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(54) Title: L-AMINO ACID PRODUCING MICROORGANISM AND A METHOD FOR PRODUCING L-AMINO ACID

(57) Abstract: An L-amino acid producing bacterium of the Enterobacteriaceae family is described, wherein the bacterium has been modified so as to not produce type I fimbrial adhesin protein is cultured in a medium to produce and excrete said L-amino acid in the medium, and collecting said L-amino acid from the medium.



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DESCRIPTION

L-AMINO ACID PRODUCING MICROORGANISM AND A METHOD FOR PRODUCING L-AMINO ACID

Technical Field

The present invention relates to the fermentation industry, and specifically to a method for producing an L-amino acid using a bacterium of the *Enterobacteriaceae* family, wherein the bacterium has been modified so as to not produce type I fimbrial adhesin protein. L-amino acids are employed as components of pharmaceuticals, animal feed additives, seasonings, and various other nutritive mixtures.

Background Art

L-amino acids are industrially produced by fermentation using L-amino-acid producing bacteria such as coryneform bacteria and bacteria of the genus *Escherichia*. To enhance productivity, artificially mutated strains of these bacteria that have been isolated from nature or transformants in which activity of an L-amino acid biosynthesis enzyme has been increased by genetic recombination have been employed as the L-amino acid producing bacteria. (US 5,661,012 US 6,040,160, US 5,827,698, US 5,932,453, and WO01/53459)

In addition to methods of increasing the level of expression of enzymes which are L-amino acid biosynthesis pathways, another methods of increasing the ability to produce L-amino acids such as L-lysine have been developed. Such methods include improving energy efficiency by modifying the energy pathway (EP1170376), increasing the ability to produce nicotinamide adenine dinucleotide phosphoric acid by amplifying the nicotinamide nucleotide transhydrogenase gene (US 5,830,716), and inactivating genes related to flagella production (WO02/097089).

Escherichia, *Salmonella*, and *Bacillus* bacteria have fimbriae. Fimbriae can be divided into types I to V which have no direct relation to sexual processes such as conjugation and gene transfer, and sexual fimbriae that are produced on the outer layers of donor bacteria and are essential to conjugate with recipient bacteria and to gene transfer (Shoji Mizushima, Kinichiro Miura, Bacterial Anatomy 129 (1979)).

There are nine genes which contribute to the formation of type I fimbriae, and these genes form an operon. The *fimH* gene, located downstream within the operon, encodes a type I fimbrial adhesin protein. Type I fimbrial adhesin protein does not contribute to the formation of fimbriae, but is known to specifically recognize the mannosylated proteins of the host. Furthermore, it is known that the capacity for bacterial aggregation can be enhanced by introducing an amino acid substitution into the gene which encodes the type I fimbrial adhesin protein (Mark A. Schenbri, Gunna Christiansen and Per Klemm: FimH-mediated autoaggregation of *Escherichia coli*: Molecular Microbiology (2001) 41 (6), 1419-1430).

However, there is no information between the effect of introducing a mutation which causes reduction in the adhesion activity of type I fimbrial adhesin protein and L-amino acid production.

SUMMARY OF THE INVENTION

Objects of the present invention include enhancing the productivity of L-amino acid producing strains and providing a method for producing an L-amino acid. The above objects were achieved by finding that the bacterium which is modified so as to not produce type I fimbrial adhesion can improve L-amino acid productivity.

It is an object of the present invention to provide an L-amino acid producing bacterium of the *Enterobacteriaceae* family, wherein the bacterium has been modified so as to not produce type I fimbrial adhesin protein.

It is a further object of the present invention to provide the bacterium as described above, wherein said bacterium has been modified so as to not produce type I

fimbrial adhesin protein by introducing a mutation into the gene encoding type I fimbrial adhesin protein on the chromosome and/or into the region regulating expression thereof.

It is a further object of the present invention to provide the bacterium as described above, wherein said bacterium has been modified so as to not produce type I fimbrial adhesin protein by attenuation of the gene encoding type I fimbrial adhesin protein.

It is a further object of the present invention to provide the bacterium as described above, wherein said bacterium has been modified so as to not produce type I fimbrial adhesin protein by inactivation of the gene encoding type I fimbrial adhesin protein on the chromosome.

It is a further object of the present invention to provide the bacterium as described above, wherein the gene encoding type I fimbrial adhesin protein is *fimH*.

It is a further object of the present invention to provide the bacterium as described above, wherein said gene encoding type I fimbrial adhesin protein is selected from the group consisting of: (a) a DNA comprising the nucleotide sequence of SEQ ID NO:1, and (b) a DNA that is able to hybridize with the complementary strand of the nucleotide sequence of SEQ ID NO:1, or a probe prepared from said nucleotide sequence under stringent conditions, and wherein the DNA encodes the type I fimbrial adhesin protein.

It is a further object of the present invention to provide the bacterium as described above, wherein said bacterium of the *Enterobacteriaceae* family is selected from the group consisting of *Escherichia*, *Pantoea*, and *Enterobacter*.

It is a further object of the present invention to provide the bacterium as described above, wherein said L-amino acid is selected from the group consisting of L-lysine, L-threonine, L-glutamic acid, and combinations thereof.

It is a further object of the present invention to provide a method for producing an L-amino acid comprising cultivating the bacterium as described above in a medium, and collecting said L-amino acid from the medium.

It is a further object of the present invention to provide the method as described

above, wherein said L-amino acid is selected from the group consisting of L-lysine, L-threonine, L-glutamic acid, and combinations thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the structure of pMW118-attL-Tc-attR.

Fig. 2 shows the structure of pMW118-attL-Cm-attR

Fig. 3 shows the structure of pMW-intxis-ts.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

<1> The bacterium of the present invention

The bacterium of the present invention is a bacterium having the ability to produce an L-amino acid and which belongs to the *Enterobacteriaceae* family. The bacterium of the present invention has been modified so as to not produce type I fimbrial adhesin protein. The ability to produce L-amino acids may include production of any single L-amino acid, or production of multiple L-amino acids. Examples of produced L-amino acids of the present invention include L-lysine, L-glutamic acid, L-threonine, L-valine, L-leucine, L-isoleucine, L-serine, L-aspartic acid, L-asparagine, L-glutamine, L-arginine, L-cysteine (cystine), L-methionine, L-phenylalanine, L-tryptophan, L-tyrosine, L-glycine, L-alanine, L-proline, L-ornithine, L-citrulline, and L-homoserine. L-lysine, L-threonine, and L-glutamic acid are particularly desirable.

The phrase "ability to produce an L-amino acid" refers to the ability of a bacterium of the *Enterobacteriaceae* family to produce and cause accumulation of an L-amino acid in the bacterial cells or into a medium to a degree which permits its recovery from the bacterial cells or the medium when the bacterium is cultured in the medium. The bacterium which has this ability may originally have had the ability to produce an L-amino acid, or may be a bacterium, such as those set forth below, that has been modified by a mutation method or recombinant DNA technique to impart the ability to produce an L-amino acid. The bacterium of the present invention may also be one that has had the ability to produce an L-amino acid imparted to it by a modification which results in

prevention of production of type I fimbrial adhesin production.

The *Enterobacteriaceae* family includes bacteria belonging to the genus *Escherichia*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Pantoea*, *Photorhabdus*, *Pseudomonas*, *Salmonella*, *Serratia*, *Shigella*, *Morganella*, and *Yersinia*, etc.. Specifically, bacteria of the *Enterobacteriaceae* family which have been classified in the database of the National Center for Biotechnology Information (NCBI) can be employed (<http://www.ncbi.nlm.nih.gov/htbin-post/Taxonomy/wgetorg?mode=Tree&id=1236&lvl=3&keep=1&srchmode=1&unlock>). Of these, the parent strain from the *Enterobacteriaceae* family employed for modification is desirably from the genus *Escherichia*, *Enterobacter*, or *Pantoea*.

The parent strain from the genus *Escherichia* which is used to obtain the bacterium of the present invention is not specifically limited. Specifically, strains described by Neidhardt et al. may be employed (Neidhardt, F.C. et al., *Escherichia coli* and *Salmonella typhimurium*, American Society for Microbiology, Washington D.C., 1029 table 1). Of these, one example is *Escherichia coli*. Specific examples of *Escherichia coli* are *Escherichia coli* W3110 (ATCC 27325) and *Escherichia coli* MG1655 (ATCC 47076), both of which are derived from the prototype wild-type strain K12.

These strains can be obtained from the American Type Culture Collection (P.O.Box 1549 Manassas, VA 20108 USA (703) 365-2700) using the assigned registration number (<http://www.atcc.org> (reference)). The registration number corresponding to each strain is listed at the American Type Culture Collection web site and catalog. Examples of strains from the genus *Enterobacter* are *Enterobacter agglomerans* and *Enterobacter aerogenes*. An example of a strain from the genus *Pantoea* is *Pantoea ananatis*. In recent years, based on 16S rRNA base sequencing analysis, *Enterobacter agglomerans* has on occasion been reclassified as *Pantoea agglomerans*, *Pantoea ananatis*, or *Pantoea stewartii*. (Int.J.Syst.Bacteriol.,43,162-173) For the present invention, any bacterium classified in the *Enterobacteriaceae* family, whether under genus *Enterobacter* or *Pantoea*, may be employed. Strains *Pantoea ananatis* AJ13355 (FERM BP-6614), AJ13356 (FERM BP-6615), AJ13601 (FERM BP-7207), or any derivative thereof may be

employed to breed *Pantoea ananatis* by genetic engineering methods. When isolated, these strains were identified and deposited as *Enterobacter agglomerans*. As stated above, analysis by 16S rRNA base sequencing has caused them to be reclassified as *Pantoea ananatis*.

Methods of imparting the ability to produce an L-amino acid to a bacterium of the *Enterobacteriaceae* family will be described below.

To impart the ability to produce an L-amino acid, an auxotrophic mutant, an analog-resistant strain, or a metabolic regulation mutant can be obtained, or a recombinant strain having enhanced expression of an L-amino acid biosynthetic enzyme can be created. Conventionally, methods that have been employed to breed coryneform bacteria and bacteria from the genus *Escherichia* can be used (See "Amino Acid Fermentation", the Japan Scientific Societies Press [Gakkai Shuppan Center], 1st Edition, published on May 30, 1986, pp.77-100). Here, in the breeding of L-amino acid producing bacteria, one or more properties such as an auxotrophic mutation, analog resistance, and a metabolic regulation mutation may be imparted. When a recombinant strain is created, the activity of one or more L-amino acid biosynthetic enzymes may be enhanced. Furthermore, imparting properties such as an auxotrophic mutation, analog resistance, and a metabolic regulation mutation may be combined with the methods for enhancing an activity of one or more L-amino acid biosynthetic enzymes.

An auxotrophic mutant strain, L-amino acid analog resistant strains, or metabolic regulation-mutated strain with the ability to produce an L-amino acid can be obtained by subjecting a parent strain or wild-type strain to a typical mutation treatment, such as exposure to X-rays or UV radiation, or treatment with a mutagenic agent such as N-methyl-N'-nitro-N-nitrosoguanidine(NTG), and selecting from among the obtained mutants those which exhibit an auxotrophic mutation, analog resistance, or a metabolic regulation-mutation and which also have the ability to produce an L-amino acid. Genetic recombination can be employed to enhance the L-amino acid biosynthetic enzyme activity of a bacterium which produces L-amino acids.

Specific examples of L-lysine analog-resistant strains and metabolic

regulation-mutated strains which are able to produce L-lysine include *Escherichia coli* strain AJ11442 (FERM BP-1543, NRRL B-12185; see JP56-18596A and U.S. 4,346,170) and *Escherichia coli* strain VL611 (JP 2000-189180A). Strain WC196 (see WO96/17930) may also be employed as an *Escherichia coli* L-lysine producing strain. Strain WC196 was originally bred by imparting resistance to S-(2-aminoethyl)cysteine (AEC) to strain W3110, which is derived from *Escherichia coli* K-12. The resulting strain was also designated as *Escherichia coli* strain AJ13069 and deposited on December 6, 1994, accession number FERM P-14690, at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology (currently the International Patent Organism Depository, National Institute of Industrial Science and Technology, an Independent Administrative Institution, Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki, Japan Postal Code 305-8566). Then, the deposit was converted to an international deposit under the provisions of the Budapest Treaty on September 29, 1995, and received an accession number of FERM BP-5252.

Furthermore, enzymatic activity relating to L-lysine biosynthesis can be enhanced to construct a bacterium which produces L-lysine. Examples of genes encoding proteins involved in L-lysine biosynthesis include, but are not limited to, genes encoding diaminopimelic acid pathway enzymes, such as the dihydrodipicolinate synthase gene (*dapA*), aspartokinase gene (*lysC*), dihydrodipicolinate reductase gene (*dapB*), diaminopimelate decarboxylase gene (*lysA*), diaminopimelate dehydrogenase gene (*ddh*) (WO96/40934), phosphoenolpyruvate carboxylase gene (*ppc*) (JP60-87788), aspartate aminotransferase gene (*aspC*) (JP 6-102028), diaminopimelate epimerase gene (*dapF*) (JP2003-135066), aspartate semialdehyde dehydrogenase gene (*asd*) (WO00/61723), and genes encoding aminoadipic acid pathway enzymes, such as the homoaconitate hydratase gene (JP2000-157276).

In addition to methods of increasing the level of expression of Lys biosynthetic enzymes, methods of increasing the level of expression of the gene involved in energy efficiency (*cyo*) (EP1170376), the nicotinamide nucleotide transhydrogenase gene (*pntAB*) (US 5,830,716), *ybjE* gene (WO2005/073390) have also been developed.

For example, the ability to produce L-lysine can be imparted by introducing a gene encoding a protein involved in L-lysine biosynthesis into a host as set forth below.

That is, a gene fragment encoding an L-lysine biosynthesis gene is ligated to a vector which is able to function in a host microorganism which has been employed to produce L-lysine. The chosen vector preferably is a multicopy-type vector, which is then used to transform the host. Since the number of copies of the genes encoding proteins involved in L-lysine biosynthesis increases in the transformed host cell, the expression level is increased and the activity of the enzymes is enhanced.

The genes encoding proteins involved in L-lysine biosynthesis are not specifically limited other than that they must be able to be expressed in the host microorganism. Examples include genes derived from *Escherichia coli* and coryneform bacteria. Since the genomic sequences of both *Escherichia coli* and *Corynebacterium glutamicum* are known, a primer can be synthesized based on the sequences of the genes, and PCR employing the chromosomal DNA of a microbe such as *Escherichia coli* K-12 can be employed to obtain the genes (Science 277 (5331), 1453-1474 (1997) Proceedings of the 9th International Symposium on the Genetics of Industrial Microorganisms: 21). The plasmids which may be used for gene cloning may be capable of autonomous replication in the bacteria from the Enterobacteriaceae family; specific examples are pBR322, pTWV228 (Takara-Bio Co.), pMW119 (Nippon Gene Corp.), pUC19, pSTV29 (Takara-Bio Co.), and RSF1010 (Gene vol. 75 (2), p. 271-288, 1989). Phage DNA vectors may also be employed.

To ligate the target gene to the above-described vector to prepare recombinant DNA, the vector is digested with a restriction enzyme matched to the end of the DNA fragment containing the target gene. The ligation is usually conducted with a ligase such as T4 DNA ligase. The target genes may be introduced into a variety of separate vectors, or introduced into a single vector. The usual methods known to those skilled in the art can be employed to digest and ligate DNA, prepare chromosomal DNA, conduct PCR, prepare plasmid DNA, transform hosts, and determine oligonucleotides for use as primers. These methods are described in Sambrook, J., Fritsch, E. F., and Maniatis, T., "Molecular

Cloning A Laboratory Manual, Second Edition", Cold Spring Harbor Laboratory Press, (1989). Any method which achieves adequate transformation efficiency can be used to introduce recombinant DNA that has been prepared as set forth above into the microbe. An example is the electroporation method (Canadian Journal of Microbiology, 43. 197 (1997)).

Enhancing expression of the genes encoding proteins involved in L-lysine biosynthesis can be achieved by introducing multiple copies of the target gene into the chromosomal DNA of the microorganism. Multiple copies of the target gene can be introduced into the chromosomal DNA of the microorganism by using a DNA in which multiple copies are present in chromosomal DNA as a target in homologous recombination. Such site-specific introduction of mutations based on genetic recombination using homologous recombination is already established. There is a method employing single strand DNA and a method employing a plasmid containing temperature-sensitive replication origin (U.S. Patent 6,303,383 or JP05-007491A). Repetitive DNA and inverted repeats present on the ends of transposable elements can be employed as sequences in which multiple copies are preset in chromosomal DNA. Alternatively, as disclosed in EP0332488, multiple copies of the target gene can be introduced into a chromosome. With either method, as a result of increasing the number of copies of the target gene in the transformant, the enzymatic activity of L-lysine biosynthesis increases.

In addition to the above-described genetic amplification, L-lysine biosynthesis enzyme activity can be enhanced by replacing the expression regulatory sequence of the promoter of the target gene with a stronger one (JP01-215280A). For example, lac promoter, trp promoter, trc promoter, tac promoter, lambda phage PR promoter, lambda phage PL promoter, tet promoter are all known as strong promoters. Substitution with these promoters enhances the enzymatic activity by increasing expression of the target gene. A paper by Goldstein (Prokaryotic promoters in biotechnology. Biotechnol. Annu. Rev., 1995, 1, 105-128) describes methods of evaluating the strength of promoters and gives examples of strong promoters.

This can also be achieved by modifying factors relating to regulation of

expression of the target gene, such as operators and repressors (Hamilton et al., J Bacteriol. 1989 Sep; 171(9): 4617-22). As is disclosed in WO00/18935, it is possible to introduce a substitution of several bases into the promoter region of a target gene to increase its strength.

Furthermore, the substitution of several nucleotides into the spacer between a ribosome binding site (RBS) and a start codon, particularly into the sequence immediately upstream from a start codon, is known to have a strong effect on mRNA translation efficiency. The expression adjustment regions of the promoters or the like of the target gene can be determined using promoter search vectors and gene analysis software such as GENETYX. Substitution of the expression regulating sequence can be conducted, for example, in the same manner as in the above-described gene substitution employing temperature-sensitive plasmids.

Furthermore, the L-lysine producing bacterium of the present invention may have reduced or deficient activity of enzymes which catalyze reactions that branch off from the L-lysine biosynthesis pathway and produce compounds other than L-lysine. Examples of such enzymes are homoserine dehydrogenase, lysine decarboxylase (cadA, ldcC), and malic enzyme. Strains in which the activity of these enzymes is reduced or eliminated are described in WO 95/23864, WO96/17930, and WO2005/010175.

Examples of methods of reducing or eliminating the intracellular activity of an enzyme include mutating or deleting a gene encoding the enzyme in cells of a microorganism so that intracellular activity is reduced or eliminated as compared to a non-mutated strain. For example, this can be achieved by using recombination to inactivate the gene encoding the enzyme on the chromosome, or to modify an expression regulating sequence such as a promoter or the Shine-Dalgarno (SD) sequence. (WO95/34672, Biotechnol Prog 1999, 15, 58-64)

This can also be achieved by introducing an amino acid substitution (missense mutation) into the region of the gene encoding the enzyme on the chromosome, introducing a stop codon (nonsense mutation), introducing or deleting one or two bases to create a frame shift mutation, or partially deleting a portion or a region of the gene, or the

entire gene (Journal of Biological Chemistry 272: 8611-8617 (1997), Journal of Antimicrobial Chemotherapy 200 46, 793-79. J. biological Chemistry vol 272 N0.13 pp8611-8617). Enzymatic activity can also be decreased or eliminated by constructing a gene encoding a mutant enzyme which lacks a coding region, using homologous recombination to replace the normal gene on the chromosome with this gene, and introducing a transposon or IS factor into the gene.

For example, the following methods may be employed to introduce a mutation causing a decrease of, or eliminating, the above enzyme activity by gene recombination. A portion of the sequence of the targeted gene is modified, a mutant gene that does not produce a normally functioning enzyme is prepared, DNA containing this gene is used to transform a microorganism from the *Enterobacteriaceae* family, and the mutant gene is made to recombine with the gene on the chromosome, which results in replacing the target gene on the chromosome with the mutant gene. Such gene substitution using homologous recombination can be conducted by methods employing linear DNA, such as the method known as "Red-driven integration" (Proc. Natl. Acad. Sci. USA, 2000, vol. 97, No. 12, p 6640-6645), and by methods employing a plasmid containing a temperature-sensitive replication (Proc. Natl. Acad. Sci. USA, 2000, vol. 97, No. 12, p 6640-6645, U.S. Patent 6,303,383 or JP05-007491A). Furthermore, the incorporation of a site-specific mutation by gene substitution using homologous recombination such as set forth above can also be conducted with a plasmid lacking the ability to replicate in the host.

The above-described methods of enhancing and decreasing the activity of enzymes relating to L-lysine biosynthesis can also be applied to the breeding of bacteria producing other L-amino acids. Methods of breeding bacteria producing other L-amino acids will be described below.

The L-glutamic acid producing bacterium employed in the present invention can be a microorganism from the *Enterobacteriaceae* family that has been modified to enhance expression of a gene encoding an enzyme related to L-glutamic acid biosynthesis, for example. Examples of enzymes related to L-glutamic acid biosynthesis are glutamate dehydrogenase (also referred to as "GDH" hereinafter), glutamine synthetase, glutamate

synthase, isocitrate dehydrogenase, aconate hydratase, citrate synthase (also referred to as "CS" hereinafter), pyruvate carboxylase, pyruvate dehydrogenase, pyruvate kinase, phosphophenol pyruvate synthase, enolase, phosphoglyceromutase, phosphoglycerate kinase, glyceraldehyde-3-phosphate dehydrogenase, triosephosphate isomerase, fructose bisphosphate aldolase, phosphofructokinase, and glucose phosphate isomerase. Among these enzymes, one or more from among CS, PEPC, and GDH are desirable, with all three being preferred.

For example, U.S. Patents 6,197,559 and 6,331,419 and European Patent 0999282 describe microbes from the *Enterobacteriaceae* family that have been modified by methods such as those set forth above to enhance expression of the citrate synthase gene, phosphoenolpyruvate carboxylase gene, and/or glutamate dehydrogenase gene.

A microorganism which has reduced or eliminated activity of enzymes which catalyze reactions that branch off from the L-glutamic acid biosynthesis pathway and produce compounds other than L-glutamic acid may also be employed as the microorganism from the *Enterobacteriaceae* family which has the ability to produce L-glutamic acid. Examples of enzymes which catalyze reactions that branch off from the L-glutamic biosynthesis pathway and produce compounds other than L-glutamic acid are: 2-oxoglutamate dehydrogenase, isocitrate lyase, phosphate acetyl transferase, acetate kinase, acetohydroxamate synthase, acetolactate synthase, formate acetyl transferase, lactate dehydrogenase, glutamate decarboxylase. Among these, the reduction or elimination of 2-oxoglutamate dehydrogenase activity is desirable.

U.S. Patents 6,197,559 and 6,331,419 describe methods of eliminating or reducing the 2-oxoglutamate dehydrogenase activity in a microorganism from the *Enterobacteriaceae* family. Specific examples of a bacterium from the *Enterobacteriaceae* family in which 2-oxoglutamate dehydrogenase activity has been eliminated or reduced are:

Pantoea ananatis AJ13601 (FERM BP-7207)

Klebsiella planticola AJ13410 (FERM BP-6617)

Pantoea ananatis AJ13355 (FERM BP-6614)

Escherichia coli AJ12949 (FERM BP-4881).

Strain AJ12949 was deposited on December 28, 1993, as depository number FERM P-14039, at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology (currently the International Patent Organism Depositary, National Institute of Industrial Science and Technology, an Independent Administrative Institution, Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki, Japan Postal Code 305-8566), and converted to a international deposit under the provisions of the Budapest Treaty on November 11, 1994, and given accession number FERM BP-4881.

Strain AJ13601 was deposited at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Ministry of International Trade and Industry on August 18, 1999 and given an accession number of FERM P-17516. The deposition was converted to an international deposition under the provisions of Budapest Treaty on July 6, 2000 and given an accession number of FERM BP-7207.

The preferred L-tryptophan producing microorganism employed in the present invention enhances the activity of one or more of the following proteins: anthranilate synthase, phosphoglycerate dehydrogenase, and tryptophan synthase. Anthranilate synthase and phosphoglycerate dehydrogenase are subject to feedback suppression by L-tryptophan and L-serine, respectively. Thus, their enzymatic activity can be enhanced by retaining a desensitized mutant enzyme. Specifically, for example, the anthranilate synthase gene (*trpE*) and/or the phosphoglycerate dehydrogenase gene (*serA*) can be mutated so as to not be subject to feedback inhibition, and the mutant gene obtained can be introduced into a microorganism of the *Enterobacteriaceae* family to obtain a microorganism retaining the desensitized enzyme. Specific examples of such microbes are transformed strains obtained by introducing plasmid pGH5 having a mutant form of *serA* encoding desensitized phosphoglycerate dehydrogenase into *Escherichia coli* SV164 which has retained a desensitized form of anthranilate synthase (WO94/08031).

A microorganism into which a recombinant DNA containing a tryptophan operon has been introduced is a further example of a desirable L-tryptophan-producing

bacteria. One specific example is *Escherichia coli* into which a tryptophan operon containing a gene encoding desensitized anthranilate synthase has been introduced (JP57-71397A and JP 62-244382A, and U.S. Patent 4,371,614). Among the tryptophan operons, enhancement of the expression of the gene (*trpBA*) encoding tryptophan synthase also enhances or imparts L-tryptophan production capability. Tryptophan synthase contains α and β subunits encoded by *trpA* and *trpB*, respectively.

Further examples of L-tryptophan producing bacteria are *Escherichia coli* strain AGX17(pGX44) (NRRL B-12263), which requires L-phenylalanine and L-tyrosine, and strain AGX6(pGX50)aroP (NRRL B-12264), which retains a plasmid pGX50 containing a tryptophan operon (see U.S. Patent 4,371,614 for both of these).

Examples of L-phenylalanine-producing strains derived from the K-12 strain include the AJ12739 strain in which the *tyrA* gene and the *tyrR* gene are disrupted (*tyrA*::Tn10, *tyrR*) (VKPM B-8197), and a strain in which the *yddG* gene and the *yedA* gene, which are involved in phenylalanine excretion, are amplified (WO 03/044192).

The strain which is modified to enhance genes which encode proteins involved in aromatic amino acids biosynthesis can be used as tryptophan and/or phenylalanine-producing strain, such genes include genes which encode proteins involved in a common pathway for aromatic acids, such as *aroF*, *aroG*, *aroH*, *aroB*, *aroD*, *aroE*, *aroK*, *aroL*, *aroA*, and *aroC* genes.

A strain having 6-dimethylaminopurine resistance (JP 5-304969A) is desirable as the L-threonine producing bacterium employed in the present invention. Examples of recombinant bacteria of the genus *Escherichia* are a strain in which a mutation producing excessive L-threonine biosynthesis has been introduced into the threonine biosynthesis gene and the gene has been amplified on a plasmid (JP 1-29559A and JP5-227977A), a strain in which a threonine operon has been amplified with a plasmid (Japanese Patent Application Publication No. Hei 2-109985), and a strain in which a gene encoding pyruvate carboxylase and a gene encoding nicotinamide nucleotide transhydrogenase have been amplified (JP2002-51787A).

A further example is *Escherichia coli* strain VKPM B-3996 (see U.S. Patent

5,175,107). Strain VKPM B-3996 was deposited on November 19, 1987, with the Russian National Collection of Industrial Microorganisms (VKPM), GNII Genetika, and given deposit number VKPM B-3996. Strain VKPM B-3966 harbors a plasmid pVIC40 (WO90/04636), which is obtained by inserting a threonine biosynthesis gene (threonine operon: thrABC) into a broad host vector plasmid pAYC32 (see Chistorerdov, A.Y., Tsygankov, Y.D. Plasmid, 1986, 16, 161-167) having a streptomycin-resistance marker. In pVIC40, feedback inhibition by L-threonine of the aspartokinase I homoserine dehydrogenase I encoded by thrA in the threonine operon is repressed.

The *Escherichia coli* VKPM B-5318 strain (EP 0593792B) may also be used as an L-threonine-producing strain. VKPM B-5318 was deposited at the Russian National Collection of Industrial Microorganisms (VKPM), GNII Genetika (Dorozhny proezd. 1, Moscow 113545, Russian Federation), with a registration number of VKPM B-5318 on November 19, 1987. The VKPM B-5318 strain is autotroph to L-isoleucine, and the threonine operon encoding the threonine biosynthesis enzyme is located downstream C1 temperature-sensitive repressor, PR-promoter and N end of Cro protein derived from λ phage, moreover, the strain harbors plasmid DNA constructed so that the expression of threonine biosynthesis gene is regulated by promoter and repressor derived from λ phage.

Preferably, the bacterium of the present invention is additionally modified to enhance expression of one or more of the following genes:

- the mutant *thrA* gene which codes for aspartokinase homoserine dehydrogenase I and is resistant to feedback inhibition by threonine;
- the *thrB* gene which codes for homoserine kinase;
- the *thrC* gene which codes for threonine synthase;
- the *rhtA* gene which codes for a putative transmembrane protein;
- the *asd* gene which codes for aspartate- β -semialdehyde dehydrogenase; and
- the *aspC* gene which codes for aspartate aminotransferase (aspartate transaminase);

The *thrA* gene which encodes aspartokinase homoserine dehydrogenase I of *Escherichia coli* has been elucidated (nucleotide positions 337 to 2799, GenBank accession NC_000913.2, gi: 49175990). The *thrA* gene is located between the *thrL* and *thrB* genes on

the chromosome of *E. coli* K-12. The *thrB* gene which encodes homoserine kinase of *Escherichia coli* has been elucidated (nucleotide positions 2801 to 3733, GenBank accession NC_000913.2, gi: 49175990). The *thrB* gene is located between the *thrA* and *thrC* genes on the chromosome of *E. coli* K-12. The *thrC* gene which encodes threonine synthase of *Escherichia coli* has been elucidated (nucleotide positions 3734 to 5020, GenBank accession NC_000913.2, gi: 49175990). The *thrC* gene is located between the *thrB* gene and the *yaaX* open reading frame on the chromosome of *E. coli* K-12. All three genes functions as a single threonine operon. The regulation sequence which encode attenuater and leader peptide present upstream threonine operon. To enhance the expression of threonine operon, a leader sequence and/or an attenuator is desirably removed from said operon.(WO2005/049808, WO2003/097839)

A mutant *thrA* gene which codes for aspartokinase homoserine dehydrogenase I and is resistant to feedback inhibition by threonine, as well as, the *thrB* and *thrC* genes can be obtained as one operon from the well-known plasmid pVIC40 which is present in the threonine producing *E. coli* strain VKPM B-3996. Plasmid pVIC40 is described in detail in U.S. Patent No. 5,705,371.

The *rhtA* gene exists at 18 min on the *E. coli* chromosome close to the *glnHPQ* operon, which encodes components of the glutamine transport system. The *rhtA* gene is identical to ORF1 (*ybiF* gene, nucleotide positions 764 to 1651, GenBank accession number AAA218541, gi:440181) and located between the *pexB* and *ompX* genes. The unit expressing a protein encoded by the ORF1 has been designated the *rhtA* gene (*rht*: resistance to homoserine and threonine). Also, it was revealed that the *rhtA23* mutation is an A-for-G substitution at position -1 with respect to the ATG start codon (ABSTRACTS of the 17th International Congress of Biochemistry and Molecular Biology in conjugation with Annual Meeting of the American Society for Biochemistry and Molecular Biology, San Francisco, California August 24-29, 1997, abstract No. 457, EP 1013765 A).

The *asd* gene of *E. coli* has already been elucidated (nucleotide positions 3572511 to 3571408, GenBank accession NC_000913.1, gi:16131307), and can be obtained by PCR (polymerase chain reaction; refer to White, T.J. et al., Trends Genet., 5,

185 (1989)) utilizing primers prepared based on the nucleotide sequence of the gene. The *asd* genes of other microorganisms can be obtained in a similar manner.

Also, the *aspC* gene of *E. coli* has already been elucidated (nucleotide positions 983742 to 984932, GenBank accession NC_000913.1, gi:16128895), and can be obtained by PCR.

The *aspC* genes of other microorganisms can be obtained in a similar manner.

Examples of L-histidine producing bacteria that are desirable for use in the present invention are strains of *Escherichia coli* FERM-P5038 and 5048, both of which incorporate a vector containing DNA encoding an L-histidine biosynthetic enzyme (JP56-005099A), a strain having the vector containing the amino acid export gene *rht* (European Patent Publication No. 1016710), and *Escherichia coli* strain 80, which is imparted with resistance to sulfaguanidine, D,L-1,2,4-triazole-3-alanine, and streptomycin (VKPM B-7270 Russian Patent Publication No. 2,119,536).

A microorganism with enhanced expression of genes encoding enzymes of the L-histidine biosynthesis pathway may be employed as a microorganism which has the ability to produce L-histidine. Examples of enzymes involved in L-histidine biosynthesis are ATP phosphoribosyltransferase (*hisG*), phosphoribosyl AMP cyclohydrolase (*hisI*), phosphoribosyl ATP pyrophosphohydrolase (*hisIE*), phosphoribosylformimino-5-aminoimidazole carboxamide ribotide isomerase (*hisA*), amidotransferase (*hisH*), histidinolphosphate aminotransferase (*hisC*), histidinolphosphatase (*hisB*), and histidinoldehydrogenase (*hisD*).

Desirable examples of the L-cysteine producing bacterium of the present invention are bacteria which have reduced cystathionine- β -lyase activity (JP2003-169668A) and *Escherichia coli* bacteria which retain serineacetyltransferase, the activity of which is decreased by feedback suppression by L-cysteine (JP11-155571A). Desirable examples of L-proline producing bacterium of the present invention are *Escherichia coli* strain 702 (VKPMB-8011), which is resistant to 3,4-dehydroxyproline and azatidine-2-carboxylate, and strain 702ilvA (VKPMB-8012), which lacks the *ilvA* of strain 702 (JP2002-300874A).

Examples of a bacterium which produces L-arginine are *Escherichia coli* strains

with resistance to α -methylmethionine, p-fluorophenylalanine, D-arginine, arginine hydroxamic acid, S-(2-aminoethyl)cysteine, α -methylserine, β -2-thienylalanine, or sulfaguanidine (JP56-106598A). *Escherichia coli* strain 237 (US Patent Application No. 2003/058315), which is an L-arginine producing bacterium harboring mutant N-acetylglutamate synthase, is also a desirable L-arginine producing strain. This strain was deposited on April 10, 2000, with the Russian National Collection of Industrial Microorganisms (VKPM), GNII Genetika under number VKPM B-7925 and was converted on May 18, 2001 to an International Deposit under the Budapest Treaty. A derivative of strain 237 that produces L-arginine and has enhanced acetic acid utilizing capability, *Escherichia coli* strain 382 (EP1170358A) can also be employed. *Escherichia coli* strain 382 was deposited on April 10, 2000, with the Russian National Collection of Industrial Microorganisms (VKPM) as deposit number VKPM B-7926.

Microorganisms with an enhanced level of expression of genes encoding enzymes related to L-arginine biosynthesis can also be employed as a microorganism having L-arginine production ability. Examples of enzymes related to L-arginine biosynthesis are one or more selected from among N-acetylglutamate synthase (*argA*), N-acetylglutamylphosphate reductase (*argC*), ornithine acetyltransferase (*argJ*), N-acetylglutamate kinase (*argB*), acetylornithine transferase (*argD*), acetylornithine deacetylase (*argE*), ornithinecarbamoyltransferase (*argF*), argininosuccinate synthase (*argG*), argininosuccinate lyase (*argH*), and carbamoylphosphate synthase (*carAB*).

L-leucine producing bacteria of the present invention include a bacterium of the genus *Escherichia* in which the branched chain amino acid transferase encoded by the *ilvE* gene is inactivated and the activity of aromatic amino acid transaminase encoded by the *tyrB* gene is enhanced (JP2004-024259), *Escherichia coli* strain H-9068 (ATCC21530), *Escherichia coli* strain H-9070 (FERM BP-4704), *Escherichia coli* strain 9072 (FERM BP-4706) which is resistant to 4-azoleucine or 5,5,5-trifluoroleucine (U.S. Patent No. 5,744,331) strains of *Escherichia coli* in which feedback inhibition of isopropylmalate synthase by L-leucine has been repressed (European Patent No. 1067191), and *Escherichia coli* strain AJ11478 which is resistant to β -2-thienylalanine and β -hydroxyleucine (U.S.

Patent 5,763,231).

The bacterium of the present invention may be improved by enhancing the expression of one or more genes involved in L-leucine biosynthesis. Examples include genes of the *leuABCD* operon, which are preferably represented by a mutant *leuA* gene coding for isopropylmalate synthase freed from feedback inhibition by L-leucine (US Patent 6,403,342). In addition, the bacterium of the present invention may be improved by enhancing the expression of one or more genes coding for proteins which excrete L-amino acid from the bacterial cell. Examples of such genes include the b2682 and b2683 genes (*ygaZH* genes) (EP 1239041 A2).

Examples of L-isoleucine producing bacteria are *Escherichia coli* variants with resistance to 6-dimethylaminopurine (JP5-304969A), *Escherichia coli* mutants with resistance to L-isoleucine hydroxamate (JP5-130882A), *Escherichia coli* strains with resistance to thiaisleucine (JP5-130882A), *Escherichia coli* mutants with resistance to DL-ethionine (JP5-130882A), and variants with resistance to arginine hydroxamate (JP5-130882A) with the ability to produce L-isoleucine. Examples of recombinant bacteria of the genus *Escherichia* are strains with genes encoding proteins involved in L-isoleucine biosynthesis, such as threonine deaminase or acetohydroxate synthase enhanced with plasmids (JP2-458A, 2-42988A, and 8-47397A).

An example of a bacterium producing L-valine include, but are not limited to, strains which have been modified to overexpress the *ilvGMEDA* operon (U.S. Patent No. 5,998,178). It is desirable to remove the region of the *ilvGMEDA* operon which is required for attenuation so that expression of the operon is not attenuated by L-valine that is produced. Furthermore, the *ilvA* gene in the operon is desirably disrupted so that threonine deaminase activity is decreased.

Examples of parent strains for deriving L-valine-producing bacteria of the present invention include also include mutants having a mutation of amino-acyl t-RNA synthetase (U.S. Patent No. 5,658,766). For example, *E. coli* VL1970, which has a mutation in the *ileS* gene encoding isoleucine tRNA synthetase, can be used. *E. coli* VL1970 has been deposited in the Russian National Collection of Industrial

Microorganisms (VKPM) (Russia, 113545 Moscow, 1 Dorozhny Proezd.) on June 24, 1988 under accession number VKPM B-4411.

Furthermore, mutants requiring lipoic acid for growth and/or lacking H⁺-ATPase can also be used as parent strains (WO96/06926).

The microorganism of the present invention can be obtained by modifying a microbe of the *Enterobacteriaceae* family which has the ability to produce L-amino acid so that it cannot produce type I fimbrial adhesin protein, as set forth above. When breeding a microbe of the *Enterobacteriaceae* family of the present invention, it makes no difference whether the ability to produce an L-amino acid is imparted first or a modification to prevent production of type I fimbrial adhesin protein is made first. Furthermore, a microbe from the *Enterobacteriaceae* family which has the ability to produce an L-amino acid may be modified so that it does not produce type I fimbrial adhesin protein, or a microbe from the *Enterobacteriaceae* family that no longer produces type I fimbrial adhesin protein may be imparted with the ability to produce an L-amino acid. It suffices for the microorganism of the present invention to be modified so that it does not normally produce type I fimbrial adhesin protein in the manner of wild-type strains or unmodified strains. However, it is desirable for the microbe of the present invention to have an ability to cause accumulation of an L-amino acid exceeding that of these strains.

The "fimbriae" of the present invention are filamentous protrusions present on the outer cell membrane of microorganism from the *Enterobacteriaceae* family. Fimbriae can be divided into two types. One type is the fimbriae which has no direct relation to sexual processes such as conjugation and gene transfer, and the second type is sexual fimbriae that are produced on the outer layer of gene-donor bacteria and are essential to conjugation with gene-recipient bacteria. The "fimbriae" referred to in the present invention are the former, and are not directly related to sexual processes such as conjugation, gene transfer, or the like (Shoji Mizushima, Kinichiro Miura, Bacterial Anatomy 129 (1979)).

In the present invention, the phrase "type I fimbrial adhesin protein" refers to a protein which belongs to a group of proteins which control the formation of fimbriae, and

furthermore, the protein has a function blocking a coagulation of red corpuscles by D-mannose.

The phrase “modified so as not to produce type I fimbrial adhesin protein” refers to when the quantity of type I fimbrial adhesin protein that is produced is lower than that in unmodified strains, and when the conformation of the protein is modified so that normal fimbriae cannot be produced by microbes from the *Enterobacteriaceae* family. For example, wild-type strains such as *Escherichia coli* strain W3110 (ATCC 27325), which is derived from the prototypical wild-type strain K12, and *Escherichia coli* MG1655 (ATCC 47076), are examples of control strains from the genus *Escherichia*. Confirmation of a decrease in the quantity of type I fimbrial adhesin protein which is produced, or the lack of production of type I fimbrial adhesin protein can be determined by the immunofluorescent antibody method, a decrease in the level of coagulation in the presence of D-mannose, or the lack of ability for coagulation (see the methods of Pallesen et al.: Microbiology 141; 2839-2848).

The type I fimbrial adhesin protein (Seq. ID No. 2) from the genus *Escherichia* is encoded by the gene *fimH* (Seq. ID No. 1). Type I fimbrial adhesin proteins from other microbes of the *Enterobacteriaceae* family include *fimH* homologs; The homologs of *fimH* from *Enterobacteriaceae* can be obtained by searching for genes having high homology to the gene denoted by Seq. ID No. 1 with BLAST (<http://blast.genome.jp>).

The GenBank Accession number of the amino acid sequence of the type I fimbrial adhesin protein from *Escherichia* microbes and the *fimH* gene encoding this protein are given in Table 1. Examples of the group of genes which encode proteins which control formation of fimbriae in the bacteria from genus *Escherichia* are *fimB*, *fimE*, *fimA*, *fimI*, *fimC*, *fimD*, *fimF*, *fimG*, and *fimH*. The amino acid sequences encoded by these genes, their gene sequences, and their GenBank Accession numbers are given in Table 1.

Table 1

Gene	Protein	Function	Amino acid sequence ID	Gene sequence ID	GenBank Accession No.
fimB	recombinase involved in phase variation; regulator for fimA	regulator; surface structures	Seq. ID No. 47	Seq. ID No. 46	NP_418732.
fimE	recombinase involved in phase variation; regulator for fimA	regulator; surface structures	Seq. ID No. 49	Seq. ID No. 48	NP_418733
fimA	major type 1 subunit fimbrin (pilin)	structural component; surface structures	Seq. ID No. 51	Seq. ID No. 50	NP_418734
fimI	fimbrial protein	structural component; surface structures	Seq. ID No. 53	Seq. ID No. 52	NP_418735
fimC	periplasmic chaperone, required for type 1 fimbriae	factor; surface structures	Seq. ID No. 55	Seq. ID No. 54	NP_418736
fimD	outer membrane protein; export and assembly of type 1 fimbriae, interrupted	membrane; outer membrane constituents	Seq. ID No. 57	Seq. ID No. 56	NP_418737.
fimF	fimbrial morphology	structural component; surface structures	Seq. ID No. 59	Seq. ID No. 58	NP_418738
fimG	fimbrial morphology	structural component; surface structures	Seq. ID No. 61	Seq. ID No. 60	NP_418739.
fimH	minor fimbrial subunit, D-mannose specific adhesin	structural component, surface structures	Seq. ID No. 2	Seq. ID No. 1	NP_418740 (AAA97216)

An example of the type I fimbrial adhesin protein is, from *Escherichia coli*, one which has the amino acid sequence of Seq. ID No. 2 in Table 1. However, as long as its activity as a type I fimbrial adhesion protein does not change, the amino acid sequence of the type I fimbrial adhesion protein may include one or several amino acid substitutions, deletions, insertions, or additions. Here, the term "one or several" means 1 to 20, desirably 1 to 10, and preferably 1 to 5. The above amino acid substitutions, deletions, insertions, or additions are conservative mutations that maintain the production and activity of type I fimbrial adhesion protein. The term "conservative mutation" means that when the substitution is an aromatic amino acid, substitution of Phe, Trp, and Tyr for each other; when the substitution is a hydrophobic amino acid, substitution of Leu, Ile, and Val for each other; in the case of a polar amino acid, substitution of Gln and Asn for each other; in the case of a basic amino acid, substitution of Lys, Arg, and His for each other; in the case of an acidic amino acid, substitution of Asp and Glu for each other; and in the case of an amino acid having an hydroxyl group, substitution of Ser and Thr for each other. A representative conservative mutation is a conservative substitution. Substitutions that are considered to be conservative substitutions are the substitution of Ala by Ser or Thr; substitution of Asp by Asn, Glu, or Gln; substitution of Cys by Ser or Ala; substitution of Gln by Asn, Glu, Lys, His, Asp, or Arg; substitution of Glu by Asn, Gln, Lys, or Asp; substitution of Gly by Pro; substitution of His by Asn, Lys, Gln, Arg, or Tyr; substitution of Ile by Leu, Met, Val, or Phe; substitution of Leu by Ile, Met, Val, or Phe; substitution of Lys by Asn, Glu, Gln, His, or Arg; substitution of Met by Ile, Leu, Val, or Phe; substitution of Phe by Trp, Tyr, Met, Ile or Leu; substitution of Ser by Thr or Ala; substitution of Thr by Ser or Ala; substitution of Trp by Phe or Tyr; substitution of Tyr by His, Phe, or Trp; and substitution of Val by Met, Ile, or Leu.

Since there may be some differences in DNA sequences between the genera or strains of the *Enterobacteriaceae* family, the gene encoding the type I fimbrial adhesin protein can be any DNA which hybridizes under stringent conditions with a probe prepared from the base sequence of SEQ.ID. No.1 or a homologous gene of the SEQ ID No.1 sequence. The term "stringent conditions" refers to conditions under which specific

hybrids form and nonspecific hybrids do not form. By way of example, these are conditions under which highly homologous fragments of DNA, for example, DNA with a degree of homology of not less than 80%, preferably not less than 90%, and most preferably not less than 95% will hybridize. A further example is Southern blot hybridization washing conditions at a temperature and salt concentration corresponding to 60°C, 1 x SSC, 0.1 percent SDS, preferably 0.1 x SSC, 0.1 percent SDS, and more preferably, 68°C, 0.1 x SSC, and 0.1 percent SDS, conducted one, two, or three times. The length of the probe can be suitably selected based on the hybridization conditions, but normally ranges from 100 bp to 1 Kbp.

The phrase “modified so as to not produce type I fimbrial adhesin protein” means a modification that does not destroy the fimbriae, but causes type I fimbrial adhesin protein to not function normally. Such a modification may be the introduction of a mutation into the protein with a drug or the like, weakening adhesion, or the use of genetic engineering or the introduction of a mutation into the gene relating to the formation of type I fimbrial adhesin so as to reduce the level of production of type I fimbrial adhesin protein, or breed a bacterium that does not produce type I fimbrial adhesin protein. For example, in microorganisms of the *Enterobacteriaceae* family that do not produce type I fimbrial adhesin protein, the transcription or translation of the gene encoding the type I fimbrial adhesin protein may be interfered with, resulting in either in the protein not being produced or being produced at a low level. A mutation may be introduced to the gene encoding the type I fimbrial adhesin protein on the chromosome and/or the region controlling expression of the gene, so that type I fimbrial adhesin proteins do not function properly. Furthermore, a mutation may be introduced into a gene encoding type I fimbrial adhesin protein as to reduce the level of expression of genes coding for type I fimbrial adhesin protein on the chromosome.

Specifically, modification so as to not produce type I fimbrial adhesin can be achieved by deleting the gene encoding type I fimbrial adhesin protein on the chromosome, or by modifying an expression regulating sequence such as a promoter or Shine-Dalgarno (SD) sequence. This can also be achieved by introducing an amino acid substitution

(missense mutation) into the coding region of the type I fimbrial adhesin gene on the chromosome, introducing a stop codon (nonsense mutation), adding or deleting one or two bases to create a frame shift mutation, or partially deleting a portion, a region, or the entire gene (Journal of Biological Chemistry 272: 8611-8617 (1997), Proceedings of the National Academy of Sciences, USA 95, 5511-5515 (1998), Journal of Biological Chemistry 266, 20833-20839 (1991)).

Furthermore, the *fimH* gene, located downstream within the operon, modification so as to not produce type I fimbrial adhesin can also be achieved by introducing the mutation into the gene in table 1 located upstream *fimH* gene.

In *Escherichia coli*, it is possible to identify the *fimH* gene shown in Table 1 as the chromosomal gene encoding type I fimbrial adhesin protein. To prevent the production of type I fimbrial adhesin protein, the *fimH* gene can be deleted or a mutation can be introduced into the region encoding *fimH*, as described above. This can also be achieved by introducing a mutation attenuating the expression of the *fimH* gene upstream from *fimH*; for example, a frame shift mutation or nonsense mutation can be introduced into one of the genes upstream from *fimH* that are indicated in Table 1, or an upstream gene can be partially deleted. More particularly, as is described further below in the embodiments, a transposon or a gene imparting resistance to an antibiotic can be incorporated in the region encoded by *fimH*, or the mutation described in the Journal of Bacteriology, July, 2001, 4099-4102 or the mutation described in Molecular Microbiology (2001) 41 (6), 1419-1430, can be incorporated. However, the present invention is not limited thereto.

For example, the following methods can be employed to introduce the above-described mutations by genetic recombination. The target gene on the chromosome can be replaced with a mutated gene by introducing a mutation of the sequence of the target gene, preparing a mutant form of the gene that does not produce a properly functioning enzyme, transforming a microorganism from the *Enterobacteriaceae* family with DNA containing the gene, and introducing the mutation of the gene to recombine with the gene on the chromosome. Such site-specific incorporation of mutations by gene substitution employing homologous recombination is already established. There exists a method

employing linear DNA and a method employing a plasmid containing a temperature-sensitive replication origin (U.S. Patent 6,303,383 or JP 05-007491). The introduction of a mutation at a specific site by gene substitution employing homologous recombination as set forth above can also be conducted using a plasmid which is not able to replicate on the chromosome.

Specifically, a gene encoding the type I fimbrial adhesin protein can be obtained by the following methods. Chromosomal DNA can be prepared from a microorganism belonging to the family *Enterobacteriaceae*, for example, by the method of Saito and Miura (see H. Saito and K. Miura, *Biochem. Biophys. Acta*, 72, 619 (1963); *Book of Bioengineering Experiments*, comp. by the Japan Bioengineering Society, p. 97-98, Baifukan, 1992). A gene encoding the type I fimbrial adhesin protein can be obtained by using a known database such as GenBank, preparing an oligonucleotide, and employing PCR. For example, it can be constructed by referring to the sequences of the genus *Escherichia* listed in Table 1.

A gene encoding the type I fimbrial adhesin protein prepared as set forth above, or a portion of such a gene, can be used for gene inactivation. However, since the gene used for gene inactivation need only have a degree of homology adequate to occur homologous recombination with the gene encoding the type I fimbrial adhesin protein on the chromosomal DNA of a microbe of the *Enterobacteriaceae* family, such a homologous gene may also be employed. Here, the term "degree of homology adequate to undergo homologous recombination" is desirably homology of 80% or more, preferably 90% or more, more preferably 95% or more, and particularly preferably, 97% or more. If the gene is a DNA that will hybridize with the above gene under stringent conditions, homologous recombination will occur. The term "stringent conditions" refers to conditions under which specific hybridization occurs but nonspecific hybridization does not. An example of such conditions is washing once, preferably two or three times, at a salt concentration corresponding to 60°C, 1 x SSC, 0.1 percent SDS, preferably 0.1 x SSC and 0.1 percent SDS.

The gene encoding type I fimbrial adhesin protein on the chromosome can be

inactivated, for example, by deleting part of the sequence of the gene so it is not able to produce a properly functioning type I fimbrial adhesin protein, transforming a group of enterobacteria with DNA containing this gene, and causing the deficient gene to recombine with the gene on the chromosome. Such gene inactivation by gene substitution using homologous recombination is already established. There exist methods employing linear DNA such as the method developed by Datsenko and Wanner called "red-driven integration" (Proc. Natl. Acad. Sci. USA, 2000, vol. 97, No. 12, p 6640-6645), and methods employing plasmids containing temperature-sensitive replication (U.S. Patent 6,303,383 or Japanese Patent Application Publication No. Hei 05-007491). Such gene destruction by gene substitution employing homologous recombination as set forth above can also be conducted using a plasmid which is not able to replicate in a host. Examples of deficient genes which have been modified so to not be able to produce type I fimbrial adhesin protein are genes from which all or a part of the region of Seq. ID No. 1 has been deleted, genes incorporating missense mutations, genes into which transposons or marker genes have been inserted, genes incorporating nonsense mutations, and genes incorporating frame shift mutations.

In addition, a method based on a combination of the method called "red-driven integration" and an excision system derived from lambda phage (J. Bacteriol. 2002 Sep; 184(18): 5200-3) Interactions between integrase and excisionase in the phage lambda excisive nucleoprotein complex. Cho EH, Gumport RI, Gardner JF.) can be used as the method for disrupting a gene on a chromosome (WO2005/010175).

According to the red-driven integration method, a gene-disrupted strain can be constructed in one step by using a PCR product, which is obtained using synthetic oligonucleotides as primers which are designed to include part of a targeted gene at its 5' terminus, and part of an antibiotic resistance gene at its 3' terminus. Furthermore, the integrated antibiotic resistance gene can be removed by introducing attL and attR, which are attachment sites of lambda phage, and the PCR product, and combining the excision system derived from lambda phage with the red-driven integration method.

Specifically, a strain in which the targeted gene is disrupted and the antibiotic

resistance gene is removed can be obtained by the following method.

A linear DNA cassette comprising an antibiotic resistance gene, attachment sites of lambda phage and a target gene is initially prepared. This is usually prepared by PCR using a suitably-prepared template.

A template in which attL and attR (SEQ ID NO: 9 (GenBank accession No. M12458 and SEQ ID NO: 10 (GenBank accession No. M12459)) are inserted at respective terminals of an antibiotic resistance gene is used as a template of the linear DNA cassette. The template may be plasmid for example pMW118-attL-Tc-attR, pMW118-attL Cm-attR, (Fig.1) , a gene inserted on a chromosome, or a synthetic oligonucleotide.

While the antibiotic resistance gene is preferably a chloramphenicol resistance gene, a streptomycin resistance gene, or an ampicillin resistance gene, any antibiotic resistance gene can be used provided that the gene functions as an antibiotic resistance gene in *Enterobacteriaceae* family and is different from a marker gene which may be contained in two helper plasmids as described below. To easily confirm the acquisition of the antibiotic resistance, the antibiotic resistance gene which is employed can be one whereby the expression is increased by replacing a promoter sequence and the like, or one in which a mutation is introduced in its structural gene sequence so that an enzyme activity is increased. The linear DNA cassette is prepared in the following order from the 5' terminus: (targeted gene 5' sequence)-(attL)-(antibiotic resistance gene)-(attR)-(targeted gene 3' sequence).

The linear DNA cassette is integrated into the chromosome. As a helper plasmid for integrating the linear DNA cassette into chromosome, pKD46 can be used (Proc. Natl. Acad. Sci. USA, 2000, 97, 6640-6645). pKD46 include temperature-sensitive replication origin and ampicillin resistance gene, a 2,154 nt DNA fragment of lambda phage (GenBank/EMBL accession No. J02459, 31088-33241), which contains the genes (gamma, beta, and exo genes) encoding Red recombinase of the lambda Red homologous recombination system and which is under the control of the arabinose-inducible ParaB promoter.

pKD46 can be introduced into a host by electroporation. The pKD46-amplified

strain is cultured with arabinose. The linear DNA cassette is introduced at the logarithmic growth phase and incubated at a high temperature to obtain a gene-disrupted strain which is resistant to an antibiotic by the antibiotic resistance gene in the linear DNA cassette. The confirmation of the gene disruption can be made by PCR or measurement of the concentration of L-lysine or L-threonine produced by the strain.

A helper plasmid for excising the antibiotic resistance gene is then introduced. The helper plasmid harbors a gene encoding integrase (Int) (SEQ ID NO: 13, GenBank accession No. J02459. B [gi:215104]) and a gene encoding excisionase (Xis) (SEQ ID NO: 15, GenBank accession No. J02459 [gi:215104]) of lambda phage and shows temperature-sensitive replication. By introduction of the helper plasmid, recombination occurs due to recognition of attL (SEQ ID NO: 11) and attR (SEQ ID NO: 12) on the chromosome. The antibiotic resistance gene between attL and attR is excised and as a result, a structure that contains only the attL or attR sequence remains on the chromosome. By incubating at a high temperature, the helper plasmid is lost. Thus, a strain in which the targeted gene is disrupted and the antibiotic gene is eliminated can be obtained.

In addition to the above-described gene manipulation, examples of methods of modification which preclude the production of type I fimbrial adhesin protein are subjecting a group of Enterobacteriaceae family to UV radiation or treating it with N-methyl-N'-nitro-N-nitrosoguanidine (NTG), nitrous acid, or some other mutagenic agent commonly employed in mutation treatments and selecting the strains that are unable to produce type I fimbrial adhesin protein.

<2> Method for Producing L-amino acids

The method for producing an L-amino acid of the present invention comprises culturing the microorganism of the present invention in a medium, causing the production and accumulation of L-amino acid in the culture or in the bacterial cell, and recovering the L-amino acid from the culture or bacterial cell. In the present invention, the cultivation, collection, and purification of an L-amino acid from the medium and the like may be performed in a manner similar to conventional fermentation methods wherein an amino acid is produced using a bacterium.

The medium employed can be one that is conventionally employed in the production of L-amino acids by fermentation. The usual medium contains a carbon source, nitrogen source, inorganic ions, and other organic components as needed. Here, exemplary carbon sources include sugar such as glucose, sucrose, lactose, galactose, fructose, or starch hydrolysis product; alcohol such as glycerol or sorbitol; and organic acids such as fumaric acid, citric acid, or succinic acid. Exemplary nitrogen sources include an inorganic ammonium salt such as ammonium sulfate, ammonium chloride, or ammonium phosphate; organic nitrogen such as a soybean hydrolysis product; ammonia gas; ammonia water; or the like. Other nutrients such as vitamin B1 and L-homoserine or yeast extract can be employed in suitable quantities as a source of a trace organic nutrient. In addition, as needed, small amounts of potassium phosphate, magnesium sulfate, iron ions, manganese ions, and the like can be added. So long as the medium employed in the present invention contains a carbon source, nitrogen source, inorganic ions and, as needed, other trace organic components, it does not matter whether it is a natural or a synthetic medium.

Culturing can be conducted for 1 to 7 days under aerobic conditions at a temperature of 24 to 37°C and at a pH of 5 to 9. Inorganic and organic acidic and alkaline substances as well as ammonia gas can be used to adjust the pH. L-amino acid can be recovered from the fermentation solution by the usual ion-exchange resin method, precipitation method, and combinations of these with other known methods. When L-amino acid accumulates within the bacterial cell, the bacterial cell can be crushed by ultrasonic waves or the like, a supernatant can be centrifugally separated from the bacterial cell, and the supernatant can be subjected to ion-exchange resin to recover the L-amino acid.

A liquid medium can be prepared under conditions which cause L-glutamic acid to precipitate, and the culture can be conducted while L-glutamic acid precipitates. An example of the conditions under which L-glutamic acid will precipitate is a pH of 5.0 to 4.0, preferably pH 4.5 to 4.0, more preferably pH 4.3 to 4.0, and particularly preferably, pH 4.0. When culturing is conducted under conditions causing L-glutamic acid to precipitate, the L-glutamic acid precipitating out into the culture solution can be collected by

centrifugation, filtration, or the like. In that case, the L-glutamic acid dissolved in the medium can be crystallized and then separated.

The present invention is further described below with reference to the following non-limiting examples.

Examples

Example 1: Construction of a bacterium which is modified to not produce type I fimbrial adhesin protein.

<1-1> Construction of a strain in which the genes *cadA* and *ldcC* encoding lysine decarboxylase are destroyed

First, a strain that does not produce lysine decarboxylase was constructed. Lysine decarboxylase is encoded by the *cadA* gene (GenBank Accession No. NP_418555, Seq. ID No. 42) and the *ldcC* gene (GenBank Accession No. NP_414728, Seq. ID No. 44) (see International Application Publication WO96/17930). The parent strain employed here was strain WC196. This strain was named *Escherichia coli* strain AJ13069 and was deposited on December 6, 1994, as deposit number FERM P-14690, at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology (currently the International Patent Organism Depositary, National Institute of Industrial Science and Technology, an Independent Administrative Institution, Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki, Japan Postal Code 305-8566). This strain was converted on September 29, 1995, to an international deposit under the provisions of the Budapest Treaty and given deposit number FERM BP-5252.

The genes *cadA* and *ldcC* encoding lysine decarboxylase were deleted by the method known as "Red-driven integration" developed by Datsenko and Wanner (Proc. Natl. Acad. Sci. USA, 2000, Vol. 97, No. 12, p 6640-6645) and by a λ phage-derived excision system (J. Bacteriol. 2002 Sep; 184 (18): 5200-3. Interactions between integrase and excisionase in the phage lambda excisive nucleoprotein complex. Cho EH, Gumport RI, Gardner JF.).(WO2005/010175) Based on the "Red-driven integration" method, a synthetic oligonucleotide designed with a part of the target gene on its 5' terminal end and

a part of a antibiotic resistance gene on its 3' terminal end was employed as a primer to obtain a PCR product, which was then employed to construct a strain having an inactivated gene in a single step. By further combining this with an excision system derived from λ phage, it was possible to remove from the gene-inactivated strain the integrated antibiotic resistance gene.

(1) Disruption of the *cadA* gene

A plasmid pMW118-attL-Cm-attR was employed as PCR template. pMW118-attL-Cm-attR was a plasmid obtained by inserting the attL and attR genes – attachment sites of λ phage – and the cat gene, a gene imparting antibiotic-resistance, into pMW118 (Takara-Bio Co.) in the order attL-cat-attR. The sequence of attL is shown by SEQ. ID No. 11 and the sequence of attR by SEQ. ID No.12.

A synthetic oligonucleotide with Seq. ID Nos. 3 and 4 on its 5' terminal, corresponding to a part of the *cadA* gene, and sequences corresponding to the both ends of attL and attR on its 3' terminal, was employed as a primer to conduct PCR.

The amplified PCR product was purified on an agarose gel and introduced by electroporation into *Escherichia coli* strain WC196, which contains plasmid pKD46 with a temperature-sensitive replication origin. Plasmid pKD46 (Proc. Natl. Acad. Sci. USA, 2000, Vol. 97, No. 12, p 6640-6645) includes a DNA fragment (GenBank/EMBL Accession No. J02459, Nos. 31088 to 33241) having a total of 2,154 bases of λ -phage containing genes (γ , β , and *exo* genes) encoding the Red recombinase of the λ Red homologous recombination system controlled by an arabinose-derived *ParaB* promoter. Plasmid pKD46 was required to introduce the PCR product into the chromosome of strain WC196.

Competent cells for electroporation were prepared as follows. The *Escherichia coli* strain WC196 that had been cultured overnight at 30°C in LB medium containing 100 mg/L of ampicillin was diluted 100-fold with 5 mL of SOB medium (Molecular Cloning: Laboratory Manual, 2nd Ed., Sambrook J. et al., Cold Spring Harbor Laboratory Press (1989)) containing L-arabinose (1 mM) and ampicillin (20 mg/L). The diluted product was cultured until reaching an OD600 of about 0.6 at 30°C, concentrated 100-fold, and readied

for electroporation by washing three times in 10 percent glycerol. Electroporation was conducted using 70 μ L of competent cells and about 100 ng of the PCR product. Following electroporation, 1 mL of SOC medium (Molecular Cloning: Laboratory Manual, 2nd Ed., Sambrook J. et al., Cold Spring Harbor Laboratory Press (1989)) was added and the cells were cultured for 2.5 hours at 37°C. They were then plate cultured on L-agar medium containing chloramphenicol (Cm, 25 mg/L) at 37°C and the Cm-resistant recombinants were selected. Next, to remove the pKD46 plasmid, cell were subcultured at 42°C on Cm-containing L-agar medium and the ampicillin resistance of the colonies obtained was tested. The ampicillin-sensitive strains without pKD46 were collected. The deletion of the *cadA* gene in mutants identified based on chloramphenicol resistance gene was confirmed by PCR. The *cadA*-deficient strain obtained was named strain WC196 Δ *cadA*::att-cat.

The above-described helper plasmid pMW-intxis-ts was employed to remove the att-cat genes that were inserted inside the *cadA* gene. pMW-intxis-ts is a plasmid which harbors a gene (SEQ. ID No. 13) encoding the integrase (Int) of the λ phage and a gene (SEQ. ID No. 15) encoding the excisionase (Xis) of the λ phage, and which has a temperature-sensitive replication origin. By introducing pMW-intxis-ts, recombination occurs due to the recognition of attL (SEQ. ID No. 11) and attR (SEQ. ID No. 12) on the chromosome, allowing gene excision between attL and attR, leaving behind only the attL or attR sequence on the chromosome.

Competent cells of the strain WC196 Δ *cadA*::att-cat obtained as set forth above were prepared by ordinary methods, transformed with helper plasmid pMW-intxis-ts, and plate cultured on L-agar medium containing 50 mg/L of ampicillin at 30°C. The ampicillin-resistant strains were selected.

Next, to remove the pMW-intxis-ts plasmid, cells were subcultured at 42°C on L agar medium. The ampicillin resistance and chloramphenicol resistance of the colonies obtained were tested and a strain sensitive to chloramphenicol and ampicillin, which was a *cadA*-deleted strain which had removed the att-cat and pMW-intxis-ts, was collected. This strain was named WC196 Δ *cadA*.

(2) Strain WC196 Δ cadA and deletion of the *ldcC* gene

Deletion of the *ldcC* gene in strain WC196 Δ cadA was conducted using primers with SEQ. ID NO. 5 and 6 to destroy the *ldcC* by the above-described method. This resulted in a strain in which *cadA* and *ldcC* had been deleted, and was designated WC196 Δ cadA Δ ldcC.

(3) Preparation of PCR template and helper plasmid

PCR template pMW118-attL-Cm-attR and helper plasmid pMW-intxis-ts were prepared as set forth below.

(3-1) pMW118-attL-Cm-attR

pMW118-attL-Cm-attR was constructed based on pMW118-attL-Tc-attR. The four following DNA fragments were spliced.

1) BglII-EcoRI DNA fragment (120 bp) (SEQ. ID No. 11) containing attL obtained by PCR amplification of a sequence corresponding to the chromosome of *E. coli* strain W3350 (ATCC 31278 containing λ prophage) using primers in the form of oligonucleotides P1 and P2 (SEQ. ID NO. 17 and 18) (these primers contained additional BglII and EcoRI endonuclease recognition sites).

2) PstI-HindIII DNA fragment (182 bp) (SEQ. ID No. 12) containing attR obtained by PCR amplification of a sequence corresponding to the chromosome of *E. coli* strain W3350 (containing λ prophage) using primers in the form of oligonucleotides P3 and P4 (SEQ. ID Nos. 19 and 20) (these primers contained additional PstI and HindIII endonuclease recognition sites).

3) BglII-HindIII large fragment (3,916 bp) of pMW118-ter_{rrnB}.
pMW118-ter_{rrn} was a fragment obtained by ligation of the following three fragments:
-A large fragment (2,359 bp) containing an AatII-EcoRI_{pol} fragment of pMW118 was obtained by cleaving pMW118 with EcoRI restriction endonuclease, processing with the Klenow fragment of DNA polymerase I, and digesting with AatII restriction endonuclease,
-AatII-BglII small fragment (1194 bp) of pUC19 containing the *bla* gene of ampicillin resistance (ApR), obtained by employing oligonucleotides P5 and P6 (SEQ. ID Nos. 21 and 22) as primers (these primers contained additional AatII and BglII endonuclease

recognition sites) in PCR amplification of the sequence corresponding to the pUC19 plasmid,

-BglII-PstIpol small fragment (363 bp) of transcription terminator_rnB obtained by employing oligonucleotides P7 and P8 (SEQ. ID Nos. 23 and 24) as primers (these primers had additional BglII and PstI endonuclease identification sites) in PCR amplification of the region corresponding to the chromosome of E. coli strain MG1655.

4) Small EcoRI-PstI fragment (1,388 bp) (SEQ. ID No. 29) of pML-Tc-ter_thrL comprising a tetracycline resistance gene and transcription terminator ter_thrL. pML-Tc-ter-thrL was obtained as follows.

pML-MCS (Mol Biol (Mosk). 2005 Sep-Oct;39(5):823-31) was digested with XbaI and BamHI restriction endonucleases and the large fragment (3342 bp) was spliced to an XbaI-BamHI fragment (68 bp) containing terminator ter_thrL. The region of the XbaI-BamHI fragment corresponding to the chromosome of E. coli strain MG1655 was amplified by PCR using oligonucleotides P9 and P10 (SEQ. ID Nos. 25 and 26) as primers (these primers contained additional XbaI and BamHI endonuclease recognition sites). The ligated reaction product was named plasmid pML-ter_thrL.

pML-ter_thrL was cleaved with KpnI and XbaI restriction endonucleases, treated with the Klenow fragment of DNA polymerase I, and then ligated to the small EcoRI-Van9II fragment (1,317 bp) of pBR322 containing a tetracycline resistance gene (pBR322 was treated with the Klenow fragment of DNA polymerase I using EcoRI and Van9II restriction endonucleases). The product of the ligation reaction was named plasmid pML-Tc-ter_thrL.

pMW118-attL-Tc-attR was obtained as set forth above.

pMW118-attL-Cm-attR was constructed by ligation the large BamHI-XbaI fragment (4,413 bp) of pMW118-attL-Tc-attR, promoter PA2 (the initial promoter of T7 phage), the cat gene of chloramphenicol resistance (CmR), and BglII-XbaI artificial DNA fragment (1,162 bp) comprising the transcription terminator ter_thrL and attR. The artificial DNA fragment (SEQ. ID No. 30) was obtained as follows.

The pML-MSC (Mol Biol (Mosk). 2005 Sep-Oct;39(5):823-31;

Biotechnologiya (Russian) No. 5: 3-20.)) was cleaved with KpnI and XbaI restriction endonucleases and ligated to the small KpnI-XbaI fragment (120 bp) containing promoter PA2 (the initial promoter of T7 phage). The KpnI-XbaI fragment was obtained by PCR amplification of the region corresponding to T7 phage DNA using oligonucleotides P11 and P12 (SEQ. ID Nos. 27 and 28) as primers (these primers had additional KpnI and XbaI endonuclease recognition sites). The product of the splicing reaction was named plasmid pML-PA2-MCS.

The XbaI site was removed from pML-PA2-MCS. The product obtained was called plasmid pML-PA2-MCS(XbaI-).

The small BglII-HindIII fragment (928 bp) of pML-PA2-MCS(XbaI-) containing promoter PA2 (the initial promoter of T7 phage) and the cat gene imparting resistance to chloramphenicol (CmR) was ligated to the small HindIII-HindIII fragment (234 bp) of pMW118-attL-Tc-attR containing transcription terminator *ter_thrL* and attR.

The targeted artificial DNA fragment (1,156 bp) was obtained by PCR amplification of the splicing reaction mixture with primers in the form of oligonucleotides P9 and P4 (SEQ. ID Nos. 25 and 20) (these primers contained additional HindIII and XbaI endonuclease recognition sites).

(3-2) pMW-intxis-ts

Initially, two DNA fragments were amplified using λ phage DNA (Fermentas) as template. The first fragment was comprised of nt 37168 to 38046 (SEQ. ID No. 39), containing CI repressor, promoters *Prm* and *Pr*, and the leader sequence of the *cro* gene. This fragment was obtained by amplification using oligonucleotides P1' and P2' (SEQ. ID NO. 31 and 32) as primers. The second fragment was comprised of nt 27801 to 29100 (SEQ. ID NO. 40), containing the *xis-int* genes of λ phage. This fragment was obtained by amplification using oligonucleotides P3' and P4' (SEQ. ID NO. 33 and 34) as primers. All the primers include suitable endonuclease recognition sites.

The PCR amplification fragment containing *ci* repressor that was obtained was cleaved with ClaI restriction endonuclease, treated with the Klenow fragment of DNA polymerase I, and digested with EcoRI restriction endonuclease. The second PCR

amplified fragment was digested with EcoRI and PstI restriction endonucleases.

Additionally, plasmid pMWPlaclacI-ts was digested with BglII endonuclease, treated with the Klenow fragment of DNA polymerase I, and cleaved with PstI restriction endonuclease. The vector fragment of pMWPladlacI-ts was eluted from agarose gel and ligated to the cut PCR amplified fragment. (Biotechnologiya (Russian) No. 5: 3-20.)

Plasmid pMWPlaclacI-ts was derived from pMWPlaclacI, which comprised the following components: 1) a BglII-HindIII artificial DNA fragment comprising PlacUV5 promoter and the lacI gene controlled by the RBS of bacteriophage T7 gene 10; 2) an AatII-BglII fragment containing an ampicillin resistance (ApR) gene, obtained by PCR amplification of the region corresponding to plasmid pUC19 using oligonucleotides P5' and P6' (SEQ. ID Nos. 35 and 36) as primers (these primers contained additional AatII and BglII endonuclease recognition sites); 3) an AatII-BglII fragment containing the AatII-PvuI fragment of recombinant plasmid pMW118-ter_rrnB.

Plasmid pMW118-ter_rrnB was constructed in the following manner. The region corresponding to the chromosome of E. coli strain MG1655 was amplified by PCR using oligonucleotides P7' and P8' (SEQ. ID Nos. 37 and 38) containing suitable endonuclease recognition sites as primers, yielding a PstI-HindIII fragment containing terminator ter_rrnB. Before ligation, the pMW118 and the ter_rrnB fragment (complement ; SEQ. ID No. 41) were restricted with PvuI and PstI, respectively, treated with the Klenow fragment of DNA polymerase to blunt the ends, and digested with AatII or HindIII endonuclease. To construct a pMWPlaclacI-ts mutant, the AatII-EcoRV fragment of the plasmid pMWPlaclacI was replaced with the AatII-EcoRV fragment of plasmid pMAN997 containing the par, ori, and repA genes of the pSC101 replicon. (Applied and Environmental Microbiology, June 2005, p. 3228-32)

<1-2> Construction of a strain which does not produce type I fimbrial adhesin protein (strain deleting the fimH gene: WC196ΔcadAΔldcCΔfimH strain) from WC196ΔcadAΔldcC

The *fimH* gene was deleted from the WC196ΔcadAΔldcC strain by the procedure of (1) above; the primers of SEQ. ID Nos. 7 and 8 were employed to delete the

fimH gene. This obtained strain did not produce type I fimbrial adhesion protein, and was designated WC196 Δ cadA Δ ldcC Δ fimH::Cm.

Strains WC196 Δ cadA Δ ldcC and WC196 Δ cadA Δ ldcC Δ fimH::Cm were transformed by the usual methods with plasmid Lys production-plasmid pCABD2 carrying *dapA*, *dapB*, and *lysC* genes (WO01/53459), obtaining strains WC196 Δ cadA Δ ldcC/pCABD2 and WC196 Δ cadA Δ ldcC Δ fimH::Cm/pCABD2. These strains were cultured at 37°C in L medium containing 20 mg/L of streptomycin to a final OD600 of about 0.6. A 40 percent glycerol solution was added in a quantity equal to that of the culture solution, the mixture was stirred, and suitable amounts were poured out and stored at -80°C. This was referred to as glycerol stock.

Example 2

Evaluation of the L-lysine production of a strain which does not produce type I fimbrial adhesin protein

The glycerol stocks of these strains were melted, 100 μ L amounts were uniformly plated onto L plates containing 20 mg/L of streptomycin, and the strain were cultured for 24 hours at 37°C. About 1/8 of the bacterial cells obtained on each plate was inoculated onto a 20 mL fermentation culture containing 20 mg/L of streptomycin in a 500 mL Sakaguchi flask and cultured for 48 hours at 37°C in a reciprocating shaking incubator. After culturing, the amount of L-lysine that had accumulated in the medium was measured by a ordinary method (Sakura Seiki, Biotech Analyzer AS210).

The composition of the fermentation medium is shown below (unit: g/L).

Glucose	40
(NH ₄) ₂ SO ₄	24
K ₂ HPO ₄	1.0
MgSO ₄ ·7H ₂ O	1.0
FeSO ₄ ·7H ₂ O	0.01
MnSO ₄ ·5H ₂ O	0.01

Yeast extract 2.0

Up to 1 L

The medium was adjusted to pH 7.0 with KOH and sterilized for 10 min at 115°C in an autoclave (the glucose and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ were separately sterilized). A 30 g/L quantity of CaCO_3 (that had been dry sterilized for 2 hours at 180°C) was added. 20 mg/L amounts of streptomycin was added as antibiotic. Culturing was conducted for 48 hours under conditions of a temperature of 37°C with stirring at 115 rpm.

The results are given in Table 2 (OD is the absorbance at 600 nm by the bacterial mass diluted 26-fold, Lys (g/L) is the amount of L-lysine accumulated in the flask, and the yield (%) is the L-lysine yield from sugar). As will be shown in Table 2, strain WC196 $\Delta\text{cadA}\Delta\text{ldcC}\Delta\text{fimH}::\text{Cm/pCABD2}$ accumulated more L-lysine than strain WC196 $\Delta\text{cadA}\Delta\text{ldcC}::\text{Cm/pCABD2}$, from which the *fimH* gene had not been deleted.

Table 2

	OD (x26)	Lys (g/L)	Yield (%)
WC196 $\Delta\text{cadA}\Delta\text{ldcC/pCABD2}$	0.712	17.54	42.21
WC196 $\Delta\text{cadA}\Delta\text{ldcC}\Delta\text{fimH/pCABD2}$	0.710	18.19	43.77

Example 3

Evaluation of the L-threonine production of a strain which does not produce type I fimbrial adhesin protein

B-5318 was employed as the L-threonine-producing parent strain which does not produce type I fimbrial adhesin protein. Strain B-5318 was deposited on November 19, 1987, under registration number VKPM B-5318 with the Russian National Collection of Industrial Microorganisms (VKPM), GNII Genetika. The strain which does not produce type I fimbrial adhesin was constructed from the L-threonine producing bacterium by the

same method as in Example 1 by employing synthetic oligonucleotides SEQ. ID Nos. 7 and 8. Specifically, the following fimH deficient strain was constructed.

B-5318 Δ fimH-cat was derived from strain B-5318 by method (1) in Example 1. Strain B-5318 and strain B-5318 Δ fimH-cat were cultured for 24 hours at 37°C on L agar medium containing 20 mg/L of streptomycin sulfate and L agar medium containing 20 mg/L of streptomycin sulfate and 25 mg/L of chloramphenicol, respectively. One-fifth of the bacterial cell was collected from each of the plates and inoculated onto 50 mL of LB liquid medium containing the above-stated antibiotics and the bacteria were precultured for 3.5 hours at a temperature of 39°C at 144 rpm.

Following preculturing, a quantity of preculture solution amounting to 10 percent of the volume of the main culture medium was inoculated into a one-liter jar fermenter containing 300 mL of main culture medium and the main culture was cultivated at 40°C at pH 7.0. The composition of the main culture medium is shown below.

Composition of Main Culture Medium

Glucose	100 g/L
Yeast extract	1.8 g/L
FeSO ₄ ·7H ₂ O	18 mg/L
MnSO ₄ ·4H ₂ O	18 mg/L
KH ₂ PO ₄	1 g/L
MgSO ₄ ·7H ₂ O	0.36 g/L
(NH ₄) ₂ SO ₄	4.5 g/L
NaCl	0.6 g/L

For strain B5318, 20 mg/L of streptomycin sulfate salt was added, and for strain B-5318 Δ fimH-cat, 20 mg/L of streptomycin sulfate salt and 25 mg/L of chloramphenicol were added.

Ammonia gas was added to adjust the culture to pH 7.0. When the glucose was exhausted, culturing was stopped and liquid chromatography was employed to measure the quantity of L-threonine. The results are shown in Table 3. OD is the absorbance at 600 nm by the bacterial mass diluted 101-fold, Thr (g/L) is the amount of L-threonine which had

accumulated in the flask, and the yield (%) is the L-threonine yield from sugar.

Use of the B-5318 Δ fimH-cat strain, which lacked the fimH gene, enhanced the yield of L-threonine compared with the B-5318 strain, which served as a control.

Table 3

Strain	OD ($\times 101$)	Thr (g/L)	Yield (%)
B-5318	0.597	32.7	32.9
B-5318 Δ fim-cat	0.617	34.4	33.4

Example 4

Evaluation of the L-glutamic acid production of a strain which does not produce type I fimbrial adhesin protein

<4-1> Evaluation of the L-glutamic acid production of a *Escherichia coli* strain which does not produce type I fimbrial adhesin protein

Escherichia coli strain AJ12949 was employed as the L-glutamic acid-producing parent strain which does not produce type I fimbrial adhesin protein. Strain AJ12949 was deposited on December 28, 1993, as depositary number FERM P-14039, at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology (currently the International Patent Organism Depositary, National Institute of Industrial Science and Technology, an Independent Administrative Institution, Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki, Japan Postal Code 305-8566), and converted on November 11, 1994, to an international deposit under the provisions of the the Budapest Treaty, and given a deposit number FERM BP-4881.

Culturing was conducted in the following specific manner. Strain AJ12949 Δ fimH-cat was derived from strain AJ12949 (BP-4881). Strain AJ12949 and strain AJ12949 Δ fimH-cat were cultured for 24 hours at 37°C in L agar medium containing 50 mg/L of ampicillin and L agar solution containing 50 mg/L of ampicillin and 25 mg/L of chloramphenicol, respectively. One-eighth of the bacterial cells was collected from each

plate and inoculated onto 20 mL of fermentation medium containing 50 mg/L of ampicillin in a 500 mL Sakaguchi flask and cultured for 24 hours at 37°C in a reciprocating shaking incubator. Following culturing, the amount of glutamic acid accumulated in the medium was measured by a known method (Sakura Seiki, Biodex Analyzer AS210).

The composition of the fermentation medium is given below (unit: g/L)

Glucose	40
Yeast extract	2
FeSO ₄ ·7H ₂ O	0.01
MnSO ₄ ·4H ₂ O	0.01
KH ₂ PO ₄	1
MgSO ₄ ·7H ₂ O	1
(NH ₄) ₂ SO ₄	20
Thiamine hydrochloride	0.01

The medium was adjusted to pH 7.0 with KOH and sterilized for 10 min at 115°C in an autoclave (the glucose and MgSO₄·7H₂O were separately sterilized). A 50 g/L quantity of CaCO₃ (that had been dry sterilized for 2 hours at 180°C) was added. A 50 mg/L quantity of ampicillin was added as an antibiotic for strain AJ12949, and a 50 mg/L quantity of ampicillin and a 50 mg/L quantity of chloramphenicol were added for AJ12949ΔfimH-cat.

The results are given in Table 4 (OD is the absorbance at 600 nm by the bacterial mass diluted 26-fold, Glu (g/L) is the amount of L-glutamine accumulated in the flask, and the yield (%) is the L-glutamic acid yield from sugar). Strain AJ12949ΔfimH-cat, the strain that did not produce type I fimbrial adhesin protein, accumulated more L-glutamic acid than strain AJ12949, from which *fimH* was not deleted.

Table 4

Strain	OD (x26)	Glu (g/L)	Yield (%)
AJ12949	0.698	20.50	51.25
AJ12949 Δ fim::Cm	0.762	21.36	53.41

<4-2> Evaluation of the L-glutamic acid production of a *Pantoea ananatis* strain which does not produce type I fimbrial adhesin protein

Pantoea ananatis strain AJ13601 can also be employed as the L-glutamic acid-producing parent strain which does not produce type I fimbrial adhesin protein. *Pantoea ananatis* strain AJ13601 was deposited on August 18, 1999, as depositary number FERM P-17516, at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Ministry of Economy, Trade and Industry (1-1-3 Higashi, Tsukuba, Ibaraki Prefecture, Postal Code 305-8566). This strain was converted on July 6, 2000, to a deposit under the provisions of the Budapest Treaty under deposit number FERM BP-7207. A strain that does not produce type I fimbrial adhesin can be constructed from the L-glutamic acid-producing microbe by the same method as in Example 1 using synthetic nucleotides having SEQ. ID Nos. 7 and 8. A mutant strain can also be constructed by introducing a temperature-sensitive plasmid having a deleted gene encoding type I fimbrial adhesin protein.

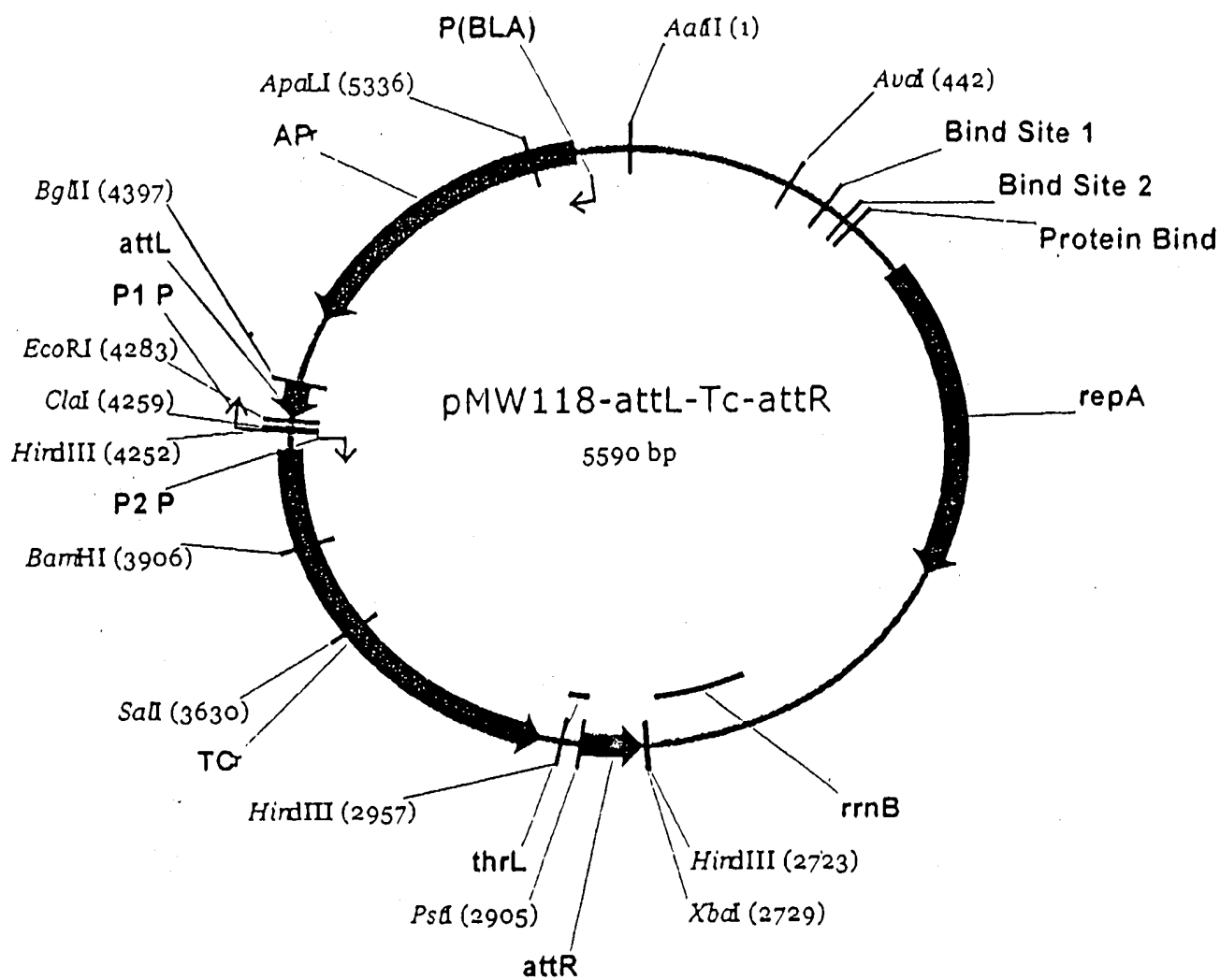
The strain not producing type I fimbrial adhesin protein can be cultured in an L-glutamic acid production medium in a reciprocating shaking incubator. Following culturing, the amount of L-glutamic acid which had accumulated in the medium is measured by an ordinary method to confirm an increase in accumulated L-glutamic acid. This method permits the obtaining of a strain that does not produce type I fimbrial adhesin protein and has an enhanced ability to produce L-glutamic acid.

CLAIMS :

1. An L-amino acid producing bacterium of the *Enterobacteriaceae* family, wherein the bacterium has been modified so as to not produce type I fimbrial adhesin protein.
2. The bacterium according to claim 1, wherein said bacterium has been modified so as to not produce type I fimbrial adhesin protein by introducing a mutation into the gene encoding type I fimbrial adhesin protein on the chromosome and/or into the region regulating expression thereof.
3. The bacterium according to claim 1, wherein said bacterium has been modified so as to not produce type I fimbrial adhesin protein by attenuation of the gene encoding type I fimbrial adhesin protein.
4. The bacterium according to claim 1, wherein said bacterium has been modified so as to not produce type I fimbrial adhesin protein by inactivation of the gene encoding type I fimbrial adhesin protein on the chromosome.
5. The bacterium according to claim 1, wherein the gene encoding type I fimbrial adhesin protein is *fimH*.
6. The bacterium according to claim 1, wherein said gene encoding type I fimbrial adhesin protein is selected from the group consisting of:
 - (a) a DNA comprising the nucleotide sequence of SEQ ID NO:1, and
 - (b) a DNA that is able to hybridize with the complementary strand of the nucleotide of SEQ ID NO:1, or a probe prepared from said nucleotide sequence under stringent conditions, and wherein the DNA encodes the type I fimbrial adhesin protein.
7. The bacterium according to claim 1 wherein said bacterium of the *Enterobacteriaceae* family is selected from the group consisting of *Escherichia*, *Pantoea*, and *Enterobacter*.
8. The bacterium according to claim 1, wherein said L-amino acid is selected from the group consisting of L-lysine, L-threonine, L-glutamic acid, and combinations thereof.
9. A method for producing an L-amino acid, comprising
 - cultivating the bacterium according to any of claim 1 to 8 in a medium, and
 - collecting said L-amino acid from the medium.

10. The method according to claim 10, wherein said L-amino acid is selected from the group consisting of L-lysine, L-threonine, L-glutamic acid, and combinations thereof.

図 1



2

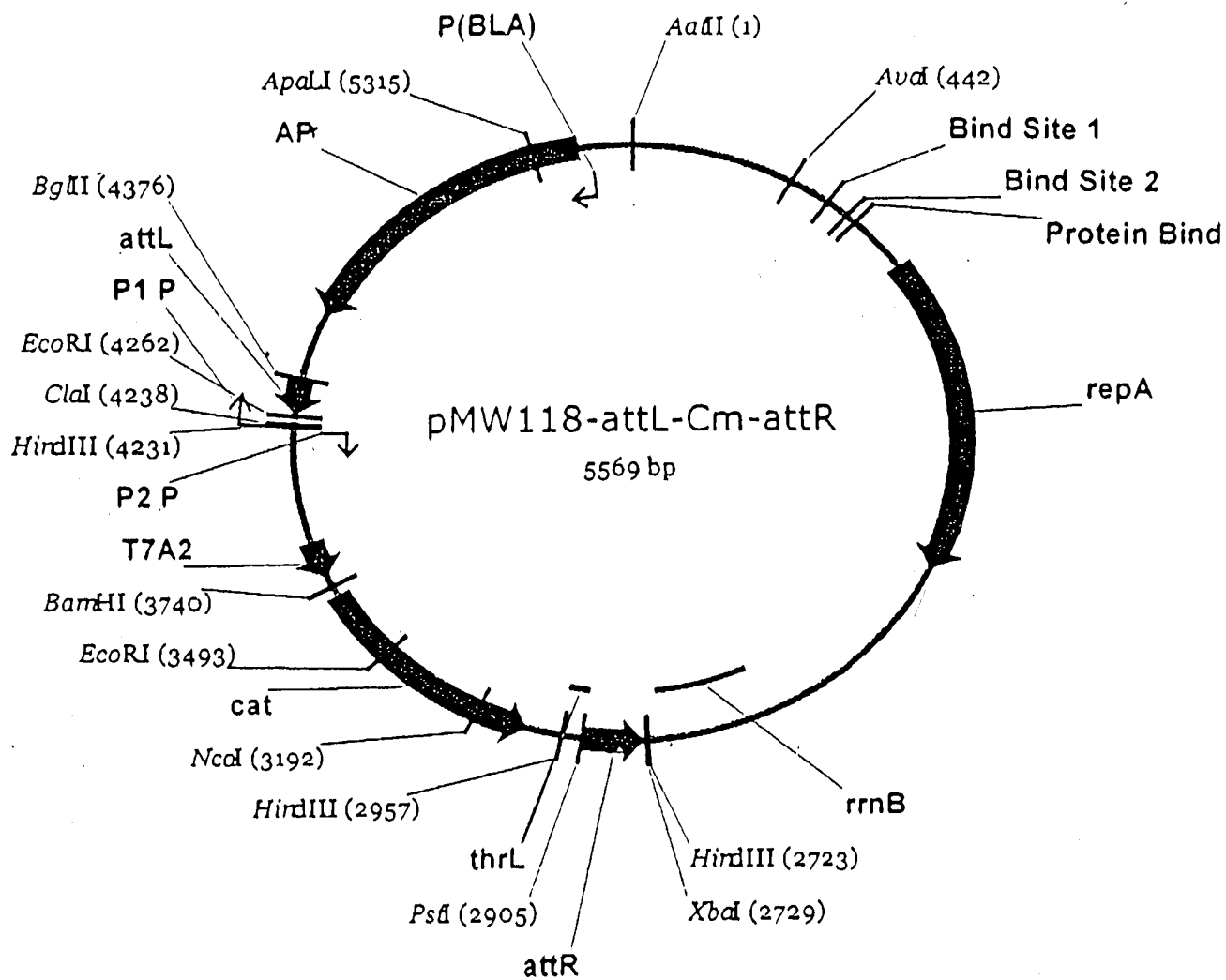
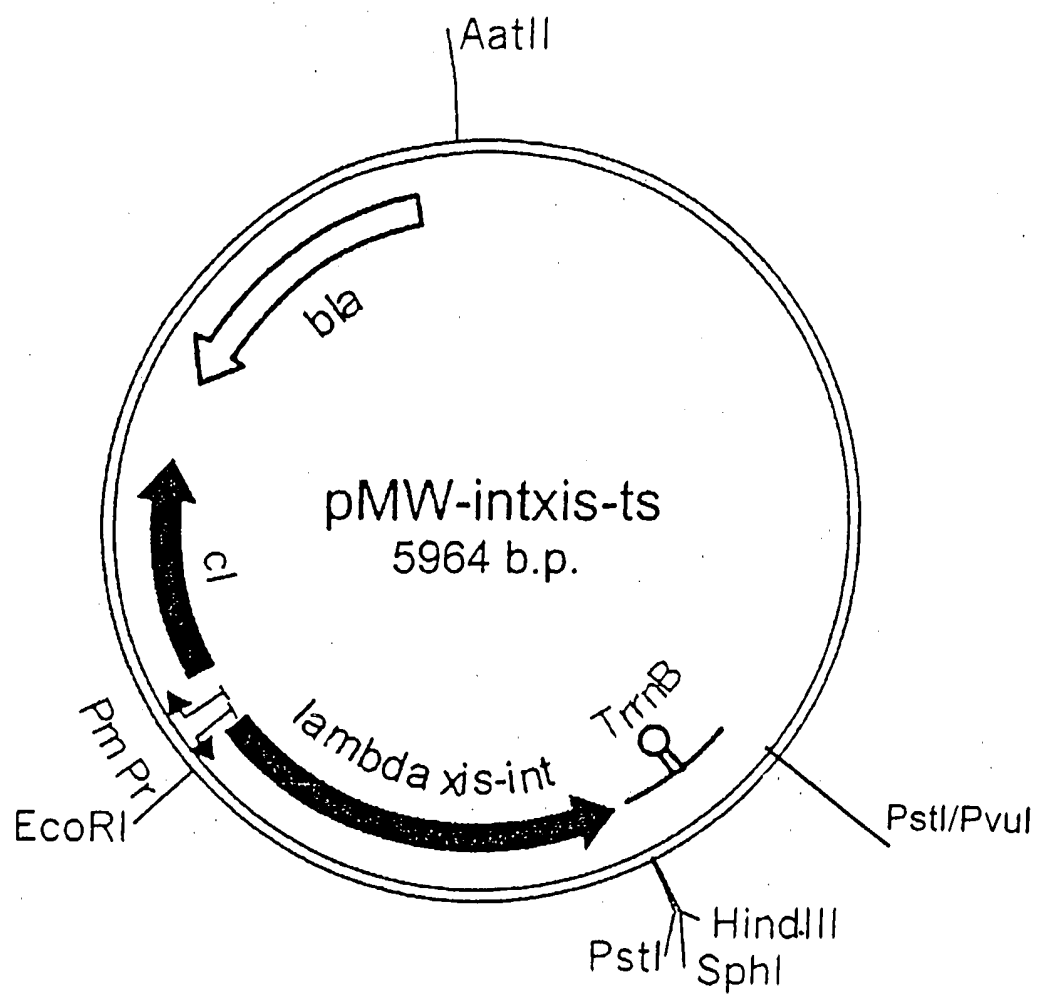


図 3



SEQUENCE LISTING

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<130> C-445

<150> JP2005-009826

<151> 2005-01-18

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<160> 61

<170> PatentIn version 3.1

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 Cys His Asn Asp Tyr Pro Glu Thr Ile Thr Asp Tyr Val Thr Leu Gln
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 85 90 95
 Lys Tyr Ser Gly Ser Ser Tyr Pro Phe Pro Thr Thr Ser Glu Thr Pro
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 Asp Asp Phe Gln Phe Val Trp Asn Ile Tyr Ala Asn Asn Asp Val Val

165 170 175
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 210 215 220
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 225 230 235 240
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Lys Glu Phe Gly Leu Gly Arg Asp Arg Arg Ile Ala Ile Thr Glu Ala
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Ser Thr Leu Ser Asp Ala Phe Arg Glu Ala Ile Ala Glu Gly His Ile
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Pro Thr Phe His Glu Leu Arg Ser Leu Ser Ala Arg Leu Tyr Glu Lys
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20      25      30
cct ccg gtt aag gat gga aga gag tat ctg ttc cac gaa tca gcg gta      144
Pro Pro Val Lys Asp Gly Arg Glu Tyr Leu Phe His Glu Ser Ala Val
35      40      45
aag gtt gac tta aat cga cca gta aca ggt ggc ctt ttg aag agg atc      192
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ttccggcgcg atcgggatgc ccgcgttgca ggccatgctg tccaggcagg tagatgacga    1080
ccatcaggga cagcttcaag gatcgctcgc ggctcttacc agcctaactt cgatcactgg    1140
accgctgacg gtcacggcga ttatgcccgc ctggcgagc acatggaacg ggttggcatg    1200
gattgtaggc gccgccctat accctgtctg cctccccgcg ttgcgtcgcg gtgcattggag    1260
ccgggccacc tcgacctgaa tgaagccgg cggcacctcg ctaacggatt caccactcca    1320
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cactgcag                                     1388

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<210> 30

<211> 1162

<212> DNA

<213> Artificial Sequence

<220>

<223> cloned DNA fragment containing artificial DNA fragment including promoter PA2 (early promoter of phage T7), cat gene for chloramphenicol resistance (CmR), transcription terminator ter_thrL and attR

<400> 30

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agatctccgg ataagtagac agcctgataa gtgcacgaa aaacaggat tgacaacatg    60
aagtaacatg cagtaagata caaatcgcta ggtaacacta gcagcgtaaa cggggcgctc    120
tagctagagc caagctagct tggccggatc cgagattttc aggagctaag gaagctaaaa    180
tggagaaaaa aatcactgga tataccaccg ttgatataac ccaatggcat gtaaaagaac    240
attttgaggc atttcagtca gttgctcaat gtacctataa ccagaccgtt cagctggata    300
ttacggcctt ttaaaagacc gtaaaagaaa ataagcaca gttttatccg gcctttattc    360
acattcttgc ccgcttgatg aatgctcatc cggatttccg tatggcaatg aaagacggtg    420
agctggatg atgggatagt gttaccctt gttaccctt tttccatgag caaactgaaa    480
cgttttcatc gctctggagt gaataccacg acgatttccg gcagtttcta cacatatatt    540
cgcaagatgt ggcgtgttac ggtgaaaacc tggcctattt ccctaagggg ttatttgaga    600
atatgttttt cgtctcagcc aatccctggg tgaatttcac cagttttgat ttaaacgttg    660
ccaatatgga caactcttc gcccccgttt tcaccatggg caaatattat acgcaaggcg    720
acaagggtgt gatgccctg gcgattcagg ttcatcatgc cgtctgtgat ggcctccatg    780
tcggcagaat gcttaatgaa ttacaacagt actgcgatga gtggcagggc ggggcgtaat    840
ttttttaagg cagttattgg tgcctttaa cgcctggteg tacgcttgaa taagtataa    900
taagcggatg aatggcagaa attcgtcgaa gcttaacaca gaaaaaagcc cgcacctgac    960
agtgcgggct ttttttttcg accactgcag tctgttacag gtcactaata ccatctaagt    1020
agttgattca tagtgactgc atatgttg ttttacagta ttatgtagtc tgttttttat    1080
gcaaaatcta atttaataa ttgatattta tatcatttta cgtttctcgt tcagcttttt    1140
tatactaact tgagcgtcta ga                                     1162

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<210> 31

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> oligonucleotide P1'

<400> 31

ctaataatcga tgaagattct tgctcaa

27

<210> 32

<211> 34
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> oligonucleotide P2'

 <400> 32
 gcgttgaatt ccatacaacc tccttagtac atgc 34

 <210> 33
 <211> 34
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> oligonucleotide P3'

 <400> 33
 gtactagaat tcgtgtaatt gcggagactt tcgc 34

 <210> 34
 <211> 41
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> oligonucleotide P4'

 <400> 34
 aatagcctgc agttatttga tttcaatttt gtcccactcc c 41

 <210> 35
 <211> 38
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> oligonucleotide P5'

 <400> 35
 ttccttagacg tcaggtagca cttttcgggg aaatgtgc 38

 <210> 36
 <211> 37
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> oligonucleotide P6'

 <400> 36
 taacagagat ctagcgaga aaaaaaggat ctcaaga 37

 <210> 37
 <211> 35
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> oligonucleotide P7'

 <400> 37
 ataaactgca gcaaaaagag tttgtagaaa cgcaa 35

 <210> 38
 <211> 34
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> oligonucleotide P8'

 <400> 38
 aacagaagct ttttccttg cggcagtagc gcgg 34

<210> 39
 <211> 879
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> cloned DNA fragment containing cl repressor gene and promoter regions

<400> 39
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 cagccaaacg tctcttcagg ccactgacta gcgataactt tccccacaac ggaacaactc 120
 tcattgcatt ggatcattgg gtactgtggg tttagtgggt gtaaaaacac ctgaccgcta 180
 tccctgatca gtttcttgaa ggtaaaacta tcaccccca gcttggtctat gcagaaatca 240
 cctggctcaa cagcctgctc agggctcaac agaatttaaca ttccgtcagg aaagcttggc 300
 ttggagcctg ttgggtcggg catggaatta cttcaacct caagccagaa tgcagaatca 360
 ctggcttttt tggttgtgct tacccatctc tccgcatcac ctttggtaaa ggttctaagc 420
 tcaggtgaga acatccctgc ctgaacatga gaaaaaacag ggtactcata ctcaacttca 480
 agtgacggct gcatactaac cgcttcatac atctcgtaga ttctcttggc gattgaaggg 540
 cttaaattct caacgctaac ttfgagaatt ttgcaagca atgcggcggt ataagcattt 600
 aatgcattga tgccattaaa taaagcacca acgcttgact gcccattccc catcttctct 660
 gcgacagatt cctgggataa gccaagttca ttttcttttt ttctataaat tgcctttaagg 720
 cgacgtgcgt cctcaagctg ctcttgtgtt aatggtttct tttttgtgct catacgttaa 780
 atctatcacc gcaaggata aatatctaac accgtgcgtg ttgactatit tacctctggc 840
 ggtgataatg gttgcatgta ctaaggagggt tgtatggaa 879

<210> 40
 <211> 1290
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> cloned DNA fragment containing int-xis genes

<400> 40
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 tgggtgccga cttatgcccg agaagatggt gagcaaacit atcgcttata tgccttctcat 120
 agagctcttc agacaaactg cgcaactcgt gaaaggtagg cggatcccc tcaaggaaaa 180
 gacctgatgc ttttcgtgcg cgataaaat accttgatac tggccggat gaaagcgggt 240
 cgcgacgagt agatgcaatt atggtttctc cgccaagaat ctctttgcat ttatcaagtg 300
 ttctcttcat tgataattcc agagcatcaa tatgcaatgc tgttgggatg gcaattttta 360
 cgctctgttt gctttgctcg acataaagat atccatctac gatatacagac cacttcattt 420
 cgcataaatc accaactcgt tgcgggtaaa caacagccag ttccattgca agtctgagcc 480
 aacatggtag tgattctgct gcttgataaa ttttcaggta ttctgcagcc gtaagtcctt 540
 atctccttac ctctgatttt gctgcgcgag tggcagcgac atggtttgtt gttatatggc 600
 ctctcagctat tgcctctcgg aatgcatcgc tcagtgttga tctgattaac ttggctgacg 660
 ccgcttggc ctgctctatg tatccattga gcattgccgc aatttctttt gttgtgatgt 720
 ctctcaagtg agcatcagg agacccctcc ttattgcttt aattttgctc atgtaattta 780
 tgagtgtctt ctgcttgatt cctctgtcgg ccaggatttt ttctgtagca tcaagccatg 840
 aatgtaacgt aacggaaata tcaactgtga ttctcgctgt cagaggcttg tgtttgtgtc 900
 ctgaaaataa ctcaatgttg gcctgtatag cttcagtgat tgcgattcgc ctgtctctgc 960
 ctaatccaaa ctctttaccg gtccctgggt ccctgtagca gtaatatcca ttgtttctta 1020
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 ctcttcaaaa ggccacctgt tactgttcga ttttaagtcaa cctttaccgc tgattcgtgg 1140
 aacagatact ctcttccatc cttaaccgga ggtgggaata tcctgcattc ccgaaccat 1200
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 aagtacatcg caaagcttcc gcaattacac 1290

<210> 41
 <211> 351
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> ter_rrnB fragment (complement)

<400> 41
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 gatgccctggc agtttatggc gggcgctcgt cccgccacc tccggccgt tgccttcgaa 120
 cgttcaaatc cgctcccggc ggatttgtcc tactcaggag agcgttcacc gacaaaacac 180
 agataaaaac aaaggccag tctttcgaat gagcctttcg ttttatttga tgcctggcag 240
 ttccctactc tcgcatgggg agacccaca ctaccatcgg cgctacggcg tttcacttct 300
 gagttcggca tggggtcagg tgggaccacc gcgctactgc cgccaggcaa a

<210> 42

<211> 2148
 <212> DNA
 <213> Escherichia coli

<220>
 <221> CDS
 <222> (1).. (2148)
 <223>

<400> 42
 atg aac gtt att gca ata ttg aat cac atg ggg gtt tat ttt aaa gaa 48
 Met Asn Val Ile Ala Ile Leu Asn His Met Gly Val Tyr Phe Lys Glu
 1 5 10 15
 gaa ccc atc cgt gaa ctt cat cgc gcg ctt gaa cgt ctg aac ttc cag 96
 Glu Pro Ile Arg Glu Leu His Arg Ala Leu Glu Arg Leu Asn Phe Gln
 20 25 30
 att gtt tac ccg aac gac cgt gac gac tta tta aaa ctg atc gaa aac 144
 Ile Val Tyr Pro Asn Asp Arg Asp Asp Leu Leu Lys Leu Ile Glu Asn
 35 40 45
 aat gcg cgt ctg tgc ggc gtt att ttt gac tgg gat aaa tat aat ctc 192
 Asn Ala Arg Leu Cys Gly Val Ile Phe Asp Trp Asp Lys Tyr Asn Leu
 50 55 60
 gag ctg tgc gaa gaa att agc aaa atg aac gag aac ctg ccg ttg tac 240
 Glu Leu Cys Glu Glu Ile Ser Lys Met Asn Glu Asn Leu Pro Leu Tyr
 65 70 75 80
 gcg ttc gct aat acg tat tcc act ctc gat gta agc ctg aat gac ctg 288
 Ala Phe Ala Asn Thr Tyr Ser Thr Leu Asp Val Ser Leu Asn Asp Leu
 85 90 95
 cgt tta cag att agc ttc ttt gaa tat gcg ctg ggt gct gct gaa gat 336
 Arg Leu Gln Ile Ser Phe Phe Glu Tyr Ala Leu Gly Ala Ala Glu Asp
 100 105 110
 att gct aat aag atc aag cag acc act gac gaa tat atc aac act att 384
 Ile Ala Asn Lys Ile Lys Gln Thr Thr Asp Glu Tyr Ile Asn Thr Ile
 115 120 125
 ctg cct ccg ctg act aaa gca ctg ttt aaa tat gtt cgt gaa ggt aaa 432
 Leu Pro Pro Leu Thr Lys Ala Leu Phe Lys Tyr Val Arg Glu Gly Lys
 130 135 140
 tat act ttc tgt act cct ggt cac atg ggc ggt act gca ttc cag aaa 480
 Tyr Thr Phe Cys Thr Pro Gly His Met Gly Gly Thr Ala Phe Gln Lys
 145 150 155 160
 agc ccg gta ggt agc ctg ttc tat gat ttc ttt ggt ccg aat acc atg 528
 Ser Pro Val Gly Ser Leu Phe Tyr Asp Phe Phe Gly Pro Asn Thr Met
 165 170 175
 aaa tct gat att tcc att tca gta tct gaa ctg ggt tct ctg ctg gat 576
 Lys Ser Asp Ile Ser Ile Ser Val Ser Glu Leu Gly Ser Leu Leu Asp
 180 185 190
 cac agt ggt cca cac aaa gaa gca gaa cag tat atc gct cgc gtc ttt 624
 His Ser Gly Pro His Lys Glu Ala Glu Gln Tyr Ile Ala Arg Val Phe
 195 200 205
 aac gca gac cgc agc tac atg gtg acc aac ggt act tcc act gcg aac 672
 Asn Ala Asp Arg Ser Tyr Met Val Thr Asn Gly Thr Ser Thr Ala Asn
 210 215 220
 aaa att gtt ggt atg tac tct gct cca gca ggc agc acc att ctg att 720
 Lys Ile Val Gly Met Tyr Ser Ala Pro Ala Gly Ser Thr Ile Leu Ile
 225 230 235 240
 gac cgt aac tgc cac aaa tgc ctg acc cac ctg atg atg atg agc gat 768
 Asp Arg Asn Cys His Lys Ser Leu Thr His Leu Met Met Met Ser Asp
 245 250 255
 gtt acg cca atc tat ttc cgc ccg acc cgt aac gct tac ggt att ctt 816
 Val Thr Pro Ile Tyr Phe Arg Pro Thr Arg Asn Ala Tyr Gly Ile Leu

260	265	270	
ggt ggt atc cca cag agt gaa ttc cag cac gct acc att gct aag cgc Gly Gly Ile Pro Gln Ser Glu Phe Gln His Ala Thr Ile Ala Lys Arg 275 280 285			864
gtg aaa gaa aca cca aac gca acc tgg ccg gta cat gct gta att acc Val Lys Glu Thr Pro Asn Ala Thr Trp Pro Val His Ala Val Ile Thr 290 295 300			912
aac tct acc tat gat ggt ctg ctg tac aac acc gac ttc atc aag aaa Asn Ser Thr Tyr Asp Gly Leu Leu Tyr Asn Thr Asp Phe Ile Lys Lys 305 310 315 320			960
aca ctg gat gtg aaa tcc atc cac ttt gac tcc gcg tgg gtg cct tac Thr Leu Asp Val Lys Ser Ile His Phe Asp Ser Ala Trp Val Pro Tyr 325 330 335			1008
acc aac ttc tca ccg att tac gaa ggt aaa tgc ggt atg agc ggt ggc Thr Asn Phe Ser Pro Ile Tyr Glu Gly Lys Cys Gly Met Ser Gly Gly 340 345 350			1056
cgt gta gaa ggg aaa gtg att tac gaa acc cag tcc act cac aaa ctg Arg Val Glu Gly Lys Val Ile Tyr Glu Thr Gln Ser Thr His Lys Leu 355 360 365			1104
ctg gcg gcg ttc tct cag gct tcc atg atc cac gtt aaa ggt gac gta Leu Ala Ala Phe Ser Gln Ala Ser Met Ile His Val Lys Gly Asp Val 370 375 380			1152
aac gaa gaa acc ttt aac gaa gcc tac atg atg cac acc acc act tct Asn Glu Glu Thr Phe Asn Glu Ala Tyr Met Met His Thr Thr Thr Ser 385 390 395 400			1200
ccg cac tac ggt atc gtg gcg tcc act gaa acc gct gcg gcg atg atg Pro His Tyr Gly Ile Val Ala Ser Thr Glu Thr Ala Ala Ala Met Met 405 410 415			1248
aaa ggc aat gca ggt aag cgt ctg atc aac ggt tct att gaa cgt gcg Lys Gly Asn Ala Gly Lys Arg Leu Ile Asn Gly Ser Ile Glu Arg Ala 420 425 430			1296
atc aaa ttc cgt aaa gag atc aaa cgt ctg aga acg gaa tct gat ggc Ile Lys Phe Arg Lys Glu Ile Lys Arg Leu Arg Thr Glu Ser Asp Gly 435 440 445			1344
tgg ttc ttt gat gta tgg cag ccg gat cat atc gat acg act gaa tgc Trp Phe Phe Asp Val Trp Gln Pro Asp His Ile Asp Thr Thr Glu Cys 450 455 460			1392
tgg ccg ctg cgt tct gac agc acc tgg cac ggc ttc aaa aac atc gat Trp Pro Leu Arg Ser Asp Ser Thr Trp His Gly Phe Lys Asn Ile Asp 465 470 475 480			1440
aac gag cac atg tat ctt gac ccg atc aaa gtc acc ctg ctg act ccg Asn Glu His Met Tyr Leu Asp Pro Ile Lys Val Thr Leu Leu Thr Pro 485 490 495			1488
ggg atg gaa aaa gac ggc acc atg agc gac ttt ggt att ccg gcc agc Gly Met Glu Lys Asp Gly Thr Met Ser Asp Phe Gly Ile Pro Ala Ser 500 505 510			1536
atc gtg gcg aaa tac ctc gac gaa cat ggc atc gtt gtt gag aaa acc Ile Val Ala Lys Tyr Leu Asp Glu His Gly Ile Val Val Glu Lys Thr 515 520 525			1584
ggt ccg tat aac ctg ctg ttc ctg ttc agc atc ggt atc gat aag acc Gly Pro Tyr Asn Leu Leu Phe Leu Phe Ser Ile Gly Ile Asp Lys Thr 530 535 540			1632
aaa gca ctg agc ctg ctg cgt gct ctg act gac ttt aaa cgt gcg ttc Lys Ala Leu Ser Leu Arg Ala Leu Thr Asp Phe Lys Arg Ala Phe 545 550 555 560			1680
gac ctg aac ctg cgt gtg aaa aac atg ctg ccg tct ctg tat cgt gaa Asp Leu Asn Leu Arg Val Lys Asn Met Leu Pro Ser Leu Tyr Arg Glu			1728

565	570	575	
gat cct gaa ttc tat gaa aac atg cgt att cag gaa ctg gct cag aat Asp Pro Glu Phe Tyr Glu Asn Met Arg Ile Gln Glu Leu Ala Gln Asn 580 585 590			1776
atc cac aaa ctg att gtt cac cac aat ctg ccg gat ctg atg tat cgc Ile His Lys Leu Ile Val His Asn Leu Pro Asp Leu Met Tyr Arg 595 600 605			1824
gca ttt gaa gtg ctg ccg acg atg gta atg act ccg tat gct gca ttc Ala Phe Glu Val Leu Pro Thr Met Val Met Thr Pro Tyr Ala Ala Phe 610 615 620			1872
cag aaa gag ctg cac ggt atg acc gaa gaa gtt tac ctc gac gaa atg Gln Lys Glu Leu His Gly Met Thr Glu Val Tyr Leu Asp Glu Met 625 630 635 640			1920
gta ggt cgt att aac gcc aat atg atc ctt ccg tac ccg ccg gga gtt Val Gly Arg Ile Asn Ala Asn Met Ile Leu Pro Tyr Pro Pro Gly Val 645 650 655			1968
cct ctg gta atg ccg ggt gaa atg atc acc gaa gaa agc cgt ccg gtt Pro Leu Val Met Pro Gly Glu Met Ile Thr Glu Glu Ser Arg Pro Val 660 665 670			2016
ctg gag ttc ctg cag atg ctg tgt gaa atc ggc gct cac tat ccg ggc Leu Glu Phe Leu Gln Met Leu Cys Glu Ile Gly Ala His Tyr Pro Gly 675 680 685			2064
ttt gaa acc gat att cac ggt gca tac cgt cag gct gat ggc cgc tat Phe Glu Thr Asp Ile His Gly Ala Tyr Arg Gln Ala Asp Gly Arg Tyr 690 695 700			2112
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<210> 43
 <211> 715
 <212> PRT
 <213> Escherichia coli

<400> 43

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

Leu Pro Pro Leu Thr Lys Ala Leu Phe Lys Tyr Val Arg Glu Gly Lys
 130 135 140
 Tyr Thr Phe Cys Thr Pro Gly His Met Gly Gly Thr Ala Phe Gln Lys
 145 150 155 160
 Ser Pro Val Gly Ser Leu Phe Tyr Asp Phe Phe Gly Pro Asn Thr Met
 165 170 175
 Lys Ser Asp Ile Ser Ile Ser Val Ser Glu Leu Gly Ser Leu Leu Asp
 180 185 190
 His Ser Gly Pro His Lys Glu Ala Glu Gln Tyr Ile Ala Arg Val Phe
 195 200 205
 Asn Ala Asp Arg Ser Tyr Met Val Thr Asn Gly Thr Ser Thr Ala Asn
 210 215 220
 Lys Ile Val Gly Met Tyr Ser Ala Pro Ala Gly Ser Thr Ile Leu Ile
 225 230 235 240
 Asp Arg Asn Cys His Lys Ser Leu Thr His Leu Met Met Met Ser Asp
 245 250 255
 Val Thr Pro Ile Tyr Phe Arg Pro Thr Arg Asn Ala Tyr Gly Ile Leu
 260 265 270
 Gly Gly Ile Pro Gln Ser Glu Phe Gln His Ala Thr Ile Ala Lys Arg
 275 280 285
 Val Lys Glu Thr Pro Asn Ala Thr Trp Pro Val His Ala Val Ile Thr
 290 295 300
 Asn Ser Thr Tyr Asp Gly Leu Leu Tyr Asn Thr Asp Phe Ile Lys Lys
 305 310 315 320
 Thr Leu Asp Val Lys Ser Ile His Phe Asp Ser Ala Trp Val Pro Tyr
 325 330 335
 Thr Asn Phe Ser Pro Ile Tyr Glu Gly Lys Cys Gly Met Ser Gly Gly
 340 345 350
 Arg Val Glu Gly Lys Val Ile Tyr Glu Thr Gln Ser Thr His Lys Leu
 355 360 365
 Leu Ala Ala Phe Ser Gln Ala Ser Met Ile His Val Lys Gly Asp Val
 370 375 380
 Asn Glu Glu Thr Phe Asn Glu Ala Tyr Met Met His Thr Thr Thr Ser
 385 390 395 400
 Pro His Tyr Gly Ile Val Ala Ser Thr Glu Thr Ala Ala Ala Met Met
 405 410 415
 Lys Gly Asn Ala Gly Lys Arg Leu Ile Asn Gly Ser Ile Glu Arg Ala
 420 425 430

Ile Lys Phe Arg Lys Glu Ile Lys Arg Leu Arg Thr Glu Ser Asp Gly
435 440 445

Trp Phe Phe Asp Val Trp Gln Pro Asp His Ile Asp Thr Thr Glu Cys
450 455 460

Trp Pro Leu Arg Ser Asp Ser Thr Trp His Gly Phe Lys Asn Ile Asp
465 470 475 480

Asn Glu His Met Tyr Leu Asp Pro Ile Lys Val Thr Leu Leu Thr Pro
485 490 495

Gly Met Glu Lys Asp Gly Thr Met Ser Asp Phe Gly Ile Pro Ala Ser
500 505 510

Ile Val Ala Lys Tyr Leu Asp Glu His Gly Ile Val Val Glu Lys Thr
515 520 525

Gly Pro Tyr Asn Leu Leu Phe Leu Phe Ser Ile Gly Ile Asp Lys Thr
530 535 540

Lys Ala Leu Ser Leu Leu Arg Ala Leu Thr Asp Phe Lys Arg Ala Phe
545 550 555 560

Asp Leu Asn Leu Arg Val Lys Asn Met Leu Pro Ser Leu Tyr Arg Glu
565 570 575

Asp Pro Glu Phe Tyr Glu Asn Met Arg Ile Gln Glu Leu Ala Gln Asn
580 585 590

Ile His Lys Leu Ile Val His His Asn Leu Pro Asp Leu Met Tyr Arg
595 600 605

Ala Phe Glu Val Leu Pro Thr Met Val Met Thr Pro Tyr Ala Ala Phe
610 615 620

Gln Lys Glu Leu His Gly Met Thr Glu Glu Val Tyr Leu Asp Glu Met
625 630 635 640

Val Gly Arg Ile Asn Ala Asn Met Ile Leu Pro Tyr Pro Pro Gly Val
645 650 655

Pro Leu Val Met Pro Gly Glu Met Ile Thr Glu Glu Ser Arg Pro Val
660 665 670

Leu Glu Phe Leu Gln Met Leu Cys Glu Ile Gly Ala His Tyr Pro Gly
675 680 685

Phe Glu Thr Asp Ile His Gly Ala Tyr Arg Gln Ala Asp Gly Arg Tyr
690 695 700

Thr Val Lys Val Leu Lys Glu Glu Ser Lys Lys
705 710 715

<210> 44
<211> 2142

<212> DNA
 <213> Escherichia coli

<220>
 <221> CDS
 <222> (1).. (2142)
 <223>

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 1 5 10 15
 gag ccc atc aaa gaa ctg gag tgc ggc ctg gtg gcg caa ggc ttt cag 96
 Glu Pro Ile Lys Glu Leu Glu Ser Ala Leu Val Ala Gln Gly Phe Gln
 20 25 30
 att atc tgg cca caa aac agc gtt gat ttg ctg aaa ttt atc gag cat 144
 Ile Ile Trp Pro Gln Asn Ser Val Asp Leu Leu Lys Phe Ile Glu His
 35 40 45
 aac cct cga att tgc ggc gtg att ttt gac tgg gat gag tac agt ctc 192
 Asn Pro Arg Ile Cys Gly Val Ile Phe Asp Trp Asp Glu Tyr Ser Leu
 50 55 60
 gat tta tgt agc gat atc aat cag ctt aat gaa tat ctc ccg ctt tat 240
 Asp Leu Cys Ser Asp Ile Asn Gln Leu Asn Glu Tyr Leu Pro Leu Tyr
 65 70 75 80
 gcc ttc atc aac acc cac tgc acg atg gat gtc agc gtg cag gat atg 288
 Ala Phe Ile Asn Thr His Ser Thr Met Asp Val Ser Val Gln Asp Met
 85 90 95
 cgg atg gcg ctc tgg ttt ttt gaa tat gcg ctg ggg cag gcg gaa gat 336
 Arg Met Ala Leu Trp Phe Phe Glu Tyr Ala Leu Gly Gln Ala Glu Asp
 100 105 110
 atc gcc att cgt atg cgt cag tac acc gac gaa tat ctt gat aac att 384
 Ile Ala Ile Arg Met Arg Gln Tyr Thr Asp Glu Tyr Leu Asp Asn Ile
 115 120 125
 aca ccg ccg ttc acg aaa gcc ttg ttt acc tac gtc aaa gag ccg aag 432
 Thr Pro Pro Phe Thr Lys Ala Leu Phe Thr Tyr Val Lys Glu Arg Lys
 130 135 140
 tac acc ttt tgt acg ccg ggg cat atg ggc ggc acc gca tat caa aaa 480
 Tyr Thr Phe Cys Thr Pro Gly His Met Gly Gly Thr Ala Tyr Gln Lys
 145 150 155 160
 agc ccg gtt ggc tgt ctg ttt tat gat ttt ttc ggc ggc aat act ctt 528
 Ser Pro Val Gly Cys Leu Phe Tyr Asp Phe Phe Gly Gly Asn Thr Leu
 165 170 175
 aag gct gat gtc tct att tgc gtc acc gag ctt ggt tgc ttg ctc gac 576
 Lys Ala Asp Val Ser Ile Ser Val Thr Glu Leu Gly Ser Leu Leu Asp
 180 185 190
 cac acc ggg cca cac ctg gaa gcg gaa gag tac atc gcg ccg act ttt 624
 His Thr Gly Pro His Leu Glu Ala Glu Glu Tyr Ile Ala Arg Thr Phe
 195 200 205
 ggc gcg gaa cag agt tat atc gtt acc aac gga aca tgc acg tgc aac 672
 Gly Ala Glu Gln Ser Tyr Ile Val Thr Asn Gly Thr Ser Thr Ser Asn
 210 215 220
 aaa att gtg ggt atg tac gcc gcg cca tcc ggc agt acg ctg ttg atc 720
 Lys Ile Val Gly Met Tyr Ala Ala Pro Ser Gly Ser Thr Leu Leu Ile
 225 230 235 240
 gac gcg aat tgt cat aaa tgc ctg gcg cat ctg ttg atg atg aac gat 768
 Asp Arg Asn Cys His Lys Ser Leu Ala His Leu Leu Met Met Asn Asp
 245 250 255
 gta gtg cca gtc tgg ctg aaa ccg acg cgt aat gcg ttg ggg att ctt 816
 Val Val Pro Val Trp Leu Lys Pro Thr Arg Asn Ala Leu Gly Ile Leu
 260 265 270

ggt ggg atc ccg cgc cgt gaa ttt act cgc gac agc atc gaa gag aaa Gly Gly Ile Pro Arg Arg Glu Phe Thr Arg Asp Ser Ile Glu Glu Lys 275 280 285	864
gtc gct gct acc acg caa gca caa tgg ccg gtt cat gcg gtg atc acc Val Ala Ala Thr Thr Gln Ala Gln Trp Pro Val His Ala Val Ile Thr 290 295 300	912
aac tcc acc tat gat ggc ttg ctc tac aac acc gac tgg atc aaa cag Asn Ser Thr Tyr Asp Gly Leu Leu Tyr Asn Thr Asp Trp Ile Lys Gln 305 310 315 320	960
acg ctg gat gtc ccg tcg att cac ttc gat tct gcc tgg gtg ccg tac Thr Leu Asp Val Pro Ser Ile His Phe Asp Ser Ala Trp Val Pro Tyr 325 330 335	1008
acc cat ttt cat ccg atc tac cag ggt aaa agt ggt atg agc ggc gag Thr His Phe His Pro Ile Tyr Gln Gly Lys Ser Gly Met Ser Gly Glu 340 345 350	1056
cgt gtt gcg gga aaa gtg atc ttc gaa acg caa tcg acc cac aaa atg Arg Val Ala Gly Lys Val Ile Phe Glu Thr Gln Ser Thr His Lys Met 355 360 365	1104
ctg gcg gcg tta tcg cag gct tcg ctg atc cac att aaa ggc gag tat Leu Ala Ala Leu Ser Gln Ala Ser Leu Ile His Ile Lys Gly Glu Tyr 370 375 380	1152
gac gaa gag gcc ttt aac gaa gcc ttt atg atg cat acc acc acc tcg Asp Glu Glu Ala Phe Asn Glu Ala Phe Met Met His Thr Thr Thr Ser 385 390 395 400	1200
ccc agt tat ccc att gtt gct tcg gtt gag acg gcg gcg gcg atg ctg Pro Ser Tyr Pro Ile Val Ala Ser Val Glu Thr Ala Ala Ala Met Leu 405 410 415	1248
cgt ggt aat ccg ggc aaa cgg ctg att aac cgt tca gta gaa cga gct Arg Gly Asn Pro Gly Lys Arg Leu Ile Asn Arg Ser Val Glu Arg Ala 420 425 430	1296
ctg cat ttt cgc aaa gag gtc cag cag ctg ccg gaa gag tct gac ggt Leu His Phe Arg Lys Glu Val Gln Arg Leu Arg Glu Glu Ser Asp Gly 435 440 445	1344
tgg ttt ttc gat atc tgg caa ccg ccg cag gtg gat gaa gcc gaa tgc Trp Phe Phe Asp Ile Trp Gln Pro Pro Gln Val Asp Glu Ala Glu Cys 450 455 460	1392
tgg ccc gtt gcg cct ggc gaa cag tgg cac ggc ttt aac gat gcg gat Trp Pro Val Ala Pro Gly Glu Gln Trp His Gly Phe Asn Asp Ala Asp 465 470 475 480	1440
gcc gat cat atg ttt ctc gat ccg gtt aaa gtc act att ttg aca ccg Ala Asp His Met Phe Leu Asp Pro Val Lys Val Thr Ile Leu Thr Pro 485 490 495	1488
ggg atg gac gag cag ggc aat atg agc gag gag ggg atc ccg gcg gcg Gly Met Asp Glu Gln Gly Asn Met Ser Glu Glu Gly Ile Pro Ala Ala 500 505 510	1536
ctg gta gca aaa ttc ctc gac gaa cgt ggg atc gta gta gag aaa acc Leu Val Ala Lys Phe Leu Asp Glu Arg Gly Ile Val Val Glu Lys Thr 515 520 525	1584
ggc cct tat aac ctg ctg ttt ctc ttt agt att ggc atc gat aaa acc Gly Pro Tyr Asn Leu Leu Phe Leu Phe Ser Ile Gly Ile Asp Lys Thr 530 535 540	1632
aaa gca atg gga tta ttg cgt ggg ttg acg gaa ttc aaa cgc tct tac Lys Ala Met Gly Leu Leu Arg Gly Leu Thr Glu Phe Lys Arg Ser Tyr 545 550 555 560	1680
gat ctc aac ctg ccg atc aaa aat atg cta ccc gat ctc tat gca gaa Asp Leu Asn Leu Arg Ile Lys Asn Met Leu Pro Asp Leu Tyr Ala Glu 565 570 575	1728

gat ccc gat ttc tac cgc aat atg cgt att cag gat ctg gca caa ggg 1776
Asp Pro Asp Phe Tyr Arg Asn Met Arg Ile Gln Asp Leu Ala Gln Gly
580 585 590
atc cat aag ctg att cgt aaa cac gat ctt ccc ggt ttg atg ttg cgg 1824
Ile His Lys Leu Ile Arg Lys His Asp Leu Pro Gly Leu Met Leu Arg
595 600 605
gca ttc gat act ttg ccg gag atg atc atg acg cca cat cag gca tgg 1872
Ala Phe Asp Thr Leu Pro Glu Met Ile Met Thr Pro His Gln Ala Trp
610 615 620
caa cga caa att aaa ggc gaa gta gaa acc att gcg ctg gaa caa ctg 1920
Gln Arg Gln Ile Lys Gly Glu Val Glu Thr Ile Ala Leu Glu Gln Leu
625 630 635 640
gtc ggt aga gta tgc gca aat atg atc ctg cct tat cca ccg ggc gta 1968
Val Gly Arg Val Ser Ala Asn Met Ile Leu Pro Tyr Pro Pro Gly Val
645 650 655
ccg ctg ttg atg cct gga gaa atg ctg acc aaa gag agc gcg aca gta 2016
Pro Leu Leu Met Pro Gly Glu Met Leu Thr Lys Glu Ser Arg Thr Val
660 665 670
ctc gat ttt cta ctg atg ctt tgt tcc gtc ggg caa cat tac ccc ggt 2064
Leu Asp Phe Leu Leu Met Leu Cys Ser Val Gly Gln His Tyr Pro Gly
675 680 685
ttt gaa acg gat att cac ggc gcg aaa cag gac gaa gac ggc gtt tac 2112
Phe Glu Thr Asp Ile His Gly Ala Lys Gln Asp Glu Asp Gly Val Tyr
690 695 700
cgc gta cga gtc cta aaa atg gcg gga taa 2142
Arg Val Arg Val Leu Lys Met Ala Gly
705 710

<210> 45
<211> 713
<212> PRT
<213> Escherichia coli

<400> 45

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

Thr Pro Pro Phe Thr Lys Ala Leu Phe Thr Tyr Val Lys Glu Arg Lys
130 135 140

Tyr Thr Phe Cys Thr Pro Gly His Met Gly Gly Thr Ala Tyr Gln Lys
145 150 155 160

Ser Pro Val Gly Cys Leu Phe Tyr Asp Phe Phe Gly Gly Asn Thr Leu
165 170 175

Lys Ala Asp Val Ser Ile Ser Val Thr Glu Leu Gly Ser Leu Leu Asp
180 185 190

His Thr Gly Pro His Leu Glu Ala Glu Glu Tyr Ile Ala Arg Thr Phe
195 200 205

Gly Ala Glu Gln Ser Tyr Ile Val Thr Asn Gly Thr Ser Thr Ser Asn
210 215 220

Lys Ile Val Gly Met Tyr Ala Ala Pro Ser Gly Ser Thr Leu Leu Ile
225 230 235 240

Asp Arg Asn Cys His Lys Ser Leu Ala His Leu Leu Met Met Asn Asp
245 250 255

Val Val Pro Val Trp Leu Lys Pro Thr Arg Asn Ala Leu Gly Ile Leu
260 265 270

Gly Gly Ile Pro Arg Arg Glu Phe Thr Arg Asp Ser Ile Glu Glu Lys
275 280 285

Val Ala Ala Thr Thr Gln Ala Gln Trp Pro Val His Ala Val Ile Thr
290 295 300

Asn Ser Thr Tyr Asp Gly Leu Leu Tyr Asn Thr Asp Trp Ile Lys Gln
305 310 315 320

Thr Leu Asp Val Pro Ser Ile His Phe Asp Ser Ala Trp Val Pro Tyr
325 330 335

Thr His Phe His Pro Ile Tyr Gln Gly Lys Ser Gly Met Ser Gly Glu
340 345 350

Arg Val Ala Gly Lys Val Ile Phe Glu Thr Gln Ser Thr His Lys Met
355 360 365

Leu Ala Ala Leu Ser Gln Ala Ser Leu Ile His Ile Lys Gly Glu Tyr
370 375 380

Asp Glu Glu Ala Phe Asn Glu Ala Phe Met Met His Thr Thr Thr Ser
385 390 395 400

Pro Ser Tyr Pro Ile Val Ala Ser Val Glu Thr Ala Ala Ala Met Leu
405 410 415

Arg Gly Asn Pro Gly Lys Arg Leu Ile Asn Arg Ser Val Glu Arg Ala
420 425 430

Leu His Phe Arg Lys Glu Val Gln Arg Leu Arg Glu Glu Ser Asp Gly
 435 440 445
 Trp Phe Phe Asp Ile Trp Gln Pro Pro Gln Val Asp Glu Ala Glu Cys
 450 455 460
 Trp Pro Val Ala Pro Gly Glu Gln Trp His Gly Phe Asn Asp Ala Asp
 465 470 475 480
 Ala Asp His Met Phe Leu Asp Pro Val Lys Val Thr Ile Leu Thr Pro
 485 490 495
 Gly Met Asp Glu Gln Gly Asn Met Ser Glu Glu Gly Ile Pro Ala Ala
 500 505 510
 Leu Val Ala Lys Phe Leu Asp Glu Arg Gly Ile Val Val Glu Lys Thr
 515 520 525
 Gly Pro Tyr Asn Leu Leu Phe Leu Phe Ser Ile Gly Ile Asp Lys Thr
 530 535 540
 Lys Ala Met Gly Leu Leu Arg Gly Leu Thr Glu Phe Lys Arg Ser Tyr
 545 550 555 560
 Asp Leu Asn Leu Arg Ile Lys Asn Met Leu Pro Asp Leu Tyr Ala Glu
 565 570 575
 Asp Pro Asp Phe Tyr Arg Asn Met Arg Ile Gln Asp Leu Ala Gln Gly
 580 585 590
 Ile His Lys Leu Ile Arg Lys His Asp Leu Pro Gly Leu Met Leu Arg
 595 600 605
 Ala Phe Asp Thr Leu Pro Glu Met Ile Met Thr Pro His Gln Ala Trp
 610 615 620
 Gln Arg Gln Ile Lys Gly Glu Val Glu Thr Ile Ala Leu Glu Gln Leu
 625 630 635 640
 Val Gly Arg Val Ser Ala Asn Met Ile Leu Pro Tyr Pro Pro Gly Val
 645 650 655
 Pro Leu Leu Met Pro Gly Glu Met Leu Thr Lys Glu Ser Arg Thr Val
 660 665 670
 Leu Asp Phe Leu Leu Met Leu Cys Ser Val Gly Gln His Tyr Pro Gly
 675 680 685
 Phe Glu Thr Asp Ile His Gly Ala Lys Gln Asp Glu Asp Gly Val Tyr
 690 695 700
 Arg Val Arg Val Leu Lys Met Ala Gly
 705 710

<210> 46
 <211> 603

<212> DNA
 <213> Escherichia coli

<220>
 <221> CDS
 <222> (1).. (603)
 <223> fimB

<400> 46
 atg aag aat aag gct gat aac aaa aaa agg aac ttc ctg acc cat agt 48
 Met Lys Asn Lys Ala Asp Asn Lys Lys Arg Asn Phe Leu Thr His Ser
 1 5 10 15
 gaa atc gaa tca ctc ctt aaa gca gca aat acc ggg cct cat gca gca 96
 Glu Ile Glu Ser Leu Leu Lys Ala Ala Asn Thr Gly Pro His Ala Ala
 20 25 30
 cgt aat tat tgt ctg act ttg ctt tgt ttt att cat ggt ttc cgg gcg 144
 Arg Asn Tyr Cys Leu Thr Leu Leu Cys Phe Ile His Gly Phe Arg Ala
 35 40 45
 agt gaa att tgt cga ttg agg att tcg gat att gat ctt aag gca aag 192
 Ser Glu Ile Cys Arg Leu Arg Ile Ser Asp Ile Asp Leu Lys Ala Lys
 50 55 60
 tgt ata tat atc cat cga tta aaa aaa ggc ttt tca aca acg cac ccg 240
 Cys Ile Tyr Ile His Arg Leu Lys Lys Gly Phe Ser Thr Thr His Pro
 65 70 75 80
 cta ttg aat aaa gaa gtt cag gct tta aaa aac tgg ttg agt atc cgt 288
 Leu Leu Asn Lys Glu Val Gln Ala Leu Lys Asn Trp Leu Ser Ile Arg
 85 90 95
 act tcg tac ccg cat gct gag agc gag tgg gta ttt tta tca cgt aag 336
 Thr Ser Tyr Pro His Ala Glu Ser Glu Trp Val Phe Leu Ser Arg Lys
 100 105 110
 ggg aat ccg ctt tct cgg caa cag ttt tac cat att atc tcg act tcc 384
 Gly Asn Pro Leu Ser Arg Gln Phe Tyr His Ile Ile Ser Thr Ser
 115 120 125
 ggt ggt aat gcc ggg ttg tca ctg gag att cat ccg cac atg tta cgc 432
 Gly Gly Asn Ala Gly Leu Ser Leu Glu Ile His Pro His Met Leu Arg
 130 135 140
 cat tcg tgt ggt ttt gct ttg gcg aat atg gga ata gat acg cga ctt 480
 His Ser Cys Gly Phe Ala Leu Ala Asn Met Gly Ile Asp Thr Arg Leu
 145 150 155 160
 atc cag gat tat ctt ggg cat cgc aat att cgt cat act gtc tgg tat 528
 Ile Gln Asp Tyr Leu Gly His Arg Asn Ile Arg His Thr Val Trp Tyr
 165 170 175
 acc gcc agc aat gca ggg cgt ttt tac ggc atc tgg gat aga gcc aga 576
 Thr Ala Ser Asn Ala Gly Arg Phe Tyr Gly Ile Trp Asp Arg Ala Arg
 180 185 190
 gga cga cag cgt cac gct gtt tta tag 603
 Gly Arg Gln Arg His Ala Val Leu
 195 200

<210> 47
 <211> 200
 <212> PRT
 <213> Escherichia coli

<400> 47
 Met Lys Asn Lys Ala Asp Asn Lys Lys Arg Asn Phe Leu Thr His Ser
 1 5 10 15
 Glu Ile Glu Ser Leu Leu Lys Ala Ala Asn Thr Gly Pro His Ala Ala
 20 25 30

Arg Asn Tyr Cys Leu Thr Leu Leu Cys Phe Ile His Gly Phe Arg Ala
35 40 45

Ser Glu Ile Cys Arg Leu Arg Ile Ser Asp Ile Asp Leu Lys Ala Lys
50 55 60

Cys Ile Tyr Ile His Arg Leu Lys Lys Gly Phe Ser Thr Thr His Pro
65 70 75 80

Leu Leu Asn Lys Glu Val Gln Ala Leu Lys Asn Trp Leu Ser Ile Arg
85 90 95

Thr Ser Tyr Pro His Ala Glu Ser Glu Trp Val Phe Leu Ser Arg Lys
100 105 110

Gly Asn Pro Leu Ser Arg Gln Gln Phe Tyr His Ile Ile Ser Thr Ser
115 120 125

Gly Gly Asn Ala Gly Leu Ser Leu Glu Ile His Pro His Met Leu Arg
130 135 140

His Ser Cys Gly Phe Ala Leu Ala Asn Met Gly Ile Asp Thr Arg Leu
145 150 155 160

Ile Gln Asp Tyr Leu Gly His Arg Asn Ile Arg His Thr Val Trp Tyr
165 170 175

Thr Ala Ser Asn Ala Gly Arg Phe Tyr Gly Ile Trp Asp Arg Ala Arg
180 185 190

Gly Arg Gln Arg His Ala Val Leu
195 200

<210> 48
<211> 597
<212> DNA
<213> Escherichia coli

<220>
<221> CDS
<222> (1).. (597)
<223> fimE

<400> 48
gtg agt aaa cgt cgt tat ctt acc ggt aaa gaa gtt cag gcc atg atg 48
Val Ser Lys Arg Arg Tyr Leu Thr Gly Lys Glu Val Gln Ala Met Met
1 5 10 15
cag gcg gtt tgt tac ggg gca acg gga gcc aga gat tat tgt ctt att 96
Gln Ala Val Cys Tyr Gly Ala Thr Gly Ala Arg Asp Tyr Cys Leu Ile
20 25 30
ctg ttg gca tat cgg cat ggg atg cgt att agt gaa ctg ctt gat ctg 144
Leu Leu Ala Tyr Arg His Gly Met Arg Ile Ser Glu Leu Leu Asp Leu
35 40 45
cat tat cag gac ctt gac ctt aat gaa ggt aga ata aat att cgc cga 192
His Tyr Gln Asp Leu Asp Leu Asn Glu Gly Arg Ile Asn Ile Arg Arg
50 55 60
ctg aag aac gga ttt tct acc gtt cac ccg tta cgt ttt gat gag cgt 240
Leu Lys Asn Gly Phe Ser Thr Val His Pro Leu Arg Phe Asp Glu Arg
65 70 75 80

gaa gcc gtg gaa cgc tgg acc cag gaa cgt gct aac tgg aaa ggc gct 288
 Glu Ala Val Glu Arg Trp Thr Gln Glu Arg Ala Asn Trp Lys Gly Ala
 85 90 95
 gac cgg act gac gct ata ttt att tct cgc cgc ggg agt cgg ctt tct 336
 Asp Arg Thr Asp Ala Ile Phe Ile Ser Arg Arg Gly Ser Arg Leu Ser
 100 105 110
 cgc cag cag gcc tat cgc att att cgc gat gcc ggt att gaa gct gga 384
 Arg Gln Gln Ala Tyr Arg Ile Ile Arg Asp Ala Gly Ile Glu Ala Gly
 115 120 125
 acc gta acg cag act cat cct cat atg tta agg cat gct tgc ggt tat 432
 Thr Val Thr Gln Thr His Pro His Met Leu Arg His Ala Cys Gly Tyr
 130 135 140
 gaa ttg gcg gag cgt ggt gca gat act cgt tta att cag gat tat ctc 480
 Glu Leu Ala Glu Arg Gly Ala Asp Thr Arg Leu Ile Gln Asp Tyr Leu
 145 150 155 160
 ggg cat cga aat att cgc cat act gtg cgt tat acc gcc agt aat gct 528
 Gly His Arg Asn Ile Arg His Thr Val Arg Tyr Thr Ala Ser Asn Ala
 165 170 175
 gct cgt ttt gcc gga tta tgg gaa aga aat aat ctc ata aac gaa aaa 576
 Ala Arg Phe Ala Gly Leu Trp Glu Arg Asn Asn Leu Ile Asn Glu Lys
 180 185 190
 tta aaa aga gaa gag gtt tga 597
 Leu Lys Arg Glu Glu Val
 195

<210> 49
 <211> 198
 <212> PRT
 <213> Escherichia coli

<400> 49

Val Ser Lys Arg Arg Tyr Leu Thr Gly Lys Glu Val Gln Ala Met Met
 1 5 10 15
 Gln Ala Val Cys Tyr Gly Ala Thr Gly Ala Arg Asp Tyr Cys Leu Ile
 20 25 30
 Leu Leu Ala Tyr Arg His Gly Met Arg Ile Ser Glu Leu Leu Asp Leu
 35 40 45
 His Tyr Gln Asp Leu Asp Leu Asn Glu Gly Arg Ile Asn Ile Arg Arg
 50 55 60
 Leu Lys Asn Gly Phe Ser Thr Val His Pro Leu Arg Phe Asp Glu Arg
 65 70 75 80
 Glu Ala Val Glu Arg Trp Thr Gln Glu Arg Ala Asn Trp Lys Gly Ala
 85 90 95
 Asp Arg Thr Asp Ala Ile Phe Ile Ser Arg Arg Gly Ser Arg Leu Ser
 100 105 110
 Arg Gln Gln Ala Tyr Arg Ile Ile Arg Asp Ala Gly Ile Glu Ala Gly
 115 120 125
 Thr Val Thr Gln Thr His Pro His Met Leu Arg His Ala Cys Gly Tyr
 130 135 140

Glu Leu Ala Glu Arg Gly Ala Asp Thr Arg Leu Ile Gln Asp Tyr Leu
145 150 155 160

Gly His Arg Asn Ile Arg His Thr Val Arg Tyr Thr Ala Ser Asn Ala
165 170 175

Ala Arg Phe Ala Gly Leu Trp Glu Arg Asn Asn Leu Ile Asn Glu Lys
180 185 190

Leu Lys Arg Glu Glu Val
195

<210> 50
<211> 549
<212> DNA
<213> Escherichia coli

<220>
<221> CDS
<222> (1).. (549)
<223> fimA

<400> 50
atg aaa att aaa act ctg gca atc gtt gtt ctg tcg gct ctg tcc ctc 48
Met Lys Ile Lys Thr Leu Ala Ile Val Val Leu Ser Ala Leu Ser Leu
1 5 10 15
agt tct aca gcg gct ctg gcc gct gcc acg acg gtt aat ggt ggg acc 96
Ser Ser Thr Ala Ala Leu Ala Ala Thr Thr Val Asn Gly Gly Thr
20 25 30
gtt cac ttt aaa ggg gaa gtt gtt aac gcc gct tgc gca gtt gat gca 144
Val His Phe Lys Gly Glu Val Val Asn Ala Ala Cys Ala Val Asp Ala
35 40 45
ggc tct gtt gat caa acc gtt cag tta gga cag gtt cgt acc gca tcg 192
Gly Ser Val Asp Gln Thr Val Gln Leu Gly Gln Val Arg Thr Ala Ser
50 55 60
ctg gca cag gaa gga gca acc agt tct gct gtc ggt ttt aac att cag 240
Leu Ala Gln Glu Gly Ala Thr Ser Ser Ala Val Gly Phe Asn Ile Gln
65 70 75 80
ctg aat gat tgc gat acc aat gtt gca tct aaa gcc gct gtt gcc ttt 288
Leu Asn Asp Cys Asp Thr Asn Val Ala Ser Lys Ala Ala Val Ala Phe
85 90 95
tta ggt acg gcg att gat gcg ggt cat acc aac gtt ctg gct ctg cag 336
Leu Gly Thr Ala Ile Asp Ala Gly His Thr Asn Val Leu Ala Leu Gln
100 105 110
agt tca gct gcg ggt agc gca aca aac gtt ggt gtg cag atc ctg gac 384
Ser Ser Ala Ala Gly Ser Ala Thr Asn Val Gly Val Gln Ile Leu Asp
115 120 125
aga acg ggt gct gcg ctg acg ctg gat ggt gcg aca ttt agt tca gaa 432
Arg Thr Gly Ala Ala Leu Thr Leu Asp Gly Ala Thr Phe Ser Ser Glu
130 135 140
aca acc ctg aat aac gga acc aat acc att ccg ttc cag gcg cgt tat 480
Thr Thr Leu Asn Asn Gly Thr Asn Thr Ile Pro Phe Gln Ala Arg Tyr
145 150 155 160
ttt gca acc ggg gcc gca acc ccg ggt gct gct aat gcg gat gcg acc 528
Phe Ala Thr Gly Ala Ala Thr Pro Gly Ala Asn Ala Asp Ala Thr
165 170 175
ttc aag gtt cag tat caa taa 549
Phe Lys Val Gln Tyr Gln
180

<210> 51
 <211> 182
 <212> PRT
 <213> Escherichia coli

<400> 51

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Met Lys Ile Lys Thr Leu Ala Ile Val Val Leu Ser Ala Leu Ser Leu
 1              5              10              15

Ser Ser Thr Ala Ala Leu Ala Ala Ala Thr Thr Val Asn Gly Gly Thr
      20              25              30

Val His Phe Lys Lys Gly Glu Val Val Asn Ala Ala Cys Ala Val Asp Ala
      35              40              45

Gly Ser Val Asp Gln Thr Val Gln Leu Gly Gln Val Arg Thr Ala Ser
 50              55              60

Leu Ala Gln Glu Gly Ala Thr Ser Ser Ala Val Gly Phe Asn Ile Gln
65              70              75              80

Leu Asn Asp Cys Asp Thr Asn Val Ala Ser Lys Ala Ala Val Ala Phe
      85              90              95

Leu Gly Thr Ala Ile Asp Ala Gly His Thr Asn Val Leu Ala Leu Gln
100              105              110

Ser Ser Ala Ala Gly Ser Ala Thr Asn Val Gly Val Gln Ile Leu Asp
115              120              125

Arg Thr Gly Ala Ala Leu Thr Leu Asp Gly Ala Thr Phe Ser Ser Glu
130              135              140

Thr Thr Leu Asn Asn Gly Thr Asn Thr Ile Pro Phe Gln Ala Arg Tyr
145              150              155              160

Phe Ala Thr Gly Ala Ala Thr Pro Gly Ala Ala Asn Ala Asp Ala Thr
165              170              175

Phe Lys Val Gln Tyr Gln
180
  
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<210> 52
 <211> 648
 <212> DNA
 <213> Escherichia coli

<220>
 <221> CDS
 <222> (1)..(648)
 <223> fimI

<400> 52

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gtg ctg cta atg cgg atg cga cct tca agg ttc agt atc aat aac cta      48
Val Leu Leu Met Arg Met Arg Pro Ser Arg Phe Ser Ile Asn Asn Leu
 1              5              10              15

ccc agg ttc agg gac gtc att acg ggc agg gat gcc cac cct tgt gcg      96
Pro Arg Phe Arg Asp Val Ile Thr Gly Arg Asp Ala His Pro Cys Ala
      20              25              30

ata aaa ata acg atg aaa agg aag aga tta ttt cta tta gcg tgg ttg      144
Ile Lys Ile Thr Met Lys Arg Lys Arg Leu Phe Leu Leu Ala Ser Leu
  
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35	40	45	
ctg cca atg ttt gct ctg gcc gga aat aaa tgg aat acc acg ttg ccc Leu Pro Met Phe Ala Leu Ala Gly Asn Lys Trp Asn Thr Thr Leu Pro 50 55 60			192
ggc gga aat atg caa ttt cag ggc gtc att att gcg gaa act tgc cgg Gly Gly Asn Met Gln Phe Gln Gly Val Ile Ile Ala Glu Thr Cys Arg 65 70 75 80			240
att gaa gcc ggt gat aaa caa atg acg gtc aat atg ggg caa atc agc Ile Glu Ala Gly Asp Lys Gln Met Thr Val Asn Met Gly Gln Ile Ser 85 90 95			288
agt aac cgg ttt cat gcg gtt ggg gaa gat agc gca ccg gtg cct ttt Ser Asn Arg Phe His Ala Val Gly Glu Asp Ser Ala Pro Val Pro Phe 100 105 110			336
gtt att cat tta cgg gaa tgt agc acg gtg gtg agt gaa cgt gta ggt Val Ile His Leu Arg Glu Cys Ser Thr Val Val Ser Glu Arg Val Gly 115 120 125			384
gtg gcg ttt cac ggt gtc gcg gat ggt aaa aat ccg gat gtg ctt tcc Val Ala Phe His Gly Val Ala Asp Gly Lys Asn Pro Asp Val Leu Ser 130 135 140			432
gtg gga gag ggg cca ggg ata gcc acc aat att ggc gta gcg ttg ttt Val Gly Glu Gly Pro Gly Ile Ala Thr Asn Ile Gly Val Ala Leu Phe 145 150 155 160			480
gat gat gaa gga aac ctc gta ccg att aat cgt cct cca gca aac tgg Asp Asp Glu Gly Asn Leu Val Pro Ile Asn Arg Pro Pro Ala Asn Trp 165 170 175			528
aaa cgg ctt tat tca ggc tct act tcg cta cat ttc atc gcc aaa tat Lys Arg Leu Tyr Ser Gly Ser Thr Ser Leu His Phe Ile Ala Lys Tyr 180 185 190			576
cgt gct acc zgg cgt cgg gtt act ggc ggc atc gcc aat gcc cag gcc Arg Ala Thr Gly Arg Arg Val Thr Gly Gly Ile Ala Asn Ala Gln Ala 195 200 205			624
tgg ttc tct tta acc tat cag taa Trp Phe Ser Leu Thr Tyr Gln 210 215			648

<210> 53
 <211> 215
 <212> PRT
 <213> Escherichia coli

<400> 53

Val Leu Leu Met Arg Met Arg Pro Ser Arg Phe Ser Ile Asn Asn Leu
1 5 10 15

Pro Arg Phe Arg Asp Val Ile Thr Gly Arg Asp Ala His Pro Cys Ala
20 25 30

Ile Lys Ile Thr Met Lys Arg Lys Arg Leu Phe Leu Leu Ala Ser Leu
35 40 45

Leu Pro Met Phe Ala Leu Ala Gly Asn Lys Trp Asn Thr Thr Leu Pro
50 55 60

Gly Gly Asn Met Gln Phe Gln Gly Val Ile Ile Ala Glu Thr Cys Arg
65 70 75 80

Ile Glu Ala Gly Asp Lys Gln Met Thr Val Asn Met Gly Gln Ile Ser
85 90 95

Ser Asn Arg Phe His Ala Val Gly Glu Asp Ser Ala Pro Val Pro Phe
100 105 110

Val Ile His Leu Arg Glu Cys Ser Thr Val Val Ser Glu Arg Val Gly
115 120 125

Val Ala Phe His Gly Val Ala Asp Gly Lys Asn Pro Asp Val Leu Ser
130 135 140

Val Gly Glu Gly Pro Gly Ile Ala Thr Asn Ile Gly Val Ala Leu Phe
145 150 155 160

Asp Asp Glu Gly Asn Leu Val Pro Ile Asn Arg Pro Pro Ala Asn Trp
165 170 175

Lys Arg Leu Tyr Ser Gly Ser Thr Ser Leu His Phe Ile Ala Lys Tyr
180 185 190

Arg Ala Thr Gly Arg Arg Val Thr Gly Gly Ile Ala Asn Ala Gln Ala
195 200 205

Trp Phe Ser Leu Thr Tyr Gln
210 215

<210> 54
<211> 726
<212> DNA
<213> Escherichia coli

<220>
<221> CDS
<222> (1) (726)
<223> fimC

<400> 21
gtg agt aat aaa aac gtc aat gta agg aaa tcg cag gaa ata aca ttc 48
Val Ser Asn Lys Asn Val Asn Val Arg Lys Ser Gln Glu Ile Thr Phe
1 5 10 15
tgc ttg ctg gca ggt atc ctg atg ttc atg gca atg atg gtt gcc gga 96
Cys Leu Leu Ala Gly Ile Leu Met Phe Met Ala Met Met Val Ala Gly
20 25 30
cgc gct gaa gcg gga gtg gcc tta ggt gcg act cgc gta att tat ccg 144
Arg Ala Glu Ala Gly Val Ala Leu Gly Ala Thr Arg Val Ile Tyr Pro
35 40 45
gca ggg caa aaa caa gag caa ctt gcc gtg aca aat aat gat gaa aat 192
Ala Gly Gln Lys Gln Glu Gln Leu Ala Val Thr Asn Asn Asp Glu Asn
50 55 60
agt acc tat tta att caa tca tgg gtg gaa aat gcc gat ggt gta aag 240
Ser Thr Tyr Leu Ile Gln Ser Trp Val Glu Asn Ala Asp Gly Val Lys
65 70 75 80
gat ggt cgt ttt atc gtg acg cct cct ctg ttt gcg atg aag gga aaa 288
Asp Gly Arg Phe Ile Val Thr Pro Pro Leu Phe Ala Met Lys Gly Lys
85 90 95
aaa gag aat acc tta cgt att ctt gat gca aca aat aac caa ttg cca 336
Lys Glu Asn Thr Leu Arg Ile Leu Asp Ala Thr Asn Asn Gln Leu Pro
100 105 110
cag gac cgg gaa agt tta ttc tgg atg aac gtt aaa gcg att ccg tca 384
Gln Asp Arg Glu Ser Leu Phe Trp Met Asn Val Lys Ala Ile Pro Ser
115 120 125

atg gat aaa tca aaa ttg act gag aat acg cta cag ctc gca att atc 432
 Met Asp Lys Ser Lys Leu Thr Glu Asn Thr Leu Gln Leu Ala Ile Ile
 130 135 140
 agc cgc att aaa ctg tac tat cgc ccg gct aaa tta gcg ttg cca ccc 480
 Ser Arg Ile Lys Leu Tyr Tyr Arg Pro Ala Lys Leu Ala Leu Pro Pro
 145 150 155 160
 gat cag gcc gca gaa aaa tta aga ttt cgt cgt agc gcg aat tct ctg 528
 Asp Gln Ala Ala Glu Lys Leu Arg Phe Arg Arg Ser Ala Asn Ser Leu
 165 170 175
 acg ctg att aac ccg aca ccc tat tac ctg acg gta aca gag ttg aat 576
 Thr Leu Ile Asn Pro Thr Pro Tyr Tyr Leu Thr Val Thr Glu Leu Asn
 180 185 190
 gcc gga acc cgg gtt ctt gaa aat gca ttg gtg cct cca atg ggc gaa 624
 Ala Gly Thr Arg Val Leu Glu Asn Ala Leu Val Pro Pro Met Gly Glu
 195 200 205
 agc acg gtt aaa ttg cct tct gat gca gga agc aat att act tac cga 672
 Ser Thr Val Lys Leu Pro Ser Asp Ala Gly Ser Asn Ile Thr Tyr Arg
 210 215 220
 aca ata aat gat tat ggc gca ctt acc ccc aaa atg acg ggc gta atg 720
 Thr Ile Asn Asp Tyr Gly Ala Leu Thr Pro Lys Met Thr Gly Val Met
 225 230 235 240
 gaattaa 726
 Glu

<210> 55
 <211> 241
 <212> PRT
 <213> Escherichia coli

<400> 55

Val Ser Asn Lys Asn Val Asn Val Arg Lys Ser Gln Glu Ile Thr Phe
1 5 10 15

Cys Leu Leu Ala Gly Ile Leu Met Phe Met Ala Met Met Val Ala Gly
20 25 30

Arg Ala Glu Ala Gly Val Ala Leu Gly Ala Thr Arg Val Ile Tyr Pro
35 40 45

Ala Gly Gln Lys Gln Glu Gln Leu Ala Val Thr Asn Asn Asp Glu Asn
50 55 60

Ser Thr Tyr Leu Ile Gln Ser Trp Val Glu Asn Ala Asp Gly Val Lys
65 70 75 80

Asp Gly Arg Phe Ile Val Thr Pro Pro Leu Phe Ala Met Lys Gly Lys
85 90 95

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

Gln Asp Arg Glu Ser Leu Phe Trp Met Asn Val Lys Ala Ile Pro Ser
115 120 125

Met Asp Lys Ser Lys Leu Thr Glu Asn Thr Leu Gln Leu Ala Ile Ile
130 135 140

Ser Arg Ile Lys Leu Tyr Tyr Arg Pro Ala Lys Leu Ala Leu Pro Pro
145 150 155 160

Asp Gln Ala Ala Glu Lys Leu Arg Phe Arg Arg Ser Ala Asn Ser Leu
165 170 175

Thr Leu Ile Asn Pro Thr Pro Tyr Tyr Leu Thr Val Thr Glu Leu Asn
180 185 190

Ala Gly Thr Arg Val Leu Glu Asn Ala Leu Val Pro Pro Met Gly Glu
195 200 205

Ser Thr Val Lys Leu Pro Ser Asp Ala Gly Ser Asn Ile Thr Tyr Arg
210 215 220

Thr Ile Asn Asp Tyr Gly Ala Leu Thr Pro Lys Met Thr Gly Val Met
225 230 235 240

Glu

<210> 56
<211> 2637
<212> DNA
<213> Escherichia coli

<220>
<221> CDS
<222> (1)... (2637)
<223> fimD

<400> 56
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Met Ser Tyr Leu Asn Leu Arg Leu Tyr Gln Arg Asn Thr Gln Cys Leu
1 5 10 15
cat att cgt aag cat cgt ttg gct ggt ttt ttt gtc cga ctc gtt gtc 96
His Ile Arg Lys His Arg Leu Ala Gly Phe Phe Val Arg Leu Val Val
20 25 30
gcc tgt gct ttt gcc gca cag gca cct ttg tca tct gcc gac ctc tat 144
Ala Cys Ala Phe Ala Ala Gln Ala Pro Leu Ser Ser Ala Asp Leu Tyr
35 40 45
ttt aat ccg cgc ttt tta gcg gat gat ccc cag gct gtg gcc gat tta 192
Phe Asn Pro Arg Phe Leu Ala Asp Asp Pro Gln Ala Val Ala Asp Leu
50 55 60
tcg cgt ttt gaa aat ggg caa gaa tta ccg cca ggg acg tat cgc gtc 240
Ser Arg Phe Glu Asn Gly Gln Glu Leu Pro Pro Gly Thr Tyr Arg Val
65 70 75 80
gat atc tat ttg aat aat ggt tat atg gca acg cgt gat gtc aca ttt 288
Asp Ile Tyr Leu Asn Asn Gly Tyr Met Ala Thr Arg Asp Val Thr Phe
85 90 95
aat acg ggc gac agt gaa caa ggg att gtt ccc tgc ctg aca cgc gcg 336
Asn Thr Gly Asp Ser Glu Gln Gly Ile Val Pro Cys Leu Thr Arg Ala
100 105 110
caa ctc gcc agt atg ggg ctg aat acg gct tct gtc gcc ggt atg aat 384
Gln Leu Ala Ser Met Gly Leu Asn Thr Ala Ser Val Ala Gly Met Asn
115 120 125
ctg ctg gcg gat gat gcc tgt gtg cca tta acc aca atg gtc cag gac 432
Leu Leu Ala Asp Asp Ala Cys Val Pro Leu Thr Thr Met Val Gln Asp
130 135 140

gct act gcg cat ctg gat gtt ggt cag cag cga ctg aac ctg acg atc Ala Thr Ala His Leu Asp Val Gly Gln Gln Arg Leu Asn Leu Thr Ile 145 150 155 160	480
cct cag gca ttt atg agt aat cgc gcg cgt ggt tat att cct cct gag Pro Gln Ala Phe Met Ser Asn Arg Ala Arg Gly Tyr Ile Pro Pro Glu 165 170 175	528
tta tgg gat ccc ggt att aat gcc gga ttg ctc aat tat aat ttc agc Leu Trp Asp Pro Gly Ile Asn Ala Gly Leu Leu Asn Tyr Asn Phe Ser 180 185 190	576
gga aat agt gta cag aat cgg att ggg ggt aac agc cat tat gca tat Gly Asn Ser Val Gln Asn Arg Ile Gly Gly Asn Ser His Tyr Ala Tyr 195 200 205	624
tta aac cta cag agt ggg tta aat att ggt gcg tgg cgt tta cgc gac Leu Asn Leu Gln Ser Gly Leu Asn Ile Gly Ala Trp Arg Leu Arg Asp 210 215 220	672
aat acc acc tgg agt tat aac agt agc gac aga tca tca ggt agc aaa Asn Thr Thr Trp Ser Tyr Asn Ser Ser Asp Arg Ser Ser Gly Ser Lys 225 230 235 240	720
aat aaa tgg cag cat atc aat acc tgg ctt gag cga gac ata ata ccg Asn Lys Trp Gln His Ile Asn Thr Trp Leu Glu Arg Asp Ile Ile Pro 245 250 255	768
tta cgt tcc cgg ctg acg ctg ggt gat ggt tat act cag gcc gat att Leu Arg Ser Arg Leu Thr Leu Gly Asp Gly Tyr Thr Gln Gly Asp Ile 260 265 270	816
ttc gat ggt att aac ttt cgc gcc gca caa ttg gcc tca gat gac aat Phe Asp Gly Ile Asn Phe Arg Gly Ala Gln Leu Ala Ser Asp Asp Asn 275 280 285	864
atg tta ccc gat agt caa aga gga ttt gcc ccg gtg atc cac ggt att Met Leu Pro Asp Ser Gln Arg Gly Phe Ala Pro Val Ile His Gly Ile 290 295 300	912
gct cgt ggt act gca cag gtc act att aaa caa aat ggg tat gac att Ala Arg Gly Thr Ala Gln Val Thr Ile Lys Gln Asn Gly Tyr Asp Ile 305 310 315 320	960
tat aat agt acg gtg cca ccg ggg cct ttt acc atc aac gat atc tat Tyr Asn Ser Thr Val Pro Pro Gly Pro Phe Thr Ile Asn Asp Ile Tyr 325 330 335	1008
gcc gca ggt aat agt ggt gac ttg cag gta acg atc aaa gag gct gac Ala Ala Gly Asn Ser Gly Asp Leu Gln Val Thr Ile Lys Glu Ala Asp 340 345 350	1056
ggc agc acg cag att ttt acc gta ccc tat tgg tca gtc ccg ctt ttg Gly Ser Thr Gln Ile Phe Thr Val Pro Tyr Ser Ser Val Pro Leu Leu 355 360 365	1104
caa cgt gaa ggg cat act cgt tat tcc att acg gca gga gaa tac cgt Gln Arg Glu Gly His Thr Arg Tyr Ser Ile Thr Ala Gly Glu Tyr Arg 370 375 380	1152
agt gga aat gcg cag cag gaa aaa acc cgc ttt ttc cag agt aca tta Ser Gly Asn Ala Gln Gln Glu Lys Thr Arg Phe Phe Gln Ser Thr Leu 385 390 395 400	1200
ctc cac gcc ctt ccg gct gcc tgg aca ata tat ggt gga acg caa ctg Leu His Gly Leu Pro Ala Gly Trp Thr Ile Tyr Gly Gly Thr Gln Leu 405 410 415	1248
gcg gat cgt tat cgt gct ttt aat ttc ggt atc ggg aaa aac atg ggg Ala Asp Arg Tyr Arg Ala Phe Asn Phe Gly Ile Gly Lys Asn Met Gly 420 425 430	1296
gca ctg gcc gct ctg tct gtg gat atg acg cag gct aat tcc aca ctt Ala Leu Gly Ala Leu Ser Val Asp Met Thr Gln Ala Asn Ser Thr Leu 435 440 445	1344

ccc gat gac agt cag cat gac gga caa tcg gtg cgt ttt ctc tat aac Pro Asp Asp Ser Gln His Asp Gly Gln Ser Val Arg Phe Leu Tyr Asn 450 455 460	1392
aaa tcg ctc aat gaa tca ggc acg aat att cag tta gtg ggt tac cgt Lys Ser Leu Asn Glu Ser Gly Thr Asn Ile Gln Leu Val Gly Tyr Arg 465 470 475 480	1440
tat tcg acc agc gga tat ttt aat ttc gct gat aca aca tac agt cga Tyr Ser Thr Ser Gly Tyr Phe Asn Phe Ala Asp Thr Thr Tyr Ser Arg 485 490 495	1488
atg aat ggc tac aac atc gaa aca cag gac gga gtt att cag gtt aag Met Asn Gly Tyr Asn Ile Glu Thr Gln Asp Gly Val Ile Gln Val Lys 500 505 510	1536
ccg aaa ttc acc gac tat tac aac ctc gct tat aac aaa cgc ggc aaa Pro Lys Phe Thr Asp Tyr Tyr Asn Leu Ala Tyr Asn Lys Arg Gly Lys 515 520 525	1584
tta caa ctc acc gtt act cag caa ctc ggc cgc aca tca aca ctg tat Leu Gln Leu Thr Val Thr Gln Gln Leu Gly Arg Thr Ser Thr Leu Tyr 530 535 540	1632
ttg agt ggt agc cat caa act tat tgg gga acg agt aat gtc gat gag Leu Ser Gly Ser His Gln Thr Tyr Trp Gly Thr Ser Asn Val Asp Glu 545 550 555 560	1680
caa ttc cag gct gga tta aat act gcg ttc gaa gat atc aac tgg acg Gln Phe Gln Ala Gly Leu Asn Thr Ala Phe Glu Asp Ile Asn Trp Thr 565 570 575	1728
ctc agc tat agc ctg acg aaa aac gcc tgg caa aaa gga cgg gat cag Leu Ser Tyr Ser Leu Thr Lys Asn Ala Trp Gln Lys Gly Arg Asp Gln 580 585 590	1776
atg tta gcg ctt aac gtc aat att cct ttc agc cac tgg ctg cgt tct Met Leu Ala Leu Asn Val Asn Ile Pro Phe Ser His Trp Leu Arg Ser 595 600 605	1824
gac agt aaa tct cag tgg cga cat gcc agt gcc agc tac agc atg tca Asp Ser Lys Ser Gln Trp Arg His Ala Ser Ala Ser Tyr Ser Met Ser 610 615 620	1872
cac gat ctc aac ggt cgg atg acc aat ctg gct ggt gta tac ggt acg His Asp Leu Asn Gly Arg Met Thr Asn Leu Ala Gly Val Tyr Gly Thr 625 630 635 640	1920
ttg ctg gaa gac aac aac ctc agc tat agc gtg caa acc ggc tat gcc Leu Leu Glu Asp Asn Asn Leu Ser Tyr Ser Val Gln Thr Gly Tyr Ala 645 650 655	1968
ggg gga ggc gat gga aat agc gga agt aca ggc tac gcc acg ctg aat Gly Gly Gly Asp Gly Asn Ser Gly Ser Thr Gly Tyr Ala Thr Leu Asn 660 665 670	2016
tat cgc ggt ggt tac ggc aat gcc aat atc ggt tac agc cat agc gat Tyr Arg Gly Gly Tyr Gly Asn Ala Asn Ile Gly Tyr Ser His Ser Asp 675 680 685	2064
gat att aag cag ctc tat tac gga gtc agc ggt ggc gta ctg gct cat Asp Ile Lys Gln Leu Tyr Tyr Gly Val Ser Gly Gly Val Leu Ala His 690 695 700	2112
gcc aat ggc gta acg ctg ggg cag ccg tta aac gat acg gtg gtg ctt Ala Asn Gly Val Thr Leu Gly Gln Pro Leu Asn Asp Thr Val Val Leu 705 710 715 720	2160
gtt aaa gcg cct ggc gca aaa gat gca aaa gtc gaa aac cag acg ggg Val Lys Ala Pro Gly Ala Lys Asp Ala Lys Val Glu Asn Gln Thr Gly 725 730 735	2208
gtg cgt acc gac tgg cgt ggt tat gcc gtg ctg cct tat gcc act gaa Val Arg Thr Asp Trp Arg Gly Tyr Ala Val Leu Pro Tyr Ala Thr Glu 740 745 750	2256

tat cgg gaa aat aga gtg gcg ctg gat acc aat acc ctg gct gat aac 2304
 Tyr Arg Glu Asn Arg Val Ala Leu Asp Thr Asn Thr Leu Ala Asp Asn
 755 760 765
 gtc gat tta gat aac gcg gtt gct aac gtt gtt ccc act cgt ggg gcg 2352
 Val Asp Leu Asp Asn Ala Val Ala Asn Val Val Pro Thr Arg Gly Ala
 770 775 780
 atc gtg cga gca gag ttt aaa gcg cgc gtt ggg ata aaa ctg ctc atg 2400
 Ile Val Arg Ala Glu Phe Lys Ala Arg Val Gly Ile Lys Leu Leu Met
 785 790 795 800
 acg ctg acc cac aat aat aag ccg ctg ccg ttt ggg gcg atg gtg aca 2448
 Thr Leu Thr His Asn Asn Lys Pro Leu Pro Phe Gly Ala Met Val Thr
 805 810 815
 tca gag agt agc cag agt agc ggc att gtt gcg gat aat ggt cag gtt 2496
 Ser Glu Ser Ser Gln Ser Ser Gly Ile Val Ala Asp Asn Gly Gln Val
 820 825 830
 tac ctc agc gga atg cct tta gcg gga aaa gtt cag gtg aaa tgg gga 2544
 Tyr Leu Ser Gly Met Pro Leu Ala Gly Lys Val Gln Val Lys Trp Gly
 835 840 845
 gaa gag gaa aat gct cac tgt gtc gcc aat tat caa ctg cca cca gag 2592
 Glu Glu Glu Asn Ala His Cys Val Ala Asn Tyr Gln Leu Pro Pro Glu
 850 855 860
 agt cag cag cag tta tta acc cag cta tca gct gaa tgt cgt taa 2637
 Ser Gln Gln Gln Leu Leu Thr Gln Leu Ser Ala Glu Cys Arg
 865 870 875

<210> 57
 <211> 878
 <212> PRT
 <213> Escherichia coli

<400> 57

Met Ser Tyr Leu Asn Leu Arg Leu Tyr Gln Arg Asn Thr Gln Cys Leu
1 5 10 15

His Ile Arg Lys His Arg Leu Ala Gly Phe Phe Val Arg Leu Val Val
20 25 30

Ala Cys Ala Phe Ala Ala Gln Ala Pro Leu Ser Ser Ala Asp Leu Tyr
35 40 45

Phe Asn Pro Arg Phe Leu Ala Asp Asp Pro Gln Ala Val Ala Asp Leu
50 55 60

Ser Arg Phe Glu Asn Gly Gln Glu Leu Pro Pro Gly Thr Tyr Arg Val
65 70 75 80

Asp Ile Tyr Leu Asn Asn Gly Tyr Met Ala Thr Arg Asp Val Thr Phe
85 90 95

Asn Thr Gly Asp Ser Glu Gln Gly Ile Val Pro Cys Leu Thr Arg Ala
100 105 110

Gln Leu Ala Ser Met Gly Leu Asn Thr Ala Ser Val Ala Gly Met Asn
115 120 125

Leu Leu Ala Asp Asp Ala Cys Val Pro Leu Thr Thr Met Val Gln Asp
130 135 140

Ala Thr Ala His Leu Asp Val Gly Gln Gln Arg Leu Asn Leu Thr Ile
 145 150 155 160
 Pro Gln Ala Phe Met Ser Asn Arg Ala Arg Gly Tyr Ile Pro Pro Glu
 165 170 175
 Leu Trp Asp Pro Gly Ile Asn Ala Gly Leu Leu Asn Tyr Asn Phe Ser
 180 185 190
 Gly Asn Ser Val Gln Asn Arg Ile Gly Gly Asn Ser His Tyr Ala Tyr
 195 200 205
 Leu Asn Leu Gln Ser Gly Leu Asn Ile Gly Ala Trp Arg Leu Arg Asp
 210 215 220
 Asn Thr Thr Trp Ser Tyr Asn Ser Ser Asp Arg Ser Ser Gly Ser Lys
 225 230 235 240
 Asn Lys Trp Gln His Ile Asn Thr Trp Leu Glu Arg Asp Ile Ile Pro
 245 250 255
 Leu Arg Ser Arg Leu Thr Leu Gly Asp Gly Tyr Thr Gln Gly Asp Ile
 260 265 270
 Phe Asp Gly Ile Asn Phe Arg Gly Ala Gln Leu Ala Ser Asp Asp Asn
 275 280 285
 Met Leu Pro Asp Ser Gln Arg Gly Phe Ala Pro Val Ile His Gly Ile
 290 295 300
 Ala Arg Gly Thr Ala Gln Val Thr Ile Lys Gln Asn Gly Tyr Asp Ile
 305 310 315 320
 Tyr Asn Ser Thr Val Pro Pro Gly Pro Phe Thr Ile Asn Asp Ile Tyr
 325 330 335
 Ala Ala Gly Asn Ser Gly Asp Leu Gln Val Thr Ile Lys Glu Ala Asp
 340 345 350
 Gly Ser Thr Gln Ile Phe Thr Val Pro Tyr Ser Ser Val Pro Leu Leu
 355 360 365
 Gln Arg Glu Gly His Thr Arg Tyr Ser Ile Thr Ala Gly Glu Tyr Arg
 370 375 380
 Ser Gly Asn Ala Gln Gln Glu Lys Thr Arg Phe Phe Gln Ser Thr Leu
 385 390 395 400
 Leu His Gly Leu Pro Ala Gly Trp Thr Ile Tyr Gly Gly Thr Gln Leu
 405 410 415
 Ala Asp Arg Tyr Arg Ala Phe Asn Phe Gly Ile Gly Lys Asn Met Gly
 420 425 430
 Ala Leu Gly Ala Leu Ser Val Asp Met Thr Gln Ala Asn Ser Thr Leu
 435 440 445

Pro Asp Asp Ser Gln His Asp Gly Gln Ser Val Arg Phe Leu Tyr Asn
450 455 460

Lys Ser Leu Asn Glu Ser Gly Thr Asn Ile Gln Leu Val Gly Tyr Arg
465 470 475 480

Tyr Ser Thr Ser Gly Tyr Phe Asn Phe Ala Asp Thr Thr Tyr Ser Arg
485 490 495

Met Asn Gly Tyr Asn Ile Glu Thr Gln Asp Gly Val Ile Gln Val Lys
500 505 510

Pro Lys Phe Thr Asp Tyr Tyr Asn Leu Ala Tyr Asn Lys Arg Gly Lys
515 520 525

Leu Gln Leu Thr Val Thr Gln Gln Leu Gly Arg Thr Ser Thr Leu Tyr
530 535 540

Leu Ser Gly Ser His Gln Thr Tyr Trp Gly Thr Ser Asn Val Asp Glu
545 550 555 560

Gln Phe Gln Ala Gly Leu Asn Thr Ala Phe Glu Asp Ile Asn Trp Thr
565 570 575

Leu Ser Tyr Ser Leu Thr Lys Asn Ala Trp Gln Lys Gly Arg Asp Gln
580 585 590

Met Leu Ala Leu Asn Val Asn Ile Pro Phe Ser His Trp Leu Arg Ser
595 600 605

Asp Ser Lys Ser Gln Trp Arg His Ala Ser Ala Ser Tyr Ser Met Ser
610 615 620

His Asp Leu Asn Gly Arg Met Thr Asn Leu Ala Gly Val Tyr Gly Thr
625 630 635 640

Leu Leu Glu Asp Asn Asn Leu Ser Tyr Ser Val Gln Thr Gly Tyr Ala
645 650 655

Gly Gly Gly Asp Gly Asn Ser Gly Ser Thr Gly Tyr Ala Thr Leu Asn
660 665 670

Tyr Arg Gly Gly Tyr Gly Asn Ala Asn Ile Gly Tyr Ser His Ser Asp
675 680 685

Asp Ile Lys Gln Leu Tyr Tyr Gly Val Ser Gly Gly Val Leu Ala His
690 695 700

Ala Asn Gly Val Thr Leu Gly Gln Pro Leu Asn Asp Thr Val Val Leu
705 710 715 720

Val Lys Ala Pro Gly Ala Lys Asp Ala Lys Val Glu Asn Gln Thr Gly
725 730 735

Val Arg Thr Asp Trp Arg Gly Tyr Ala Val Leu Pro Tyr Ala Thr Glu
740 745 750

Tyr Arg Glu Asn Arg Val Ala Leu Asp Thr Asn Thr Leu Ala Asp Asn
755 760 765

Val Asp Leu Asp Asn Ala Val Ala Asn Val Val Pro Thr Arg Gly Ala
770 775 780

Ile Val Arg Ala Glu Phe Lys Ala Arg Val Gly Ile Lys Leu Leu Met
785 790 795 800

Thr Leu Thr His Asn Asn Lys Pro Leu Pro Phe Gly Ala Met Val Thr
805 810 815

Ser Glu Ser Ser Gln Ser Ser Gly Ile Val Ala Asp Asn Gly Gln Val
820 825 830

Tyr Leu Ser Gly Met Pro Leu Ala Gly Lys Val Gln Val Lys Trp Gly
835 840 845

Glu Glu Glu Asn Ala His Cys Val Ala Asn Tyr Gln Leu Pro Pro Glu
850 855 860

Ser Gln Gln Gln Leu Leu Thr Gln Leu Ser Ala Glu Cys Arg
865 870 875

<210> 58
<211> 531
<212> DNA
<213> Escherichia coli

<220>
<221> CDS
<222> (1) (531)
<223> fimF

<400> 58
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Met Arg Asn Lys Pro Phe Tyr Leu Leu Cys Ala Phe Leu Trp Leu Ala
5 10 15

gtg agt cac gct ttg gct gcg gat agc acg att act atc cgc gcc tat 96
Val Ser His Ala Leu Ala Ala Asp Ser Thr Ile Thr Ile Arg Gly Tyr
20 25 30

gtc cgg gat aac ggc tgt agt gtg gcc gct gaa tca acc aat ttt act 144
Val Arg Asp Asn Gly Cys Ser Val Ala Ala Glu Ser Thr Asn Phe Thr
35 40 45

gtt gat ctg atg gaa aac gcg gcg aag caa ttt aac aac att ggc gcg 192
Val Asp Leu Met Glu Asn Ala Ala Lys Gln Phe Asn Asn Ile Gly Ala
50 55 60

acg act cct gtt gtt cca ttt cgt att ttg ctg tca ccc tgt ggt aat 240
Thr Thr Pro Val Val Pro Phe Arg Ile Leu Leu Ser Pro Cys Gly Asn
65 70 75 80

gcc gtt tct gcc gta aag gtt ggg ttt act ggc gtt gca gat agc cac 288
Ala Val Ser Ala Val Lys Val Gly Phe Thr Gly Val Ala Asp Ser His
85 90 95

aat gcc aac ctg ctt gca ctt gaa aat acg gtg tca gcg gct tcg gga 336
Asn Ala Asn Leu Leu Ala Leu Glu Asn Thr Val Ser Ala Ala Ser Gly
100 105 110

ctg gga ata cag ctt ctg aat gag cag caa aat caa ata ccc ctt aat 384
Leu Gly Ile Gln Leu Leu Asn Glu Gln Gln Asn Gln Ile Pro Leu Asn
115 120 125

gct cca tcg tcc gcg ctt tcg tgg acg acc ctg acg ccg ggt aaa cca 432

Ala Pro Ser Ser Ala Leu Ser Trp Thr Thr Leu Thr Pro Gly Lys Pro
 130 135 140
 aat acg ctg aat ttt tac gcc cgg cta atg gcc aca cag gtc cct gtc 480
 Asn Thr Leu Asn Phe Tyr Ala Arg Leu Met Ala Thr Gln Val Pro Val
 145 150 155 160
 act gcc ggg cat atc aat gcc acg gct acc ttc act ctt gaa tat cag 528
 Thr Ala Gly His Ile Asn Ala Thr Ala Thr Phe Thr Leu Glu Tyr Gln
 165 170 175
 taa 531

<210> 59
 <211> 176
 <212> PRT
 <213> Escherichia coli

<400> 59

Met Arg Asn Lys Pro Phe Tyr Leu Leu Cys Ala Phe Leu Trp Leu Ala
 1 5 10 15

Val Ser His Ala Leu Ala Ala Asp Ser Thr Ile Thr Ile Arg Gly Tyr
 20 25 30

Val Arg Asp Asn Gly Cys Ser Val Ala Ala Glu Ser Thr Asn Phe Thr
 35 40 45

Val Asp Leu Met Glu Asn Ala Ala Lys Gln Phe Asn Asn Ile Gly Ala
 50 55 60

Thr Thr Pro Val Val Pro Phe Arg Ile Leu Leu Ser Pro Cys Gly Asn
 65 70 75 80

Ala Val Ser Ala Val Lys Val Gly Phe Thr Gly Val Ala Asp Ser His
 85 90 95

Asn Ala Asn Leu Leu Ala Leu Glu Asn Thr Val Ser Ala Ala Ser Gly
 100 105 110

Leu Gly Ile Gln Leu Leu Asn Glu Gln Gln Asn Gln Ile Pro Leu Asn
 115 120 125

Ala Pro Ser Ser Ala Leu Ser Trp Thr Thr Leu Thr Pro Gly Lys Pro
 130 135 140

Asn Thr Leu Asn Phe Tyr Ala Arg Leu Met Ala Thr Gln Val Pro Val
 145 150 155 160

Thr Ala Gly His Ile Asn Ala Thr Ala Thr Phe Thr Leu Glu Tyr Gln
 165 170 175

<210> 60
 <211> 504
 <212> DNA
 <213> Escherichia coli

<220>
 <221> CDS
 <222> (1) (504)
 <223> fimG

<400> 60

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 Met Lys Trp Cys Lys Arg Gly Tyr Val Leu Ala Ala Ile Leu Ala Leu
 1 5 10 15
 gca agt gcg acg ata cag gca gcc gat gtc acc atc acg gtg aac ggt 96
 Ala Ser Ala Thr Ile Gln Ala Ala Asp Val Thr Ile Thr Val Asn Gly
 20 25 30
 aag gtc gtc gcc aaa ccg tgt acg gtt tcc acc acc aat gcc acg gtt 144
 Lys Val Val Ala Lys Pro Cys Thr Val Ser Thr Thr Asn Ala Thr Val
 35 40 45
 gat ctc ggc gat ctt tat tct ttc agt ctt atg tct gcc ggg gcg gca 192
 Asp Leu Gly Asp Leu Tyr Ser Phe Ser Leu Met Ser Ala Gly Ala Ala
 50 55 60
 tcg gcc tgg cat gat gtt gcg ctt gag ttg act aat tgt ccg gtg gga 240
 Ser Ala Trp His Asp Val Ala Leu Glu Leu Thr Asn Cys Pro Val Gly
 65 70 75 80
 acg tgc agg gtc act gcc agc ttc agc ggg gca gcc gac agt acc gga 288
 Thr Ser Arg Val Thr Ala Ser Phe Ser Gly Ala Ala Asp Ser Thr Gly
 85 90 95
 tat tat aaa aac cag ggg acc gcg caa aac atc cag tta gag cta cag 336
 Tyr Tyr Lys Asn Gln Gly Thr Ala Gln Asn Ile Gln Leu Glu Leu Gln
 100 105 110
 gat gac agt ggc aac aca ttg aat act ggc gca acc aaa aca gtt cag 384
 Asp Asp Ser Gly Asn Thr Leu Asn Thr Gly Ala Thr Lys Thr Val Gln
 115 120 125
 gtg gat gat tcc tca caa tca gcg cac ttc ccg tta cag gtc aga gca 432
 Val Asp Asp Ser Ser Gln Ser Ala His Phe Pro Leu Gln Val Arg Ala
 130 135 140
 ttg aca gta aat ggc gga gcc act cag gga acc att cag gca gtg att 480
 Leu Thr Val Asn Gly Gly Ala Thr Gln Gly Thr Ile Gln Ala Val Ile
 145 150 155 160
 agc atc acc tat acc tac agc tga 504
 Ser Ile Thr Tyr Thr Tyr Ser
 165

<210> 61
 <211> 167
 <212> PRT
 <213> Escherichia coli

<400> 61

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

Tyr Tyr Lys Asn Gln Gly Thr Ala Gln Asn Ile Gln Leu Glu Leu Gln
100 105 110

Asp Asp Ser Gly Asn Thr Leu Asn Thr Gly Ala Thr Lys Thr Val Gln
115 120 125

Val Asp Asp Ser Ser Gln Ser Ala His Phe Pro Leu Gln Val Arg Ala
130 135 140

Leu Thr Val Asn Gly Gly Ala Thr Gln Gly Thr Ile Gln Ala Val Ile
145 150 155 160

Ser Ile Thr Tyr Thr Tyr Ser
165

PCT

Original (for SUBMISSION)

0-1	Form PCT/RO/134 (SAFE) Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)	
0-1-1	Prepared Using	PCT-SAFE [EASY mode] Version 3.50 (Build 0002.175)
0-2	International Application No.	
0-3	Applicant's or agent's file reference	C-445
1	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
1-1	page	15
1-2	line	9
1-3	Identification of deposit	
1-3-1	Name of depositary institution	VKPM Russian National Collection of Industrial Microorganisms
1-3-2	Address of depositary institution	GNII Genetika, Dorozhny proezd. 1, Moscow 113545, Russian Federation
1-3-3	Date of deposit	03 May 1990 (03.05.1990)
1-3-4	Accession Number	VKPM B-5318
1-5	Designated States for Which Indications are Made	all designations
2	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
2-1	page	13
2-2	line	2
2-3	Identification of deposit	
2-3-1	Name of depositary institution	IPOD International Patent Organism Depositary, National Institute of Advanced Industrial Science and Technology (AIST)
2-3-2	Address of depositary institution	AIST Tsukuba Central 6, 1-1, Higashi 1-chome Tsukuba-shi, Ibaraki-ken 305-8566 Japan
2-3-3	Date of deposit	28 December 1993 (28.12.1993)
2-3-4	Accession Number	IPOD FERM BP-4881
2-5	Designated States for Which Indications are Made	all designations

PCT

Original (for SUBMISSION)

3	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
3-1	page	7
3-2	line	7
3-3	Identification of deposit	
3-3-1	Name of depositary institution	IPOD International Patent Organism Depositary, National Institute of Advanced Industrial Science and Technology (AIST)
3-3-2	Address of depositary institution	AIST Tsukuba Central 6, 1-1, Higashi 1-chome Tsukuba-shi, Ibaraki-ken 305-8566 Japan
3-3-3	Date of deposit	06 December 1994 (06.12.1994)
3-3-4	Accession Number	IPOD FERM BP-5252
3-5	Designated States for Which Indications are Made	all designations
4	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
4-1	page	13
4-2	line	9
4-3	Identification of deposit	
4-3-1	Name of depositary institution	IPOD International Patent Organism Depositary, National Institute of Advanced Industrial Science and Technology (AIST)
4-3-2	Address of depositary institution	AIST Tsukuba Central 6, 1-1, Higashi 1-chome Tsukuba-shi, Ibaraki-ken 305-8566 Japan
4-3-3	Date of deposit	18 August 1999 (18.08.1999)
4-3-4	Accession Number	IPOD FERM BP-7207
4-5	Designated States for Which Indications are Made	all designations

FOR RECEIVING OFFICE USE ONLY

0-4	This form was received with the international application: (yes or no)	
0-4-1	Authorized officer	

FOR INTERNATIONAL BUREAU USE ONLY

0-5	This form was received by the international Bureau on:	08 FEB 2006
0-5-1	Authorized officer	山崎 子

Appendix 3
page 24
Budapest treaty on the international
recognition of the deposit of microorganisms
for the purposes of patent procedure

To
Ajinomoto Co.,Inc

1-15-1 Kyobashi, Chuo-ku
Tokyo 104-8315
Japan

INTERNATIONAL FORM
VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified on the following page

name and address of the party
to whom the viability statement
is issued

I. DEPOSITOR

Ajinomoto Co., Inc

II. IDENTIFICATION OF THE
MICROORGANISM

Accession number given by the
INTERNATIONAL DEPOSITARY
AUTHORITY
VKPM B-5318
Date of deposit
03/05/90

III. VIABILITY STATEMENT

The viability of the microorganism identified under above was tested
On July. 03 On that date the said microorganism was viable

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: Russian National Collection
of Industrial Microorganisms (VKPM)
Depositary
АИИГ3GosNIIGenetika
Address: Russia 117545 Moscow
1 Dorozhny proezd 1

Signature (s) of person(s) having the
power to represent the International

Authority or authorized official(s):

Date: 16/07/03 Sineoky S.P.



INTERNATIONAL FORM
receipt in the case of an original deposit
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITORY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM

Accession number given by the
INTERNATIONAL DEPOSITARY-
AUTHORITY:
B-5318

The microorganism identified under I above was accompanied by:

 x a scientific description producer of threonine
_____ a proposed taxonomic designation
(Mark with a cross where applicable)

**This International Depositary Authority accepts the microorganism identified under I above,
which was received by it on 03.05.90 (date of original deposit)**

The microorganism identified under I above was received by this International Depository Authority on _____ (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on _____ (date of receipt of request for conversion)

**Name: Russian National Collection
of Industrial Microorganisms (VKPM)**

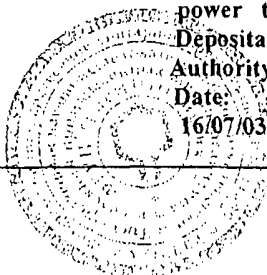
FGUPGosNIIGenetika
Address: Russia 117545 Moscow
1 Dorozhny proezd 1

Signature (s) of person(s) having the power to represent the International Depositary

Authority or authorized official(s):
Date: Simeoky S.P.

Date: 16/07/03

~~Sineoky S.P.~~



国際様式 INTERNATIONAL FORM

〔 特許手続上の微生物の寄託の国際的承認
に関するブダペスト条約 〕

下記国際寄託当局によって規則 7. 1 に従い
発行される

原寄託についての受託証

氏名 (名称) 味の素株式会社
代表取締役社長 鳥羽 董
寄託者 殿
あて名 ⑤ 104
東京都中央区京橋一丁目 15 番 1 号

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF
MICROORGANISMS FOR THE PURPOSES OF
PATENT PROCEDURE

RECEIPT IN THE CASE OF AN ORIGINAL
DEPOSIT

issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this
page.

I. 微生物の表示

(寄託者が付した識別のための表示)
Escherichia coli AJ12949

(受託番号)
FERM BP- 4881

II. 科学的性質及び分類学上の位置

I 欄の微生物には、次の事項を記載した文書が添付されていた。

- ☐ 科学的性質
☒ 分類学上の位置

III. 受領及び受託

本国際寄託当局は、平成 5 年 12 月 28 日 (原寄託日) に受領した I 欄の微生物を受託する。

IV. 移管請求の受領

本国際寄託当局は、平成 5 年 12 月 28 日 (原寄託日) に I 欄の微生物を受領した。
そして、平成 6 年 11 月 11 日に原寄託よりブダペスト条約に基づく寄託への移管請求を受領した。
(平成 5 年 12 月 28 日に寄託された微工研菌寄第 P- 14039 号より移管)

V. 国際寄託当局

通商産業省工業技術院生命工学工業技術研究所

名称: National Institute of Advanced Industrial Science and Human-Technology
Agency for Industrial Science and Technology

所長 鈴木 修 Osamu Suzuki, DIRECTOR GENERAL.

あて名: 日本国茨城県 1 丁目 1 番 3 号 (郵便番号 305)
1-3, Higashi 1 chome Tsukuba-shi Ibaraki-ken
305, JAPAN

平成 6 年 (1994) 11 月 11 日

国際様式 INTERNATIONAL FORM

〔 特許手続上の微生物の寄託の国際的承認
に関するブダペスト条約 〕

下記国際寄託当局によって規則7.1に従い
発行される

原寄託についての受託証

氏名 (名称) 味の素株式会社
代表取締役社長 稲森 俊介
寄託者 あて名 ⑤ 104
東京都中央区京橋一丁目15番1号

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF
MICROORGANISMS FOR THE PURPOSES OF
PATENT PROCEDURE

RECEIPT IN THE CASE OF AN ORIGINAL
DEPOSIT

issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this
page.

I. 微生物の表示

(寄託者が付した識別のための表示)
Escherichia coli AJ13069

(受託番号)
FERM BP- 5252

II. 科学的性質及び分類学上の位置

I 欄の微生物には、次の事項を記載した文書が添付されていた。

- ☐ 科学的性質
☒ 分類学上の位置

III. 受領及び受託

本国際寄託当局は、平成 6年 12月 6日 (原寄託日) に受領した I 欄の微生物を受託する。

IV. 移管請求の受領

本国際寄託当局は、平成 6年 12月 6日 (原寄託日) に I 欄の微生物を受領した。
そして、平成 7年 9月 29日に原寄託よりブダペスト条約に基づく寄託への移管請求を受領した。
(平成 6年 12月 6日に寄託された微工研菌寄第 P- 14690 号より移管)

V. 国際寄託当局

通商産業省工業技術院生命工学工業技術研究所

名称: National Institute of Bioscience and Human-Technology
Agency of Industrial Science and Technology

所長 大石 道夫
Michio Oishi, Ph. D., DIRECTOR GENERAL.

あて名: 日本国茨城県つくば市東1丁目1番3号 (郵便番号 305)
1-3, Higashi 1 chome Tsukuba-shi Ibaraki-ken
305, JAPAN

平成 7年 (1995) 9月 29日

特許手続上の微生物の寄託の国際的承認
に関するブダペスト条約

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF
MICROORGANISMS FOR THE PURPOSES OF
PATENT PROCEDURE

RECEIPT IN THE CASE OF AN ORIGINAL
DEPOSIT

下記国際寄託当局によって規則7.1に従い
発行される。

issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this
page.

原寄託についての受託証

氏名 (名称)

味の素株式会社

代表取締役社長 江頭 邦雄

寄託者

あ て 名 所

東京都中央区京橋一丁目15番1号

殿

1. 微生物の表示

(寄託者が付した識別のための表示)

Enterobacter agglomerans AJ13601

(受託番号)

FERM BP- 7207

2. 科学的性質及び分類学上の位置

1欄の微生物には、次の事項を記載した文書が添付されていた。

☐ 科学的性質

☒ 分類学上の位置

3. 受領及び受託

本国際寄託当局は、平成11年 8月18日(原寄託日)に受領した1欄の微生物を受託する。

4. 移管請求の受領

本国際寄託当局は、平成11年 8月18日(原寄託日)に1欄の微生物を受領した。
そして、平成12年 7月 6日に原寄託よりブダペスト条約に基づく寄託への移管請求を受領した。
(平成11年 8月18日に寄託された微工研菌寄第P- 17516 号より移管)

5. 国際寄託当局

通商産業省工業技術院生命工学工業技術研究所

名称: National Institute of Bioscience and Human-Technology
Agency for Industrial Science and Technology

所長 大箸 信一

Dr. Shinichi Ohsashi Director-General

あて名: 日本国茨城県つくば市東1丁目1番3号(郵便番号305-8566)

1-3, Higashi 1 chome Tsukuba-shi Ibaraki-ken
305-8566, JAPAN

平成12年(2000) 7月 6日

INTERNATIONAL SEARCH REPORT

International application No
PCT/JP2006/301071

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K14/24 C12P13/04 C12P13/08 C12P13/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ORNDORFF P E ET AL: "Immunoglobulin-mediated agglutination of and biofilm formation by Escherichia coli K-12 require the type 1 pilus fiber" INFECTION AND IMMUNITY, vol. 72, no. 4, April 2004 (2004-04), pages 1929-1938, XP002384921 ISSN: 0019-9567 abstract; table 1	1-8
A	EP 0 796 912 A (AJINOMOTO CO., INC) 24 September 1997 (1997-09-24) claims	1-10



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *G* document member of the same patent family

Date of the actual completion of the international search

12 June 2006

Date of mailing of the international search report

28/06/2006

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Sommer, B

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/JP2006/301071

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0796912	A	24-09-1997	
		AU 703308 B2	25-03-1999
		AU 3994895 A	26-06-1996
		BR 9509896 A	30-12-1997
		CA 2207271 A1	13-06-1996
		CN 1175280 A	04-03-1998
		CZ 9701746 A3	12-11-1997
		DK 0796912 T3	10-04-2006
		HU 77752 A2	28-07-1998
		WO 9617930 A1	13-06-1996
		JP 3692538 B2	07-09-2005
		PL 320645 A1	13-10-1997
		RU 2188235 C2	27-08-2002
		SK 70297 A3	14-01-1998
		US 5827698 A	27-10-1998
		ZA 9510442 A	19-06-1996