or reduces the incidence of disease, the progression of the disease and/or the symptoms of the disease. Alternatively, a protective immune response or protective immunity may be useful in the treatment of disease including infectious disease. The protective effects may be complete

or partial, as long as the benefits of the treatment outweigh any disadvantages thereof.

Administration of the attenuated enterobacteria and compositions of the invention can be by any means known in the art, including oral, rectal, topical, buccal (*e.g.*, sub-lingual), vaginal, intra-ocular, parenteral (*e.g.*, subcutaneous, intramuscular including skeletal muscle, cardiac muscle, diaphragm muscle and smooth muscle, intradermal, intravenous, intraperitoneal), topical (*e.g.,* mucosal surfaces including airway surfaces), intranasal, transmucosal, intratracheal, transdermal, intraventricular, intraarticular, intrathecal and inhalation administration.

The most suitable route in any given case will depend on the nature and severity of the condition being treated, the FNR deficient strain enterobacterium, and the composition being administered.

The FNR deficient enterobacterium strain can be formulated for administration in a pharmaceutical carrier in accordance with known techniques. *See, e.g.,* Remington, *The Science and Practice of Pharmacy* (9th Ed. 1995). In the manufacture of a pharmaceutical formulation according to the invention, the FNR deficient enterobacterium strain is typically admixed with, *inter alia*, an acceptable carrier. The carrier can be a solid or a liquid, or both, and is optionally formulated as a unit-dose formulation, which can be prepared by any of the well-known techniques of pharmacy.

For injection, the carrier is typically a liquid, such as sterile pyrogen-free water, pyrogen-free phosphate-buffered saline solution, bacteriostatic water, or Cremophor EL[R] (BASF, Parsippany, N.J.). For other methods of administration, the carrier can be either solid or liquid.

For oral administration, the FNR deficient enterobacterium strain can be administered in solid dosage forms, such as capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, and suspensions. The FNR deficient strain enterobacterium can be encapsulated in gelatin capsules together with inactive ingredients and powdered carriers, such as glucose, lactose, sucrose, mannitol, starch, cellulose or cellulose derivatives, magnesium stearate, stearic acid, sodium saccharin, talcum, magnesium carbonate and the like. Examples of additional inactive ingredients that can be added to provide desirable color, taste, stability, buffering capacity, dispersion

or other known desirable features are red iron oxide, silica gel, sodium lauryl sulfate, titanium dioxide, edible white ink and the like. Similar diluents can be used to make compressed tablets. Both tablets and capsules can be manufactured as sustained release products to provide for continuous release of medication over a period of hours. Compressed tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric- coated for selective disintegration in the gastrointestinal tract. Liquid dosage forms for oral administration can contain coloring and flavoring to increase patient acceptance.

Formulations suitable for buccal (sub-lingual) administration include lozenges comprising the FNR deficient enterobacterium strain in a flavored base, usually sucrose and acacia or tragacanth; and pastilles comprising the FNR deficient enterobacterium strain in an inert base such as gelatin and glycerin or sucrose and acacia.

Formulations of the present invention suitable for parenteral administration can comprise sterile aqueous and non-aqueous injection solutions of the FNR deficient enterobacterium strain, which preparations are generally isotonic with the blood of the intended recipient. These preparations can contain anti-oxidants, buffers, bacteriostats and solutes, which render the formulation isotonic with the blood of the intended recipient. Aqueous and non-aqueous sterile suspensions can include suspending agents and thickening agents. The formulations can be presented in unit\dose or multi-dose containers, for example sealed ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline or water-for-injection immediately prior to use.

Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules and tablets. For example, in one aspect of the present invention, there is provided an injectable, stable, sterile composition comprising a FNR deficient enterobacterium strain of the invention, in a unit dosage form in a sealed container. Optionally, the composition is provided in the form of a lyophilizate, which is capable of being reconstituted with a suitable pharmaceutically acceptable carrier to form a liquid composition suitable for injection thereof into a subject.

Formulations suitable for rectal or vaginal administration can be presented as suppositories. These can be prepared by admixing the FNR deficient enterobacterium strain with one or more conventional excipients or carriers, for example, cocoa butter, polyethylene glycol or a suppository wax, which are solid at room temperature, but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the FNR deficient FNR deficient enterobacterium strain.

Formulations suitable for topical application to the skin can take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers that can be used include petroleum jelly, lanoline, polyethylene glycols, alcohols, transdermal enhancers, and combinations of two or more thereof.

Formulations suitable for transdermal administration can be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Formulations suitable for transdermal administration can also be delivered by iontophoresis [*see*, for example, *Pharmaceutical Research* 3 (6):318 (1986)] and typically take the form of an optionally buffered aqueous solution. Suitable formulations comprise citrate or bis\tris buffer (pH 6) or ethanol/water.

The FNR deficient enterobacterium strain can be formulated for nasal administration or otherwise administered to the lungs of a subject by any suitable means, for example, by an aerosol suspension of respirable particles comprising the FNR deficient enterobacterium strain, which the subject inhales. The respirable particles can be liquid or solid. The term "aerosol" includes any gas-borne suspended phase, which is capable of being inhaled into the bronchioles or nasal passages. Specifically, an aerosol includes a gas-borne suspension of droplets, as can be produced in a metered dose inhaler or nebulizer, or in a mist sprayer. An aerosol also includes a dry powder composition suspended in air or other carrier gas, which can be delivered by insufflation from an inhaler device, for example. See Ganderton & Jones, *Drug Delivery to the Respiratory Tract*, Ellis Horwood (1987); Gonda (1990) *Critical Reviews in Therapeutic Drug Carrier Systems* 6:273-313; and Raeburn et al. (1992) *J. Pharmacol. Toxicol. Methods* 27:143-159. Aerosols of liquid particles can be produced by any suitable means, such as with a pressure-driven aerosol nebulizer or an ultrasonic nebulizer, as is known to

those of skill in the art. *See, e.g.*, U.S. Patent No. 4,501,729. Aerosols of solid particles comprising the FNR deficient enterobacterium strain can likewise be produced with any solid particulate medicament aerosol generator, by techniques known in the pharmaceutical art.

In particular embodiments of the invention, administration is by subcutaneous or intradermal administration. Subcutaneous and intradermal administration can be by any method known in the art, including but not limited to injection, gene gun, powderject device, bioject device, microenhancer array, microneedles, and scarification (*i.e.*, abrading the surface and then applying a solution comprising the FNR deficient enterobacterium strain).

In other embodiments, the FNR deficient enterobacterium strain is administered intramuscularly, for example, by intramuscular injection.

The examples which follow are set forth to illustrate the present invention, and are not to be construed as limiting thereof.

EXAMPLE 1

MATERIALS AND METHODS

Bacterial strains. Wild-type (WT) serovar Typhimurium (ATCC 14028s) and its isogenic *fnr* mutant (NC 983) were used throughout the studies described herein. The mutant strain was constructed by transducing the *fnr*::Tn*10* mutation from serovar Typhimurium [SL2986/TN 2958 (*fnr*::Tn*10*)] to strain 14028s using P22 phage (all from the culture collection of S. Libby). The transductants were plated on Evans blueuranine agar, and the tetracycline marker was eliminated (Bochner et al., (1980) *J. Bacteriol.* 143:926-933). The Tet^S and FNR^- phenotypes were confirmed by the inability of NC983 (*fnr* mutant) to grow on media containing tetracycline (10μg/ml) and by its inability to grow anaerobically on M9 minimal medium containing glycerol plus nitrate, respectively. Sequence analysis of *fnr* and neighboring genes (*i.e.*, *ogt* and *ydaA*, respectively) in NC 893 showed that the remnant of Tn*10* (*tnpA*) interrupts *fnr* between bp 106 and 107 and has no polar effect on *ogt* or *ydaA* (**Fig. 1**).

For complementation studies, a low-copy-number plasmid expressing *fnr* (pfnr) was constructed. The complete *fnr* sequence starting from the stop codon of *ogtA* (TGA [indicated in boldface type]) to 21 bp downstream of *fnr* (*i.e.*, a 972 bp fragment) was amplified from WT strain 14028s with the following primers: *fnr*-Forward, 5'-ATATCCATGG**TGA**ATATACAGGAAAAAGTGC-3' (an NcoI site is underlined; **SEQ ID NO:1**); *fnr*-Reverse, 5'-ATATATTCAGCTGCATCAATGGTTTAGCTGACG-3' (a PvuII site is underlined; **SEQ ID NO:2**). The PCR product was digested with NcoI and PvuII and ligated into the low-copy-number vector pACYC184 cut with NcoI and PvuII. Thus, in the new plasmid (pfnr) the Cmr gene in pACYC184 is replaced with the *fnr* gene. The plasmid (pfnr) was electroporated and maintained in *E. coli* DH5α. Transformants were confirmed for Tet^r (15 μg/ml) and Cms (20 μg/ml) on Luria-Bertani (LB) plates, and the presence of the *fnr* gene was confirmed by restriction analysis using EcoRI and HindIII. The plasmid isolated from DH5α was used to complement the *fnr* mutant. Transformants were selected on LB plates containing tetracycline (15 μg/ml).

Growth conditions. The WT and the *fnr* mutant were grown anaerobically at 37°C in MOPS (morpholinepropanesulfonic acid)-buffered (100 mM, pH 7.4) LB broth supplemented with 20 mM D-xylose (LB-MOPS-X). This medium was used in order to avoid the indirect effects of pH and catabolite repression. A Coy anaerobic chamber (Coy, Ann Arbor, MI) and anaerobic gas mixture (10% H2, 5% CO_2, and 85% N_2) were used. All solutions were preequilibrated for 48 h in the chamber. Cells from frozen stocks were used to inoculate LB-MOPS-X broth. Cultures were grown for 16 h and used to inoculate fresh anoxic media. The anaerobic growth kinetics of the mutant and the WT strains were similar, and the doubling times of the *fnr* mutant and the WT were 53.9 ± 1.2 and 45.4 ± 2.9 min, respectively.

RNA isolation. Anaerobic cultures were used to inoculate three independent flasks each containing 150 ml of anoxic LB-MOPS-X. The three independent cultures were grown to an optical density at 600 nm (OD600) of

0.25 to 0.35, pooled, and treated with RNAlater (QIAGEN, Valencia, CA) to fix the cells and preserve the quality of the RNA. Total RNA was extracted with the Rneasy RNA extraction kit (QIAGEN), and the samples were treated with RNase-free DNase (Invitrogen, Carlsbad, CA). The absence of contaminating DNA and the quality of the RNA was confirmed by PCR amplification of known genes and by using agarose gel electrophoresis. Aliquots of the RNA samples were kept at -80°C for use in the microarray and quantitative real-time reverse transcription-PCR (qRT-PCR) studies.

Microarray studies. Serovar Typhimurium microarray slides were prepared and used as previously described in Porwollik, S. et al., "The delta *uvrB* mutations in the Ames strains of *Salmonella* span 15-119 gene" *Mutat. Res.* 483:1-11 (2001). The SuperScript Indirect cDNA labeling system (Invitrogen) was used to synthesize the cDNA for the hybridizations. Each experiment consisted of two hybridizations, on two slides, and was carried out in Corning Hybridization Chambers at 42°C overnight. Dye swapping was performed to avoid dye-associated effects on cDNA synthesis. The slides were washed at increasing stringencies (2X SSC [1X SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 0.1% sodium dodecyl sulfate [SDS], 42°C; 0.1% SSC, 0.1% SDS, room temperature; 0.1% SSC, room temperature). Following hybridization, the microarrays were scanned for the Cy3 and Cy5 fluorescent signals with a ScanArray 4000 microarray scanner from GSI Lumonics (Watertown, MA). The intensity of every spot was codified as the sum of the intensities of all the pixels within a circle positioned over the spot itself and the background as the sum of the intensities of an identical number of pixels in the immediate surroundings of the circled spot.

Data analysis. Cy3 and Cy5 values for each spot were normalized over the total intensity for each dye to account for differences in total intensity between the two scanned images. The consistency of the data obtained from the microarray analysis was evaluated by two methods: (i) a pair-wise comparison, calculated with a two-tailed Student's *t* test and analyzed by the MEAN and TTEST procedures of SAS-STAT statistical software (SAS Institute, Cary, NC) (the effective degrees of freedom for the *t* test were

calculated as described previously in Satterthwaite, F.E. "An approximate distribution of estimates of variance components" *Biometrics Bull.* 2:110-114 (1946)); and (ii) a regularized *t* test followed by a posterior probability of differential expression [PPDE (*p*)] method. These statistical analyses are implemented in the Cyber-T software package available online at the website of the Institute for Genomics and Bioinformatics of the University of California, Irvine. The signal intensity at each spot from the FNR mutant and the WT were background subtracted, normalized, and used to calculate the ratio of gene expression between the two strains. All replicas were combined, and the median expression ratios and standard deviations were calculated for open reading frames (ORFs) showing ≥2.5-fold change.

qRT-PCR. qRT-PCR was used to validate the microarray data, where 19 genes were randomly chosen from the differentially expressed genes. This technique was also used to confirm the expression of a set of selected genes. qRT-PCRs were carried out with the QuantiTect SYBR green RT-PCR kit (QIAGEN) and an iCycler (Bio-Rad, Hercules, CA), and the data were analyzed by the Bio-Rad Optical System software, version 3.1, according to manufacturer specifications. To ensure accurate quantification of the mRNA levels, three amplifications for each gene were made with 1:5:25 dilutions of the total RNA. Measured mRNA levels were normalized to the mRNA levels of the housekeeping gene *rpoD* (σ70). Normalized values were used to calculate the ratios of the expression levels in the *fnr* mutant relative to the WT.

Logo graph and promoter analysis. The information matrix for the generation of the FNR logo was produced by using the alignment of the *E. coli* FNR binding sequences, available at http://arep.med.harvard.edu/ecoli_matrices/. The alignment of the FNR motifs from this website did not include the motifs present in the *sodA* and *mutM* promoters; therefore, they were included in our analysis. To account for differences in nucleotide usage or slight variations in consensus sequences, a second alignment was built for serovar Typhimurium using the 5' regions of

the homologous genes originally used to build the *E. coli* information matrix. The alignment was used to prepare a new information matrix using the Patser software (version 3d), available at http://rsat.ub.ac.be/rsat/. A graphical representation (**Fig. 2**) of the matrices through a logo graph was obtained with Weblogo software (version 2.8.1, 18 October 2004), available at http://weblogo.berkeley.edu/.

Motility assay and electron microscopy. The motilities of the WT, the *fnr* mutant, and the complemented mutant/pfnr were evaluated under anoxic conditions. Ten microliters of anaerobically grown (16 h) cells were spotted onto LB-MOPS-X agar (0.6% agar) plates and incubated at 37°C for 24 h. The diameter of the growth halo was used as a measure of motility. Scanning electron microscopy (SEM) was used to examine the morphology of the extracellular surfaces. WT and *fnr* cultures were grown anaerobically (OD600, 0.3 to 0.4) and centrifuged, and the pellets were resuspended in a fixative solution (3% glutaraldehyde in 0.1 M phosphate-buffered saline [PBS] [pH 7.4]) under anaerobic conditions. The fixed samples were rinsed in 0.1 M PBS buffer, postfixed with 1% osmium tetroxide in 0.1 M PBS for 2 h, and rinsed with PBS, all at 4°C. An aliquot of each sample was filtered through a 0.1-μm filter. Each filter was dehydrated through a graded ethanol series (up to 100%), brought to room temperature, critical point dried with liquid CO_2 (Tousimis Research, Rockville, MD), placed on stubs, and sputter coated with Au/Pd (Anatech Ltd., Denver, NC). Samples were viewed at 15 kV with a JEOL 5900LV SEM (JEOL USA, Peabody, MA). Transmission electron microscopy (TEM) and negative staining were used to visualize the flagella. WT and *fnr* cultures were grown anaerobically (OD600, 0.3 to 0.4), and a 20-μl aliquot of each sample was separately placed on a Formvar-carbon grid. The grids were washed with 0.1 M sodium acetate (pH 6.6), negatively stained with 2% phosphotungstic acid (PTA), and air dried for 5 min before being viewed at 80 kV with a JEOL JEM-100S TEM (JEOL USA, Peabody, MA).

Pathogenicity assays. Immunocompetent 6- to 8-week-old C57BL/6 mice and their congenic iNOS-/- and pg91*phox*$^{-/-}$ immunodeficient mice (bred in the University of Colorado Health Science Center [UCHSC] animal facility according to Institutional Animal Care and Use Committee guidelines) were used in this study. Stationary-phase serovar Typhimurium (WT and *fnr* mutant) cultures grown aerobically in LB-MOPS-X broth were used, and the cells were diluted in PBS. For oral (p.o.) challenge, groups of 10 mice were gavaged with 5 x 10^6 or 5 x 10^7 CFU in 200 μl of PBS/mouse. For intraperitoneal (i.p.) challenge, groups of five mice were inoculated with 250 CFU in 500 μl of PBS/mouse. Mortality was scored over a 15- to 30-day period.

Macrophage assay. Peritoneal macrophages were harvested from C57BL/6 mice and pg91*phox*$^{-/-}$ immunodeficient mice (bred in the UCHSC animal facility) 4 days after intraperitoneal inoculation with 1 mg/ml sodium periodate and used as previously described in DeGroote, M.A. et al. "Periplasmic superoxide dismutase protects *Salmonella* from products of phagocyte NADPH-oxidase and nitric oxide synthase" *Proc. Natl. Acad. Sci.* 94:13997-14001 (1997). Macrophages were challenged (multiplicity of infection of 2) for 25 min with the different test strains. Stationary-phase cultures grown aerobically in LB-MOPS-X broth were used as outlined above. Prior to infection, each strain was opsonized with 10% normal mouse serum for 20 min. After the challenge, extracellular bacteria were removed from the monolayers by washing with prewarmed RPMI medium (Cellgro, Herndon, VA) containing gentamicin (6 mg/ml) (Sigma), the *Salmonella*-infected macrophages were lysed at indicated time points, and the surviving bacteria were enumerated on LB agar plates. The results are expressed as percent survival relative to the number of viable intracellular bacteria recovered at time zero (*i.e.*, after washing and removal of the extracellular bacteria, 25 min after infection).

Microarray data. The microarray data are accessible via GEO accession number GSE3657 at http://www.ncbi.nlm.nih.gov/geo (the disclosure of which is incorporated herein by reference in its entirety).

EXAMPLE 2

TRANSCRIPTOME PROFILING

Out of 4,579 genes, the two-tailed Student *t* test produced a set of 1,664 coding sequences showing significant differences ($P < 0.05$) between the *fnr* mutant and the WT. The analysis was restricted to include highly affected genes (*i.e.*, having a ratio of $\geq$ 2.5-fold). Under this constraint, 311 genes were differentially expressed in the *fnr* mutant relative to the WT; of these, 189 genes were up-regulated and 122 genes were down-regulated by FNR (**Table 3**). The 311 FNR-regulated genes were classified into clusters of orthologous groups (COGs) as defined at http://www.ncbi.nlm.nih.gov/COG. Throughout the study levels of transcription in the *fnr* mutant were compared to that in the WT strain. Thus, genes repressed by FNR possess values of >1, while genes activated by FNR have values of <1.

In order to globally validate the microarray data, 19 of the 311 differentially expressed genes for qRT-PCR were selected. The measured levels of mRNA were normalized to the mRNA levels of the housekeeping gene *rpoD*. The specific priers used for qRT-PCR and the normalized mRNA levels are shown in **Table 1**. The microarray and qRT-PCR data were $\log_2$ transformed and plotted (**Fig. 3**). The correlation between the two sets of data was 0.94 ($P< 0.05$).

To determine whether a binding site for FNR might be present in the region upstream of the candidate FNR-regulated genes, 5' regions of these genes were searched for the presence of a putative FNR-binding motif using a *Salmonella* logo graph (**Fig. 2**). One hundred ten out of the 189 genes activated by FNR (58%) and 59 out of the 122 genes repressed by FNR (48%) contained at least one putative FNR-binding site.

EXAMPLE 3

FNR AS A REPRESSOR

Transcription of the genes required for aerobic metabolism, energy generation, and nitric oxide detoxification was repressed by FNR. In particular, the genes coding for cytochrome *c* oxidase (*cyoABCDE*), cytochrome *cd* complex (*cydAB*), NADH-dehydrogenase (*nuoBCEFJLN*), succinyl-coenzyme A (CoA) metabolism (*sucBCD*), fumarases (*fumB*, stm0761, and stm0762), and the NO·-detoxifying flavohemoglobin (*hmpA*) were expressed at higher levels in the *fnr* mutant than in the WT (**Table 3**). Also, genes required for L-lactate metabolism (*lld-PRD*) and for the production of phosphoenolpyruvate (*pykF*), oxaloacetate (*ppc*), and acetoacetyl-CoA (*yqeF*) were expressed at higher levels in the mutant than in the WT (**Table 3**).

EXAMPLE 4

FNR AS AN ACTIVATOR

Several genes associated with anaerobic metabolism, flagellar biosynthesis, motility, chemotaxis, and *Salmonella* pathogenesis were activated by FNR. The genes constituting the *dms* operon, *dmsABC* (encoding the anaerobic dimethyl sulfoxide reductase), required for the use of dimethyl sulfoxide (DMSO) as an anaerobic electron acceptor, had the lowest expression levels (*i.e.*, -200-, -62-, and -23-fold, respectively) in the *fnr* mutant relative to the WT (**Table 3**). Two other operons coding for putative anaerobic DMSO reductases (STM4305 to STM4307 and STM2528 to STM2530) were also under positive control by FNR. The genes required for the conversion of pyruvate to phosphoenolpyruvate (*pps*), Ac-CoA (*aceF*), Ac-P (*pta*), and OAc (*ackA*), as well as those for the production of formate (*tdcE*, *yfiD*, *focA*) and D-lactate (*ldhA*), were expressed at lower levels in the *fnr* mutant than in the WT. In addition, the genes coding for a universal stress protein (*ynaF*), a ferritinlike protein (*ftnB*), an ATP-dependent helicase (*hrpA*), and aerotaxis/redox sensing (*aer*) were also positively regulated by FNR (**Table 3**).

The genes for ethanolamine utilization (*eut* operon) had lower transcript levels in the *fnr* mutant (**Fig. 4B**). Although the FNR-dependent

genes for tetrathionate utilization (*ttrABCSR*), a major anaerobic electron acceptor, were not affected by the lack of FNR, this was not surprising since tetrathionate is also needed to induce expression.

Several of the middle flagellar (class 2) genes (*e.g.*, *flgNMDEFGKL* and *fliZADSTHJLM*) and late flagellar (class 3) genes (*e.g.*, *cheZYBRMWA*, *motBA*, *aer*, *trg*, and *tsr*) had lower transcript levels in the *fnr* mutant than in the WT (**Fig. 4C to E**). There was no significant difference in the transcript levels of the early flagellar genes (class 1) *flhD* and *flhC*, whose gene products FlhD/FlhC are the master regulators of flagellar biosynthesis (**Fig. 4D**). In addition, many newly identified flagellar genes (*i.e.*, *mcpA*, *mcpC*, and *cheV*) had lower expression levels in the *fnr* mutant, while the expression of *mcpB* was not affected.

Several genes in SPI-1 (*e.g.*, *prgKJIH*, *iagB*, *sicA*, *spaPO*, *invJICBAEGF*) had lower levels of expression in the *fnr* mutant than in the WT (**Fig. 4A**). This region contains genes coding for a type three secretion system and for proteins required for invasion and interaction with host cells. The data also show that genes belonging to the other SPIs were unaffected by the lack of FNR. However, the virulence operon *srfABC*, which is located outside SPI-2 and regulated by a two-component regulatory system (SsrAB) located on SPI-2 (Waterman et al. (2003) *Cell. Microbiol.* 5:501-511; Worley et al., (2000) *Mol. Microbiol.* 36:749-761), was differentially regulated by FNR. The effects of FNR on a subset of the above-mentioned invasion and virulence genes were further confirmed by measuring the levels of mRNA in the *fnr* mutant and the WT strains by qRT-PCR (**Table 2**).

EXAMPLE 5

EFFECTS OF FNR ON MOTILITY AND FLAGELLA

Expression of the flagellar biosynthesis, motility, and chemotaxis genes was lower in the *fnr* mutant than in the WT. Therefore, the WT, *fnr* mutant was compared to the mutant cells harboring pfnr for motility in soft agar under anaerobic conditions. The data indicate that the *fnr* mutant was nonmotile and that the lack of motility was complemented (~75%) by the inclusion of pfnr. The 100% complementation by pfnr is probably due to extra copies of the global regulator FNR. The WT was also compared to the mutant for the

presence of flagella by SEM and TEM. Taken together, these data show that the *fnr* mutant is nonmotile due to the lack of flagella.

EXAMPLE 6

EFFECTS OF FNR ON PATHOGENICITY AND KILLING BY MACROPHAGES

FNR positively regulates the expression of various loci (see **Table 3**), such as motility and SPI-1 genes that are important determinants for *Salmonella* pathogenesis, so the virulence of *fnr* in a murine model of mucosal and acute infection was tested. In immunocompetent C57BL/6 mice, the *fnr* mutant was completely attenuated over a 15-day period following an oral challenge with 5 x 10^6 or 5 x 10^7 CFU/mouse, while the WT strain killed all mice within 10 or 12 days, respectively (**Fig. 5A**). The mutant strain was also 100% attenuated when 250 CFU/mouse were inoculated i.p. (**Fig. 5B**). The different *Salmonella* strains were also tested for the ability to survive killing by macrophages (**Fig. 6**). Similar numbers of *fnr* mutant and WT cells were recovered from the macrophages 25 min after infection (designated as time zero postinfection). Data in **Fig. 6A** indicate that the lack of FNR resulted in a dramatic reduction in the ability of *Salmonella* to replicate in macrophages. Interestingly, most of the killing of the WT by macrophages took place during the first 2 h postinfection (*i.e.*, the WT resisted further killing beyond 2 h), while the viability of the *fnr* mutant continued to decline by 1 log between 2 and 20 h postinfection (**Fig. 6A and B**). Data in **Fig. 6B** also show that this phenotype is complemented in *fnr* mutant cells harboring pfnr. Congenic iNOS-/- mice (unable to make NO·) and pg91*phox*$^{-/-}$ mice (defective in oxidative burst oxidase) were used to examine the roles of reactive nitrogen and oxygen species (RNS and ROS), respectively, in resistance to an acute systemic infection with FNR-deficient or WT *Salmonella*. The *fnr* mutant was as attenuated in iNOS-/- mice as in congenic WT C57BL/6 controls. In sharp contrast, the *fnr* mutant killed pg91*phox*$^{-/-}$ mice, albeit at a lower rate than the WT strain (**Fig. 7A and B**). Consistent with the *in vivo* data, the WT and the isogenic *fnr* mutant survived to similar extents in NADPH oxidase-deficient macrophages isolated from pg91*phox*$^{-/-}$ mice (**Fig. 7C**).

The foregoing is illustrative of the present invention, and is not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

TABLE 1. Validation of microarray data using qRT-PCR of randomly selected genes relative to the housekeeping gene, *rpoD*[a].

Locus[b]	Name[c]	Primer sequence [d]	SEQ ID NO:	Fragment (bp)[e]	*S.* Typhimurium Gene Function[f]	Ratio of *fnr* mutant/WT		Log_2 ratio	
						qRT-PCR[g]	Microarray[h]	qRT-PCR[i]	Microarray[j]
STM3217	*aer*	CGTACAACATCTTAATCGTAGC TTCGTTCAGATCATTATTACCC	3 4	163	Aerotaxis sensor receptor; senses cellular redox state or proton motive force	0.190	0.210	-2.4	-2.3
STM1781	*cheM*	GCCAATTTCAAAAATATGACG GTCCAGAAACTGAATAAGTTCG	5 6	114	Methyl-accepting chemotaxis protein II; aspartate sensor-receptor	0.036	0.120	-4.8	-3.1
STM0441	*cyoC*	TATTTAGCTCCATTACCTACGG GGAATTCATAGAGTTCCATCC	7 8	134	Cytochrome *o* ubiquinol oxidase subunit III	153.967	7.096	7.3	2.8
STM1803	*dadA*	TAACCTTTCGCTTTAATACTCC GATATCAACAATGCCTTTAAGC	9 10	155	D-Amino acid dehydrogenase subunit	2.835	3.169	1.5	1.7
STM0964	*dmsA*	AGCGTCTTATCAAAGAGTATGG TCACCGTAGTGATTAAGATAACC	11 12	154	Anaerobic dimethyl sulfoxide reductase, subunit A	0.001	0.005	-9.8	-7.6
STM2892	*invJ*	TTGCTATCGTCTAAAAATAGGC TTGATATTATCGTCAGAGATTCC	13 14	128	Surface presentation of antigens; secretory proteins	0.246	0.182	-2.0	-2.5
STM2324	*nuoF*	GGATATCGAGACACTTGAGC GATTAAATGGGTATTACTGAACG	15 16	163	NADH dehydrogenase I, chain F	2.894	2.600	1.5	1.4
STM0650	STM0650	CAACAGCTTATTGATTTAGTGG CTAACGATTTTTCTTCAATGG	17 18	130	Putative hydrolase, C terminus	0.476	0.219	-1.1	-2.2
STM2787	STM2787	AAGCGAATACAGCTATGAACC ATTAGCTTTTGCAGAACATGG	19 20	144	Tricarboxylic transport	28.241	6.892	4.8	2.8
STM4463	STM4463	AAGGTATCAGCCAGTCTACG CGTATGGATAAGGATAAATTCG	21 22	142	Putative arginine repressor	0.325	0.181	-1.6	-2.5
STM4535	STM4535	TAAGCCAGCAGGTAGATACG CGACATAAAGAGATCGATAACC	23 24	139	Putative PTS permease	6.053	8.217	2.6	3.0
STM2464	*eutN*	AGGACAAATCGTATGTACCG ACCAGCAGTACCCACTCTCC	25 26	153	Putative detox protein in ethanolamine utilization	0.062	0.125	-4.0	-3.0
STM2454	*eutR*	GGTAAAAGAGCAGCATAAAGC ATTATCACTCAAGACCTTACGC	27 28	118	Putative regulator; ethanolamine operon (AraC/XylS family)	0.043	0.195	-4.6	-2.4
STM2470	*eutS*	AATAAAGAACGCATTATTCAGG GTTAAAGTCATAATGCCAATCG	29 30	137	Putative carboxysome structural protein; ethanol utilization	0.049	0.073	-4.3	-3.8

Locus[b]	Name[c]	Primer sequence [d]	SEQ ID NO:	Fragment (bp)[e]	*S.* Typhimurium Gene Function[f]	Ratio of *fnr* mutant/WT		Log_2 ratio	
						qRT-PCR[g]	Microarray[h]	qRT-PCR[i]	Microarray[j]
STM1172	*flgM*	AGCGACATTAATATGGAACG TTTACTCTGTAAGTAGCTCTGC	31 32	126	Anti-FliA (anti-sigma) factor; also known as RflB protein	0.050	0.174	-4.3	-2.5
STM3692	*lldP*	TGATTAAACTCAAGCTGAAAGG CCGAAATTTTATAGACAAAGACC	33 34	189	LctP transporter; L-lactate permease	76.492	16.003	6.3	4.0
STM3693	*lldR*	GAACAGAATATCGTGCAACC GAGTCTGATTTTCTCTTTGTCG	35 36	153	Putative transcriptional regulator for *lct* operon (GntR family)	68.378	30.597	6.1	4.9
STM1923	*motA*	GGTTATCGGTACAGTTTTCG TAGATTTTGTGTATTTCGAACG	37 38	194	Proton conductor component of motor; torque generator	0.048	0.092	-4.4	-3.4
STM4277	*nrfA*	GACTAACTCTCTGTCGAAAACC ATTTTATGGTCGGTGTAGAGC	39 40	159	Nitrite reductase; periplasmic cytochrome c_{552}	0.051	0.324	-4.3	-1.6

[a]STM3211 (*rpoD*) was used as the reference gene where no significant change in expression level was observed. The primer sequences (5' to 3') used for *rpoD* were as follows: CGATGTCTCTGAAGAAGTGC (forward; SEQ ID NO:41) and TTCAACCATCTCTTTCTTCG (reverse; SEQ ID NO:42). The size of the fragment generated is 150 bp.
[b]Location of the open reading frame (ORF) in the *S.* Typhimurium LT2 genome.
[c]Respective gene name or symbol.
[d]For each set, the first primer is the forward primer and the second primer is the reverse primer.
[e]Size of the amplified PCR product.
[f]Functional classification according to the KEGG (Kyoto Encyclopedia of Genes and Genomes) database.
[g]Expression levels of quantitative reverse transcriptase polymerase chain reaction - values shown as the ratio between the *fnr* mutant and the wild-type; where values <1 indicate that FNR acts as an activator, and values >1 indicate FNR acts as a repressor.
[h]Expression levels from the microarray data - values shown as the ratio between the *fnr* mutant and the wild-type; where values <1 indicate that FNR acts as an activator, and values >1 indicate FNR acts as a repressor.
[i]Expression levels of quantitative reverse transcriptase polymerase chain reaction comparing the *fnr* mutant versus the wild-type - shown in signal to log_2 ratio (SLR).
[j]Expression levels of microarray data comparing the *fnr* mutant versus the wild-type - shown in signal to log_2 ratio (SLR).

TABLE 2. qRT-PCR of Selected Invasion and Virulence Genes[a]

Locus[b]	Name[c]	Primer sequence[d]	SEQ ID NO:	Fragment size (bp)[e]	Ratio[f]
STM2893	*invI*	5'-CTTCGCTATCAGGATGAGG-3' 5'-CGAACAATAGACTGCTTACG-3'	43 44	161	-9.27
STM2874	*prgH*	5'-GGCTCGTCAGGTTTTAGC-3' 5'-CTTGCTCATCGTGTTTCG-3'	45 46	190	-8.45
STM2871	*prgK*	5'-ATTCGCTGGTATCGTCTCC-3' 5'-GAACCTCGTTCATATACGG-3'	47 48	199	-8.56
STM2886	*sicA*	5'-GATTACACCATGGGACTGG-3' 5'-CAGAGACTCATCTTCAGTACG-3'	49 50	207	-3.92
STM1593	*srfA*	5'-AGGCGGCATTTAGTCAGG-3' 5'-GACAGGTAAGCTCCACAGC-3'	51 52	176	-4.33
STM1594	*srfB*	5'-GGTACCAGAAATACAGATGG-3' 5'-GCCGATATCAATCGATGC-3'	53 54	190	-6.55

[a]STM3211 (*rpoD*) was used as the reference gene where no significant change in expression level was observed. The primer sequences used for *rpoD* were as follows: 5'-CGATGTCTCTGAAGAAGTGC-3' (forward; SEQ ID NO:41) and 5'-TTCAACCATCTCTTTCTTCG-3' (reverse; SEQ ID NO:42). The size of the fragment generated was 150 bp.

[b]Location of the open reading frame (ORF) in the *S.* Typhimurium LT2 genome.

[c]Respective gene name or symbol.

[d]For each set, the first primer is the forward and the second primer is the reverse.

[e]Size of the amplified PCR product.

[f]Ratio of the transcription levels in the *fnr* mutant relative to the wild-type.

TABLE 3. Differentially expressed genes and the presence/absence of putative FNR-binding motifs in their 5' regions.

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
PSLT018	-	pefA	plasmid-encoded fimbriae; major fimbrial subunit	-6.11	5.92	9.16E-04	-2.56						
PSLT019	-	pefB	plasmid-encoded fimbriae;regulation	-9.86	6.59	3.46E-05	-4.59	R	-337	-316	tttcTTTTTTGATATATGTCTTTTCAtgta	5.41	-7.58
STM0002	E	thrA	aspartokinase I , bifunctional enxyme N-terminal is aspartokinasel and C-terminal is homoserine dehydrogenase I	-11.82	7.27	5.20E-06	-3.33	R	-351	-330	GGAATTGGTTGAAAATAAATATatcg	5.71	-7.83
STM0041	G	STM0041	putative glycosyl hydrolase	-9.21	5.78	1.15E-04	-3.19						
STM0042	G	STM0042	putative sodium galactoside symporter	-12.79	5.16	4.17E-05	-3.32						
STM0153	C	aceF	pyruvate dehydrogenase, dihydrolipoyltransacetylase component	-7.07	5.72	4.93E-04	-3.83	D	-52	-31	gaatAATGGCTATCGAAATCAAAGTAccgg	4.98	-7.24
STM0178	G	yadI	putative PTS enzyme	-6.35	5.43	1.05E-03	-7.00	D	-263	-242	tttaTAATATATATTTAATCAATTATtttg	5.19	-7.41
STM0439	H	cyoE	protohaeme IX farnesyltransferase (haeme O biosynthesis)	9.48	5.20	1.77E-04	7.71	D	-102	-81	tctgGATTATGTGGAACCTCAACTACaaca	4.54	-6.9
STM0440	C	cyoD	cytochrome o ubiquinol oxidase subunit IV	13.11	5.21	3.45E-05	7.05	R	-326	-305	tatcGGGATGGAACTCTATGAATTCCatca	4.64	-6.98

WO 2008/108805 PCT/US2007/016357

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM0441	C	cyoC	cytochrome o ubiquinol oxidase subunit III	36.33	5.46	9.96E-08	7.10						
STM0442	C	cyoB	cytochrome o ubiquinol oxidase subunit I	22.84	5.64	8.89E-07	5.05	R	-206	-185	aaccTGATTTGTTCAAGGACGTTATTaaca	4.18	-6.63
STM0443	C	cyoA	cytochrome o ubiquinol oxidase subunit II	22.81	5.46	1.25E-06	4.51	D	-68	-47	ccgtGGAATTGAGGTCGTTAAATGAGactc	5.84	-7.94
STM0465	S	ybaY	glycoprotein/polysaccharide metabolism	-15.14	5.29	1.46E-05	-4.75	R	-75	-54	ctggTGCATTGATGATAAGGAGAATTgaat	5.34	-7.53
STM0467	-	ffs	signal recognition particle, RNA component	-9.58	5.21	1.67E-04	-4.45						
STM0650	G	STM0650	putative hydrolase C-terminus	-9.87	5.46	1.10E-04	-4.56	D	-55	-34	aaggGAAAATGATTATGAGCAATGAGactt	6.95	-8.95
STM0659	O	hscC	putative heatshock protein, homolog of hsp70 in Hsc66 subfamily	-15.49	8.16	2.46E-07	-2.82	R	-128	-107	gcgtGACATTGATAAAGATCACCACGccag	5.25	-7.46
STM0662	E	gltL	ABC superfamily (atp_bind), glutamate/aspartate transporter	12.96	5.46	2.61E-05	4.39	R	-309	-288	actgGCAGTCGATGAAGCTCATTATTctgc	5.97	-8.06

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM0663	E	gltK	ABC superfamily (membrane), glutamate/aspartate transporter	12.08	8.19	1.67E-06	2.81						
STM0664	E	gltJ	ABC superfamily (membrane), glutamate/aspartate transporter	12.47	7.16	4.09E-06	2.67	D	-62	-41	tttcGGAGTAGATGTATGTCAATAGActgg	4.58	-6.93
STM0665	E	gltI	ABC superfamily (bind_prot), glutamate/aspartate transporter	10.65	5.77	5.20E-05	4.24	D	-216	-195	taggATTTTTGCCTCTGAACGGTGCGgcgc	5.67	-7.8
STM0699	R	STM0699	putative cytoplasmic protein	-15.11	6.35	3.25E-06	-4.22	R	-30	-9	ggaaGTCACTGATATAGCAGAAATACtggc	6.99	-8.99
STM0728	L	nei	endonuclease VIII removing oxidized pyrimidines may also remove oxidized purines in absence of MutY and Fpg [EC:3.2.-.-]	16.01	6.47	1.90E-06	2.57	D	-80	-59	tccaTTAATCAACTGTTAACAAAGGAtatt	5.12	-7.35
STM0737	C	sucB	2-oxoglutarate dehydrogenase (dihydrolipoyltranssuccinase E2 component)	14.92	9.44	7.01E-08	2.75						
STM0738	C	sucC	succinyl-CoA synthetase, beta subunit	21.50	5.79	9.65E-07	4.03	D	-39	-18	acatGAATATCAGGCAAAACAACTTTttgc	6.69	-8.71

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM0739	C	sucD	succinyl-CoA synthetase, alpha subunit	23.03	5.62	8.87E-07	4.66						
STM0740	C	cydA	cytochrome d terminal oxidase, polypeptide subunit I [EC:1.10.3.-]	17.22	6.09	2.13E-06	2.55	R	-307	-286	gccaTAAATTGATCGCTGTCGAAAAAagca	10.38	-13.11
STM0741	C	cydB	cytochrome d terminal oxidase polypeptide subunit II	21.16	5.67	1.30E-06	3.74	D	-169	-148	cagaATTGTTCCTGATGTTCAAATTTgcac	5.62	-7.76
STM0742	S	ybgT	putative outer membrane lipoprotein	11.38	7.56	5.01E-06	4.34	R	-165	-144	tgttCGTACCGATCATTCTGATCTACacca	4.83	-7.12
STM0743	S	ybgE	putative inner membrane lipoprotein	13.92	9.36	1.44E-07	3.48	R	-102	-81	cacgTGCACTGTTGGAAGAGGTTATCcgac	4.31	-6.72
STM0759	-	ybgS	putative homeobox protein	-14.35	5.04	2.80E-05	-4.60						
STM0761	C	STM0761	fumarate hydratase Class I anaerobic	6.55	5.53	8.38E-04	4.48						
STM0762	C	STM0762	fumarate hydratase, alpha subunit	8.26	5.15	3.67E-04	5.69	D	-340	-319	agttTATTTTTCTTTCTATCAAATAAtgtc	6.33	-8.37
STM0781	P	modA	ABC superfamily (peri_perm), molybdate transporter	-22.42	7.25	5.78E-08	-3.12	R	-140	-119	tttaATCGTTAATGGGTATGAATAACcgct	6.43	-8.47

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM0790	-	hutU	pseudogene; frameshift relative to *Pseudomonas putida* urocanate hydratase (HUTU) (SW:P25080)	9.49	5.45	1.36E-04	5.44						
STM0791	E	hutH	histidine ammonia lyase	19.06	6.84	3.51E-07	4.55						
STM0828	E	glnQ	ABC superfamily (atp_bind), glutamine high-affinity transporter	8.23	6.06	1.66E-04	2.86						
STM0830	E	glnH	ABC superfamily (bind_prot), glutamine high-affinity transporter	9.60	5.47	1.26E-04	3.70						
STM0853	-	yliH	putative cytoplasmic protein	-15.57	6.53	2.08E-06	-3.33						
STM0907	R	aSTM0907	Fels-1 prophage; putative chitinase	-6.72	5.39	8.16E-04	-3.12						
STM0912	O	aSTM0912	Fels-1 prophage; protease subunits of ATP-dependent proteases, ClpP family	11.94	7.50	3.81E-06	3.40						

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM0964	C	dmsA	anaerobic dimethyl sulfoxide reductase, subunit A	-18.73	5.00	7.95E-06	-200.85	D	-151	-130	ctacTTTTTCGATATATATCAGACTTtata	7.44	-9.43
STM0965	C	dmsB	anaerobic dimethyl sulfoxide reductase, subunit B	-53.46	5.29	1.93E-08	-62.55						
STM0966	R	dmsC	anaerobic dimethyl sulfoxide reductase, subunit C	-28.93	5.04	8.52E-07	-23.60	R	-260	-239	gtaaGAAACCGATTTGCGTCGAATCCtgcc	8.79	-10.93
STM0972	-	STM0972	homologous to secreted protein sopD	5.62	6.48	1.04E-03	3.07	D	-256	-235	aataATTCTCAACATAATTCAGATGTgtcc	4.68	-7.01
STM0974	P	focA	putative FNT family, formate transporter (formate channel 1)	-7.49	5.77	3.53E-04	-3.19	R	-129	-108	ggcgAGATATGATCTATATCAAATTCtcat	8.35	-10.41
STM0989	-	STM0989	mukF protein (killing factor KicB)	-10.99	5.11	9.47E-05	-4.90	R	-279	-258	cgtgCGCGCTGAAATGCGTTAACATGatcc	4	-6.5
STM1118	-	yccJ	putative cytoplasmic protein	-9.37	5.50	1.38E-04	-4.94						
STM1119	R	wraB	trp-repressor binding protein	-10.54	5.13	1.14E-04	-6.45	D	-167	-146	attaATTATTGTTATAAATCAAAGAAatgg	9.3	-11.57
STM1123	S	STM1123	putative periplasmic protein	4.28	6.28	4.69E-03	3.13						

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM1124	-	putA	bifunctional in plasma membrane proline dehydrogenase and pyrroline-5-carboxylate dehydrogenase OR in cytoplasm a transcriptional repressor	24.50	6.20	2.07E-07	7.57						
STM1125	E	putP	SSS family, major sodium/proline symporter	14.50	5.46	1.44E-05	7.16	D	-195	-174	tgtaAATGGTGTGTTAAATCGATTGTgaat	7.78	-9.79
STM1126	-	phoH	PhoB-dependent, ATP-binding pho regulon component	9.41	6.88	3.56E-05	2.63	NA	NA	NA	NA	NA	NA
STM1128	E	STM1128	putative sodium/glucose cotransporter	-18.20	6.39	9.58E-07	-4.78	R	-289	-268	cctgAAGCCTGTTTGAACGCAATATCggat	5.25	-7.45
STM1129	G	STM1129	putative inner membrane protein	-15.72	5.52	8.59E-06	-6.50						
STM1130	S	STM1130	putative inner membrane protein	-9.85	5.15	1.56E-04	-11.85						
STM1131	-	STM1131	putative outer membrane protein	-10.50	5.84	5.25E-05	-4.67	D	-58	-37	cggaGTATTTTATGAAAATCAACAAAtatc	7.06	-9.06
STM1132	G	STM1132	putative sugar transort protein	-9.42	5.29	1.67E-04	-6.20	R	-120	-99	ttcgGCAATTGATATGACTTAAAAATtaat	10.03	-12.57

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM1133	R	STM1133	putative dehydrogenases and related proteins	-14.98	5.29	1.56E-05	-5.56	D	-90	-69	cgtgAAAGTTTTCAATCAACAAAAGAattt	4.6	-6.94
STM1138	-	ycdZ	putative inner membrane protein	-9.09	5.03	2.62E-04	-11.00	D	-106	-85	agaaTAATGTGATGTAAATCACCCTTaact	5.45	-7.62
STM1171	N	flgN	flagellar biosynthesis: belived to be export chaperone for FlgK and FlgL	-12.69	5.24	3.93E-05	-7.85						
STM1172	K	flgM	anti-FliA (anti-sigma) factor; also known as RflB protein	-13.69	5.30	2.46E-05	-5.75	R	-72	-51	cgtaACCCTCGATGAGGATAAATAAAtgag	5.44	-7.61
STM1176	N	flgD	flagellar biosynthesis, initiation of hook assembly	-11.76	5.49	4.21E-05	-2.94						
STM1177	N	flgE	flagellar biosynthesis, hook protein	-14.54	5.88	7.83E-06	-3.56						
STM1178	N	flgF	flagellar biosynthesis, cell-proximal portion of basal-body rod	-7.59	6.06	2.58E-04	-2.81						
STM1179	N	flgG	flagellar biosynthesis, cell-distal portion of basal-body rod	-7.52	5.56	4.10E-04	-3.50						

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM1183	N	flgK	flagellar biosynthesis, hook-filament junction protein 1	-11.80	5.20	5.95E-05	-5.67						
STM1184	N	flgL	flagellar biosynthesis; hook-filament junction protein	-11.04	5.33	7.16E-05	-4.18						
STM1227	E	pepT	putative peptidase T(aminotripeptidase)	-13.20	6.24	8.57E-06	-2.67						
STM1254	-	STM1254	putative outer membrane lipoprotein	-8.06	5.65	2.64E-04	-3.82						
STM1271	P	yeaR	putative cytoplasmic protein	13.89	5.66	1.37E-05	4.25	R	-284	-263	gcaaTTCTTTGATTGGCCTTCTTTTCgtcg	4.81	-7.1
STM1272	-	yoaG	putative cytoplasmic protein	6.92	7.98	1.23E-04	2.87	D	-225	-204	cggaAGAGATCATGGTGATCAATGCCggcg	8.55	-10.65
STM1300	-	STM1300	putative periplasmic protein	-8.78	5.08	2.93E-04	-4.83	D	-256	-235	ggttGTATTTGCGTTTTATCAGAATAtgta	6.36	-8.4
STM1301	L	STM1301	putative mutator MutT protein	-9.26	5.65	1.27E-04	-3.36						
STM1349	G	pps	phosphoenolpyruvate synthase	-19.01	5.69	2.28E-06	-3.45	D	-58	-37	aggaTTGTTCGATGTCCAACAATGGCtcgt	5.74	-7.86
STM1378	G	pykF	pyruvate kinase I (formerly F), fructose stimulated	14.46	5.51	1.36E-05	2.55						
STM1489	H	ynfK	putative dethiobiotin synthase	-11.20	5.23	7.52E-05	-8.40	R	-140	-119	aactCAAGCTGATTGCCCTTGCCATAtctt	4.73	-7.04

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM1498	C	STM1498	putative dimethyl sulphoxide reductase	-21.41	5.10	3.44E-06	-27.55	R	-177	-156	atacAAATCTGGTGGAAATCGAAAAAatct	4.87	-7.15
STM1499	C	STM1499	putative dimethyl sulphoxide reductase, chain A1	-19.08	5.35	3.98E-06	-8.27	D	-101	-80	ataaTTTCGTTATAGTTATCAATATAtagc	4.38	-6.78
STM1509	-	ydfZ	putative cytoplasmic protein	-14.41	5.58	1.25E-05	-7.62	R	-161	-140	ccgtGAGCTTGATCAAAAACAAAAAAaatt	8.84	-10.98
STM1538	C	STM1538	putative hydrogenase-1 large subunit	11.55	5.78	3.28E-05	3.96						
STM1539	C	STM1539	putative hydrogenase-1 small subunit	8.66	5.44	2.21E-04	3.53						
STM1562	-	STM1562	putative periplasmic transport protein	-11.30	5.28	6.68E-05	-4.99	D	-217	-196	tgctTTATTCATCAAACATCAAAATCagtc	6.71	-8.73
STM1564	-	yddX	putative cytoplasmic protein	-10.75	8.27	3.83E-06	-2.93						
STM1568	C	fdnI	formate dehydrogenase-N, cytochrome B556(Fdn) gamma subunit, nitrate-inducible	-28.97	6.32	5.81E-08	-3.98	R	-119	-98	tcgcCGGTCTGATTTACCACTACATCggta	7.14	-9.14
STM1569	C	fdnH	formate dehydrogenase, iron-sulfur subunit (formate dehydrogenase beta subunit) [EC:1.2.1.2]	-9.33	8.93	6.68E-06	-2.50						

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM1593	-	srfA	ssrAB activated gene	-5.82	6.28	9.63E-04	-2.52	D	-185	-164	acccTGATTTAACTTACGTCAAGTGGaaac	5.8	-7.91
STM1594	S	srfB	ssrAB activated gene	-14.70	6.14	5.14E-06	-2.88	D	-58	-37	tgccTGATTTTATGTTGGTCAATCTGtgtg	5.31	-7.5
STM1626	N	trg	methyl-accepting chemotaxis protein III, ribose and galactose sensor receptor	-13.30	5.46	2.28E-05	-5.72						
STM1640	S	ydcF	putative inner membrane protein	-11.44	5.63	4.15E-05	-5.72						
STM1641	L	hrpA	helicase, ATP-dependent	-21.19	6.25	4.68E-07	-20.97	R	-217	-196	gtgcCCCGTTGCTTGTTGACACTTTAttca	4.72	-7.04
STM1642	I	acpD	acyl carrier protein phosphodiesterase	-11.32	7.81	4.05E-06	-4.01	D	-107	-86	tgaaTAAAGTGTCAACAAGCAACGGGgcac	4.72	-7.04
STM1647	C	ldhA	fermentative D-lactate dehydrogenase, NAD-dependent	-16.29	5.95	3.65E-06	-24.42	D	-139	-118	tcatTATATGTATGACTATCAATTATtttt	5.05	-7.29
STM1648	O	hslJ	heat shock protein hslJ	-8.34	5.41	2.75E-04	-5.00	R	-74	-53	ttaaCTATCAGATTACAGAGAATATCaatg	4.11	-6.57
STM1650	-	STM1650	putative reverse transcriptase	-5.38	7.56	7.97E-04	-3.52						
STM1651	C	nifJ	putative pyruvate-flavodoxin oxidoreductase	-8.67	6.43	8.87E-05	-4.97						
STM1652	T	ynaF	putative universal stress protein	-20.67	5.01	4.81E-06	-116.15	R	-282	-261	ttatTGAATTAAACGGTAACATCTCTtttt	4.7	-7.02

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM1653	P	STM1653	putative membrane transporter of cations	-10.25	5.85	5.92E-05	-6.56						
STM1657	N	STM1657	putative methyl-accepting chemotaxis protein	-9.41	6.29	6.15E-05	-4.01	D	-52	-31	caaaAATGTTGAGAAATATCAGCGTCagga	8.58	-10.68
STM1658	S	ydaL	putative Smr domain	-28.29	6.75	2.87E-08	-4.01						
STM1659	L	ogt	O-6-alkylguanine-DNA/cysteine-protein methyltransferase	-6.79	6.25	4.20E-04	-2.92						
STM1660	-	fnr	transcriptional regulation of aerobic, anaerobic respiration, osmotic balance (CRP family)	-8.24	5.04	4.11E-04	-6.55	D	-88	-67	tgttAAAATTGACAAATATCAATTACggct	11.43	-14.93
STM1688	K	pspC	phage shock protein; regulatory gene, activates expression of psp operon with PspB	11.96	6.86	7.63E-06	3.23	R	-45	-24	gggtGGAATCAATCTGAATAAAAAACtatg	6.11	-8.18
STM1706	J	yciH	putative translation initiation factor SUI1	9.58	5.71	9.91E-05	4.32						

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM1732	M	ompW	outer membrane protein W; colicin S4 receptor; putative transporter	-6.72	5.07	1.05E-03	-8.36	R	-172	-151	gttcTAAATTAATCTGGATCAATAAAtgtt	8.33	-10.39
STM1746	-	oppA	ABC superfamily (periplasm), oligopeptide transport protein with chaperone properties	16.43	6.25	2.23E-06	3.83	R	-290	-269	atttCACATTGTTGATAAGTATTTTCattt	5.36	-7.54
STM1767	T	narL	response regulator in two-component regulatory system with NarX (or NarQ), regulates anaerobic respiration and fermentation (LuxR/UhpA familiy)	11.78	6.53	1.22E-05	2.78						
STM1781	P	ychM	putative SulP family transport protein	-10.77	6.07	3.49E-05	-3.33	R	-230	-209	acgaAGAATCGATTTCCGCCATGTTCgagc	5.29	-7.49
STM1795	E	STM1795	putative homologue of glutamic dehyrogenase	12.42	5.46	3.28E-05	5.33	R	-302	-281	acatAACATTGATACATGTCGTTATCataa	6.57	-8.6
STM1798	-	ycgR	putative inner membrane protein	-14.17	5.94	8.40E-06	-4.09	D	-267	-246	tggcGATAACGCCGGCAATCAAACCAaaaa	6.66	-8.68

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM1803	E	dadA	D-amino acid dehydrogenase subunit	10.10	8.96	3.40E-06	3.16	R	-261	-240	cctcCACATTGAACGGCAAAAAATCGggta	4.58	-6.92
STM1831	G	manY	Sugar Specific PTS family, mannose-specific enzyme IIC	15.20	5.98	5.28E-06	3.73	R	-146	-125	atccGAAACTGAAAATGATGGATTTAattg	4.99	-7.24
STM1832	G	manZ	Sugar Specific PTS family, mannose-specific enzyme IID	14.15	9.24	1.42E-07	2.96	D	-277	-256	ttggTTATGCGATGGTTATCAATATGatgc	7.21	-9.2
STM1915	N	cheZ	chemotactic response; CheY protein phophatase	-9.59	5.76	9.42E-05	-3.56						
STM1916	T	cheY	chemotaxis regulator, transmits chemoreceptor signals to flagelllar motor components	-9.32	5.69	1.18E-04	-3.74	D	-111	-90	agcaGATGTTGGCGAAAATCAGTGCCggac	8.6	-10.7
STM1917	N	cheB	methyl esterase, response regulator for chemotaxis (cheA sensor)	-8.37	5.74	2.00E-04	-4.33						
STM1918	N	cheR	glutamate methyltransferase, response regulator for chemotaxis	-22.41	8.99	3.40E-09	-3.32						

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM1919	N	cheM	methyl accepting chemotaxis protein II, aspartate sensor-receptor	-18.86	5.13	6.12E-06	-8.31						
STM1920	N	cheW	purine-binding chemotaxis protein; regulation	-14.43	5.32	1.82E-05	-4.31	D	-296	-275	gggcATTGTTGTGATCCTGCAAAGCGcggg	4.39	-6.78
STM1921	N	cheA	sensory histitine protein kinase, transduces signal between chemo- signal receptors and CheB and CheY	-16.27	6.02	3.32E-06	-4.67	D	-39	-18	ggatATTAGCGATTTTTATCAGACATtttt	4.84	-7.12
STM1922	N	motB	enables flagellar motor rotation, linking torque machinery to cell wall	-15.03	5.33	1.43E-05	-7.00	R	-177	-156	atgcGCCGCCGATTGCCGTGGAATTTggtc	5.72	-7.84
STM1923	N	motA	proton conductor component of motor, torque generator	-5.97	5.07	1.80E-03	-10.82						
STM1932	P	ftnB	ferritin-like protein	-21.51	5.01	3.92E-06	-21.11	D	-95	-74	tctcGTCTGTCATGCACATCAACACTttct	4.24	-6.67
STM1955	-	fliZ	putative regulator of FliA	-15.76	5.35	1.09E-05	-6.25	D	-331	-310	acagGAAAACCCGTTACATCAACTGCtgga	5.78	-7.9

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM1956	K	fliA	sigma F (sigma 28) factor of RNA polymerase, transcription of late flagellar genes (class 3a and 3b operons)	-21.86	8.81	5.60E-09	-5.96	R	-64	-43	acgcAGGGCTGTTTATCGTGAATTCActgt	4.87	-7.15
STM1960	N	fliD	flagellar biosynthesis; filament capping protein; enables filament assembly	-15.69	6.81	1.35E-06	-4.45	R	-82	-61	cttaACTACTGTTTGCAATCAAAAAGgaag	4.63	-6.97
STM1961	O	fliS	flagellar biosynthesis; repressor of class 3a and 3b operons (RflA activity)	-9.80	5.27	1.40E-04	-5.10	R	-231	-210	acgcCACGCTGAAAAGCCTGACAAAAcagt	4.08	-6.56
STM1962	-	fliT	flagellar biosynthesis; possible export chaperone for FliD	-7.66	5.16	5.25E-04	-6.03						
STM1971	N	fliH	flagellar biosynthesis; possible export of flagellar proteins	-8.61	6.32	1.01E-04	-2.94						
STM1973	N	fliJ	flagellar fliJ protein	-7.71	6.36	1.88E-04	-4.27						
STM1975	N	fliL	flagellar biosynthesis	-8.94	5.55	1.68E-04	-2.79						

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM1976	N	fliM	flagellar biosynthesis, component of motor switch and energizing	-9.19	5.26	1.95E-04	-3.07	R	-161	-140	aacaAAAACTGATTGCCGCCATTAAAgaga	5.62	-7.76
STM1978	N	fliO	flagellar biosynthesis	-5.21	6.02	1.98E-03	-2.76						
STM2059	S	yeeX	putative cytoplasmic protein	8.96	6.90	4.79E-05	2.75						
STM2183	F	cdd	cytidine/deoxycytidine deaminase	-17.85	5.47	4.66E-06	-7.71	R	-157	-136	atttTTCATTGAAGTTTCACAAGTTGcata	5.31	-7.51
STM2186	E	STM2186	putative NADPH-dependent glutamate synthase beta chain or related oxidoreductase	-15.60	5.64	7.43E-06	-4.18	D	-269	-248	cttcTTTTTTATCGTTAATCTATTTAttat	5.46	-7.63
STM2187	F	yeiA	putative dihydropyrimidine dehydrogenase	-15.69	5.44	9.74E-06	-4.05						
STM2277	F	nrdA	ribonucleoside diphosphate reductase 1, alpha subunit	26.74	5.26	8.07E-07	11.15	D	-60	-39	ggtaGAAAACCACATGAATCAGAGTCtgct	4.2	-6.64
STM2278	F	nrdB	ribonucleoside-diphosphate reductase 1, beta subunit	29.51	5.69	1.92E-07	9.57	D	-68	-47	tcccATAAAGGATTCACTTCAATGGCatac	6.02	-8.11
STM2279	C	yfaE	putative ferredoxin	6.33	5.30	1.17E-03	3.37	R	-297	-276	acggTTCGATGATCGGCCTGAATAAAgata	5.02	-7.27
STM2280	G	STM2280	putative permease	11.04	6.42	2.06E-05	4.32						

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM2287	-	STM2287	putative cytoplasmic protein	4.59	6.33	3.24E-03	3.46	R	-279	-258	ggggATAACTGAATATCCCCAATAATaatt	4.46	-6.84
STM2314	T	STM2314	putative chemotaxis signal transduction protein	-25.76	5.48	6.21E-07	-6.99						
STM2315	R	yfbK	putative von Willebrand factor, vWF type A domain	-15.60	9.01	7.88E-08	-2.84						
STM2316	-	nuoN	NADH dehydrogenase I chain N	8.29	6.13	1.50E-04	2.67	R	-334	-313	tggtGCCGGTGATTACCGTGATCTCCacct	4.26	-6.69
STM2318	C	nuoL	NADH dehydrogenase I chain L	8.02	7.14	8.05E-05	2.54	R	-261	-240	tgttTATGCTGATTGGGCTGGAAATCatga	5.57	-7.71
STM2320	C	nuoJ	NADH dehydrogenase I chain J [EC:1.6.5.3]	11.27	6.55	1.58E-05	2.54						
STM2324	C	nuoF	NADH dehydrogenase I chain F	9.85	7.93	1.01E-05	2.60						
STM2325	C	nuoE	NADH dehydrogenase I chain E	8.07	8.32	3.28E-05	2.65						
STM2326	C	nuoC	NADH dehydrogenase I chain C,D	22.56	6.50	2.04E-07	3.34						
STM2327	C	nuoB	NADH dehydrogenase I chain B [EC:1.6.5.3]	11.71	6.20	1.86E-05	2.52						

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM2334	R	yfbT	putative phosphatase	18.20	6.34	1.03E-06	3.49	R	-76	-55	gagcGCGAATGAAATCAATCAAATCAttaa	5.55	-7.7
STM2335	S	yfbU	putative cytoplasmic protein	6.74	5.61	6.87E-04	3.39						
STM2337	C	ackA	acetate kinase A (propionate kinase 2)	-16.97	6.34	1.60E-06	-3.51	R	-147	-126	tcctGCGCATGATGTTAATCATAAATgtca	4.76	-7.07
STM2338	C	pta	phosphotransacetylase	-6.43	5.94	6.96E-04	-3.17						
STM2340	G	STM2340	putative transketolase	11.33	5.19	7.42E-05	6.60	D	-82	-61	gctcAATGAGGCCATTCATCAACTGGaggt	4.18	-6.62
STM2341	G	STM2341	putative transketolase	23.38	5.42	1.19E-06	6.47						
STM2342	S	STM2342	putative inner membrane protein	9.98	5.52	9.77E-05	5.22	R	-276	-255	aaaaAGTATTAAAGAAACTCAATATTgacg	6.82	-8.83
STM2343	G	STM2343	putative cytoplasmic protein	10.35	6.09	4.30E-05	3.34	D	-64	-43	tttaAAAGGTGACAATAATGAAAATCatgg	4.64	-6.97
STM2409	F	nupC	NUP family, nucleoside transport	-15.30	5.45	1.09E-05	-3.91	D	-347	-326	gtttATTGATAATGATTATCAAGTGCattt	5.87	-7.97
STM2454	K	eutR	putative regulator ethanolamine operon (AraC/XylS family)	-12.08	5.35	4.34E-05	-5.13						
STM2455	Q	eutK	putative carboxysome structural protein, ethanolamine utilization	-9.27	5.21	1.97E-04	-6.77	D	-61	-40	aacgGAGGCTGCCAATGATCAATGCCctgg	4.45	-6.83

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM2456	E	eutL	putative carboxysome structural protein, ethanolamine utilization	-9.22	5.22	1.99E-04	-6.68						
STM2457	E	eutC	ethanolamine ammonia-lyase, light chain	-16.21	5.56	6.85E-06	-7.07						
STM2458	E	eutB	ethanolamine ammonia-lyase, heavy chain	-17.30	5.54	4.94E-06	-6.33	D	-315	-294	ccgcATTACTCACGGTCATCAACGCGctga	5.38	-7.56
STM2459	E	eutA	CPPZ-55 prophage; chaperonin in ethanolamine utilization	-19.26	5.16	5.24E-06	-6.17						
STM2460	E	eutH	putative transport protein, ethanolamine utilization	-17.75	5.32	6.09E-06	-6.29						
STM2462	E	eutJ	paral putative heatshock protein (Hsp70)	-7.74	5.05	5.52E-04	-7.30						
STM2463	C	eutE	putative aldehyde oxidoreductase in ethanolamine utilization	-18.52	5.34	4.73E-06	-7.02	R	-64	-43	aaatAGGATTGAACATCATGAATCAAcagg	4.88	-7.15
STM2464	Q	eutN	putative detox protein in ethanolamine utilization	-14.11	5.14	2.65E-05	-8.00	D	-194	-173	tggaAGAAGTGTTCCCGATCAGCTTCaaag	5.21	-7.42

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM2465	Q	eutM	putative detox protein in ethanolamine utilization	-28.26	5.11	8.21E-07	-11.02	R	-250	-229	ctatCGCGCTGTTGGGCCGCTAATTCaggg	4.58	-6.93
STM2466	C	eutD	putative phosphotransacetylase in ethanolamine utilization	-15.83	5.11	1.53E-05	-10.36						
STM2467	E	eutT	putative cobalamin adenosyltransferase, ethanolamine utilization	-16.88	5.92	3.13E-06	-6.59	R	-237	-216	cgtgGACGCTGAACTACGACGAAATCgaca	6.89	-8.9
STM2468	E	eutQ	putative ethanolamine utilization protein	-18.58	5.26	5.33E-06	-7.60						
STM2469	E	eutP	putative ethanolamine utilization protein	-20.06	5.13	4.47E-06	-9.38	R	-247	-226	aaacGGCGATGATCGCTGGCGATTTAgcga	4.71	-7.03
STM2470	E	eutS	putative carboxysome structural protein, ethanol utilization	-10.43	5.05	1.32E-04	-13.70	R	-154	-133	ttctCTTAGTGATCTACCTCACCTTTtaca	5.95	-8.05
STM2479	E	aegA	putative oxidoreductase	-7.82	7.39	7.90E-05	-3.37	R	-187	-166	gaaaTAAATTGATCTGCCACAGGTTCtgga	7.26	-9.26
STM2530	C	STM2530	putative anaerobic dimethylsulfoxide reductase	-10.14	7.00	1.96E-05	-3.67	D	-158	-137	ttatGAATTTCATTTAATTTAAAGTTaatg	6.79	-8.8

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM2556	C	hmpA	dihydropteridine reductase 2 and nitric oxide dioxygenase activity	15.99	5.95	4.09E-06	3.01	R	-95	-74	agatGCATTTGATATACATCATTAGAtttt	6.24	-8.3
STM2558	E	cadB	APC family, lysine/cadaverine transport protein	-6.65	5.19	1.01E-03	-6.43	D	-219	-198	tatgTTAATTCAAAAAAATCAATCTAtcag	6.78	-8.8
STM2559	E	cadA	lysine decarboxylase 1	-10.67	5.18	1.02E-04	-5.58	R	-221	-200	tcgtCAGTCTGATCATCCTGATGTTCtacg	5.74	-7.86
STM2646	R	yfiD	putative formate acetyltransferase	-26.43	5.69	3.54E-07	-5.08	D	-179	-158	ggttTTTATTGATTTAAATCAAAGAAtgaa	10.76	-13.71
STM2733	-	STM2733	Fels-2 prophage: similar to *E. coli* retron Ec67	-5.44	8.92	4.26E-04	-2.72						
STM2786	S	STM2786	tricarboxylic transport	19.84	5.84	1.38E-06	8.21						
STM2787	-	STM2787	tricarboxylic transport	10.54	9.96	1.01E-06	6.89						
STM2788	S	STM2788	tricarboxylic transport	11.85	5.46	4.19E-05	3.46	R	-107	-86	ttgaCCGGCTGCTTGATGTCACCTTAcctc	4.26	-6.68
STM2795	S	ygaU	putative LysM domain	-3.59	5.59	1.31E-02	-2.98	D	-55	-34	aggtGAATATGGGACTTTTCAATTTTgtaa	5.43	-7.6
STM2851	C	hycC	hydrogenase 3, membrane subunit (part of FHL complex)	-5.41	8.55	5.07E-04	-3.49						
STM2855	O	hypB	hydrogenase-3 accessory protein, assembly of metallocenter	-14.31	6.06	6.74E-06	-3.12	R	-143	-122	cataGAGATTGATGAAACTGAAGATTaatg	9.23	-11.47

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM2856	O	hypC	putative hydrogenase expression/formation protein	-9.09	6.18	8.40E-05	-2.56						
STM2857	O	hypD	putative hydrogenase expression/formation protein	-8.01	5.29	3.76E-04	-2.97						
STM2871	U	prgK	cell invasion protein; lipoprotein, may link inner and outer membranes	-15.27	7.06	1.15E-06	-3.25						
STM2872	-	prgJ	cell invasion protein; cytoplasmic	-9.60	5.35	1.43E-04	-4.65	R	-344	-323	agccCACTTTAATTTAACGTAAATAAggaa	5.69	-7.82
STM2873	-	prgI	cell invasion protein; cytoplasmic	-12.42	5.80	2.13E-05	-4.16	R	-83	-62	agccCACTTTAATTTAACGTAAATAAggaa	5.69	-7.82
STM2874	-	prgH	cell invasion protein	-16.99	6.31	1.65E-06	-3.85						
STM2877	M	iagB	cell invasion protein	-4.52	5.49	5.01E-03	-3.55	R	-86	-65	ccgcTTGATTAAATTACGGTAAAATCtgag	4.68	-7
STM2886	R	sicA	surface presentation of antigens; secretory proteins	-10.24	5.19	1.24E-04	-2.80	D	-57	-36	ggagTAAGTAATGGATTATCAAAATAatgt	4.34	-6.75
STM2890	U	spaP	surface presentation of antigens; secretory proteins	-5.39	5.53	2.17E-03	-4.27	D	-88	-67	cttaGGCGTTGAGATCCATGAATGGCtgag	4.61	-6.95

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM2891	N	spaO	surface presentation of antigens; secretory proteins	-8.43	5.86	1.72E-04	-3.67						
STM2892	-	invJ	surface presentation of antigens; secretory proteins	-7.06	5.22	7.34E-04	-5.51	D	-239	-218	tttaGAACTCCAGATTATACAAATTCagga	4.22	-6.66
STM2893	-	invI	surface presentation of antigens; secretory proteins	-12.02	5.46	3.91E-05	-4.44	R	-190	-169	gcttTTCATTGACTTGGGAGAATATCgtcc	6.09	-8.16
STM2894	N	invC	surface presentation of antigens; secretory proteins	-6.62	6.04	5.54E-04	-2.79						
STM2895	-	invB	surface presentation of antigens; secretory proteins	-10.44	5.50	7.85E-05	-3.88	R	-267	-246	ccgcTAATTTGATGGATCTCATTACActta	8.41	-10.48
STM2896	U	invA	invasion protein	-6.84	5.81	5.50E-04	-3.34						
STM2897	-	invE	invasion protein	-5.52	6.12	1.40E-03	-3.09	R	-29	-8	tccgGTATTTCATTTTCCAGAATATTgtcc	4.35	-6.76
STM2898	N	invG	invasion protein; outer membrane	-6.82	5.89	5.30E-04	-3.48	D	-126	-105	cacaTTTTTCTAGTGAGATCAAAGAGctga	7.49	-9.49
STM2899	K	invF	invasion protein	-5.45	5.63	1.95E-03	-3.57						
STM2983	-	ygdI	putative lipoprotein	-7.97	6.00	2.09E-04	-3.64						
STM3019	I	yqeF	putative acetyl-CoA acetyltransferase	10.03	6.84	2.46E-05	2.58	R	-249	-228	ctatTGATTTGCTGTGGAACAAGAAAacgc	4.54	-6.9

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM3131	S	STM3131	putative cytoplasmic protein	-17.74	9.67	1.07E-08	-7.24	R	-77	-56	actgCCACCTGATCAACAAGGAGATAaatc	5.09	-7.32
STM3136	G	STM3136	putative D-mannonate oxidoreductase	9.94	5.61	8.96E-05	2.88						
STM3138	N	STM3138	putative methyl-accepting chemotaxis protein	-8.98	5.43	1.84E-04	-4.76						
STM3155	-	STM3155	putative cytoplasmic protein	-7.23	7.42	1.31E-04	-2.81	R	-78	-57	gtatACCACTGATCGTAAAGGATATTtagt	7.28	-9.28
STM3216	N	STM3216	putative methyl-accepting chemotaxis protein	-15.35	7.19	9.30E-07	-3.37	R	-280	-259	tctaCCTATTAATAGGTATAAACTCAgtta	5.59	-7.73
STM3217	T	aer	aerotaxis sensor receptor, senses cellular redox state or proton motive force	-12.23	5.32	4.29E-05	-4.77	D	-238	-217	aaagGTTGTCCACGCTAAACAATTTCataa	8.02	-10.04
STM3225	E	ygjU	putative dicarboxylate permease	17.69	9.71	1.04E-08	3.68						
STM3238	S	yhaN	putative inner membrane protein	-6.92	6.10	4.20E-04	-3.18	R	-146	-125	aaggCGCTTCGTTGTACCTGATTATTatta	4.55	-6.9
STM3240	E	tdcG	L-serine deaminase	-16.72	5.57	5.68E-06	-4.80	R	-249	-228	ggatGCGATTGAACATCCGGAAAACTaccc	6.02	-8.11
STM3241	C	tdcE	pyruvate formate-lyase 4/ 2-ketobutyrate formate-lyase	-9.34	10.00	2.95E-06	-2.66						

WO 2008/108805 PCT/US2007/016357

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM3242	C	tdcD	propionate kinase/acetate kinase II, anaerobic	-14.83	7.09	1.35E-06	-4.10	R	-147	-126	tgacCATCCTGAATATTGTCTACAAAttgt	4.53	-6.89
STM3245	K	tdcA	transcriptional activator of tdc operon (LysR family)	-17.03	5.41	6.59E-06	-6.04	D	-239	-218	cctgTTTTTTGATTGAAATCAGGCTAagtt	8.48	-10.57
STM3248	I	garR	tartronate semialdehyde reductase (TSAR)	15.78	5.93	4.53E-06	4.16						
STM3273	I	yhbT	putative lipid carrier protein	-9.33	5.49	1.43E-04	-2.71	D	-237	-216	acgcAAAATTGTTAACGAACAGGGATttta	5.52	-7.68
STM3274	O	yhbU	putative protease	-10.06	5.51	9.38E-05	-10.68	R	-103	-82	taaaATCCCTGTTCGTTAACAATTTTgcgt	5.52	-7.68
STM3275	-	yhbV	putative protease	-13.35	5.95	1.16E-05	-10.88						
STM3334	F	STM3334	putative cytosine deaminase	-8.46	6.65	8.47E-05	-2.58	D	-277	-256	agtgATTATTGCCGACTATCTGTTGAaccg	4	-6.5
STM3338	G	nanT	MFS family, sialic acid transport protein	-19.92	5.67	1.83E-06	-3.02						
STM3545	R	yhhX	putative oxidoreductase	14.13	5.91	8.84E-06	3.21						
STM3547	-	STM3547	putative transcriptional regulator of sugar metabolism	54.21	5.60	7.77E-09	6.06						
STM3548	-	STM3548	putative cytoplasmic protein	40.36	5.24	9.82E-08	9.29	R	-327	-306	gcttGCTTATGATGGCACACAATTCAtcta	4.98	-7.24

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM3549	S	STM3549	putative inner membrane protein	13.96	5.28	2.25E-05	7.22						
STM3550	R	STM3550	putative phosphotriesterase	22.45	5.17	2.34E-06	7.40						
STM3576	P	zntA	P-type ATPase family, Pb/Cd/Zn/Hg transporting ATPase [EC:3.6.3.3 3.6.3.5]	9.90	5.54	9.92E-05	2.55	R	-261	-240	cgacGCTGCTGTTCATCGGCAATATCgtct	5.02	-7.27
STM3577	N	tcp	methyl-accepting transmembrane citrate/phenol chemoreceptor	-22.22	5.71	9.23E-07	-5.64	R	-126	-105	agcgTGATTTGATGTAAGGTTAATTTttat	6.53	-8.56
STM3598	E	STM3598	putative L-asparaginase	-8.27	6.47	1.13E-04	-3.70						
STM3599	R	STM3599	putative inner membrane protein	-7.19	5.11	7.38E-04	-9.14	D	-108	-87	cacaATAGGTTACGTCCCTCAATGTAaagc	4.29	-6.71
STM3600	G	STM3600	putative sugar kinases, ribokinase family	-9.76	5.07	1.77E-04	-12.63	R	-322	-301	aacgCGCGTTAAACTTCCTGAAAAAAtatg	5	-7.25
STM3601	M	STM3601	putative phosphosugar isomerase	-26.75	5.09	1.12E-06	-11.94	R	-266	-245	cgcaACAGTTGATTGTCGGCGATAAAatac	7.59	-9.59
STM3611	T	yhjH	putative Diguanylate cyclase/phosphodiesterase domain 3	-16.54	5.11	1.23E-05	-7.89						

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM3626	E	dppF	ABC superfamily (atp_bind), dipeptide transport protein	9.03	5.90	1.14E-04	5.86						
STM3627	E	dppD	ABC superfamily (atp_bind), dipeptide transport protein	8.88	5.24	2.36E-04	7.65	D	-45	-24	ggcgTTATTAAATGTAGATCAATTATcggt	8.18	-10.23
STM3628	E	dppC	ABC superfamily (membrane), dipeptide transport protein 2	16.86	5.71	4.35E-06	4.09						
STM3629	E	dppB	ABC superfamily (membrane), dipeptide transport protein 1	11.76	5.16	6.34E-05	12.45	D	-55	-34	ttcgGGTTATGTTGCAGTTCATTCTCcgac	4.22	-6.66
STM3630	E	dppA	ABC superfamily (peri_perm), dipeptide transport protein	9.06	5.06	2.56E-04	9.90						
STM3690	-	STM3690	putative inner membrane lipoprotein	-15.85	9.97	2.15E-08	-5.67	R	-305	-284	ccgcAAAATTAAATAACATTATCATCcctg	4.96	-7.22
STM3692	C	lldP	LctP transporter, L-lactate permease	9.84	5.02	1.81E-04	16.00	D	-160	-139	tgtcATTATCCATACACAACAATATTggca	6.37	-8.41

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM3693	K	lldR	putative transcriptional regulator for lct operon (GntR family)	12.30	5.04	5.98E-05	30.60						
STM3694	C	lldD	L-lactate dehydrogenase	27.93	5.02	1.05E-06	28.33						
STM3695	J	yibK	putative tRNA/rRNA methyltransferase	9.64	9.95	2.31E-06	2.95						
STM3708	E	tdh	threonine 3-dehydrogenase	7.11	6.53	2.66E-04	3.16						
STM3709	H	kbl	2-amino-3-ketobutyrate CoA ligase (glycine acetyltransferase)	12.63	6.75	6.02E-06	2.86	D	-298	-277	taacGATATTGCTGCCGATAAAGCCCgcgc	5.12	-7.34
STM3750	G	yicJ	putative GPH family transport protein	-13.67	7.06	2.44E-06	-2.67						
STM3801	G	dsdX	putative Gnt family transport protein	14.70	7.68	6.70E-07	3.42	R	-22	-1	gcacGCTGCTGATCAGCATCGTGTT	5.63	-7.77
STM3802	E	dsdA	D-serine deaminase (dehydratase)	17.71	5.05	9.69E-06	7.90						
STM3808	-	ibpB	small heat shock protein	6.72	5.53	7.44E-04	2.85						
STM3820	P	STM3820	putative cytochrome c peroxidase	-12.03	6.07	1.83E-05	-5.87	D	-165	-144	tgtaATTATTGATACCAATCAATATCcatg	11	-14.14

WO 2008/108805 PCT/US2007/016357

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM3831	R	yidA	putative hydrolase of the HAD superfamily	19.09	7.62	1.03E-07	4.38	D	-220	-199	acggTATTTTCTGTTTGATTAATGAGgtta	7.22	-9.21
STM3861	M	glmS	L-glutamine:D-fructose-6-phosphate aminotransferase	8.04	6.11	1.80E-04	2.89	R	-24	-3	cgcgCAGCGTGATGTAGCTGAAATCCt	4.19	-6.63
STM3862	M	glmU	N-acetyl glucosamine-1-phosphate uridyltransferase and glucosamine-1-phosphate acetyl transferase	7.97	5.70	2.67E-04	2.78						
STM3909	E	ilvC	ketol-acid reductoisomerase	10.62	5.70	5.68E-05	3.38						
STM4004	H	hemN	O2-independent coproporphyrinogen III oxidase	10.06	5.66	8.07E-05	3.82						
STM4007	E	glnA	glutamine synthetase	19.35	5.32	3.91E-06	5.97						
STM4034	O	fdhE	putative formate dehydrogenase formation protein ? Mn_fn	9.93	6.01	5.99E-05	3.12						
STM4035	C	fdoI	formate dehydrogenase, cytochrome B556 (FDO) subunit	8.84	5.80	1.40E-04	3.60						

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM4036	C	fdoH	formate dehydrogenase-O, Fe-S subunit	21.65	8.93	5.02E-09	2.85						
STM4037	C	fdoG	formate dehydrogenase	7.75	5.52	3.62E-04	2.73						
STM4062	G	pfkA	6-phosphofructokinase I	12.62	9.16	4.23E-07	2.97	R	-181	-160	cattTGGCCTGACCTGAATCAATTCAgcag	5.19	-7.4
STM4078	G	yneB	putative fructose-1,6-bisphosphate aldolase	15.91	7.71	3.57E-07	2.57	R	-51	-30	aagaATGGCTGATTTAGATGATATTAaaga	5.53	-7.68
STM4085	G	glpX	unknown function in glycerol metabolism	6.62	5.50	8.16E-04	3.20	R	-60	-39	ctacGAGTTTGTTATGAGACGAGAACttgc	5.56	-7.71
STM4109	G	talC	putative transaldolase	19.34	8.13	4.38E-08	4.72	D	-221	-200	tcatTATGCTGACGCTTAACAAACACgccg	4.79	-7.09
STM4110	G	ptsA	General PTS family, enzyme I	7.39	6.01	3.14E-04	2.66	D	-257	-236	tactGGATTTTTGTAATATCAGTATAaaaa	5.49	-7.65
STM4113	G	frwB	PTS system fructose-like IIB component 1	5.65	5.64	1.62E-03	2.89	D	-83	-62	tttaGATTTTGAGATGAATTAAGCGAggaa	4.79	-7.09
STM4119	C	ppc	phosphoenolpyruvate carboxylase	9.54	5.26	1.62E-04	3.40						
STM4126	C	udhA	soluble pyridine nucleotide transhydrogenase	13.35	6.46	6.04E-06	3.17						

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM4229	G	malE	ABC superfamily (bind_prot) maltose transport protein, substrate recognition for transport and chemotaxis	56.71	9.34	3.52E-13	7.00						
STM4231	G	lamB	phage lambda receptor protein; maltose high-affinity receptor, facilitates diffusion of maltose and maltoseoligosaccharides	26.26	5.36	7.19E-07	11.38						
STM4240	S	yjbJ	putative cytoplasmic protein	-6.01	5.16	1.64E-03	-4.24						
STM4277	P	nrfA	nitrite reductase periplasmic cytochrome c(552)	-7.36	5.12	6.58E-04	-3.09	R	-198	-177	acttACAATTGATTAAAGACAACATTttaa	11.55	-15.16
STM4278	-	nrfB	formate-dependent nitrite reductase; a penta-haeme cytochrome c	-7.18	7.31	1.46E-04	-3.69	D	-262	-241	gtttGAATATGCAACAAATCAACGCGgaga	6.12	-8.19
STM4298	G	melA	alpha-galactosidase	25.01	5.44	7.96E-07	7.21						
STM4299	G	melB	GPH family, melibiose permease II	24.57	5.24	1.29E-06	6.66	D	-327	-306	cgatGATGCAGACCAACATCAACGTGcaaa	4.94	-7.21

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM4300	C	fumB	fumarase B (fumarate hydratase class I), anaerobic isozyme	9.38	5.99	8.37E-05	2.69	R	-216	-195	tgccGGGTTTGATTGGCGTGAGCGTCtcct	4.17	-6.62
STM4301	R	dcuB	Dcu family, anaerobic C4-dicarboxylate transporter	12.46	5.83	2.01E-05	3.38	R	-155	-134	ctggCGCATTGAATATTCGCCATTTCctga	5.55	-7.7
STM4305	-	STM4305	putative anaerobic dimethyl sulfoxide reductase, subunit A	-15.43	5.16	1.62E-05	-10.14						
STM4306	C	STM4306	putative anaerobic dimethyl sulfoxide reductase, subunit B	-7.45	5.19	5.86E-04	-7.59	R	-65	-44	cttaAGGAGTGATGTACGATGAAACAgtat	4.33	-6.73
STM4307	R	STM4307	putative anaerobic dimethyl sulfoxide reductase, subunit C	-13.21	9.88	1.33E-07	-2.90						
STM4308	R	STM4308	putative component of anaerobic dehydrogenases	-7.84	6.73	1.27E-04	-2.55						
STM4398	E	cycA	APC family, D-alanine/D-serine/glycine transport protein	13.44	6.79	3.81E-06	2.54	D	-151	-130	gccgATTCTTACCTAATATCGATGAGtcct	5.02	-7.27

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM4399	D	ytfE	putative cell morphogenesis	7.64	6.16	2.31E-04	2.96						
STM4439	C	cybC	cytochrome b(562)	23.97	7.61	1.92E-08	4.26	R	-317	-296	attcTGGGTTGAAAATGGTGAAATCCagta	5.06	-7.3
STM4452	F	nrdD	anaerobic ribonucleoside-triphosphate reductase	-12.74	6.51	7.62E-06	-3.17	R	-304	-283	ttttTACCTTGTTCTACATCAATAAAattg	7.97	-9.99
STM4462	-	yjgG	putative cytoplasmic protein	-5.83	5.39	1.64E-03	-3.63						
STM4463	E	STM4463	putative arginine repressor	-8.53	5.33	2.64E-04	-5.52						
STM4469	E	argI	ornithine carbamoyltransferase 1	-12.54	9.04	5.08E-07	-3.36						
STM4510	M	STM4510	putative aspartate racemase	-6.50	5.16	1.14E-03	-6.35	D	-115	-94	gcatTTTTTATATACACATCAAGTTGatag	6.58	-8.61
STM4511	K	yjiE	putative transcriptional regulator, LysR family	-8.28	5.18	3.54E-04	-6.79						
STM4512	-	iadA	isoaspartyl dipeptidase	-16.84	5.28	8.66E-06	-6.59	R	-75	-54	gcagCTTATTGTTTAATAAGGAGTTAtcat	4.52	-6.88
STM4513	S	yjiG	putative permease	-12.81	5.10	4.53E-05	-8.61						
STM4514	-	yjiH	putative inner membrane protein	-31.77	6.17	4.45E-08	-6.56	R	-338	-317	gcgtGAAATTGACTAACGTCAAATTTattt	9.1	-11.31
STM4526	V	hsdR	endonuclease R, host restriction	-10.25	5.85	5.93E-05	-3.28	R	-153	-132	attgTTCGTTGATCACACACAATATGaagt	5.93	-8.02

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM4533	N	tsr	methyl-accepting chemotaxis protein I, serine sensor receptor	-4.07	6.18	6.19E-03	-2.75	D	-301	-280	cgcgTAAAGTTAGGTAAATCAGTGAGtggt	7.31	-9.31
STM4535	G	STM4535	putative PTS permease	18.00	6.01	1.87E-06	8.22	D	-179	-158	agaaCTTATCGAGCAAGATCAACAGTttta	4.23	-6.66
STM4536	G	STM4536	putative PTS permease	15.55	5.53	8.94E-06	4.50						
STM4537	G	STM4537	putative PTS permease	29.73	6.56	3.04E-08	6.57	R	-172	-151	gttaGCGGATGAAATGACTCAACTTCggga	5.44	-7.61
STM4538	G	STM4538	putative PTS permease	12.87	5.17	4.03E-05	7.25						
STM4539	M	STM4539	putative glucosamine-fructose-6-phosphate aminotransferase	7.10	5.07	8.07E-04	8.20	R	-277	-256	cggcCTCCATGATTGATATCACCATTccca	5.1	-7.33
STM4540	-	STM4540	putative glucosamine-fructose-6-phosphate aminotransferase	10.51	5.26	9.95E-05	4.89						
STM4561	R	osmY	hyperosmotically inducible periplasmic protein, RpoS-dependent stationary phase gene	-11.35	5.80	3.54E-05	-5.19	D	-163	-142	tcacGAATGTGATGCCAGTCATTGACttca	4.12	-6.59
STM4565	O	yjjW	pyruvate formate lyase activating enzyme	-15.96	9.55	3.30E-08	-3.71	D	-34	-13	gcgcTTTAGTCAGTAAGATCATTGCGtttt	5.28	-7.48

TABLE 3 (Cont'd.)

Locus[a]	Category[b]	Name[c]	STM Gene Function[d]	t value[e]	DF[f]	Prob t[g]	Ratio[h]	Strand[i]	Start[j]	End[k]	Sequence[l]	Score[m]	ln(P)[n]
STM4566	-	yjjI	putative cytoplasmic protein	-20.43	5.09	4.43E-06	-17.28	R	-181	-160	actgTAATGAGATCTGAATCAAATTAtccc	4.68	-7
STM4567	F	deoC	2-deoxyribose-5-phosphate aldolase	-6.83	6.15	4.34E-04	-2.91	D	-184	-163	gggaTAATTTGATTCAGATCTCATTAcagt	4.68	-7
STM4568	F	deoA	thymidine phosphorylase	-8.83	6.49	7.51E-05	-2.76						

[a]Location of the open reading frame (ORF) in the *S.* Typhimurium LT2 genome.

[b]Functional category assigned to the gene by the National Center for Biotechnology Information, Cluster of Orthologous Genes (COGs). The designations of functional categories are as follows:

C, energy production and conversion, D, cell cycle control and mitosis, E, amino acid metabolism and transport, F, nucleotide metabolism and transport, G, carbohydrate metabolism and transport,

H, coenzyme metabolism and transport, I, lipid metabolism and transport, J, translation, K, transcription, L, replication, recombination, and repair, M, cell wall/membrane/envelope biogenesis,

N, Cell motility, O, post-translational modification, protein turnover, chaperone functions, P, inorganic ion transport and metabolism, Q, secondary metabolites biosynthesis, transport, and catabolism,

R, general functional prediction only (typically, prediction of biochemical activity), S, function unknown, T, signal transduction mechanisms, U, intracellular trafficking and secretion,

V, defense mechanisms, -, not in COGs.

[c]Respective gene name or symbol.

[d]Functional classification according to the KEGG (Kyoto Encyclopedia of Genes and Genomes) database.

[e]The numerical value of t for the t test (statistical method).

[f]The degrees of freedom employed for the analysis of each gene.

[g]The probability associated with the t test for each gene.

[h]Ratio between the expression level of the *fnr* mutant relative to the wild-type.

[i]The strand on which the motif has been localized. R, reverse; D, direct; NA, not available in the Regulatory Sequence Analysis Tools (RSAT) database (the locus identity was not recognized),

WO 2008/108805 PCT/US2007/016357

TABLE 3 (Cont'd.)

and a blank cell indicates that no motif was present.

[j]The starting position of the putative motif. The positions are relative to the region searched and span from -300 to +50 relative to the starting ATG.

[k]The ending position of the putative motif. The positions are relative to the region searched and span from -300 to +50 relative to the starting ATG.

[l]The sequence of the HIGHEST RANKING putative motif (capitalized letters) and 4 base pairs (bps) flanking either side of the region (lower case letters). A blank cell indicates that no motif was present.

All of the sequences are reported from the 5' to the 3' end of the ORF (Open Reading Frame) analyzed.

[m]The score indicating the similarity of the motif to the information matrix. The cutoff used was a score higher than 4.00 or a ln(P) lower than -6.5.

[n]The natural logarithm of the probability that the putative motif is randomly similar to the information matrix. The cutoff used was a score higher than 4.00 or a ln(P) lower than -6.5.

We claim:

1. A pharmaceutical composition comprising an attenuated enterobacterium comprising an attenuating mutation in the *fnr* gene in a pharmaceutically acceptable carrier.

2. The pharmaceutical composition of claim 1, wherein the attenuating mutation is an attenuating deletion mutation.

3. The pharmaceutical composition of claim 1, wherein the attenuated enterobacterium is an attenuated *Salmonella*.

4. The pharmaceutical composition of claim 3, wherein the attenuated *Salmonella* is an attenuated *S. enterica* serovar Typhimurium.

5. The pharmaceutical composition of claim 1, wherein the attenuated enterobacterium is an attenuated *Shigella*.

6. The pharmaceutical composition of claim 1, wherein the attenuated enterobacterium is an attenuated *Escherichia coli*.

7. The pharmaceutical composition of claim 6, wherein the attenuated *E. coli* is an attenuated *E. coli* strain O157:H7.

8. An attenuated enterobacterium comprising an attenuating mutation in the *fnr* gene and a heterologous nucleic acid sequence encoding a foreign antigen.

9. The attenuated enterobacterium of claim 8, wherein the attenuating mutation is an attenuating deletion mutation.

10. The attenuated enterobacterium of claim 8, wherein the attenuated enterobacterium is an attenuated *Salmonella*.

11. The attenuated enterobacterium of claim 10, wherein the attenuated *Salmonella* is an attenuated *S. enterica* serovar Typhimurium.

12. The attenuated enterobacterium of claim 8, wherein the attenuated enterobacterium is an attenuated *Shigella*.

13. The attenuated enterobacterium of claim 8, wherein the attenuated enterobacterium is an attenuated *Escherichia coli*.

14. The attenuated enterobacterium of claim 13, wherein the attenuated *E. coli* is an attenuated *E. coli* strain O157:H7.

15. The attenuated enterobacterium of claim 8, wherein the heterologous nucleic acid sequence is incorporated into the bacterial genomic nucleic acid.

16. The attenuated enterobacterium of claim 9, wherein the heterologous nucleic acid sequence is incorporated into the deleted FNR region.

17. The attenuated enterobacterium of claim 8, wherein the heterologous nucleic acid sequence is incorporated into a plasmid.

18. A pharmaceutical composition comprising the attenuated enterobacterium of claim 8 in a pharmaceutically acceptable carrier.

19. A method of inducing an immune response in a subject comprising administering to the subject an immunogenically effective amount of the pharmaceutical composition of claim 1.

20. The method of claim 19, wherein an immune response is induced against one or more *Salmonella* spp.

21. The method of claim 20, wherein an immune response is induced against *S. enterica* serovar Typhimurium.

22. The method of claim 19, wherein an immune response is induced against one or more *Shigella* spp.

23. The method of claim 19, wherein an immune response is induced against one or more *E. coli* spp.

24. The method of claim 23, wherein an immune response is induced against *E. coli* strain O157:H7.

25. A method of inducing an immune response in a subject comprising administering to the subject an immunogenically effective amount of the attenuated enterobacterium of claim 8.

26. The method of claim 25, wherein the immune response is induced against the foreign antigen and the enterobacterium.

27. The method of claim 26, wherein an immune response is induced against one or more *Salmonella* spp.

28. The method of claim 27, wherein an immune response is induced against *S. enterica* serovar Typhimurium.

29. The method of claim 26, wherein an immune response is induced against one or more *Shigella* spp.

30. The method of claim 26, wherein an immune response is induced against one or more *E. coli* spp.

31. The method of claim 30, wherein an immune response is induced against *E. coli* strain O157:H7.

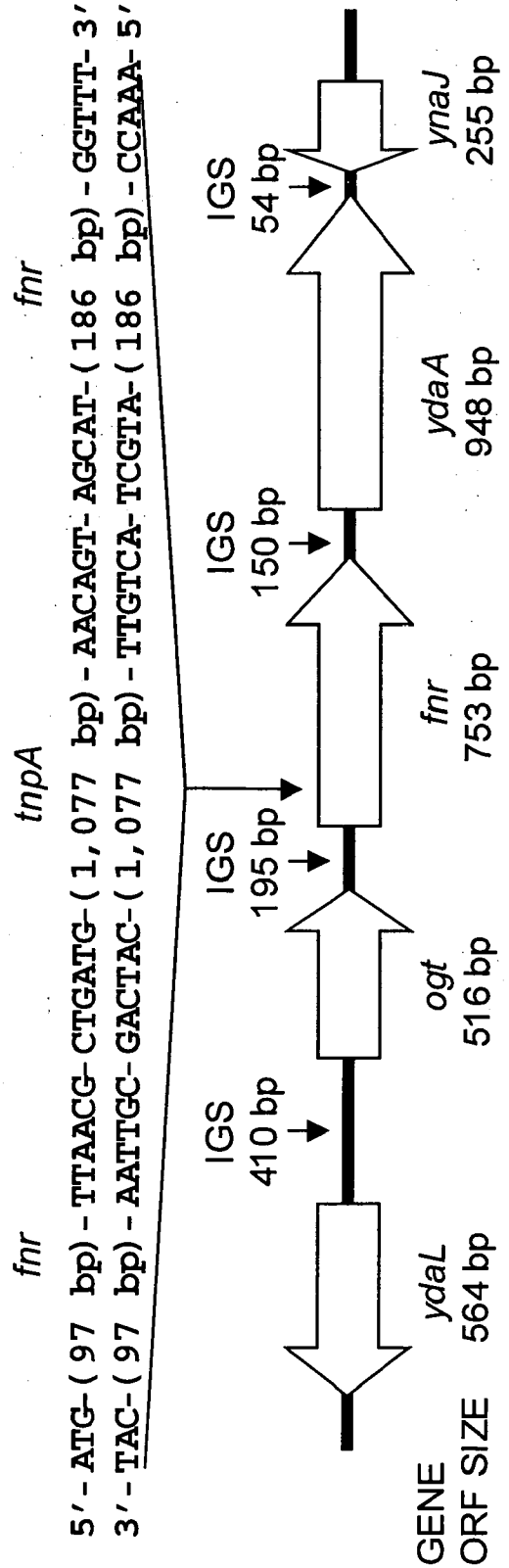
5′ - ATG- (97 bp) - TTAACG- CTGATG- (1, 077 bp) - AACAGT- AGCAT- (186 bp) - GGTTT- 3′
3′ - TAC- (97 bp) - AATTGC- GACTAC- (1, 077 bp) - TTGTCA- TCGTA- (186 bp) - CCAAA- 5′
fnr
tnpA
fnr
IGS
410 bp
IGS
195 bp
IGS
150 bp
IGS
54 bp
ydaL
564 bp
ogt
516 bp
fnr
753 bp
ydaA
948 bp
ynaJ
255 bp
GENE
ORF SIZE

FIG. 1

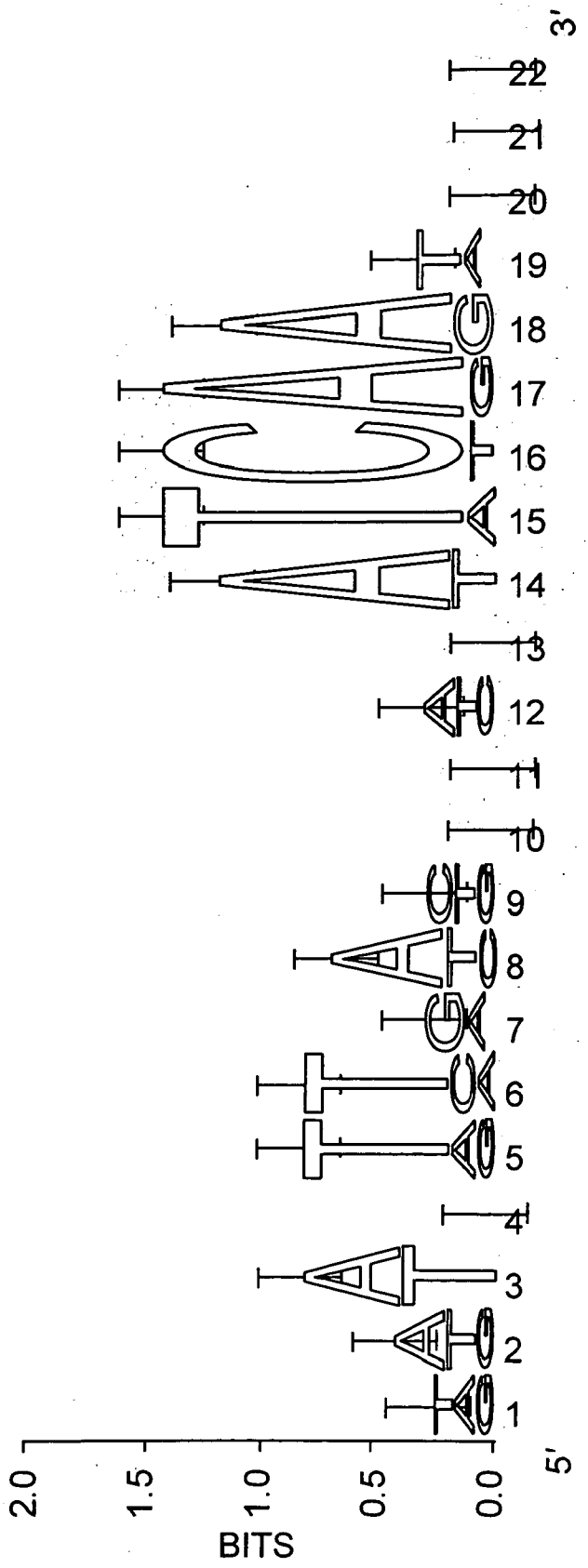
3'
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
5'
INFORMATION CONTENT: 9.054
2.0
1.5
1.0
0.5
0.0
BITS

FIG. 2

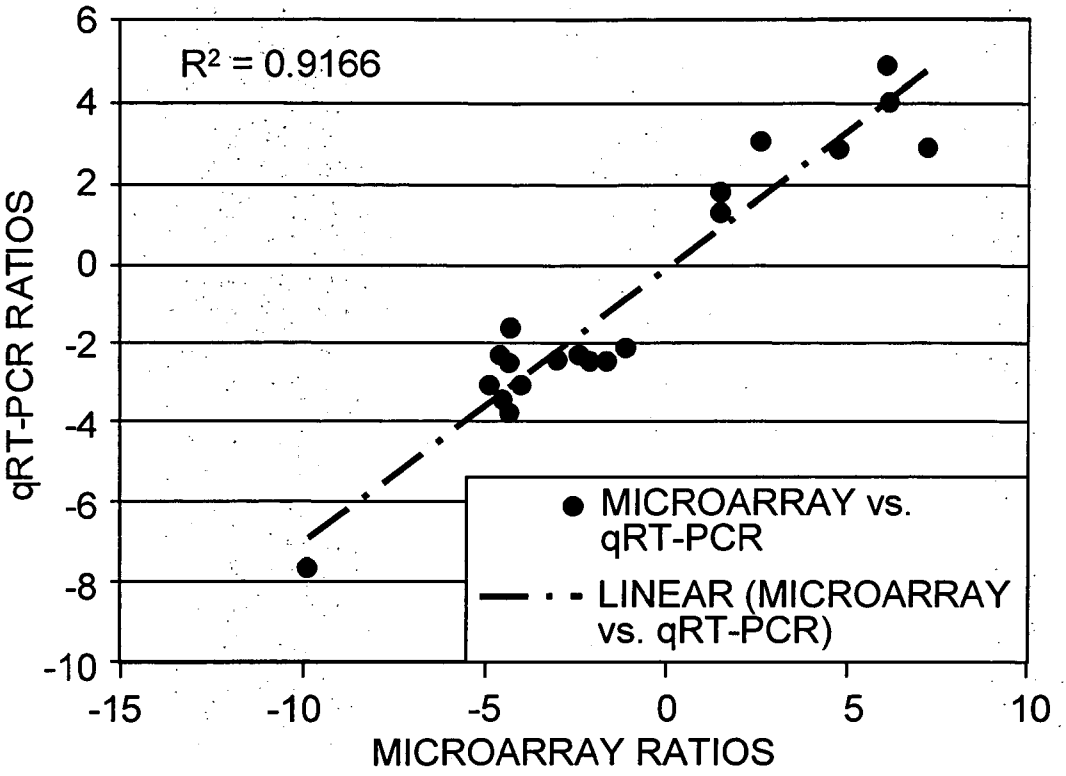
R² = 0.9166
qRT-PCR RATIOS
MICROARRAY RATIOS
6
4
2
0
-2
-4
-6
-8
-10
-15
-10
-5
0
5
10
MICROARRAY vs. qRT-PCR
LINEAR (MICROARRAY vs. qRT-PCR)

FIG. 3

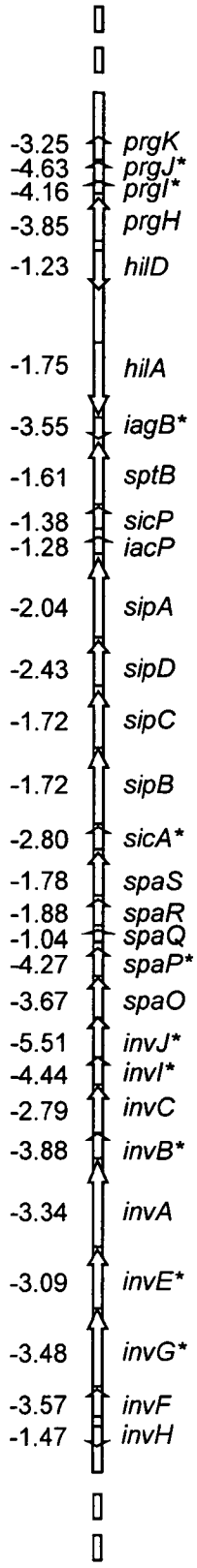

FIG. 4A

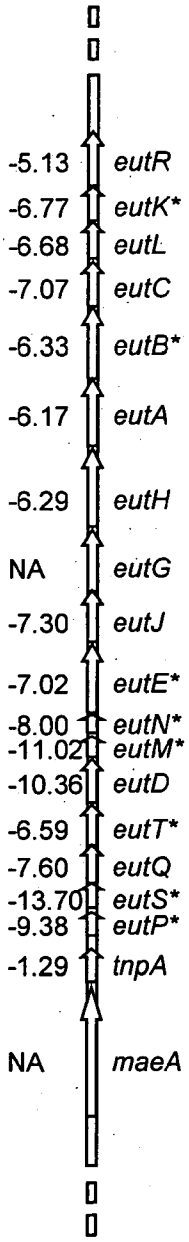

FIG. 4B

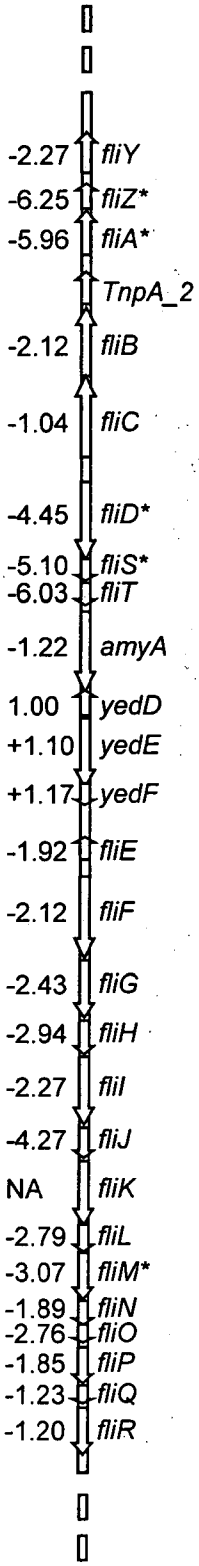

FIG. 4C

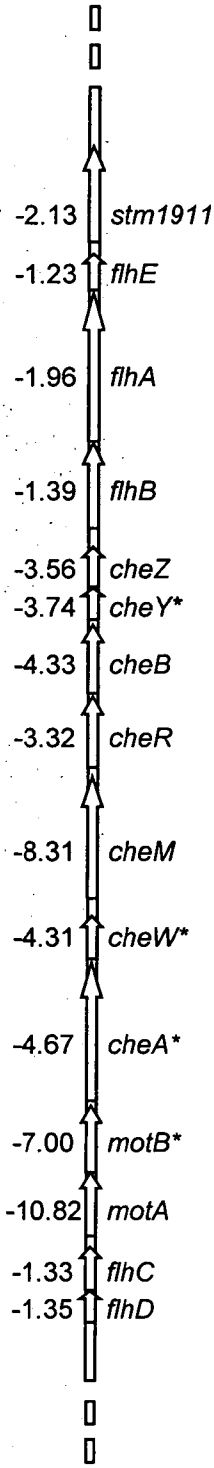

FIG. 4D

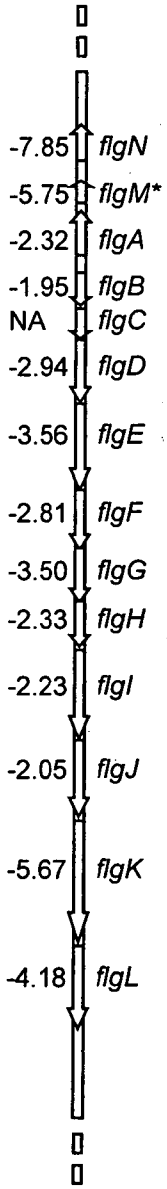

FIG. 4E

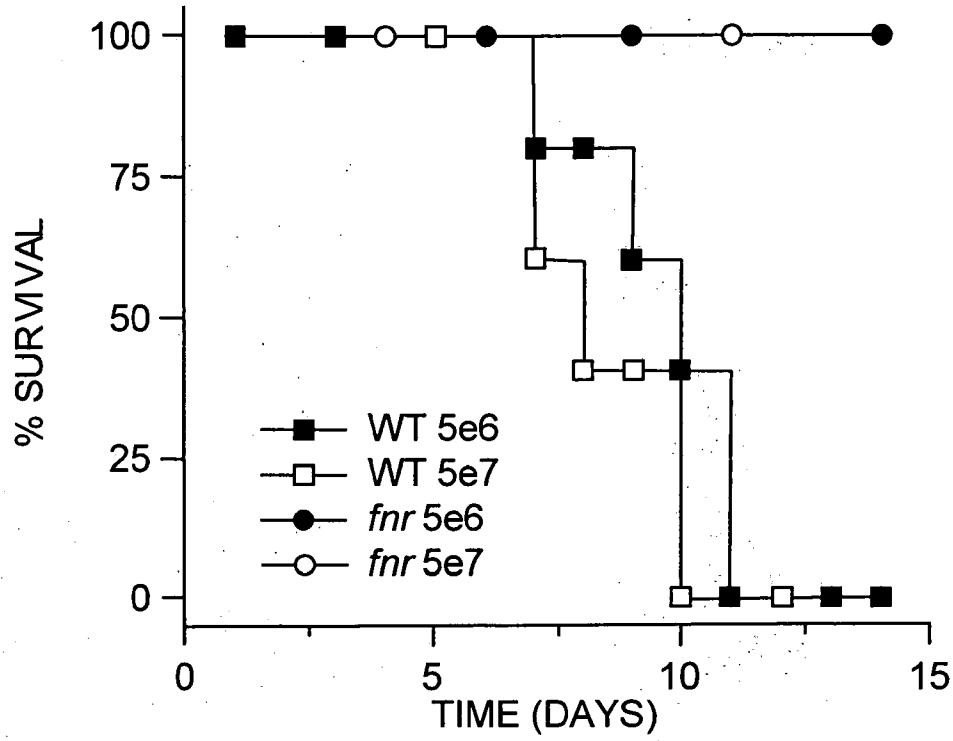
100
75
50
25
0
% SURVIVAL
0
5
10
15
TIME (DAYS)
WT 5e6
WT 5e7
fnr 5e6
fnr 5e7

FIG. 5A

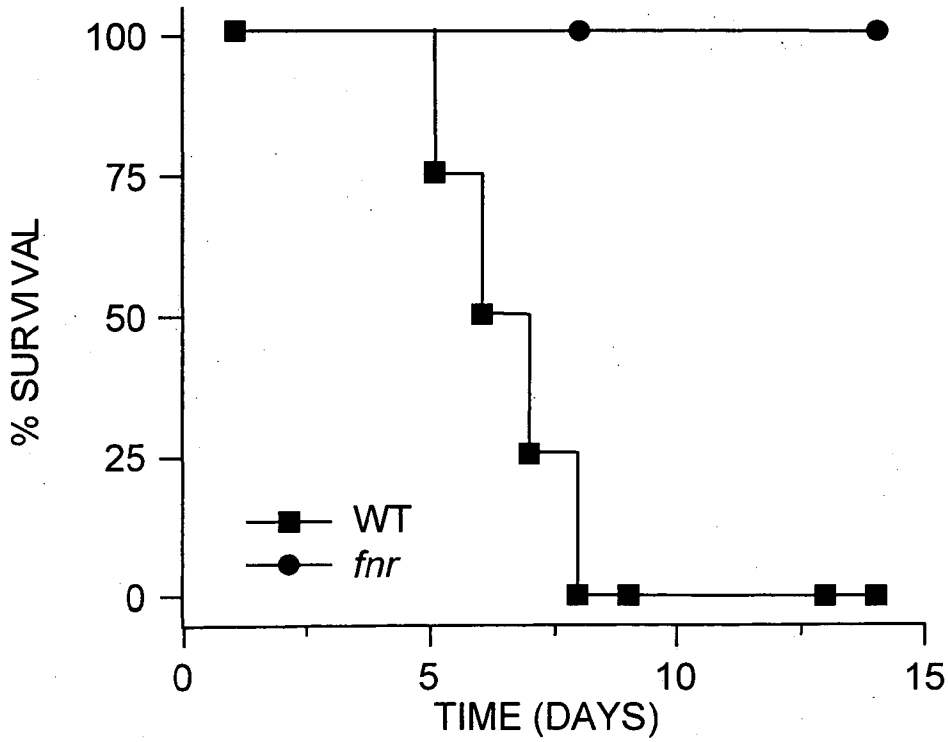
100
75
50
25
0
% SURVIVAL
0
5
10
15
TIME (DAYS)
WT
fnr

FIG. 5B

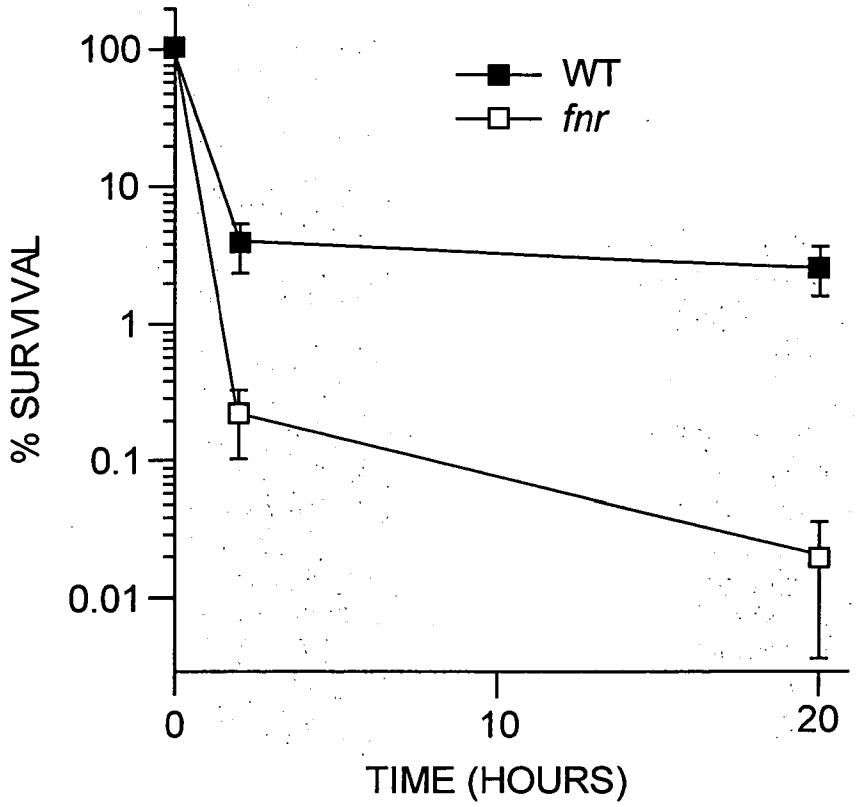
100
10
1
0.1
0.01
% SURVIVAL
WT
fnr
0
10
20
TIME (HOURS)

FIG. 6A

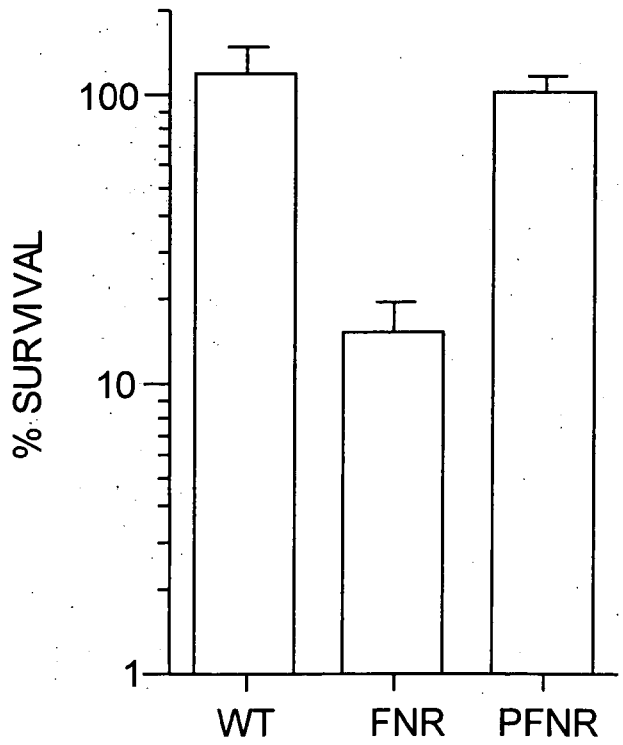
100
10
1
% SURVIVAL
WT
FNR
PFNR

FIG. 6B

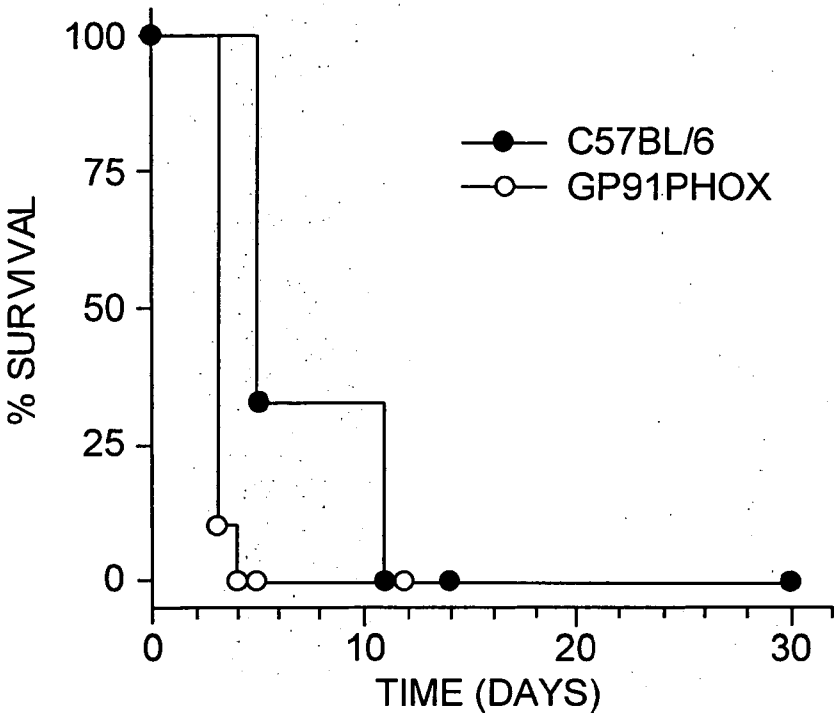
100
75
50
25
0
% SURVIVAL
0
10
20
30
TIME (DAYS)
C57BL/6
GP91PHOX

FIG. 7A

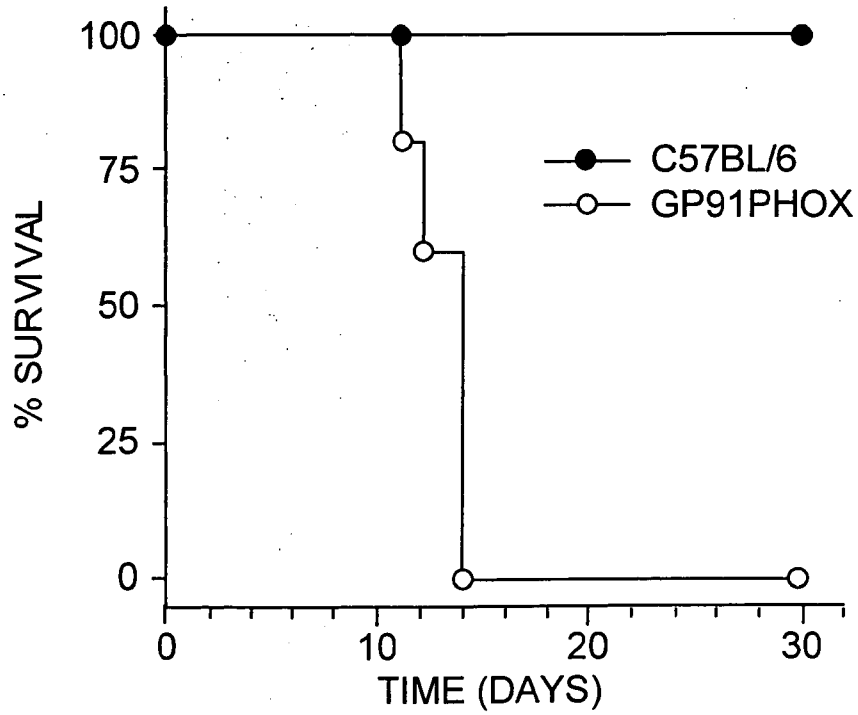
100
75
50
25
0
% SURVIVAL
0
10
20
30
TIME (DAYS)
C57BL/6
GP91PHOX

FIG. 7B

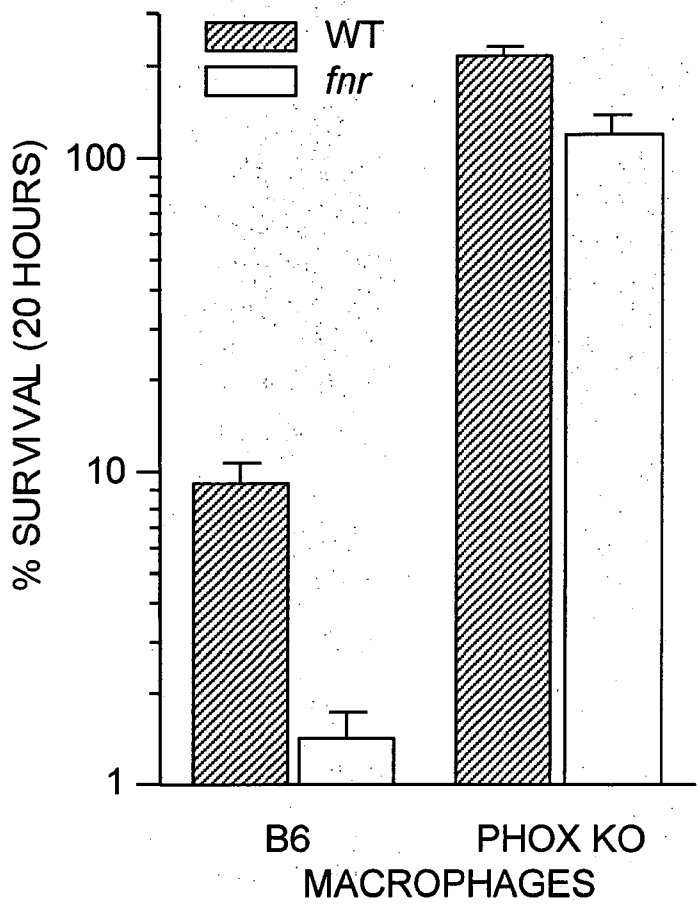

FIG. 7C