



US008278075B2

(12) **United States Patent**  
**Nonaka et al.**

(10) **Patent No.:** **US 8,278,075 B2**  
(45) **Date of Patent:** **Oct. 2, 2012**

(54) **L-CYSTEINE-PRODUCING BACTERIUM AND A METHOD FOR PRODUCING L-CYSTEINE**

(75) Inventors: **Gen Nonaka**, Kawasaki (JP); **Hiroshi Takagi**, Ikoma (JP); **Iwao Ohtsu**, Ikoma (JP)

(73) Assignees: **Ajinomoto Co., Inc.**, Tokyo (JP); **National University Corporation Nara Institute of Science and Technology**, Nara (JP)

(\* ) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **12/858,590**

(22) Filed: **Aug. 18, 2010**

(65) **Prior Publication Data**

US 2011/0033902 A1 Feb. 10, 2011

**Related U.S. Application Data**

(63) Continuation of application No. PCT/JP2009/053021, filed on Feb. 20, 2009.

(30) **Foreign Application Priority Data**

Feb. 21, 2008 (JP) ..... 2008-040167

(51) **Int. Cl.**

**C12P 13/12** (2006.01)  
**C12P 13/06** (2006.01)  
**C12N 1/20** (2006.01)  
**C12N 15/00** (2006.01)  
**C07H 21/04** (2006.01)

(52) **U.S. Cl.** ..... **435/113**; 435/116; 435/252.3; 435/320.1; 536/23.2

(58) **Field of Classification Search** ..... None  
See application file for complete search history.

(56) **References Cited**

**U.S. PATENT DOCUMENTS**

5,972,663	A	10/1999	Winterhalter et al.
6,218,168	B1	4/2001	Leinfelder et al.
7,312,058	B2	12/2007	Kashiwagi et al.
2003/0077766	A1	4/2003	Takagi et al.
2005/0112731	A1	5/2005	Kashiwagi et al.
2005/0221453	A1	10/2005	Takagi et al.
2008/0076163	A1	3/2008	Takagi et al.
2009/0226983	A1	9/2009	Nonaka et al.
2009/0226984	A1	9/2009	Nonaka et al.
2010/0093045	A1	4/2010	Takagi et al.
2010/0209977	A1	8/2010	Takumi et al.

**FOREIGN PATENT DOCUMENTS**

JP	11-155571	6/1999
JP	11-221080	8/1999
JP	2992010	10/1999
JP	2002-233384	8/2002
JP	2003-169668	6/2003
JP	2005-245311	9/2005
JP	2005-287333	10/2005
WO	WO01/27307	4/2001
WO	WO2008/096837	8/2008

**OTHER PUBLICATIONS**

Chica et al. *Curr Opin Biotechnol.* Aug. 2005;16(4):378-84.\*  
Sen et al. *Appl Biochem Biotechnol.* Dec. 2007;143(3):212-23.\*  
BioCyc Home Page, Summary of *Escherichia coli*, Strain K-12, version 11.6, *E. coli* K-12 Gene: tolC, pp. 1-9.  
Daßler, T., et al., "Identification of a major facilitator protein from *Escherichia coli* involved in efflux of metabolites of the cysteine pathway," *Mol. Microbiol.* 2000;36(5):1101-1112.  
Li, Z., et al., "Functional analysis of the *Escherichia coli* outer membrane protein porin TolC and its application to L-cysteine production," *Seikagaku, BMB2008*, with its full English translation.  
Wiriyanathanawudhiwong, N., et al., "The outer membrane TolC is involved in cysteine tolerance and overproduction in *Escherichia coli*," *Appl. Microbiol. Biotechnol.* 2009;81:903-913.  
International Search Report for PCT Patent App. No. PCT/JP2009/053021 (Apr. 28, 2009).  
U.S. Appl. No. 12/711,299, filed Feb. 24, 2010, Nonaka et al.  
U.S. Appl. No. 12/722,094, filed Mar. 11, 2010, Nonaka et al.  
Supplementary European Search Report for EP Patent App. No. 09713597.4 (Jun. 15, 2012).

\* cited by examiner

*Primary Examiner* — Christian Fronda

(74) *Attorney, Agent, or Firm* — Shelly Guest Cermak; Cermak Nakajima LLP

(57) **ABSTRACT**

L-Cysteine, L-cystine, a derivative or precursor thereof, or a mixture thereof is produced by culturing a bacterium belonging to the family Enterobacteriaceae, which has L-cysteine-producing ability and has been modified so that the activity of a protein encoded by a tolC gene, for example, a protein defined in the following (a) or (b), is increased in a medium, and by collecting L-cysteine, L-cystine, a derivative or precursor thereof, or a mixture thereof from the medium:

- (a) a protein comprising the amino acid sequence of SEQ ID NO: 2,
- (b) a protein comprising the amino acid sequence of SEQ ID NO: 2, but wherein one or several amino acid residues are substituted, deleted, inserted or added, increase of which activity in the bacterium improves the ability of the bacterium to produce L-cysteine.

**16 Claims, 2 Drawing Sheets**

Fig. 1

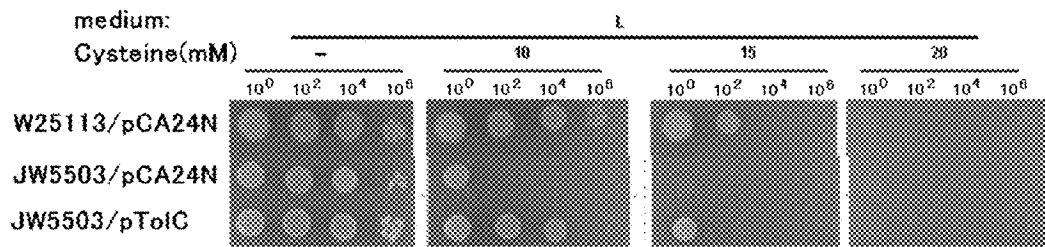


Fig. 2

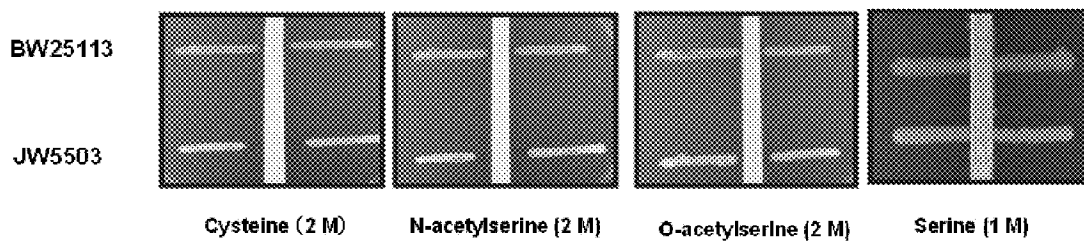


Fig. 3

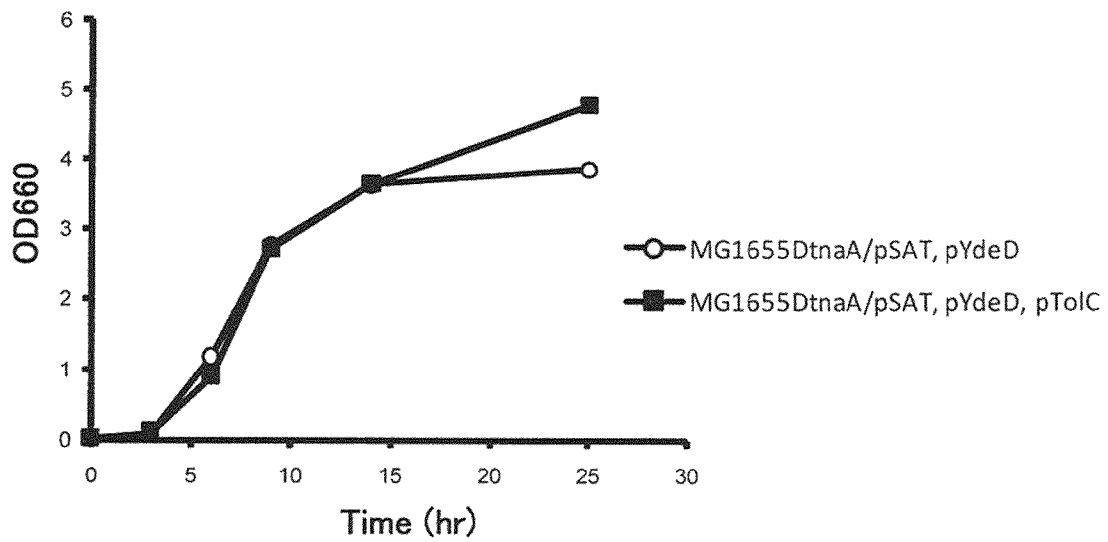
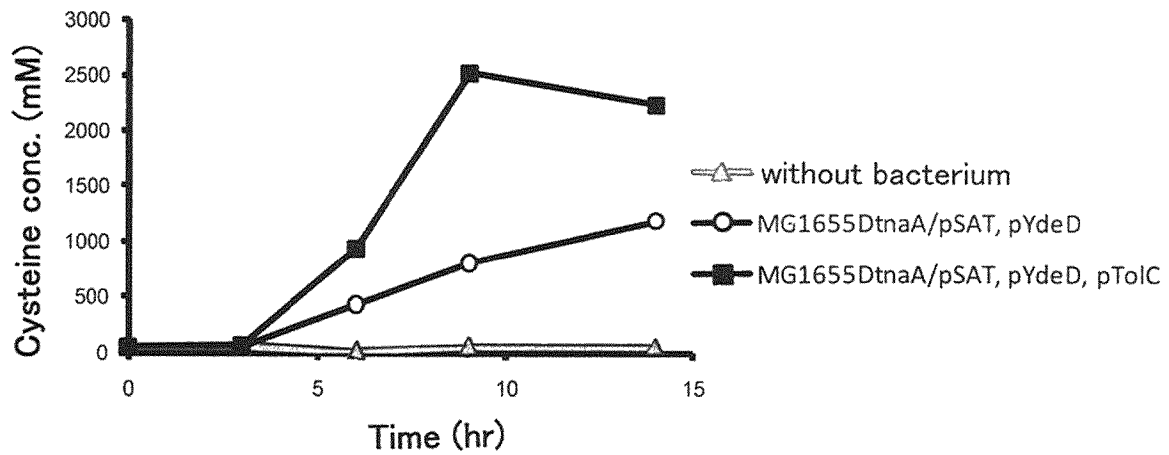


Fig. 4



## L-CYSTEINE-PRODUCING BACTERIUM AND A METHOD FOR PRODUCING L-CYSTEINE

This application is a continuation under 35 U.S.C. §120 of PCT Patent Application No. PCT/JP2009/053021, filed Feb. 20, 2009, which claims priority under 35 U.S.C. §119 to Japanese Patent Application No. 2008-040167, filed on Feb. 21, 2008, which are incorporated in their entireties by reference. The Sequence Listing in electronic format filed herewith is also hereby incorporated by reference in its entirety (File Name: 2010-08-18T\_US-441\_Seq\_List; File Size: 120 KB; Date Created: Aug. 18, 2010).

### BACKGROUND OF THE INVENTION

#### 1. Field of the Invention

The present invention relates to a method for producing L-cysteine and related substances. Specifically, the present invention relates to a bacterium suitable for the production of L-cysteine and related substances and a method for producing L-cysteine and related substances utilizing the bacterium. L-cysteine and L-cysteine-related substances are utilized in the fields of drugs, cosmetics, and foods.

#### 2. Brief Description of the Related Art

L-cysteine can be obtained by extraction from keratin-containing substances such as hair, horns and feathers, or by the conversion of the precursor DL-2-aminothiazoline-4-carboxylic acid using a microbial enzyme. L-cysteine has also been produced on a large scale by using an immobilized enzyme method and a novel enzyme. Furthermore, production of L-cysteine by fermentation utilizing a microorganism has also been attempted.

Microorganisms, which are able to produce L-cysteine, are also known. For example, a coryneform bacterium with increased intracellular serine acetyltransferase activity produces cysteine (Japanese Patent Laid-open (Kokai) No. 2002-233384). The ability to produce L-cysteine can also be increased by incorporating serine acetyltransferase which has been mutated to attenuate feedback inhibition by L-cysteine (Japanese Patent Laid-open No. 11-155571; U.S. Patent Published Application No. 20050112731; U.S. Pat. No. 6,218,168).

Furthermore, the ability to produce L-cysteine in a microorganism can be enhanced by suppressing the L-cysteine decomposition system. Examples of such microorganisms include coryneform bacteria or *Escherichia* bacteria in which the activity of cystathionine- $\beta$ -lyase (Japanese Patent Laid-open No. 11-155571), tryptophanase (Japanese Patent Laid-open No. 2003-169668), or O-acetylserine sulfhydrylase B (Japanese Patent Laid-open No. 2005-245311) is attenuated or deleted.

Furthermore, the ydeD gene which encodes the YdeD protein participates in excretion of the metabolic products of the cysteine pathway (Dabler et al., Mol. Microbiol., 36, 1101-1112 (2000)). Also, techniques are known for enhancing L-cysteine-producing ability by increasing expression of the mar-locus, emr-locus, acr-locus, cmr-locus, mex-gene, bmr-gene or qacA-gene. These loci and/or genes encode proteins which cause secretion of toxic substances from cells (U.S. Pat. No. 5,972,663). The emrAB, emrKY, yojIH, acrEF, bcr, and cusA genes are further examples (Japanese Patent Laid-open No. 2005-287333).

An *Escherichia coli* has been reported which produces L-cysteine, and which has increased activity of the positive transcriptional control factor of the cysteine regulon encoded by the cysB gene (International Patent Publication WO01/27307).

Although the tolC gene (BioCyc Home Page, Summary of *Escherichia coli*, Strain K-12, version 11.6, *E. coli* K-12 Gene: tolC [searched on Feb. 11, 2008], Internet URL: biocyc.org/ECOLI/NEW-IMAGE?type=GENE&object=EG 11009) is known as a gene coding for a porin (outer membrane channel), its relation to L-cysteine production is not known.

### SUMMARY OF THE INVENTION

The present invention provides novel techniques for improving the ability to produce bacterial L-cysteine, and thereby providing an L-cysteine-producing bacterium, as well as a method for producing L-cysteine, L-cystine, their derivatives or precursors or a mixture of these by using such a bacterium.

The ability of a bacterium to produce L-cysteine is enhanced by modifying the bacterium so that the activity of the protein encoded by the tolC gene is increased.

It is an aspect of the present invention to provide a bacterium belonging to the family Enterobacteriaceae, which has the ability to produce L-cysteine and has been modified so that the activity of the protein encoded by a tolC gene is increased.

It is a further aspect of the present invention to provide the bacterium as described above, wherein the activity of the protein is increased by increasing expression amount of the tolC gene, increasing translation amount of the tolC gene, or combinations thereof.

It is a further aspect of the present invention to provide the bacterium as described above, wherein expression amount of the tolC gene is increased by increasing copy number of the tolC gene, or by modifying an expression control sequence of the gene.

It is a further aspect of the present invention to provide the bacterium as described above, wherein the protein is selected from the group consisting of:

(a) a protein comprising the amino acid sequence of SEQ ID NO: 2,

(b) a protein comprising the amino acid sequence of SEQ ID NO: 2, but wherein one or several amino acid residues substituted, deleted, inserted or added, wherein the increase of the activity in the bacterium improves the ability to produce L-cysteine of the bacterium.

It is a further aspect of the present invention to provide the bacterium as described above, wherein the tolC gene is selected from the group consisting of:

(a) a DNA comprising the nucleotide sequence of SEQ ID NO: 1,

(b) a DNA which hybridizes with the nucleotide sequence of SEQ ID NO: 1, or a probe prepared from the nucleotide sequence, under stringent conditions, and codes for a protein, wherein the increase of the activity in the bacterium improves the ability to produce L-cysteine of the bacterium.

It is a further aspect of the present invention to provide the bacterium as described above, which contains a mutant serine acetyltransferase in which feedback inhibition by L-cysteine has been attenuated.

It is a further aspect of the present invention to provide the bacterium as described above, wherein the activity of the protein encoded by the ydeD gene is increased.

It is a further aspect of the present invention to provide the bacterium as described above, wherein an activity of a protein having cysteine desulfhydrase activity decreases.

It is a further aspect of the present invention to provide the bacterium as described above, wherein the activity of the protein encoded by the ydeD gene is increased.

3

It is a further aspect of the present invention to provide the bacterium as described above, wherein an activity of a protein having cysteine desulfhydrase activity is decreased.

It is a further aspect of the present invention to provide the bacterium as described above, wherein an activity of a protein having cysteine desulfhydrase activity is decreased.

It is a further aspect of the present invention to provide the bacterium as described above, wherein activity of a protein having the cysteine desulfhydrase activity decreases.

It is a further aspect of the present invention to provide the bacterium as described above, wherein the protein having the cysteine desulfhydrase activity is tryptophanase.

It is a further aspect of the present invention to provide the bacterium as described above, which is an *Escherichia* bacterium.

It is a further aspect of the present invention to provide the bacterium as described above, which is *Escherichia coli*.

It is a further aspect of the present invention to provide a method for producing L-cysteine, L-cystine, a derivative or precursor thereof, or a mixture thereof, which comprises culturing the bacterium as described above in a medium and collecting L-cysteine, L-cystine, a derivative or precursor thereof, or a mixture thereof from the medium.

It is a further aspect of the present invention to provide the method as described above, wherein the derivative of L-cysteine is a thiazolidine derivative.

It is a further aspect of the present invention to provide the method as described above, wherein the precursor of L-cysteine is O-acetylserine or N-acetylserine.

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the cysteine sensitivity of a strain which is deficient in a part of or the entire *tolC* gene and complementation (recovery of growth) with a *tolC* plasmid (photograph).

FIG. 2 shows the sensitivity (antibacterial activity) of a strain which is deficient in a part of or the entire *tolC* gene to O-acetylserine and N-acetylserine (photograph).

FIG. 3 shows the growth curve of a *TolC*-enhanced cysteine-producing bacterium.

FIG. 4 shows cysteine production by a *TolC*-enhanced cysteine-producing bacterium.

#### DETAILED DESCRIPTION OF EXEMPLARY EMBODIMENTS

##### <1> Bacterium

The bacterium belongs to the family Enterobacteriaceae, and is able to produce L-cysteine. Furthermore, the bacterium has been modified so that the activity of the protein encoded by the *tolC* gene is increased. The "ability to produce L-cysteine" or the "L-cysteine-producing ability" can mean an ability of the bacterium to produce L-cysteine and cause accumulation of L-cysteine in a medium or the bacterial cells in such an amount that the L-cysteine can be collected from the medium or cells when the bacterium is cultured in the medium. A bacterium having L-cysteine-producing ability can mean a bacterium which can produce and cause accumulation of a larger amount of L-cysteine as compared with a wild-type, parent, or unmodified strain, and can be a bacterium which can produce and cause accumulation of L-cysteine in a medium in an amount of, for example, 0.05 g/L or more, 0.1 g/L or more, or 0.2 g/L or more.

The L-cysteine produced by the bacterium can change into L-cystine in the medium by the formation of a disulfide bond. Furthermore, as described below, S-sulfocysteine can be generated by the reaction of L-cysteine and thiosulfuric acid in

4

the medium (Szczepkowski T. W., Nature, vol. 182 (1958)). Moreover, the L-cysteine generated in bacterial cells can be condensed with a ketone, aldehyde, or, for example, pyruvic acid, which is present in the cells, to produce a thiazolidine derivative via a hemithioketal intermediate (refer to Japanese Patent No. 2992010). The thiazolidine derivative and hemithioketal can be present as an equilibrated mixture. Therefore, the ability to produce L-cysteine is not limited to the ability to accumulate only L-cysteine in the medium or cells, but also includes the ability to accumulate, L-cystine or its derivative or precursor, or a mixture thereof. Examples of the aforementioned derivative of L-cysteine or L-cystine include, for example, S-sulfocysteine, thiazolidine derivatives, hemithioketal, and so forth. Examples of the precursor of L-cysteine or L-cystine include, for example, O-acetylserine, which is a precursor of L-cysteine. The precursors of L-cysteine or L-cystine also include derivatives of the precursors, for example, N-acetylserine, which is a derivative of O-acetylserine, and so forth.

O-Acetylserine (OAS) is a precursor of L-cysteine biosynthesis. OAS is a metabolite of bacteria and plants, and is produced by acetylation of L-serine by an enzymatic reaction catalyzed by serine acetyltransferase (SAT). OAS is further converted into L-cysteine in cells.

The ability to produce L-cysteine can be inherent to the bacterium, or it can be imparted by modifying a microorganism such as those described below by mutagenesis or recombinant DNA techniques. Unless specially mentioned, the term L-cysteine refers to the reduced-type L-cysteine, L-cystine, a derivative or precursor such as those mentioned above, or a mixture thereof.

The bacterium is not particularly limited so long as the bacterium belongs to the family Enterobacteriaceae and has the ability to produce L-cysteine. Such bacteria include those of the genera *Escherichia*, *Enterobacter*, *Pantoea*, *Klebsiella*, *Serratia*, *Erwinia*, *Salmonella* and *Morganella*. Specifically, those classified into the family Enterobacteriaceae according to the taxonomy used in the NCBI (National Center for Biotechnology Information) database ([www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=91347](http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=91347)) can be used. As the parent strain of the family Enterobacteriaceae, a bacterium of the genus *Escherichia*, *Enterobacter*, *Pantoea*, *Erwinia*, or *Klebsiella* can be used.

Although the *Escherichia* bacteria are not particularly limited, specifically, those described in the work of Neidhardt et al. (Backmann B. J., 1996, Derivations and Genotypes of some mutant derivatives of *Escherichia coli* K-12, p. 2460-2488, Table 1, In F. D. Neidhardt (ed.), *Escherichia coli* and *Salmonella* Cellular and Molecular Biology/Second Edition, American Society for Microbiology Press, Washington, D.C.) can be used. Among these, *Escherichia coli* is one example. Examples of *Escherichia coli* include *Escherichia coli* W3110 (ATCC 27325), *Escherichia coli* MG1655 (ATCC 47076), and so forth, and include those derived from the prototype wild-type strain, K12 strain.

These strains are available from, for example, American Type Culture Collection (Address: P.O. Box 1549, Manassas, Va. 20108, United States of America). That is, registration numbers are given to each of the strains, and the strains can be ordered by using these registration numbers (refer to [www.atcc.org/](http://www.atcc.org/)). The registration numbers of the strains are listed in the catalogue of the American Type Culture Collection.

Examples of the *Enterobacter* bacteria include *Enterobacter agglomerans*, *Enterobacter aerogenes* and so forth, and examples of the *Pantoea* bacteria include *Pantoea ananatis*. Some strains of *Enterobacter agglomerans* were recently

reclassified into *Pantoea agglomerans*, *Pantoea ananatis*, or *Pantoea stewartii* on the basis of nucleotide sequence analysis of 16S rRNA etc. A bacterium belonging to either *Enterobacter* or *Pantoea* can be used so long as it is classified as the family Enterobacteriaceae.

In particular, *Pantoea* bacteria, *Erwinia* bacteria, and *Enterobacter* bacteria are classified as  $\gamma$ -proteobacteria, and they are taxonomically very close to one another (J. Gen. Appl. Microbiol., 1997, 43, 355-361; International Journal of Systematic Bacteriology, October 1997, pp. 1061-1067). In recent years, some bacteria belonging to the genus *Enterobacter* were reclassified as *Pantoea agglomerans*, *Pantoea dispersa*, or the like, on the basis of DNA-DNA hybridization experiments etc. (International Journal of Systematic Bacteriology, July 1989, 39(3), pp. 337-345). Furthermore, some bacteria belonging to the genus *Erwinia* were reclassified as *Pantoea ananas* or *Pantoea stewartii* (refer to International Journal of Systematic Bacteriology, January 1993, 43(1), pp. 162-173).

Examples of the *Enterobacter* bacteria include, but are not limited to, *Enterobacter agglomerans*, *Enterobacter aerogenes*, and so forth. Specifically, the strains exemplified in European Patent Publication No. 952221 can be used.

A typical strain of the genus *Enterobacter* is the *Enterobacter agglomerans* ATCC 12287 strain.

Typical strains of the *Pantoea* bacteria include, but are not limited to, *Pantoea ananatis*, *Pantoea stewartii*, *Pantoea agglomerans*, and *Pantoea citrea*.

Specific examples of *Pantoea ananatis* include the *Pantoea ananatis* AJ13355 strain and SC17 strain. The SC17 strain was selected as a low phlegm-producing mutant strain from the AJ13355 strain (FERM BP-6614), which was isolated from soil in Iwata-shi, Shizuoka-ken, Japan for its ability to proliferate in a low pH medium containing L-glutamic acid and a carbon source (U.S. Pat. No. 6,596,517).

The *Pantoea ananatis* AJ13355 strain was deposited at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Ministry of International Trade and Industry (currently, the National Institute of Advanced Industrial Science and Technology, International Patent Organism Depository, Address: Tsukuba Central 6, 1-1, Higashi 1-Chome, Tsukuba-shi, Ibaraki-ken, 305-8566, Japan) on Feb. 19, 1998 and assigned an accession number of FERMP-16644. It was then converted to an international deposit under the provisions of Budapest Treaty on Jan. 11, 1999 and assigned an accession number of FERM BP-6614. This strain was identified as *Enterobacter agglomerans* when it was isolated and deposited as the *Enterobacter agglomerans* AJ13355 strain. However, it was recently reclassified as *Pantoea ananatis* on the basis of nucleotide sequencing of 16S rRNA and so forth.

Examples of the *Erwinia* bacteria include, but are not limited to, *Erwinia amylovora* and *Erwinia carotovora*, and examples of the *Klebsiella* bacteria include *Klebsiella planticola*.

Impartation or Enhancement of L-Cysteine-Producing Ability

Hereinafter, methods for imparting the ability to produce L-cysteine to bacteria belonging to Enterobacteriaceae, or methods for enhancing the ability to produce L-cysteine of such bacteria, are described.

To impart the ability to produce L-cysteine, methods conventionally employed in the breeding of coryneform bacteria or bacteria of the genus *Escherichia* (see "Amino Acid Fermentation", Gakkai Shuppan Center (Ltd.), 1st Edition, published May 30, 1986, pp. 77-100) can be used. Such methods include acquiring the properties of an auxotrophic mutant, an

analogue-resistant strain, or a metabolic regulation mutant, or constructing a recombinant strain so that it overexpresses an L-cysteine biosynthesis enzyme. Here, in the breeding of L-cysteine-producing bacteria, one or more of the above-described properties such as auxotrophy, analogue resistance, and metabolic regulation mutation can be imparted. The expression of L-cysteine biosynthesis enzyme(s) can be enhanced alone or in combinations of two or more. Furthermore, the methods of imparting properties such as an auxotrophy, analogue resistance, or metabolic regulation mutation can be combined with enhancement of the biosynthesis enzymes.

An auxotrophic mutant strain, L-cysteine analogue-resistant strain, or metabolic regulation mutant strain with the ability to produce L-cysteine can be obtained by subjecting a parent, wild-type, or unmodified strain to conventional mutagenesis, such as exposure to X-rays or UV irradiation, or by treating them with a mutagen such as N-methyl-N'-nitro-N-nitrosoguanidine or ethyl methanesulfonate (EMS), then selecting those which exhibit autotrophy, analogue resistance, or a metabolic regulation mutation and which also have the ability to produce L-cysteine from the obtained mutant strains.

Specific examples of L-cysteine-producing bacteria include, but are not limited to, *E. coli* JM15 transformed with multiple kinds of *cysE* gene alleles encoding serine acetyltransferase (SAT) resistant to feedback inhibition (U.S. Pat. No. 6,218,168), *E. coli* W3110 in which a gene encoding a protein responsible for excretion of cytotoxic substances is overexpressed (U.S. Pat. No. 5,972,663), an *E. coli* strain having decreased cysteine desulfhydrase activity (Japanese Patent Laid-open No. 11-155571), and *E. coli* W3110 with increased activity of the positive transcriptional control factor of the cysteine regulon encoded by the *cysB* gene (WO01/27307).

The following proteins are known to have the cysteine desulfhydrase activity of *E. coli*: cystathionine- $\beta$ -lyase (metC product, Japanese Patent Laid-open No. 11-155571, Chandra et al., Biochemistry, 21 (1982) 3064-3069), tryptophanase (tnaA product, Japanese Patent Laid-open No. 2003-169668, Austin Newton et al., J. Biol. Chem., 240 (1965) 1211-1218), O-acetylserine sulfhydrylase B (*cysM* gene product, Japanese Patent Laid-open No. 2005-245311) and the *malY* gene product (Japanese Patent Laid-open No. 2005-245311). By decreasing the activities of these proteins, L-cysteine-producing ability is improved.

The phrase "decreasing activity of a protein" can mean that activity of the protein is decreased as compared with a non-modified strain such as a wild-type or parent strain, and also can mean the complete disappearance of the activity.

Decreasing the activity of a protein having the cysteine desulfhydrase activity can be attained by, for example, reducing the expression of a gene coding for the protein. Specifically, for example, intracellular activity of the protein can be reduced by deleting a part of or the entire coding region of the target gene on the chromosome. Expression of a target gene can also be decreased by modifying an expression control sequence of the gene such as the promoter and the Shine-Dalgarno (SD) sequences. Furthermore, the expression of the gene can also be reduced by modifying a non-translated region other than the expression control sequence. Additionally, the entire gene as well as the sequences on both sides of the gene on the chromosome can be deleted. Moreover, modification can also be attained by introducing an amino acid substitution (missense mutation), a stop codon (nonsense mutation), or a frame shift mutation which adds or deletes one or two nucleotides into the coding region of the target gene on

the chromosome (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, modification can be caused by a conventional mutagenesis based on X-ray or ultraviolet irradiation or the use of a mutagen such as N-methyl-N'-nitro-N-nitrosoguanidine, as long as the activity of the target protein is decreased.

Modification of an expression control sequence is performed, for example, for one or more nucleotides, two or more nucleotides, or three or more nucleotides. When a coding region is deleted, the region to be deleted can be an N-terminus region, an internal region or a C-terminus region, or even the entire coding region, so long as the function of the target protein is decreased or deleted. Deletion of a longer region is more likely to inactivate a gene. Furthermore, reading frames upstream and downstream of the region to be deleted can be dissimilar.

When another sequence is inserted into a coding region of a target gene, the sequence can be inserted into any region of the gene, and insertion of a longer sequence is more likely to inactivate the gene. Reading frames upstream and downstream of the insertion site can be dissimilar. The other sequence is not particularly limited so long as a sequence which decreases or deletes function of the encoded protein is chosen, and examples include a transposon carrying an antibiotic resistance gene, a gene useful for L-cysteine production, and so forth.

A target gene on the chromosome can be modified as described above by, for example, preparing a deletion-type version of the gene in which a partial sequence of the gene is deleted so that the deletion-type gene does not produce a normally-functioning protein. Then, a bacterium can be transformed with a DNA containing the deletion-type gene to cause homologous recombination between the deletion-type gene and the native gene on the chromosome, which results in the substitution of the deletion-type gene for the gene on the genome. The protein encoded by the deletion-type gene has a conformation different from that of the wild-type enzyme protein, if it is even produced, and thus, the function is reduced or deleted. Such gene disruption based on gene substitution utilizing homologous recombination is known, and examples include Red-driven integration (Datsenko, K. A., and Wanner, B. L., Proc. Natl. Acad. Sci. USA, 97:6640-6645 (2000)), methods using a linear DNA such as the method of utilizing Red driven integration in combination with an excision system derived from  $\lambda$  phage (Cho, E. H., Gumport, R. I., Gardner, J. F., J. Bacteriol., 184:5200-5203 (2002)) (refer to WO2005/010175), methods using a plasmid containing a temperature sensitive replication origin or a plasmid capable of conjugative transfer, methods utilizing a suicide vector without a replication origin in a host (U.S. Pat. No. 6,303,383, Japanese Patent Laid-open No. 05-007491), and so forth.

Decrease of the expression of a target gene can be confirmed by comparing the amount of mRNA transcribed from the gene with that in a wild-type strain or non-modified strain. The expression amount can be confirmed by Northern hybridization, RT-PCR (Molecular Cloning (Cold Spring Harbor Laboratory Press, Cold Spring Harbor (USA), 2001)), and the like.

A decrease in the amount of a target protein can be confirmed by Western blotting using antibodies (Molecular Cloning (Cold Spring Harbor Laboratory Press, Cold Spring Harbor (USA), 2001)).

The L-cysteine-producing bacterium can have a SAT which has been mutated to be resistant to feedback inhibition. The following mutations in SAT are known to induce resis-

tance to feedback inhibition and are derived from *Escherichia coli*: when the methionine residue at position 256 is replaced with a glutamate residue (Japanese Patent Laid-open No. 11-155571), when the methionine residue at position 256 is replaced with an isoleucine residue (Denk, D. and Boeck, A., J. General Microbiol., 133, 515-525 (1987)), a mutation in the region from the amino acid residue at position 97 to the amino acid residue at position 273 or a deletion of the C-terminus region from the amino acid residue at position 227 (International Patent Publication WO97/15673, U.S. Pat. No. 6,218, 168), when the amino acid sequence corresponding to positions 89 to 96 of the wild-type SAT contains one or more mutations (U.S. Patent Published Application No. 20050112731(A1)), and so forth. In the *cysE5* gene which encodes the mutant SAT described in the examples, the Val residue and the Asp residue at positions 95 and 96 of the wild-type SAT are replaced with an Arg residue and a Pro residue, respectively.

The SAT gene is not limited to the gene of *Escherichia coli*, but can be any gene encoding a protein having the SAT activity. For example, a SAT isozyme of *Arabidopsis thaliana* desensitized to feedback inhibition by L-cysteine is known, and the gene encoding this SAT can also be used (FEMS Microbiol. Lett., 179 (1999) 453-459).

If a gene encoding a mutant SAT is introduced into a bacterium, the ability to produce L-cysteine is imparted to the bacterium. To introduce a mutant SAT gene into a bacterium, various vectors which are typically used for protein expression can be used. Examples of such vectors include pUC19, pUC18, pHSG299, pHSG399, pHSG398, RSF1010, pBR322, pACYC184, pMW219, and so forth.

In order to introduce a recombinant vector containing a SAT gene into a bacterium, methods which are typically used to transform bacteria can be used, such as the method of D. A. Morrison (Methods in Enzymology, 68, 326 (1979)), treating recipient cells with calcium chloride to increase permeability of the cells for DNA (Mandel, M. and Higa, A., J. Mol. Biol., 53, 159 (1970)), and a method based on electroporation.

Furthermore, the SAT activity can also be enhanced by increasing the copy number of the SAT gene. The copy number of the SAT gene can be increased by introducing the SAT gene into a bacterium by using a vector such as those described above, or by introducing multiple copies of the SAT gene onto the chromosomal DNA of a bacterium. Multiple copies of the SAT gene are introduced by homologous recombination which targets a sequence present on the chromosomal DNA in multiple copies. A repetitive DNA or inverted repeat present at the end of a transposable element can be used as a sequence which is present on the chromosomal DNA in multiple copies. Alternatively, as disclosed in Japanese Patent Laid-open No. 2-109985, multiple copies of the SAT gene can be introduced into the chromosomal DNA by incorporating them into a transposon and transferring it.

Moreover, it is known that the *ydeD* gene coding for the YdeD protein participates in secretion of metabolic products of the cysteine pathway, and the ability to produce L-cysteine can also be improved by enhancing the activity of the YdeD protein (Japanese Patent Laid-open No. 2002-233384). Modification for increasing the activity of the YdeD protein can be attained, for example, by improving expression of the *ydeD* gene. Improvement of the expression of the *ydeD* gene can be attained in the same manner as that of the improvement of expression of the *tolC* gene described later.

The *ydeD* gene of *Escherichia coli* can be obtained from *Escherichia coli* chromosomal DNA by PCR using, for example, the primers having the nucleotide sequences of SEQ ID NOS: 9 and 10.

Furthermore, by incorporating 3-phosphoglycerate dehydrogenase (PGD) desensitized to the feedback inhibition by serine, the ability to produce L-cysteine can also be improved. The serA5 gene is known as a gene coding for such a mutant PGD (described in U.S. Pat. No. 6,180,373).

Additionally, an L-cysteine-producing *Escherichia* bacterium which has been modified to enhance expression of the cysPTWAM cluster genes coding for the sulfate/thiosulfate transport system proteins (Japanese Patent Laid-open No. 2005-137369, EP 1528108) can also be used.

Moreover, an *Escherichia* bacterium which has the ability to produce L-cysteine and has been modified to increase expression of the emrAB, emrKY, yojIH, acrEF, ber or cusA gene (Japanese Patent Laid-open No. 2005-287333) can also be used.

Particular examples of the bacteria having the ability to produce L-cysteine include a bacterium containing a mutant SAT resistant to feedback inhibition, a bacterium having enhanced activity of the YdeD protein, a bacterium deficient in the cysteine desulfhydrase activity, a bacterium containing a mutant SAT resistant to feedback inhibition and having enhanced activity of the YdeD protein, a bacterium containing a mutant SAT resistant to feedback inhibition and deficient in the cysteine desulfhydrase activity, a bacterium having enhanced activity of the YdeD protein and deficient in the cysteine desulfhydrase activity, and a bacterium containing a mutant SAT resistant to feedback inhibition, deficient in the cysteine desulfhydrase activity, and having enhanced activity of the YdeD protein. The cysteine desulfhydrase activity can be the tryptophanase activity.

The bacterium can be obtained by modifying a bacterium belonging to the family Enterobacteriaceae, which has the ability to produce L-cysteine such as those described above, so that the activity of the protein encoded by tolC gene (henceforth also referred to as "TolC") is increased. Alternatively, after the performance of such a modification where the activity of the TolC protein is increased, the ability to produce L-cysteine can be imparted.

The tolC gene is the same as ECK3026, weeA, b3035, colE1-i, mtcB, mukA, ref1 and toc genes.

The phrase "the activity of the protein encoded by the tolC gene is increased" can mean that the activity of the TolC protein encoded by the tolC gene is increased as compared with a non-modified strain such as a wild-type or parent strain.

Specifically, the activity of the TolC protein can mean an activity in which an increase in the bacterium improves its ability to produce L-cysteine. Furthermore, the TolC protein increases cysteine resistance as compared with a non-modified strain when expression of the protein is enhanced, as described in the example section. Therefore, according to another definition, the activity of the TolC protein can mean such an activity of increasing cysteine resistance.

Modification for increasing the activity of the TolC protein encoded by the tolC gene is attained, for example, by increasing expression of the tolC gene.

To enhance the expression of the tolC gene, the copy number of the tolC gene can be increased by using a gene recombination technique. For example, a recombinant DNA can be prepared by ligating a gene fragment containing the tolC gene with a vector functioning in a host bacterium, such as a multi-copy type vector, and then introduced into the bacterium to transform it.

Examples of the vector include vectors which are autonomously replicable in host bacterium cells. Examples of the vectors autonomously replicable in *Escherichia coli* cells include pUC19, pUC18, pHSG299, pHSG399, pHSG398,

pACYC184 (pHSG and pACYC series vectors are available from Takara Bio), RSF1010, pBR322, pMW219 (pMW219 is available from NIPPON GENE), pSTV29 (available from Takara Bio), and so forth.

To introduce such a recombinant DNA into a bacterium, any known reported transformation methods can be employed. For instance, the method of treating recipient cells with calcium chloride so as to increase permeability thereof for DNA, has been reported for *Escherichia coli* K-12 (Mandel, M. and Higa, A., J. Mol. Biol., 53, 159 (1970)), and the method of preparing competent cells from cells which are at the growth phase followed by introducing the DNA thereinto, has been reported for *Bacillus subtilis* (Duncan, C. H., Wilson, G. A. and Young, F. E., Gene, 1, 153 (1977)). In addition to these is the method of making DNA-recipient cells into protoplasts or spheroplasts, which can easily take up recombinant DNA, followed by introducing the recombinant DNA into the DNA recipient cells, which is known to be applicable to *Bacillus subtilis*, actinomycetes and yeasts (Chang, S. and Choen, S. N., Mol. Gen. Genet., 168, 111 (1979); Bibb, M. J., Ward, J. M. and Hopwood, O. A., Nature, 274, 398 (1978); Hinnen, A., Hicks, J. B. and Fink, G. R., Proc. Natl. Sci. USA, 75, 1929 (1978)).

Increase of the copy number of the tolC gene can also be achieved by introducing multiple copies of the tolC gene into a genomic DNA of a bacterium. In order to introduce multiple copies of the tolC gene into a genomic DNA of a bacterium, homologous recombination is carried out by using a sequence whose multiple copies are present in the genomic DNA as targets. Sequences whose multiple copies are present in genomic DNA can be used, such as repetitive DNA, and inverted repeats existing at the end of a transposable element. Another tolC gene can be introduced beside the tolC gene existing on a genome in tandem, or it can be introduced into an unnecessary gene on a genome in a plural number. Such gene transfer can be attained by using a temperature sensitive vector or an integration vector.

Alternatively, as disclosed in Japanese Patent Laid-open No. 2-109985, it is also possible to incorporate the tolC gene into a transposon, and allow it to transfer to introduce multiple copies of the genes into a genomic DNA. Transfer of the gene to the genome can be confirmed by performing Southern hybridization using a part of the tolC gene as a probe.

Furthermore, in addition to the aforementioned increase of the gene copy number, expression of the tolC gene can also be enhanced by replacing an expression control sequence such as a promoter of the tolC gene on a genome DNA or plasmid with a stronger one, by making the -35 and -10 regions of the gene closer to the consensus sequence, by amplifying a regulator that increases expression of the tolC gene, or by deleting or attenuating a regulator that decreases expression of the tolC gene according to the methods described in International Patent Publication WO00/18935. For example, the lac promoter, trp promoter, trc promoter, tac promoter, araBA promoter, lambda phage PR promoter and PL promoter, tet promoter, T7 promoter,  $\Phi$ 10 promoter, and so forth, are known as strong promoters. Furthermore, the promoter of the threonine operon of *E. coli* can also be used. A promoter or SD region of the tolC gene can also be modified so as to become stronger by introducing a nucleotide substitution or the like. Examples of methods for evaluating strength of a promoter and strong promoters are described in the paper of Goldstein et al. (Prokaryotic promoters in biotechnology, Biotechnol. Annu. Rev., 1, 105-128 (1995)), and so forth. Additionally, it is known that substitution of several nucleotides in a spacer between the ribosome-binding site (RBS) and the translation initiation codon, especially a sequence immediately upstream



from the initiation codon, greatly affects mRNA translation efficiency, and therefore, this sequence can be modified. Expression control regions such as the promoter of the *tolC* gene can also be identified by using a promoter probe vector or gene analysis software such as GENETYX. By such substitution or modification of the promoter as described above, expression of the *tolC* gene is enhanced. Substitution of an expression control sequence can also be attained, for example, by a method using a temperature sensitive plasmid or Red-driven integration (WO2005/010175).

The nucleotide sequence of the *tolC* gene of *Escherichia coli* and the amino acid sequence encoded by this gene are shown in SEQ ID NOS: 1 and 2, respectively.

Since the nucleotide sequence of the *tolC* gene can be different depending on the species or strain of the bacterium, the *tolC* gene to be modified can be a variant of the nucleotide sequence of SEQ ID NO: 1. Homologues of *TolC* are known for many bacteria, and can be found by a search of databases. When proteins highly homologous to the *TolC* protein of the *E. coli* K-12 strain are searched for on the basis of sequence information, the search can be performed, for example, as a BLAST search ([www.ncbi.nlm.nih.gov/blast/Blast.cgi](http://www.ncbi.nlm.nih.gov/blast/Blast.cgi)). Furthermore, when homologues are searched for with a keyword, if the search engine of Entrez ([www.ncbi.nlm.nih.gov/sites/gquery](http://www.ncbi.nlm.nih.gov/sites/gquery)) is used, and a term "tolC" or "outer membrane channel protein" is entered as a keyword, for example, candidate sequences are retrieved from plural databases. By scrutinizing these candidates, objective homologue sequences can be found. Nucleotide sequences of genes and amino acid sequences of *TolC* homologues of the following bacteria are shown in SEQ ID NOS: 11 to 30, as among the many *TolC* homologues found by such a method. The accession numbers in the NCBI (National Center for Biotechnology Information) database and identity (%) with respect to the amino acid sequence of SEQ ID NO: 2 are shown in the parentheses.

*Shigella boydii* Sb227 (NCBI accession: YP\_409239, 99%)  
*Shigella flexneri* 2a str. 2457T (NCBI accession: NP\_838556, Identity: 99%)

*Salmonella enterica* subsp. *enterica* serovar *Typhi* Ty2 (NCBI accession: NP\_806790, 89%)

*Citrobacter koseri* ATCC BAA-895 (NCBI accession: YP\_001455919, 89%)

*Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 (NCBI accession: YP\_001337075, 83%)

*Enterobacter sakazakii* ATCC BAA-894 (NCBI accession: YP\_001436507, 80%)

*Erwinia carotovora* subsp. *atroseptica* SCRI1043 (NCBI accession: YP\_048456, 76%)

*Serratia proteamaculans* 568 (NCBI accession: YP\_001480490, 73%)

*Aeromonas salmonicida* subsp. *salmonicida* A449 (NCBI accession: ABO88689, 51%)

*Vibrio vulnificus* YJ016 (NCBI accession: NP\_933376, 45%)

The *tolC* gene can also be a gene encoding a protein having a sequence corresponding to the amino acid sequence of the aforementioned *TolC* protein or *TolC* homologue, but which includes substitutions, deletions, insertions, additions, or the like, of one or several amino acid residues at one or several positions. Although the number of the "one or several" amino acid residues can differ depending on their position in the three-dimensional structure or the types of amino acid residues of the proteins, specifically, for example, it can be 1 to 20, 1 to 10, or 1 to 5. These substitutions, deletions, insertions, or additions of one or several amino acid residues can be conservative mutations so as to preserve the normal function of the protein. Typical examples of the conservative muta-

tions are conservative substitutions. Conservative substitutions include a mutation wherein substitution takes place mutually among Phe, Trp and Tyr, if the substitution site is an aromatic amino acid; among Leu, Ile and Val, if the substitution site is a hydrophobic amino acid; between Gln and Asn, if it is a polar amino acid; among Lys, Arg and His, if it is a basic amino acid; between Asp and Glu, if it is an acidic amino acid; and between Ser and Thr, if it is an amino acid having a hydroxyl group. Specific examples of conservative substitutions include: substitution of Ser or Thr for Ala; substitution of Gln, His or Lys for Arg; substitution of Glu, Gln, Lys, His or Asp for Asn; substitution of Asn, Glu or Gln for Asp; substitution of Ser or Ala for Cys; substitution of Asn, Glu, Lys, His, Asp or Arg for Gln; substitution of Gly, Asn, Gln, Lys or Asp for Glu; substitution of Pro for Gly; substitution of Asn, Lys, Gln, Arg or Tyr for His; substitution of Leu, Met, Val or Phe for Ile; substitution of Ile, Met, Val or Phe for Leu; substitution of Asn, Glu, Gln, His or Arg for Lys; substitution of Ile, Leu, Val or Phe for Met; substitution of Trp, Tyr, Met, Ile or Leu for Phe; substitution of Thr or Ala for Ser; substitution of Ser or Ala for Thr; substitution of Phe or Tyr for Trp; substitution of His, Phe or Trp for Tyr; and substitution of Met, Ile or Leu for Val. The above-mentioned amino acid substitution, deletion, insertion, addition, inversion, etc., can be a result of a naturally occurring mutation or variation due to an individual difference, or a difference of species of a microorganism as an origin of gene (mutant or variant).

Furthermore, the gene having such a conservative mutation as mentioned above can be a gene encoding a protein showing a homology, for example, of 80% or more, 90% or more, 95% or more, 97% or more, 98% or more, or 99% or more, to the entire encoded amino acid sequence, and having a function equivalent to that of a wild-type *TolC* protein.

The *tolC* gene can be a DNA which hybridizes with a probe prepared from known gene sequences, for example, the aforementioned nucleotide sequence, or sequences complementary to the sequences under stringent conditions and which encodes a protein which is a functional equivalent to the *TolC* protein. The term "stringent conditions" refers to conditions where a so-called specific hybrid is formed and a non-specific hybrid is not formed. Examples thereof include conditions where DNAs having high a homology, for example, of 80% or more, 90% or more, 95% or more, 97% or more, 98% or more, or 99% or more, hybridize with each other and DNAs having a homology less than the value do not hybridize with each other; and specifically include conditions corresponding to a salt concentration and temperature of washing which are typical of Southern hybridization, that is, washing once or 2 or 3 times, at a salt concentration and temperature corresponding to 1×SSC, 0.1% SDS at 60° C., 0.1×SSC, 0.1% SDS at 60° C., or 0.1×SSC, 0.1% SDS at 68° C., for example.

The probe can be a partial sequence of a complementary sequence of the gene. Such a probe can be prepared by PCR using oligonucleotides prepared based on the known nucleotide sequences of genes as primers, and a DNA fragment containing these nucleotide sequences as the template. When a DNA fragment of a length of about 300 bp is used as the probe, the conditions of washing after hybridization can be, for example, 50° C., 2×SSC, and 0.1% SDS.

The above descriptions about variants of genes and proteins are similarly applied to enzymes such as serine acetyltransferase and cysteine desulfhydrase, the YdeD protein, and the genes that code for them.

<2> Method for Producing L-Cysteine, L-Cystine, Derivatives or Precursors Thereof or Mixture Thereof

These compounds can be produced by culturing the bacterium obtained as described above in a medium, and collecting L-cysteine, L-cystine, derivatives or precursors thereof or a mixture thereof from the medium. Examples of a derivative or precursor of L-cysteine include S-sulfocysteine, a thiazolidine derivative, a hemithioacetal corresponding to the thiazolidine derivative mentioned above, O-acetylserine, N-acetylserine, and so forth.

Examples of the medium used for the culture can include ordinary media containing a carbon source, nitrogen source, sulfur source, inorganic ions, and other organic components as required.

As the carbon source, saccharides such as glucose, fructose, sucrose, molasses and starch hydrolysate, and organic acids such as fumaric acid, citric acid and succinic acid can be used.

As the nitrogen source, inorganic ammonium salts such as ammonium sulfate, ammonium chloride and ammonium phosphate, organic nitrogen such as soybean hydrolysate, ammonia gas, aqueous ammonia and so forth can be used.

As the sulfur source, inorganic sulfur compounds, such as sulfates, sulfites, sulfides, hyposulfites and thiosulfates can be examples.

As organic trace amount nutrients, required substances such as vitamin B<sub>1</sub>, yeast extract and so forth can be added in appropriate amounts. Other than these, potassium phosphate, magnesium sulfate, iron ions, manganese ions and so forth are added in small amounts.

The culture can be performed under aerobic conditions for 30 to 90 hours. The culture temperature can be controlled to be at 25° C. to 37° C., and pH can be controlled to be 5 to 8 during the culture. To adjust the pH, inorganic or organic, acidic or alkaline substances, ammonia gas, and so forth, can be used. Collection of L-cysteine from the culture can be attained by, for example, any combination of usual ion exchange resin methods, precipitation, and other known methods.

L-cysteine obtained as described above can be used to produce L-cysteine derivatives. The cysteine derivatives include methylcysteine, ethylcysteine, carbocysteine, sulfo-cysteine, acetylcysteine, and so forth.

Furthermore, when a thiazolidine derivative of L-cysteine is accumulated in the medium, L-cysteine can be produced by collecting the thiazolidine derivative from the medium to break the reaction equilibrium between the thiazolidine derivative and L-cysteine so that L-cysteine is excessively produced.

Moreover, when S-sulfocysteine is accumulated in the medium, it can be converted into L-cysteine by reduction with a reducing agent such as dithiothreitol.

As shown in the example section described later, a tolC gene-deficient strain is more sensitive to L-cysteine as compared to a non-modified strain. Furthermore, a tolC gene-deficient strain also shows sensitivity to O-acetylserine (OAS) and N-acetylserine (NAS). On the basis of these results, TolC is considered to be an outer membrane secretion factor for secreting not only L-cysteine, but also NAS and OAS. Therefore, enhancement of the TolC activity is considered to provide high production of not only L-cysteine, but also NAS and OAS.

Methods for producing OAS by fermentation are described in Japanese Patent Laid-open Nos. 11-56381 and 2002-262896. In order to increase OAS production by fermentation, a mutant SAT in which feedback inhibition is reduced can be incorporated into a bacterium, and the activity of an

inner membrane secretion pump the YdeD can be increased thereby excreting OAS from inside of the cells to outside of the cells via an inner membrane (Dabler, T. et al., Mol. Microbiol., 36, 1101-1112 (2000)). Therefore, a bacterium having a mutant SAT and showing increased activity of the YdeD protein is also suitable for production of OAS (Japanese Patent Laid-open No. 2002-262896), and such a bacterium especially constitutes an embodiment of the bacterium in accordance with the presently disclosed subject matter. A bacterium showing increased TolC activity, having a mutant SAT and showing increased activity of YdeD protein is more suitable for production of OAS. An example of such a bacterium includes the *E. coli* MG1655ΔtnaA::Km<sup>r</sup>/pCEM256I/pYdeD/pLSTolC, shown in the example section. Although a factor of the inner membrane relating to the provision of high concentration and secretion of intracellular OAS was known, any effective factor for making OAS efficiently penetrate the outer membrane and secrete it in a medium has not been known so far. This is also the same for L-cysteine. Development of an effective means for enabling efficient penetration through the outer membrane has been a common objective for L-cysteine and OAS, and it is considered that it can be achieved for both by enhancement of the TolC activity.

Since OAS is a relatively unstable compound, it can be converted into NAS by an irreversible chemical reaction during culture. Therefore, in fermentation performed under neutral or approximately neutral conditions, NAS formed from OAS by the natural reaction can also be accumulated in the medium together with OAS in an intermingled state. When OAS is mainly produced by fermentation, for example, a method of maintaining pH of the medium to be in an acidic region can be used (Japanese Patent Laid-open No. 2002-262896). Furthermore, when NAS is mainly produced, NAS can be produced from OAS by the natural reaction, by maintaining pH of the medium to be in the alkali region.

By culturing the bacterium in accordance with the presently disclosed subject matter in a medium under suitable conditions, and collecting NAS and/or OAS accumulating in the medium, NAS and/or OAS can be produced. As the medium used for the culture, such media as described above, for example, the L-cysteine production medium described in the example section and the production medium described in Japanese Patent Laid-open No. 2002-262896, can be used. A substance that promotes the intracellular reaction for converting OAS to L-cysteine, such as thiosulfuric acid, cannot be added to the medium, in order to produce more OAS. Conditions suitable for the production can be determined by measuring the quantity of NAS and/or OAS accumulated in the medium. NAS and/or OAS can be quantified by HPLC using a hydrophobic column and a UV detector, or the like. As described above, OAS can be converted into NAS during the culture or quantification. Therefore, for evaluation of fermentation result, the fermentation products can be determined as the sum of OAS and NAS by converting all OAS produced by fermentation into NAS, and measuring the amount of NAS by HPLC. In order to convert all OAS into NAS, for example, the medium can be adjusted to an alkali pH by mixing the medium with 200 mM Tris buffer (pH 9.0) (Japanese Patent Laid-open No. 2002-262896).

#### EXAMPLES

Hereinafter, the present invention will be explained more specifically with reference to the following non-limiting examples. In the following descriptions, cysteine means L-cysteine.

## (1) Screening of Clones Showing Cysteine Sensitivity

In order to comprehensively search for genes participating in cysteine resistance, the Keio collection (single gene-knock out library except for essential genes of *E. coli* BW25113, Baba, T, et al., 2006, Mol. Syst. Biol., 2:2006.0008) was screened for clones showing sensitivity to cysteine.

## (1-1) Screening of Keio Collection for Clones Showing Cysteine Sensitivity

The 3,985 clones of the Keio collection were cultured at 37° C. for 15 hours in 0.5 ml of LB liquid medium. This culture medium was stamped on LB agar media containing cysteine at different concentrations (0, 15, 20, 25 mM), and culture was performed overnight at 37° C. Clones that were sensitive to cysteine at a concentration not higher than the growth inhibition concentration of cysteine for wild-type strains (20 mM) were visually selected. Specifically, clones that did not form colonies on the LB plate containing 15 mM cysteine were selected as candidates. A strain which is deficient in a part of or the entire *tolC* gene was obtained as a clone showing particularly strong and distinctive cysteine sensitivity among the above candidates. TolC is an example of a protein called a porin, which localizes in the outer membrane and forms a channel for substance transportation via the outer membrane. Although the presence of many other porins was known for *E. coli*, TolC was the only porin selected by this screening, among the several candidates considered showing strong cysteine sensitivity.

Strains deficient in *OmpA*, *OmpC*, *OmpF*, *OmpG*, *OmpN*, *OmpT*, *OmpX*, *LamB* or *BtuB*, which are also known examples of porins, did not show cysteine sensitivity at all. Since cysteine is a highly toxic amino acid, a possibility is estimated that TolC can promote transportation (secretion) of cysteine and cysteine-related substances, and thereby cysteine resistance is acquired. Most of the factors known so far to participate in transportation of cysteine and cysteine-related substances, *YdeD* (Dassler, T. et al., Mol. Microbiol., 2000; 36:1101-1112), *YfiK* (Franke, I. et al., J. Bacteriol., 2003; 185:1161-1166), *CydDC* (Pittman, Marc S. et al., J. Biol. Chem., December 2002; 277:49841-49849), and multidrug efflux pump (Yamada, S., et al., Appl. Environ. Microbiol., July 2006; 72:4735-4742), are factors of the inner membrane, and it was known that a secretion factor was required for penetration of the inner membrane. However, it is not known whether a porin (outer membrane channel), such as TolC, is required for penetration of a low molecule amino acid, such as cysteine, through the outer membrane. Moreover, it was an unexpected result that only TolC was particularly selected as a candidate by the screening among many porins, and a possible explanation was because only TolC was a central factor of the transportation of cysteine.

(1-2) Cysteine Sensitivity Induced by *tolC* Gene Deficiency

Since a strain which is deficient in a part of or the entire *tolC* gene was obtained by the screening of the Keio Collection, growth of the gene-deficient strain was observed on the agar medium containing cysteine of different concentrations in order to analyze the sensitivity of that strain to cysteine in more detail. The strain which is deficient in a part of or the entire *tolC* gene used here was the JW5503 strain (Keio collection), and the parent strain thereof was the BW25113 strain (Andreas Haldimann, A. and Wanner, B. L., J. Bacteriol., 2001 November; 183 (21):6384-6393). The plasmid carrying the *tolC* gene for a complementation experiment was pTolC (ASKA clone, Kitagawa, M, et al., 2005; DNA Res., 12:291-299), and the vector used as the base thereof was pCA24 (vector for ASKA clone, Kitagawa, M, et al., 2005, DNA Res., 12:291-299).

The bacteria containing each of the plasmids were each inoculated into 5 ml of L medium (10 g/L of Bacto trypton, 5 g/L of Bacto yeast extract, 5 g/L of NaCl), and cultured overnight at 37° C. The culture was serially diluted 10 times for every dilution with 0.9% physiological saline to prepare serially diluted cell suspensions ( $10^{-2}$  to  $10^{-6}$ ), and the cell suspensions were spotted (5  $\mu$ l) onto L agar medium (10 g/L of Bacto trypton, 5 g/L of Bacto yeast extract, 5 g/L of NaCl, 15 g/L of agar) containing various concentrations (10, 15, 20 mM) of cysteine. Culture was performed at 37° C. overnight, and a growth test of the strain which is deficient in a part of or the entire *tolC* gene in the cysteine medium, and a complementation (recovery of growth) test with the *tolC* plasmid were performed. The results are shown in FIG. 1. The strain which is deficient in a part of or the entire *tolC* gene JW5503/pCA24 showed marked cysteine sensitivity as compared with the control strain BW25113/pCA24, and when the *tolC* gene was introduced as a plasmid (JW5503/pTolC strain), the strain recovered from the sensitivity. Therefore, it was found that TolC was involved in the cysteine resistance.

(1-3) Sensitivity to N-Acetylserine (NAS) and O-Acetylserine (OAS) Induced by *tolC* Gene Deficiency

Influence of a *tolC* gene deficiency on N-acetylserine (NAS) and O-acetylserine (OAS) sensitivity was investigated in a strain which is deficient in a part of or the entire *tolC* gene by the cross streak method. In order to compare growth inhibition by NAS (2 M), OAS (2 M), L-cysteine (2 M) and L-serine (1 M), the *tolC*-deficient JW5503 strain, and the wild-type BW25113 strain used as a control, were cultured overnight in the L liquid medium, and each culture medium was streaked on the L agar medium with a platinum loop. A strip-shaped filter paper onto which each of the aforementioned reagents was dropped, was placed on each of the strains in a direction perpendicular to the streaking direction, and the strains were cultured overnight at 30° C. After the culture, lengths of the filter paper on which growth of the bacteria was inhibited (antibacterial widths) were measured, and the antibacterial activities of the reagents on both the strains were compared. The results are shown in FIG. 2. The antibacterial widths are shown in Table 1.

It was found that the strain which is deficient in a part of or the entire *tolC* gene showed sensitivity to L-cysteine as described above. Also in this experiment, a larger antibacterial width was seen for the strain which is deficient in a part of or the entire *tolC* gene as compared to the wild-type strain, and sensitivity of the strain which is deficient in a part of or the entire *tolC* gene to L-cysteine was observed. The strain which is deficient in a part of or the entire *tolC* gene similarly showed large antibacterial widths for N-acetylserine (NAS) and O-acetylserine (OAS), and it became clear that it showed sensitivity to these substances.

TABLE 1

	Growth inhibition width (mm)	
	BW25113	BW25113 $\Delta$ tolC
L-Cysteine (2 M)	<1	5
O-Acetylserine (2 M)	<1	4
N-Acetylserine (2 M)	<2	10
L-Serine (1 M)	0	0

(2) Construction of Cysteine-Producing Bacterium (*E. coli* MG1655tnaA::Km<sup>r</sup>pCEM2561/pYdeD)

A strain in which a tryptophanase gene was deleted, a mutant SAT gene was contained, and *ydeD* gene expression was enhanced was constructed from the *E. coli* MG1655 strain.

(2-1) Construction of Strain which is Deficient in a Part of or the Entire *tnaA* Gene of *E. coli* MG1655

A strain which is deficient in a part of or the entire *tnaA* gene of *E. coli* MG1655 was constructed by transducing *tnaA::Km<sup>r</sup>* of the *E. coli* JW3686 strain (Keio collection) into the MG1655 strain (ATCC No. 47076) using the P1kc phage. Preparation of a phage suspension and transduction were carried out as follows according to the method of Miller et al. (Miller, J. H., Experiments in molecular genetics, Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory; 1972, Generalized transduction: use of P1 in strain construction; pp. 201-205).

The JW3686 strain was cultured overnight at 37° C. in 3 ml of the L medium. To 3 ml of soft agar (0.5% agar), 100 µl of the culture medium, 100 µl of P1kc phage suspension and 100 µl of CaCl<sub>2</sub> (100 mM) were added, and the mixture was overlaid on the L medium containing 2.5 mM CaCl<sub>2</sub>. After the soft agar solidified, culture was performed overnight at 37° C. On the soft agar on which plaques appeared, 2 ml of the L medium was added, the agar was disrupted, and the grown P1kc phages were collected. Chloroform was added in a volume of 100 µl to this L medium, they were mildly mixed, and the mixture was left standing at room temperature for 15 minutes. The cells and the soft agar were removed by centrifugation (4° C., 2,000xg, 5 minutes), and the supernatant was collected as a phage suspension. The *E. coli* MG1655 strain was cultured overnight at 37° C. in 3 ml of the L medium, and the culture was used as a preculture suspension of the recipient. The preculture suspension was inoculated in an amount of 1% into the L medium containing 5 mM CaCl<sub>2</sub>, and culture was carried out at 37° C. with shaking until the OD<sub>660</sub> became 0.5. To this culture medium in a volume of 150 µl, an equivalent volume of the phage suspension diluted so that m.o.i. was 0.1 to 0.01 was added, and the mixture was kept at 37° C. for 30 minutes. After the phage particles were adsorbed, 100 µl of trisodium citrate solution (1 M) was added, and the mixture was kept at 37° C. for 60 minutes. The mixture was applied in a volume of 0.2 ml each to a selection medium, and culture was performed overnight at 37° C. The formed colonies were obtained as transductants. Transduction of the *tnaA::Km<sup>r</sup>* gene at the target position was confirmed by PCR and activity staining.

(2-2) Construction of Plasmid pCEM256I Carrying Feedback Inhibition-Resistant Mutant SAT Gene

A plasmid having the same structure as that of pCEM256I described in literatures (Japanese Patent Laid-open No. 11-155571, Nakamori, S, et al., Appl. Environ. Microbiol., 1998, 64, 1607-1611) was used as a plasmid carrying a mutant SAT gene. pCEM256I had a mutant SAT gene obtained by introducing a mutation into the wild-type SAT gene (*cysE*) of *E. coli*. This mutant SAT gene includes substitution of isoleucine for the methionine at the 256-position, and shows resistance to the feedback inhibition by cysteine because of that mutation (Japanese Patent Laid-open No. 11-155571). Specifically, pCEM256I was obtained as follows.

In order to isolate the *cysE* gene including the promoter region and the terminator region, PCR was performed by using the chromosome of *E. coli* JM240 as a template, as well as a sense primer (5'-GGGAATTCATCGCTTCGGCGT-TGAAA-3', Primer 1, SEQ ID NO: 3) and an antisense primer (5'-GGCTCTAGAAGCGGTATTGAGAGAGATTA-3', Primer 2, SEQ ID NO: 4), which were prepared on the basis of the sequence of the *cysE* gene (coding for SAT) determined by Denk et al. (Denk, D. and Bock, A. J., General Microbiol., 133, 515-525 (1987)). PCR was performed by repeating a cycle consisting of reactions at 94° C. for 1 minute, 55° C. for

1 minute and 72° C. for 3 minutes, 25 times using DNA Thermal Cycler 480 (Perkin Elmer Co.) and Ex Taq polymerase. The specifically amplified DNA fragment of about 1.2 kb was ligated to the plasmid vector pBluscriptII SK<sup>+</sup> treated with EcoRV by a TA cloning technique to obtain pCE. It was confirmed by sequencing that the region amplified by PCR was the same as that of the wild-type.

Site-specific mutagenesis of the *cysE* gene was performed as follows. By using 5'-CAGGAAACAGCTATGAC-3' (Primer 3, SEQ ID NO: 5), 5'-CTGCAATCTGTGACGCT-3' (Primer 4, SEQ ID NO: 6), 5'-AATGGATATAGACCAGC-3' (Primer 5, SEQ ID NO: 7), and 5'-GCTGGTCTATATC-CATT-3' (Primer 6, SEQ ID NO: 8), isoleucine was substituted for the methionine residue at the 256th position of SAT. Primer 3 and Primer 4 were designed so that they are complementary to the 140 bp upstream region from the PstI site of the plasmid pCE, and the 50 bp downstream region from the BstEII site of the same, respectively. Primer 4 and Primer 5 were used as primers for site-specific mutagenesis. First, PCR was performed in separate tubes by using pCE as a template and Primer 3 and Primer 5, and Primer 4 and Primer 6, respectively. The obtained PCR products were subjected to agarose gel electrophoresis, and then collected from the gel. PCR was performed again by using the collected DNA fragments of 270 bp and 250 bp as templates, as well as Primer 3 and Primer 4. After the second PCR, the amplified DNA fragment of 500 bp was treated with the restriction enzymes PstI and BstEII, the obtained fragment of 310 bp was ligated with the large fragment of pCE similarly treated with the restriction enzymes to obtain pCEM256I. It was confirmed by sequencing that the intended mutation had been introduced. It was also confirmed that the other region was the same as that of the wild-type.

(2-3) Cloning of the *ydeD* Gene (Construction of Plasmid pYdeD for Enhancement of *ydeD* Gene)

*E. coli* *ydeD* gene coding for the cysteine secretion pump was cloned as follows. First, PCR was performed by using the genomic DNA of the *E. coli* MG1655 strain (ATCC No. 47076) as a template, a sense primer (5'-CGCGGATC-CAATGGTCATAAATGGCAGCGTAGCGC-3', Primer 7, SEQ ID NO: 9) and an antisense primer (5'-CGCGGATCCG-CAGGGCGTTGCGGAACAAAC-3', Primer 8, SEQ ID NO: 10). PCR was performed by using Pyrobest DNA polymerase (Takara) according to the protocol attached to the polymerase to obtain a *ydeD* gene fragment of about 1.5 kb including a region of about 300 bp upstream from the *ydeD* gene and a region of about 200 bp downstream from the *ydeD* gene. The BamHI site was designed in both the primers. The PCR fragment was digested with BamHI, and then inserted into the pSTV29 (Takara) at the BamHI site, and the obtained plasmid, in which the *ydeD* gene fragment was inserted in the same direction as the *lacZ* gene on the pSTV29 vector, was designated plasmid pYdeD. The portion amplified by PCR was sequenced to confirm that it did not contain PCR error.

(2-4) Construction of Cysteine-Producing Bacterium, MG1655Δ*tnaA::Km<sup>r</sup>*/pCEM256I/pYdeD Strain

pCEM256I and pYdeD were introduced into the MG1655Δ*tnaA::Km<sup>r</sup>* strain in a conventional manner to construct a cysteine-producing bacterium MG1655Δ*tnaA::Km<sup>r</sup>*/pCEM256I/pYdeD strain, in which the mutant SAT and cysteine secretion pump YdeD were enhanced, and the cysteine decomposition system, *TnaA*, was deleted.

(3) Construction of Cysteine-Producing Bacterium in which TolC is Enhanced

In order to investigate the effect of enhancement of the *tolC* gene in a cysteine-producing bacterium, a plasmid for

enhancement of the *tolC* gene was constructed, and introduced into the aforementioned cysteine-producing bacterium.

### (3-1) Construction of Plasmid pLSToIC for Enhancement of *TolC*

First, the plasmid vector pMW219 (3,923 bp, NIPPON GENE) was digested with *Cl*I, and the 5' end was blunt-ended by using T4 DNA polymerase. Then, the kanamycin resistance gene of about 0.6 kb was excised with *Eco*T14I, and a large fragment of 3.2 kbp was collected. Then, the plasmid pFW5 (2,726 bp, Podbielski, A., et al., Gene, 1996, 177, 137-147) was digested with *Hind*III, then the 5' end was blunt-ended, and then the *aad9* gene (spectinomycin resistance gene) of 1.2 kb was collected with *Eco*T14I. The plasmid constructed by ligating both the recovered fragments was designated pLS219 (4,444 bp). The *tolC* gene including the promoter region and the terminator region (2.6 kbp) was excised from the plasmid pUX (5208 bp, Aono, R., et al., J. Bacteriol., 1998, 180, 938-944) with *Hind*III and *Eco*RI. This excised *tolC* gene fragment was ligated to pLS219 at the *Hind*III-*Eco*RI site in the multi-cloning site (pLSToIC, 6,966 bp).

### (3-2) Construction of *TolC*-Enhanced Cysteine-Producing Bacterium, *E. coli* MG1655Δ*tnaA*::*Km*<sup>r</sup>/pCEM256I/pYdeD/pLSToIC

pLSToI was introduced into the cysteine-producing bacterium, MG1655Δ*tnaA*::*Km*<sup>r</sup>/pCEM256I/pYdeD, to construct the MG1655Δ*tnaA*::*Km*<sup>r</sup>/pCEM256I/pYdeD/pLSToIC strain. The transformation was performed by electroporation in a conventional manner.

### (4) Production of Cysteine by *TolC*-Enhanced Cysteine-Producing Bacterium

The *TolC*-enhanced cysteine-producing bacterium (*E. coli* MG1655 Δ*tnaA*::*Km*<sup>r</sup>/pCEM256I/pYdeD/pLSToIC) and a control strain in which *TolC* was not enhanced (*E. coli* MG1655Δ*tnaA*::*Km*<sup>r</sup>/pCEM256I/pYdeD) were each inoculated into 5 ml of the L medium (chloramphenicol (40 μg/mL), kanamycin (50 μg/mL) and ampicillin (50 μg/mL) were added, and spectinomycin (100 μg/mL) was further added for the strain having pLSToIC), and cultured overnight at 37° C. (preculture). Each cell suspension of the overnight culture was taken in a volume of 250 μl, and added to 25 ml of fresh medium (SM1+10% L medium), and culture was performed at 37° C. with shaking at 140 rpm. The culture medium was taken after 0, 3, 6, 9, 14 and 25 hours of the culture, and the cell number (OD<sub>660</sub>) and the amount of produced cysteine were investigated. The composition of the SM1 medium used for the culture was as follows: 0.1 M KH<sub>2</sub>PO<sub>4</sub>—K<sub>2</sub>HPO<sub>4</sub> buffer (pH 7.0), 30 g/L of glucose, 10 g/L of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.1 g/L of NaCl, 7.2 μM FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.6 μM Na<sub>2</sub>MoO<sub>4</sub>, 40.4 μM H<sub>3</sub>BO<sub>3</sub>, 2.9 μM CoCl<sub>2</sub>, 1 μM CuSO<sub>4</sub>, 8.1 μM MnCl<sub>2</sub>, 1 mM MgSO<sub>4</sub>, and 0.1 mM CaCl<sub>2</sub> (Dassler, T., et al., Mol. Microbiol., 2000, 36, 1101-1112). The SM1+10% L medium was obtained by adding L medium components of 1/10 concentrations to the above SM1 medium.

Cysteine, cystine and cysteine-related compounds were quantified as follows according to the method of Gaitonde (Gaitonde, M. K., Biochem. J., 1967, 104, 627-633). To 100 μl of the culture medium, 200 μl of the Gaitonde reagent (250 mg of ninhydrin, 6 ml of acetic acid, 4 ml of hydrochloric acid) was added. The color developing reaction was performed at 100° C. for 5 minutes, and 400 μl of 100% ethanol was added to the mixture, and the OD<sub>560</sub> was measured. The growth curves are shown in FIG. 3, and the change in the amount of cysteine accumulated in the medium (amount quantified by the Gaitonde method) is shown in FIG. 4. It was found that the growth of the *TolC*-enhanced strain was sub-

stantially equivalent to that of the control strain, and it showed markedly increased cysteine amount. Thus, it became clear that enhancement of *TolC* had an effect of increasing cysteine production amount.

### EXPLANATION OF SEQUENCE LISTING

- SEQ ID NO: 1: Nucleotide sequence of *E. coli* *tolC* gene  
 SEQ ID NO: 2: Amino acid sequence of *E. coli* *TolC*  
 SEQ ID NOS: 3 to 10: PCR primers  
 SEQ ID NO: 11: Nucleotide sequence of *Shigella boydii* *tolC* gene homologue  
 SEQ ID NO: 12: Amino acid sequence of *Shigella boydii* *TolC* homologue  
 SEQ ID NO: 13: Nucleotide sequence of *Shigella flexneri* *tolC* gene homologue  
 SEQ ID NO: 14: Amino acid sequence of *Shigella flexneri* *TolC* homologue  
 SEQ ID NO: 15: Nucleotide sequence of *Salmonella enterica* *tolC* gene homologue  
 SEQ ID NO: 16: Amino acid sequence of *Salmonella enterica* *TolC* homologue  
 SEQ ID NO: 17: Nucleotide sequence of *Citrobacter koseri* *tolC* gene homologue  
 SEQ ID NO: 18: Amino acid sequence of *Citrobacter koseri* *TolC* homologue  
 SEQ ID NO: 19: Nucleotide sequence of *Klebsiella pneumoniae* *tolC* gene homologue  
 SEQ ID NO: 20: Amino acid sequence of *Klebsiella pneumoniae* *TolC* homologue  
 SEQ ID NO: 21: Nucleotide sequence of *Enterobacter sakazakii* *tolC* gene homologue  
 SEQ ID NO: 22: Amino acid sequence of *Enterobacter sakazakii* *TolC* homologue  
 SEQ ID NO: 23: Nucleotide sequence of *Erwinia carotovora* *tolC* gene homologue  
 SEQ ID NO: 24: Amino acid sequence of *Erwinia carotovora* *TolC* homologue  
 SEQ ID NO: 25: Nucleotide sequence of *Serratia proteamaculans* *tolC* gene homologue  
 SEQ ID NO: 26: Amino acid sequence of *Serratia proteamaculans* *TolC* homologue  
 SEQ ID NO: 27: Nucleotide sequence of *Aeromonas salmonicida* *tolC* gene homologue  
 SEQ ID NO: 28: Amino acid sequence of *Aeromonas salmonicida* *TolC* homologue  
 SEQ ID NO: 29: Nucleotide sequence of *Vibrio vulnificus* *tolC* gene homologue  
 SEQ ID NO: 30: Amino acid sequence of *Vibrio vulnificus* *TolC* homologue

### Industrial Applicability

According to the present invention, the ability of bacteria to produce L-cysteine can be improved. Moreover, according to the present invention, L-cysteine, L-cystine, derivatives and precursors thereof, or mixtures thereof can be efficiently produced.

While the invention has been described in detail with reference to preferred embodiments thereof, it will be apparent to one skilled in the art that various changes can be made, and equivalents employed, without departing from the scope of the invention. Each of the aforementioned documents is incorporated by reference herein in its entirety.

## SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 30

<210> SEQ ID NO 1

<211> LENGTH: 1482

<212> TYPE: DNA

<213> ORGANISM: Escherichia coli

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1)..(1482)

<400> SEQUENCE: 1

```

atg aag aaa ttg ctc ccc att ctt atc ggc ctg agc ctt tct ggg ttc      48
Met Lys Lys Leu Leu Pro Ile Leu Ile Gly Leu Ser Leu Ser Gly Phe
 1          5          10          15

agt tcg ttg agc cag gcc gag aac ctg atg caa gtt tat cag caa gca      96
Ser Ser Leu Ser Gln Ala Glu Asn Leu Met Gln Val Tyr Gln Gln Ala
 20          25          30

cgc ctt agt aac ccg gaa ttg cgt aag tct gcc gcc gat cgt gat gct     144
Arg Leu Ser Asn Pro Glu Leu Arg Lys Ser Ala Ala Asp Arg Asp Ala
 35          40          45

gcc ttt gaa aaa att aat gaa gcg cgc agt cca tta ctg cca cag cta     192
Ala Phe Glu Lys Ile Asn Glu Ala Arg Ser Pro Leu Leu Pro Gln Leu
 50          55          60

ggg tta ggt gca gat tac acc tat agc aac ggc tac cgc gac gcg aac     240
Gly Leu Gly Ala Asp Tyr Thr Tyr Ser Asn Gly Tyr Arg Asp Ala Asn
 65          70          75          80

ggc atc aac tct aac gcg acc agt gcg tcc ttg cag tta act caa tcc     288
Gly Ile Asn Ser Asn Ala Thr Ser Ala Ser Leu Gln Leu Thr Gln Ser
 85          90          95

att ttt gat atg tcg aaa tgg cgt gcg tta acg ctg cag gaa aaa gca     336
Ile Phe Asp Met Ser Lys Trp Arg Ala Leu Thr Leu Gln Glu Lys Ala
100         105         110

gca ggg att cag gac gtc acg tat cag acc gat cag caa acc ttg atc     384
Ala Gly Ile Gln Asp Val Thr Tyr Gln Thr Asp Gln Gln Thr Leu Ile
115         120         125

ctc aac acc gcg acc gct tat ttc aac gtg ttg aat gct att gac gtt     432
Leu Asn Thr Ala Thr Ala Tyr Phe Asn Val Leu Asn Ala Ile Asp Val
130         135         140

ctt tcc tat aca cag gca caa aaa gaa gcg atc tac cgt caa tta gat     480
Leu Ser Tyr Thr Gln Ala Gln Lys Glu Ala Ile Tyr Arg Gln Leu Asp
145         150         155         160

caa acc acc caa cgt ttt aac gtg ggc ctg gta gcg atc acc gac gtg     528
Gln Thr Thr Gln Arg Phe Asn Val Gly Leu Val Ala Ile Thr Asp Val
165         170         175

cag aac gcc cgc gca cag tac gat acc gtg ctg gcg aac gaa gtg acc     576
Gln Asn Ala Arg Ala Gln Tyr Asp Thr Val Leu Ala Asn Glu Val Thr
180         185         190

gca cgt aat aac ctt gat aac gcg gta gag cag ctg cgc cag atc acc     624
Ala Arg Asn Asn Leu Asp Asn Ala Val Glu Gln Leu Arg Gln Ile Thr
195         200         205

ggg aac tac tat ccg gaa ctg gct gcg ctg aat gtc gaa aac ttt aaa     672
Gly Asn Tyr Tyr Pro Glu Leu Ala Ala Leu Asn Val Glu Asn Phe Lys
210         215         220

acc gac aaa cca cag ccg gtt aac gcg ctg ctg aaa gaa gcc gaa aaa     720
Thr Asp Lys Pro Gln Pro Val Asn Ala Leu Leu Lys Glu Ala Glu Lys
225         230         235         240

cgc aac ctg tcg ctg tta cag gca cgc ttg agc cag gac ctg gcg cgc     768
Arg Asn Leu Ser Leu Leu Gln Ala Arg Leu Ser Gln Asp Leu Ala Arg
245         250         255

gag caa att cgc cag gcg cag gat ggt cac tta ccg act ctg gat tta     816

```

-continued

Glu	Gln	Ile	Arg	Gln	Ala	Gln	Asp	Gly	His	Leu	Pro	Thr	Leu	Asp	Leu		
			260					265					270				
acg	gct	tct	acc	ggg	att	tct	gac	acc	tct	tat	agc	ggg	tcg	aaa	acc		864
Thr	Ala	Ser	Thr	Gly	Ile	Ser	Asp	Thr	Ser	Tyr	Ser	Gly	Ser	Lys	Thr		
			275				280					285					
cgt	ggg	gcc	gct	ggg	acc	cag	tat	gac	gat	agc	aat	atg	ggc	cag	aac		912
Arg	Gly	Ala	Ala	Gly	Thr	Gln	Tyr	Asp	Asp	Ser	Asn	Met	Gly	Gln	Asn		
	290					295					300						
aaa	ggt	ggc	ctg	agc	ttc	tcg	ctg	ccg	att	tat	cag	ggc	gga	atg	ggt		960
Lys	Val	Gly	Leu	Ser	Phe	Ser	Leu	Pro	Ile	Tyr	Gln	Gly	Gly	Met	Val		
	305				310					315					320		
aac	tcg	cag	gtg	aaa	cag	gca	cag	tac	aac	ttt	gtc	ggg	gcc	agc	gag		1008
Asn	Ser	Gln	Val	Lys	Gln	Ala	Gln	Tyr	Asn	Phe	Val	Gly	Ala	Ser	Glu		
				325						330					335		
caa	ctg	gaa	agt	gcc	cat	cgt	agc	gtc	gtg	cag	acc	gtg	cgt	tcc	tcc		1056
Gln	Leu	Glu	Ser	Ala	His	Arg	Ser	Val	Val	Gln	Thr	Val	Arg	Ser	Ser		
				340				345							350		
ttc	aac	aac	att	aat	gca	tct	atc	agt	agc	att	aac	gcc	tac	aaa	caa		1104
Phe	Asn	Asn	Ile	Asn	Ala	Ser	Ile	Ser	Ser	Ile	Asn	Ala	Tyr	Lys	Gln		
				355				360					365				
gcc	gta	ggt	tcc	gct	caa	agc	tca	tta	gac	gcg	atg	gaa	gcg	ggc	tac		1152
Ala	Val	Val	Ser	Ala	Gln	Ser	Ser	Leu	Asp	Ala	Met	Glu	Ala	Gly	Tyr		
				370			375				380						
tcg	gtc	ggg	acg	cgt	acc	att	ggt	gat	gtg	ttg	gat	gcg	acc	acc	acg		1200
Ser	Val	Gly	Thr	Arg	Thr	Ile	Val	Asp	Val	Leu	Asp	Ala	Thr	Thr	Thr		
						390					395				400		
ttg	tac	aac	gcc	aag	caa	gag	ctg	gcg	aat	gcg	cgt	tat	aac	tac	ctg		1248
Leu	Tyr	Asn	Ala	Lys	Gln	Glu	Leu	Ala	Asn	Ala	Arg	Tyr	Asn	Tyr	Leu		
				405						410					415		
att	aat	cag	ctg	aat	att	aag	tca	gct	ctg	ggg	acg	ttg	aac	gag	cag		1296
Ile	Asn	Gln	Leu	Asn	Ile	Lys	Ser	Ala	Leu	Gly	Thr	Leu	Asn	Glu	Gln		
				420				425							430		
gat	ctg	ctg	gca	ctg	aac	aat	gcg	ctg	agc	aaa	ccg	ggt	tcc	act	aat		1344
Asp	Leu	Leu	Ala	Leu	Asn	Asn	Ala	Leu	Ser	Lys	Pro	Val	Ser	Thr	Asn		
				435				440							445		
ccg	gaa	aac	ggt	gca	ccg	caa	acg	ccg	gaa	cag	aat	gct	att	gct	gat		1392
Pro	Glu	Asn	Val	Ala	Pro	Gln	Thr	Pro	Glu	Gln	Asn	Ala	Ile	Ala	Asp		
				450			455								460		
ggg	tat	gcg	cct	gat	agc	ccg	gca	cca	gtc	ggt	cag	caa	aca	tcc	gca		1440
Gly	Tyr	Ala	Pro	Asp	Ser	Pro	Ala	Pro	Val	Val	Gln	Gln	Thr	Ser	Ala		
				465		470					475				480		
cgc	act	acc	acc	agt	aac	ggg	cat	aac	cct	ttc	cgt	aac	tga				1482
Arg	Thr	Thr	Thr	Ser	Asn	Gly	His	Asn	Pro	Phe	Arg	Asn					
				485						490							

&lt;210&gt; SEQ ID NO 2

&lt;211&gt; LENGTH: 493

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Escherichia coli

&lt;400&gt; SEQUENCE: 2

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

-continued

65	70	75	80
Gly Ile Asn Ser Asn Ala Thr Ser Ala Ser Leu Gln Leu Thr Gln Ser	85	90	95
Ile Phe Asp Met Ser Lys Trp Arg Ala Leu Thr Leu Gln Glu Lys Ala	100	105	110
Ala Gly Ile Gln Asp Val Thr Tyr Gln Thr Asp Gln Gln Thr Leu Ile	115	120	125
Leu Asn Thr Ala Thr Ala Tyr Phe Asn Val Leu Asn Ala Ile Asp Val	130	135	140
Leu Ser Tyr Thr Gln Ala Gln Lys Glu Ala Ile Tyr Arg Gln Leu Asp	145	150	160
Gln Thr Thr Gln Arg Phe Asn Val Gly Leu Val Ala Ile Thr Asp Val	165	170	175
Gln Asn Ala Arg Ala Gln Tyr Asp Thr Val Leu Ala Asn Glu Val Thr	180	185	190
Ala Arg Asn Asn Leu Asp Asn Ala Val Glu Gln Leu Arg Gln Ile Thr	195	200	205
Gly Asn Tyr Tyr Pro Glu Leu Ala Ala Leu Asn Val Glu Asn Phe Lys	210	215	220
Thr Asp Lys Pro Gln Pro Val Asn Ala Leu Leu Lys Glu Ala Glu Lys	225	230	240
Arg Asn Leu Ser Leu Gln Ala Arg Leu Ser Gln Asp Leu Ala Arg	245	250	255
Glu Gln Ile Arg Gln Ala Gln Asp Gly His Leu Pro Thr Leu Asp Leu	260	265	270
Thr Ala Ser Thr Gly Ile Ser Asp Thr Ser Tyr Ser Gly Ser Lys Thr	275	280	285
Arg Gly Ala Ala Gly Thr Gln Tyr Asp Asp Ser Asn Met Gly Gln Asn	290	295	300
Lys Val Gly Leu Ser Phe Ser Leu Pro Ile Tyr Gln Gly Gly Met Val	305	310	320
Asn Ser Gln Val Lys Gln Ala Gln Tyr Asn Phe Val Gly Ala Ser Glu	325	330	335
Gln Leu Glu Ser Ala His Arg Ser Val Val Gln Thr Val Arg Ser Ser	340	345	350
Phe Asn Asn Ile Asn Ala Ser Ile Ser Ser Ile Asn Ala Tyr Lys Gln	355	360	365
Ala Val Val Ser Ala Gln Ser Ser Leu Asp Ala Met Glu Ala Gly Tyr	370	375	380
Ser Val Gly Thr Arg Thr Ile Val Asp Val Leu Asp Ala Thr Thr Thr	385	390	400
Leu Tyr Asn Ala Lys Gln Glu Leu Ala Asn Ala Arg Tyr Asn Tyr Leu	405	410	415
Ile Asn Gln Leu Asn Ile Lys Ser Ala Leu Gly Thr Leu Asn Glu Gln	420	425	430
Asp Leu Leu Ala Leu Asn Asn Ala Leu Ser Lys Pro Val Ser Thr Asn	435	440	445
Pro Glu Asn Val Ala Pro Gln Thr Pro Glu Gln Asn Ala Ile Ala Asp	450	455	460
Gly Tyr Ala Pro Asp Ser Pro Ala Pro Val Val Gln Gln Thr Ser Ala	465	470	480
Arg Thr Thr Thr Ser Asn Gly His Asn Pro Phe Arg Asn	485	490	



-continued

---

<210> SEQ ID NO 3  
<211> LENGTH: 26  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 3

gggaattcat cgcttcggcg ttgaaa 26

<210> SEQ ID NO 4  
<211> LENGTH: 29  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 4

ggctctagaa gcggtattga gagagatta 29

<210> SEQ ID NO 5  
<211> LENGTH: 17  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 5

caggaaacag ctatgac 17

<210> SEQ ID NO 6  
<211> LENGTH: 17  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 6

ctgcaatctg tgacgct 17

<210> SEQ ID NO 7  
<211> LENGTH: 17  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 7

aatggatata gaccagc 17

<210> SEQ ID NO 8  
<211> LENGTH: 17  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 8

gctggtctat atccatt 17

<210> SEQ ID NO 9  
<211> LENGTH: 35  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: primer

-continued

&lt;400&gt; SEQUENCE: 9

cgcggatcca atggtcataa atggcagcgt agcgc 35

&lt;210&gt; SEQ ID NO 10

&lt;211&gt; LENGTH: 30

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: primer

&lt;400&gt; SEQUENCE: 10

cgcggatccg cagggcgctg cggaacaaac 30

&lt;210&gt; SEQ ID NO 11

&lt;211&gt; LENGTH: 1488

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Shigella boydii

&lt;220&gt; FEATURE:

&lt;221&gt; NAME/KEY: CDS

&lt;222&gt; LOCATION: (1)..(1488)

&lt;400&gt; SEQUENCE: 11

atg caa atg aag aaa ttg ctc ccc att ctt atc ggc ctg agc ctt tct 48  
 Met Gln Met Lys Lys Leu Leu Pro Ile Leu Ile Gly Leu Ser Leu Ser  
 1 5 10 15

ggg ttc agt tcg ttg agc cag gcc gag aac ctg atg caa gtt tat cag 96  
 Gly Phe Ser Ser Leu Ser Gln Ala Glu Asn Leu Met Gln Val Tyr Gln  
 20 25 30

caa gca cgc ctt agt aac ccg gaa ttg cgt aag tct gcc gcc gat cgt 144  
 Gln Ala Arg Leu Ser Asn Pro Glu Leu Arg Lys Ser Ala Ala Asp Arg  
 35 40 45

gat gct gcc ttt gaa aaa att aat gaa gcg cgc agt cca tta ctg cca 192  
 Asp Ala Ala Phe Glu Lys Ile Asn Glu Ala Arg Ser Pro Leu Leu Pro  
 50 55 60

cag cta ggt tta ggt gca gat tac acc tat agc aac gcc tac cgc gac 240  
 Gln Leu Gly Leu Gly Ala Asp Tyr Thr Tyr Ser Asn Gly Tyr Arg Asp  
 65 70 75 80

gcg aac gcc atc aac tcg aac gcg acc agt gcg tcc ctg cag tta act 288  
 Ala Asn Gly Ile Asn Ser Asn Ala Thr Ser Ala Ser Leu Gln Leu Thr  
 85 90 95

caa tcc att ttt gat atg tcg aaa tgg cgt gcg tta acg ctg cag gaa 336  
 Gln Ser Ile Phe Asp Met Ser Lys Trp Arg Ala Leu Thr Leu Gln Glu  
 100 105 110

aaa gca gca ggg att cag gac atc aca tat cag acc gat cag caa acc 384  
 Lys Ala Ala Gly Ile Gln Asp Ile Thr Tyr Gln Thr Asp Gln Gln Thr  
 115 120 125

ttg atc ctc aac acc gcg acc gct tat ttc aac gtg ttg aat gct att 432  
 Leu Ile Leu Asn Thr Ala Thr Ala Tyr Phe Asn Val Leu Asn Ala Ile  
 130 135 140

gac gtt ctt tcc tat aca cag gca caa aaa gaa gcg atc tac cgt caa 480  
 Asp Val Leu Ser Tyr Thr Gln Ala Gln Lys Glu Ala Ile Tyr Arg Gln  
 145 150 155 160

tta gat caa acc acc caa cgt ttt aac gtg gcc ctg gta gcg atc acc 528  
 Leu Asp Gln Thr Thr Gln Arg Phe Asn Val Gly Leu Val Ala Ile Thr  
 165 170 175

gac gtg cag aac gcc cgc gcg cag tac gat acc gtg ctg gcg aac gaa 576  
 Asp Val Gln Asn Ala Arg Ala Gln Tyr Asp Thr Val Leu Ala Asn Glu  
 180 185 190

gtg acc gca cgt aat aac ctt gat aac gcg gta gag cag ctg cgc cag 624  
 Val Thr Ala Arg Asn Asn Leu Asp Asn Ala Val Glu Gln Leu Arg Gln  
 195 200 205

atc acc ggt aac tac tat ccg gaa ctg gcg gcg ctg aat gtc gaa aac 672

-continued

Ile	Thr	Gly	Asn	Tyr	Tyr	Pro	Glu	Leu	Ala	Ala	Leu	Asn	Val	Glu	Asn				
	210					215					220								
ttt	aaa	acc	gac	aaa	cca	cag	ccg	ggt	aac	gcg	ctg	ctg	aaa	gaa	gcc			720	
Phe	Lys	Thr	Asp	Lys	Pro	Gln	Pro	Val	Asn	Ala	Leu	Leu	Lys	Glu	Ala				
225				230					235					240					
gaa	aaa	cgc	aac	ctg	tcg	ctg	tta	cag	gca	gcg	ttg	agc	cag	gac	ctg			768	
Glu	Lys	Arg	Asn	Leu	Ser	Leu	Leu	Gln	Ala	Arg	Leu	Ser	Gln	Asp	Leu				
			245					250						255					
gcg	cgc	gag	caa	att	cgc	cag	gcg	cag	gat	ggt	cac	tta	ccg	acg	ctg			816	
Ala	Arg	Glu	Gln	Ile	Arg	Gln	Ala	Gln	Asp	Gly	His	Leu	Pro	Thr	Leu				
			260					265					270						
gat	tta	acg	gct	tct	acc	ggg	att	tct	gac	acc	tct	tat	agc	ggt	tcg			864	
Asp	Leu	Thr	Ala	Ser	Thr	Gly	Ile	Ser	Asp	Thr	Ser	Tyr	Ser	Gly	Ser				
	275					280						285							
aaa	act	cgt	ggt	gcc	gct	ggt	acc	cag	tat	gac	gac	agc	aat	atg	ggc			912	
Lys	Thr	Arg	Gly	Ala	Ala	Gly	Thr	Gln	Tyr	Asp	Asp	Ser	Asn	Met	Gly				
	290					295					300								
cag	aac	aaa	gtg	ggc	ctg	agc	ttc	tcg	ctg	ccg	att	tat	cag	ggc	gga			960	
Gln	Asn	Lys	Val	Gly	Leu	Ser	Phe	Ser	Leu	Pro	Ile	Tyr	Gln	Gly	Gly				
305				310						315				320					
atg	ggt	aac	tcg	cag	gtg	aaa	cag	gcc	cag	tac	aac	ttt	ggt	ggt	gcc			1008	
Met	Val	Asn	Ser	Gln	Val	Lys	Gln	Ala	Gln	Tyr	Asn	Phe	Val	Gly	Ala				
			325					330						335					
agc	gag	caa	ctg	gaa	agc	gcg	cat	cgt	agc	atc	gtg	caa	acc	gta	cgt			1056	
Ser	Glu	Gln	Leu	Glu	Ser	Ala	His	Arg	Ser	Ile	Val	Gln	Thr	Val	Arg				
			340					345						350					
tcc	tcc	ttc	aac	aac	att	aat	gca	tct	atc	agt	agc	att	aac	gcc	tac			1104	
Ser	Ser	Phe	Asn	Asn	Ile	Asn	Ala	Ser	Ile	Ser	Ser	Ile	Asn	Ala	Tyr				
		355					360						365						
aaa	caa	gcc	gta	ggt	tcc	gct	caa	agc	tca	tta	gac	gcg	atg	gaa	gcg			1152	
Lys	Gln	Ala	Val	Val	Ser	Ala	Gln	Ser	Ser	Leu	Asp	Ala	Met	Glu	Ala				
	370					375						380							
ggc	tac	tcg	gtc	ggt	acg	cgt	acc	att	ggt	gat	gtg	ttg	gat	gca	acc			1200	
Gly	Tyr	Ser	Val	Gly	Thr	Arg	Thr	Ile	Val	Asp	Val	Leu	Asp	Ala	Thr				
385				390						395				400					
acc	acg	ctg	tac	aac	gct	aag	caa	gag	ctg	gca	aat	gcg	cgt	tat	aac			1248	
Thr	Thr	Leu	Tyr	Asn	Ala	Lys	Gln	Glu	Leu	Ala	Asn	Ala	Arg	Tyr	Asn				
				405					410					415					
tac	ctg	att	aat	cag	ctg	aat	att	aag	tca	gcc	ctg	ggt	acg	ttg	aac			1296	
Tyr	Leu	Ile	Asn	Gln	Leu	Asn	Ile	Lys	Ser	Ala	Leu	Gly	Thr	Leu	Asn				
			420					425						430					
gag	cag	gat	ctg	ctg	gca	ctg	aac	aat	gcg	ctg	agc	aaa	ccg	ggt	tcc			1344	
Glu	Gln	Asp	Leu	Leu	Ala	Leu	Asn	Asn	Ala	Leu	Ser	Lys	Pro	Val	Ser				
		435					440					445							
act	aat	ccg	gaa	aac	ggt	gcc	ccg	caa	acg	ccg	gaa	cag	aat	gct	att			1392	
Thr	Asn	Pro	Glu	Asn	Val	Ala	Pro	Gln	Thr	Pro	Glu	Gln	Asn	Ala	Ile				
	450					455						460							
gct	gat	ggt	tat	gcg	cct	gat	agc	ccg	gca	ccc	gtc	ggt	cag	caa	aca			1440	
Ala	Asp	Gly	Tyr	Ala	Pro	Asp	Ser	Pro	Ala	Pro	Val	Val	Gln	Gln	Thr				
465				470						475				480					
tcc	gca	cgc	act	acc	acc	agt	aac	ggt	cat	aac	cct	ttc	cgt	aac	tga			1488	
Ser	Ala	Arg	Thr	Thr	Thr	Ser	Asn	Gly	His	Asn	Pro	Phe	Arg	Asn					
			485						490					495					

<210> SEQ ID NO 12  
 <211> LENGTH: 495  
 <212> TYPE: PRT  
 <213> ORGANISM: Shigella boydii  
  
 <400> SEQUENCE: 12

Met Gln Met Lys Lys Leu Leu Pro Ile Leu Ile Gly Leu Ser Leu Ser

-continued

1	5	10	15
Gly Phe Ser Ser Leu Ser Gln Ala Glu Asn Leu Met Gln Val Tyr Gln	20	25	30
Gln Ala Arg Leu Ser Asn Pro Glu Leu Arg Lys Ser Ala Ala Asp Arg	35	40	45
Asp Ala Ala Phe Glu Lys Ile Asn Glu Ala Arg Ser Pro Leu Leu Pro	50	55	60
Gln Leu Gly Leu Gly Ala Asp Tyr Thr Tyr Ser Asn Gly Tyr Arg Asp	65	70	75
Ala Asn Gly Ile Asn Ser Asn Ala Thr Ser Ala Ser Leu Gln Leu Thr	85	90	95
Gln Ser Ile Phe Asp Met Ser Lys Trp Arg Ala Leu Thr Leu Gln Glu	100	105	110
Lys Ala Ala Gly Ile Gln Asp Ile Thr Tyr Gln Thr Asp Gln Gln Thr	115	120	125
Leu Ile Leu Asn Thr Ala Thr Ala Tyr Phe Asn Val Leu Asn Ala Ile	130	135	140
Asp Val Leu Ser Tyr Thr Gln Ala Gln Lys Glu Ala Ile Tyr Arg Gln	145	150	155
Leu Asp Gln Thr Thr Gln Arg Phe Asn Val Gly Leu Val Ala Ile Thr	165	170	175
Asp Val Gln Asn Ala Arg Ala Gln Tyr Asp Thr Val Leu Ala Asn Glu	180	185	190
Val Thr Ala Arg Asn Asn Leu Asp Asn Ala Val Glu Gln Leu Arg Gln	195	200	205
Ile Thr Gly Asn Tyr Tyr Pro Glu Leu Ala Ala Leu Asn Val Glu Asn	210	215	220
Phe Lys Thr Asp Lys Pro Gln Pro Val Asn Ala Leu Leu Lys Glu Ala	225	230	235
Glu Lys Arg Asn Leu Ser Leu Leu Gln Ala Arg Leu Ser Gln Asp Leu	245	250	255
Ala Arg Glu Gln Ile Arg Gln Ala Gln Asp Gly His Leu Pro Thr Leu	260	265	270
Asp Leu Thr Ala Ser Thr Gly Ile Ser Asp Thr Ser Tyr Ser Gly Ser	275	280	285
Lys Thr Arg Gly Ala Ala Gly Thr Gln Tyr Asp Asp Ser Asn Met Gly	290	295	300
Gln Asn Lys Val Gly Leu Ser Phe Ser Leu Pro Ile Tyr Gln Gly Gly	305	310	315
Met Val Asn Ser Gln Val Lys Gln Ala Gln Tyr Asn Phe Val Gly Ala	325	330	335
Ser Glu Gln Leu Glu Ser Ala His Arg Ser Ile Val Gln Thr Val Arg	340	345	350
Ser Ser Phe Asn Asn Ile Asn Ala Ser Ile Ser Ser Ile Asn Ala Tyr	355	360	365
Lys Gln Ala Val Val Ser Ala Gln Ser Ser Leu Asp Ala Met Glu Ala	370	375	380
Gly Tyr Ser Val Gly Thr Arg Thr Ile Val Asp Val Leu Asp Ala Thr	385	390	395
Thr Thr Leu Tyr Asn Ala Lys Gln Glu Leu Ala Asn Ala Arg Tyr Asn	405	410	415
Tyr Leu Ile Asn Gln Leu Asn Ile Lys Ser Ala Leu Gly Thr Leu Asn	420	425	430

-continued

Glu Gln Asp Leu Leu Ala Leu Asn Asn Ala Leu Ser Lys Pro Val Ser  
 435 440 445

Thr Asn Pro Glu Asn Val Ala Pro Gln Thr Pro Glu Gln Asn Ala Ile  
 450 455 460

Ala Asp Gly Tyr Ala Pro Asp Ser Pro Ala Pro Val Val Gln Gln Thr  
 465 470 475 480

Ser Ala Arg Thr Thr Thr Ser Asn Gly His Asn Pro Phe Arg Asn  
 485 490 495

<210> SEQ ID NO 13  
 <211> LENGTH: 1482  
 <212> TYPE: DNA  
 <213> ORGANISM: Shigella flexneri  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (1)..(1482)

<400> SEQUENCE: 13

atg aag aaa ttg ctc ccc att ctt atc ggc ctg agc ctt tct ggg ttc 48  
 Met Lys Lys Leu Leu Pro Ile Leu Ile Gly Leu Ser Leu Ser Gly Phe  
 1 5 10 15

agt tcg ttg agc cag gcc gag aac ctg atg caa gtt tat cag caa gca 96  
 Ser Ser Leu Ser Gln Ala Glu Asn Leu Met Gln Val Tyr Gln Gln Ala  
 20 25 30

cgc ctt agt aac ccg gaa ttg cgt aag tct gcc gcc gat cgt gat gct 144  
 Arg Leu Ser Asn Pro Glu Leu Arg Lys Ser Ala Ala Asp Arg Asp Ala  
 35 40 45

gcc ttt gaa aaa att aat gaa gcg cgc agt cca tta ctg cca cag cta 192  
 Ala Phe Glu Lys Ile Asn Glu Ala Arg Ser Pro Leu Leu Pro Gln Leu  
 50 55 60

ggc tta ggt gca gat tac acc tat agc aac ggc tac cgc gac gcg aac 240  
 Gly Leu Gly Ala Asp Tyr Thr Tyr Ser Asn Gly Tyr Arg Asp Ala Asn  
 65 70 75 80

ggc atc aac tcg aac gcg acc agt gcg tcc ctg cag tta act caa tcc 288  
 Gly Ile Asn Ser Asn Ala Thr Ser Ala Ser Leu Gln Leu Thr Gln Ser  
 85 90 95

att ttt gat atg tcg aaa tgg cgt gcg tta acg ctg cag gaa aaa gca 336  
 Ile Phe Asp Met Ser Lys Trp Arg Ala Leu Thr Leu Gln Glu Lys Ala  
 100 105 110

gca ggg att cag gac gtc aca tat cag acc gat cag caa acc ttg atc 384  
 Ala Gly Ile Gln Asp Val Thr Tyr Gln Thr Asp Gln Gln Thr Leu Ile  
 115 120 125

ctc aac acc gcg acc gct tat ttc aac gtg ttg aat gct att gac gtt 432  
 Leu Asn Thr Ala Thr Ala Tyr Phe Asn Val Leu Asn Ala Ile Asp Val  
 130 135 140

ctt tcc tat aca cag gca caa aaa gaa gcg atc tac cgt caa tta gat 480  
 Leu Ser Tyr Thr Gln Ala Gln Lys Glu Ala Ile Tyr Arg Gln Leu Asp  
 145 150 155 160

caa acc acc caa cgt ttt aac gtg ggc ctg gta gcg atc acc gac gtg 528  
 Gln Thr Thr Gln Arg Phe Asn Val Gly Leu Val Ala Ile Thr Asp Val  
 165 170 175

cag aac gcc cgc gcg cag tac gat acc gtg ctg gcg aac gaa gtg acc 576  
 Gln Asn Ala Arg Ala Gln Tyr Asp Thr Val Leu Ala Asn Glu Val Thr  
 180 185 190

gca cgt aat aac ctt gat aac gcg gta gag cag ctg cgc cag atc acc 624  
 Ala Arg Asn Asn Leu Asp Asn Ala Val Glu Gln Leu Arg Gln Ile Thr  
 195 200 205

ggc aac tac tat ccg gaa ctg gcg gcg ctg aat gtc gaa aac ttt aaa 672  
 Gly Asn Tyr Tyr Pro Glu Leu Ala Ala Leu Asn Val Glu Asn Phe Lys  
 210 215 220

acc gac aaa cca cag ccg gtt aac gcg ctg ctg aaa gaa gcc gaa aaa 720



-continued

---

Arg Leu Ser Asn Pro Glu Leu Arg Lys Ser Ala Ala Asp Arg Asp Ala  
           35                                  40                                  45  
 Ala Phe Glu Lys Ile Asn Glu Ala Arg Ser Pro Leu Leu Pro Gln Leu  
           50                                  55                                  60  
 Gly Leu Gly Ala Asp Tyr Thr Tyr Ser Asn Gly Tyr Arg Asp Ala Asn  
   65                                  70                                  75                                  80  
 Gly Ile Asn Ser Asn Ala Thr Ser Ala Ser Leu Gln Leu Thr Gln Ser  
                                   85                                  90  
 Ile Phe Asp Met Ser Lys Trp Arg Ala Leu Thr Leu Gln Glu Lys Ala  
           100                                  105                                  110  
 Ala Gly Ile Gln Asp Val Thr Tyr Gln Thr Asp Gln Gln Thr Leu Ile  
           115                                  120                                  125  
 Leu Asn Thr Ala Thr Ala Tyr Phe Asn Val Leu Asn Ala Ile Asp Val  
   130                                  135                                  140  
 Leu Ser Tyr Thr Gln Ala Gln Lys Glu Ala Ile Tyr Arg Gln Leu Asp  
   145                                  150                                  155                                  160  
 Gln Thr Thr Gln Arg Phe Asn Val Gly Leu Val Ala Ile Thr Asp Val  
                                   165                                  170                                  175  
 Gln Asn Ala Arg Ala Gln Tyr Asp Thr Val Leu Ala Asn Glu Val Thr  
           180                                  185                                  190  
 Ala Arg Asn Asn Leu Asp Asn Ala Val Glu Gln Leu Arg Gln Ile Thr  
   195                                  200                                  205  
 Gly Asn Tyr Tyr Pro Glu Leu Ala Ala Leu Asn Val Glu Asn Phe Lys  
   210                                  215                                  220  
 Thr Asp Lys Pro Gln Pro Val Asn Ala Leu Leu Lys Glu Ala Glu Lys  
   225                                  230                                  235                                  240  
 Arg Asn Leu Ser Leu Leu Gln Ala Arg Leu Ser Gln Asp Leu Glu Arg  
           245                                  250                                  255  
 Glu Gln Ile Arg Gln Ala Gln Asp Gly His Leu Pro Thr Leu Asp Leu  
           260                                  265                                  270  
 Thr Ala Ser Thr Gly Ile Ser Asp Thr Ser Tyr Ser Gly Ser Lys Thr  
   275                                  280                                  285  
 Arg Gly Ala Ala Gly Thr Gln Tyr Asp Asp Ser Asn Met Gly Gln Asn  
   290                                  295                                  300  
 Lys Val Gly Leu Ser Phe Ser Leu Pro Ile Tyr Gln Gly Gly Met Val  
   305                                  310                                  315                                  320  
 Asn Ser Gln Val Lys Gln Ala Gln Tyr Asn Phe Val Gly Ala Ser Glu  
           325                                  330                                  335  
 Gln Leu Glu Ser Ala His Arg Ser Val Val Gln Thr Val Arg Ser Ser  
   340                                  345                                  350  
 Phe Asn Asn Ile Asn Ala Ser Ile Ser Ser Ile Asn Ala Tyr Lys Gln  
   355                                  360                                  365  
 Ala Val Val Ser Ala Gln Ser Ser Leu Asp Ala Met Glu Ala Gly Tyr  
   370                                  375                                  380  
 Ser Val Gly Thr Arg Thr Ile Val Asp Val Leu Asp Ala Thr Thr Thr  
   385                                  390                                  395                                  400  
 Leu Tyr Asn Ala Lys Gln Glu Leu Ala Asn Ala Arg Tyr Asn Tyr Leu  
           405                                  410                                  415  
 Ile Asn Gln Leu Asn Ile Lys Ser Ala Leu Gly Thr Leu Asn Glu Gln  
           420                                  425                                  430  
 Asp Leu Leu Ala Leu Asn Asn Ala Leu Ser Lys Pro Val Ser Thr Asn  
   435                                  440                                  445  
 Pro Glu Asn Val Ala Pro Gln Thr Pro Glu Gln Asn Ala Ile Ala Asp

-continued

450	455	460	
Gly Tyr Ala Pro Asp Ser Pro Ala Pro Val Val Gln Gln Thr Ser Ala			
465	470	475	480
Arg Thr Thr Thr Ser Asn Gly His Asn Pro Phe Arg Asn			
	485	490	
 <210> SEQ ID NO 15			
<211> LENGTH: 1476			
<212> TYPE: DNA			
<213> ORGANISM: Salmonella enterica			
<220> FEATURE:			
<221> NAME/KEY: CDS			
<222> LOCATION: (1)..(1476)			
 <400> SEQUENCE: 15			
atg caa atg aag aaa ttg ctc ccc atc ctt atc ggc ctg agc ctg tcg			48
Met Gln Met Lys Lys Leu Leu Pro Ile Leu Ile Gly Leu Ser Leu Ser			
1	5	10	15
ggg ttc agc aca cta agc cag gca gag aac ctg atg caa gtt tat cag			96
Gly Phe Ser Thr Leu Ser Gln Ala Glu Asn Leu Met Gln Val Tyr Gln			
	20	25	30
caa gca cgc ctg agc aac ccg gaa ttg cgt aaa tcc gct gcc gat cgc			144
Gln Ala Arg Leu Ser Asn Pro Glu Leu Arg Lys Ser Ala Ala Asp Arg			
	35	40	45
gat gct gca ttc gaa aaa att aac gaa gca cgt agt cct tta ctg ccg			192
Asp Ala Ala Phe Glu Lys Ile Asn Glu Ala Arg Ser Pro Leu Leu Pro			
	50	55	60
caa ctg ggt tta ggt gcc gac tac acc tac agc aac ggt tat cgc gat			240
Gln Leu Gly Leu Gly Ala Asp Tyr Thr Tyr Ser Asn Gly Tyr Arg Asp			
	65	70	80
gcg aac ggt atc aac tcc aat gaa acc agc gct tct ctg caa tta acg			288
Ala Asn Gly Ile Asn Ser Asn Glu Thr Ser Ala Ser Leu Gln Leu Thr			
	85	90	95
cag acg cta ttt gat atg tcg aaa tgg cgt ggg ctc acc ctg caa gaa			336
Gln Thr Leu Phe Asp Met Ser Lys Trp Arg Gly Leu Thr Leu Gln Glu			
	100	105	110
aaa gca gca ggc att cag gat gtc acc tat cag acc gat cag cag acg			384
Lys Ala Ala Gly Ile Gln Asp Val Thr Tyr Gln Thr Asp Gln Gln Thr			
	115	120	125
ctg atc ctc aat acc gcg aac gcg tat ttt aag gta ttg aac gct att			432
Leu Ile Leu Asn Thr Ala Asn Ala Tyr Phe Lys Val Leu Asn Ala Ile			
	130	135	140
gat gtg ctt tcc tat acc cag gcg caa aaa gag gct atc tac cgt cag			480
Asp Val Leu Ser Tyr Thr Gln Ala Gln Lys Glu Ala Ile Tyr Arg Gln			
	145	150	155
tta gat caa acg acg caa cgt ttt aac gtg ggt ctg gtc gcc att acc			528
Leu Asp Gln Thr Thr Gln Arg Phe Asn Val Gly Leu Val Ala Ile Thr			
	165	170	175
gac gtg caa aac gcc cgt gcg caa tat gat acc gta ctg gcg aat gaa			576
Asp Val Gln Asn Ala Arg Ala Gln Tyr Asp Thr Val Leu Ala Asn Glu			
	180	185	190
gtg acc gcc cgc aac aac ctg gat aac gcg gta gaa gag ctg cgc cag			624
Val Thr Ala Arg Asn Asn Leu Asp Asn Ala Val Glu Glu Leu Arg Gln			
	195	200	205
gta acc ggc aat tat tac ccg gag ctg gcg tcg ctt aac gtc gag cat			672
Val Thr Gly Asn Tyr Tyr Pro Glu Leu Ala Ser Leu Asn Val Glu His			
	210	215	220
ttt aaa acc gac aaa ccc aaa gct gtt aat gcg ctg ctg aag gaa gcg			720
Phe Lys Thr Asp Lys Pro Lys Ala Val Asn Ala Leu Leu Lys Glu Ala			
	225	230	235
gaa aac cgt aac ctg tcg ctg ttg cag gcg cgt tta agt cag gat ctg			768



-continued

Glu	Asn	Arg	Asn	Leu	Ser	Leu	Leu	Gln	Ala	Arg	Leu	Ser	Gln	Asp	Leu		
			245						250					255			
gcg	cgc	gag	caa	atc	cgT	cag	gcg	cag	gat	ggt	cat	ctg	ccg	acg	ctg		816
Ala	Arg	Glu	Gln	Ile	Arg	Gln	Ala	Gln	Asp	Gly	His	Leu	Pro	Thr	Leu		
		260					265					270					
aat	tta	acg	gcc	tca	acc	ggc	att	tct	gat	acc	tct	tat	agc	ggt	tct		864
Asn	Leu	Thr	Ala	Ser	Thr	Gly	Ile	Ser	Asp	Thr	Ser	Tyr	Ser	Gly	Ser		
		275					280					285					
aaa	acc	aac	tcc	gcc	cag	tac	gac	gat	agc	aac	atg	ggg	cag	aat	aaa		912
Lys	Thr	Asn	Ser	Ala	Gln	Tyr	Asp	Asp	Ser	Asn	Met	Gly	Gln	Asn	Lys		
	290					295				300							
atc	ggc	ctg	aac	ttc	tcc	ctg	ccg	ctg	tat	caa	ggc	ggg	atg	ggt	aac		960
Ile	Gly	Leu	Asn	Phe	Ser	Leu	Pro	Leu	Tyr	Gln	Gly	Gly	Met	Val	Asn		
305				310						315					320		
tcg	cag	gta	aaa	cag	gcg	cag	tat	aac	ttc	gtc	ggc	gca	agc	gaa	cag		1008
Ser	Gln	Val	Lys	Gln	Ala	Gln	Tyr	Asn	Phe	Val	Gly	Ala	Ser	Glu	Gln		
			325						330					335			
ctg	gaa	agc	gcg	cac	cgT	agc	gtg	gtg	cag	acc	gta	cgt	tct	tcc	ttt		1056
Leu	Glu	Ser	Ala	His	Arg	Ser	Val	Val	Gln	Thr	Val	Arg	Ser	Ser	Phe		
			340				345						350				
aac	aat	att	aac	gcc	tcc	atc	agc	agc	atc	aac	gcg	tat	aaa	cag	gca		1104
Asn	Asn	Ile	Asn	Ala	Ser	Ile	Ser	Ser	Ile	Asn	Ala	Tyr	Lys	Gln	Ala		
		355				360						365					
gtc	gtt	tcc	gcg	caa	agt	tct	ttg	gat	gca	atg	gaa	gcc	ggt	tac	tcg		1152
Val	Val	Ser	Ala	Gln	Ser	Ser	Leu	Asp	Ala	Met	Glu	Ala	Gly	Tyr	Ser		
	370				375						380						
gtc	ggt	aca	cgT	acc	att	gtt	gac	gta	ctg	gat	gcc	acc	acc	act	ctg		1200
Val	Gly	Thr	Arg	Thr	Ile	Val	Asp	Val	Leu	Asp	Ala	Thr	Thr	Thr	Leu		
	385				390					395					400		
tat	gat	gcc	aag	cag	caa	ctg	gcc	aac	gcg	cgT	tat	acc	tat	ttg	att		1248
Tyr	Asp	Ala	Lys	Gln	Gln	Leu	Ala	Asn	Ala	Arg	Tyr	Thr	Tyr	Leu	Ile		
			405						410					415			
aat	cag	tta	aat	atc	aaa	tat	gcg	ctc	ggt	acg	ctg	aac	gag	cag	gat		1296
Asn	Gln	Leu	Asn	Ile	Lys	Tyr	Ala	Leu	Gly	Thr	Leu	Asn	Glu	Gln	Asp		
			420					425					430				
ctg	ctc	gcg	ctt	aac	agt	acg	ttg	ggt	aaa	cct	atc	ccg	acg	tcg	ccg		1344
Leu	Leu	Ala	Leu	Asn	Ser	Thr	Leu	Gly	Lys	Pro	Ile	Pro	Thr	Ser	Pro		
		435					440					445					
gaa	agc	gta	gcg	ccg	gaa	acg	cca	gag	cag	gat	gct	gcc	gca	gac	ggt		1392
Glu	Ser	Val	Ala	Pro	Glu	Thr	Pro	Glu	Gln	Asp	Ala	Ala	Ala	Asp	Gly		
		450				455					460						
tat	aat	gcc	cat	agc	gcc	gcg	ccg	gca	gta	cag	ccg	acc	gcc	gct	cgC		1440
Tyr	Asn	Ala	His	Ser	Ala	Ala	Pro	Ala	Val	Gln	Pro	Thr	Ala	Ala	Arg		
	465				470					475					480		
gcc	aac	agc	aat	aac	ggc	aat	cca	ttc	cgC	cat	tga						1476
Ala	Asn	Ser	Asn	Asn	Gly	Asn	Pro	Phe	Arg	His							
			485					490									

&lt;210&gt; SEQ ID NO 16

&lt;211&gt; LENGTH: 491

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Salmonella enterica

&lt;400&gt; SEQUENCE: 16

Met	Gln	Met	Lys	Lys	Leu	Leu	Pro	Ile	Leu	Ile	Gly	Leu	Ser	Leu	Ser		
1			5						10					15			

Gly	Phe	Ser	Thr	Leu	Ser	Gln	Ala	Glu	Asn	Leu	Met	Gln	Val	Tyr	Gln		
		20					25					30					

Gln	Ala	Arg	Leu	Ser	Asn	Pro	Glu	Leu	Arg	Lys	Ser	Ala	Ala	Asp	Arg		
		35					40					45					

-continued

---

Asp Ala Ala Phe Glu Lys Ile Asn Glu Ala Arg Ser Pro Leu Leu Pro  
 50 55 60

Gln Leu Gly Leu Gly Ala Asp Tyr Thr Tyr Ser Asn Gly Tyr Arg Asp  
 65 70 75 80

Ala Asn Gly Ile Asn Ser Asn Glu Thr Ser Ala Ser Leu Gln Leu Thr  
 85 90 95

Gln Thr Leu Phe Asp Met Ser Lys Trp Arg Gly Leu Thr Leu Gln Glu  
 100 105 110

Lys Ala Ala Gly Ile Gln Asp Val Thr Tyr Gln Thr Asp Gln Gln Thr  
 115 120 125

Leu Ile Leu Asn Thr Ala Asn Ala Tyr Phe Lys Val Leu Asn Ala Ile  
 130 135 140

Asp Val Leu Ser Tyr Thr Gln Ala Gln Lys Glu Ala Ile Tyr Arg Gln  
 145 150 155 160

Leu Asp Gln Thr Thr Gln Arg Phe Asn Val Gly Leu Val Ala Ile Thr  
 165 170 175

Asp Val Gln Asn Ala Arg Ala Gln Tyr Asp Thr Val Leu Ala Asn Glu  
 180 185 190

Val Thr Ala Arg Asn Asn Leu Asp Asn Ala Val Glu Glu Leu Arg Gln  
 195 200 205

Val Thr Gly Asn Tyr Tyr Pro Glu Leu Ala Ser Leu Asn Val Glu His  
 210 215 220

Phe Lys Thr Asp Lys Pro Lys Ala Val Asn Ala Leu Leu Lys Glu Ala  
 225 230 235 240

Glu Asn Arg Asn Leu Ser Leu Leu Gln Ala Arg Leu Ser Gln Asp Leu  
 245 250 255

Ala Arg Glu Gln Ile Arg Gln Ala Gln Asp Gly His Leu Pro Thr Leu  
 260 265 270

Asn Leu Thr Ala Ser Thr Gly Ile Ser Asp Thr Ser Tyr Ser Gly Ser  
 275 280 285

Lys Thr Asn Ser Ala Gln Tyr Asp Asp Ser Asn Met Gly Gln Asn Lys  
 290 295 300

Ile Gly Leu Asn Phe Ser Leu Pro Leu Tyr Gln Gly Gly Met Val Asn  
 305 310 315 320

Ser Gln Val Lys Gln Ala Gln Tyr Asn Phe Val Gly Ala Ser Glu Gln  
 325 330 335

Leu Glu Ser Ala His Arg Ser Val Val Gln Thr Val Arg Ser Ser Phe  
 340 345 350

Asn Asn Ile Asn Ala Ser Ile Ser Ser Ile Asn Ala Tyr Lys Gln Ala  
 355 360 365

Val Val Ser Ala Gln Ser Ser Leu Asp Ala Met Glu Ala Gly Tyr Ser  
 370 375 380

Val Gly Thr Arg Thr Ile Val Asp Val Leu Asp Ala Thr Thr Thr Leu  
 385 390 395 400

Tyr Asp Ala Lys Gln Gln Leu Ala Asn Ala Arg Tyr Thr Tyr Leu Ile  
 405 410 415

Asn Gln Leu Asn Ile Lys Tyr Ala Leu Gly Thr Leu Asn Glu Gln Asp  
 420 425 430

Leu Leu Ala Leu Asn Ser Thr Leu Gly Lys Pro Ile Pro Thr Ser Pro  
 435 440 445

Glu Ser Val Ala Pro Glu Thr Pro Glu Gln Asp Ala Ala Ala Asp Gly  
 450 455 460

Tyr Asn Ala His Ser Ala Ala Pro Ala Val Gln Pro Thr Ala Ala Arg  
 465 470 475 480

-continued

---

Ala Asn Ser Asn Asn Gly Asn Pro Phe Arg His  
 485 490

<210> SEQ ID NO 17  
 <211> LENGTH: 1470  
 <212> TYPE: DNA  
 <213> ORGANISM: Citrobacter koseri  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (1)..(1470)

<400> SEQUENCE: 17

atg caa atg aag aaa ttg ctc ccc atc ctt atc ggc ctg agc ctg acg 48  
 Met Gln Met Lys Lys Leu Leu Pro Ile Leu Ile Gly Leu Ser Leu Thr  
 1 5 10 15

ggg ttc agc aca ctg agc cag gca gag aac ctg atg caa gtt tat cag 96  
 Gly Phe Ser Thr Leu Ser Gln Ala Glu Asn Leu Met Gln Val Tyr Gln  
 20 25 30

caa gca cgc ctg agc aac ccg gaa ttg cgt aaa tcc gcc gcc gat cgc 144  
 Gln Ala Arg Leu Ser Asn Pro Glu Leu Arg Lys Ser Ala Ala Asp Arg  
 35 40 45

gat gct gca ttc gaa aaa att aac gaa gcg cgt agt cct tta ctg ccg 192  
 Asp Ala Ala Phe Glu Lys Ile Asn Glu Ala Arg Ser Pro Leu Leu Pro  
 50 55 60

caa ctg ggt tta ggt gcc gat tac acc tac agc aac ggc tat cgt gat 240  
 Gln Leu Gly Leu Gly Ala Asp Tyr Thr Tyr Ser Asn Gly Tyr Arg Asp  
 65 70 75 80

gcg aat ggc atc aac tcc aac gcc acc agc gcc tct ctg caa tta acc 288  
 Ala Asn Gly Ile Asn Ser Asn Ala Thr Ser Ala Ser Leu Gln Leu Thr  
 85 90 95

cag acc ctt ttt gat atg tca aaa tgg cgc gcg ctg acg ttg cag gaa 336  
 Gln Thr Leu Phe Asp Met Ser Lys Trp Arg Ala Leu Thr Leu Gln Glu  
 100 105 110

aaa tcc gca ggt atc cag gac gtc acg ttc cag acc gat cag caa acg 384  
 Lys Ser Ala Gly Ile Gln Asp Val Thr Phe Gln Thr Asp Gln Gln Thr  
 115 120 125

ctg atc ctc aat acg gcg agc gcc tac ttt aaa gtc ctg aac gcc att 432  
 Leu Ile Leu Asn Thr Ala Ser Ala Tyr Phe Lys Val Leu Asn Ala Ile  
 130 135 140

gac gtt ctc tct tat acg cag gcg cag aaa gaa gcc gtt tat cgt cag 480  
 Asp Val Leu Ser Tyr Thr Gln Ala Gln Lys Glu Ala Val Tyr Arg Gln  
 145 150 155 160

tta gat caa acc acc cag cgt ttt aac gtc ggc ctg gtc gct atc act 528  
 Leu Asp Gln Thr Thr Gln Arg Phe Asn Val Gly Leu Val Ala Ile Thr  
 165 170 175

gac gtg caa aac gcc cgt gca caa tac gat acc gtg ctg gcg aac gaa 576  
 Asp Val Gln Asn Ala Arg Ala Gln Tyr Asp Thr Val Leu Ala Asn Glu  
 180 185 190

gtc acc gcc cgc aac aat ctg gat aac gcc gta gaa gaa ctg cgc cag 624  
 Val Thr Ala Arg Asn Asn Leu Asp Asn Ala Val Glu Glu Leu Arg Gln  
 195 200 205

gtc acc ggt aac tac tac ccg gaa ctg gct tcg ctg aat gtc aca aac 672  
 Val Thr Gly Asn Tyr Tyr Pro Glu Leu Ala Ser Leu Asn Val Thr Asn  
 210 215 220

ttt aaa acc gac aag ccg cag gcc gtt aac gcg ctg ctg aaa gag gcc 720  
 Phe Lys Thr Asp Lys Pro Gln Ala Val Asn Ala Leu Leu Lys Glu Ala  
 225 230 235 240

gaa aac cgt aac ctg acg ctg ttg cag gcg cgt ctg agc cag gat ctg 768  
 Glu Asn Arg Asn Leu Thr Leu Leu Gln Ala Arg Leu Ser Gln Asp Leu  
 245 250 255

gcg cgc gag caa atc cgc cag gcg cag gac ggc cat ctg cca acg ctg 816



-continued

65	70	75	80
Ala Asn Gly Ile Asn Ser Asn Ala Thr Ser Ala Ser Leu Gln Leu Thr	85	90	95
Gln Thr Leu Phe Asp Met Ser Lys Trp Arg Ala Leu Thr Leu Gln Glu	100	105	110
Lys Ser Ala Gly Ile Gln Asp Val Thr Phe Gln Thr Asp Gln Gln Thr	115	120	125
Leu Ile Leu Asn Thr Ala Ser Ala Tyr Phe Lys Val Leu Asn Ala Ile	130	135	140
Asp Val Leu Ser Tyr Thr Gln Ala Gln Lys Glu Ala Val Tyr Arg Gln	145	150	160
Leu Asp Gln Thr Thr Gln Arg Phe Asn Val Gly Leu Val Ala Ile Thr	165	170	175
Asp Val Gln Asn Ala Arg Ala Gln Tyr Asp Thr Val Leu Ala Asn Glu	180	185	190
Val Thr Ala Arg Asn Asn Leu Asp Asn Ala Val Glu Glu Leu Arg Gln	195	200	205
Val Thr Gly Asn Tyr Tyr Pro Glu Leu Ala Ser Leu Asn Val Thr Asn	210	215	220
Phe Lys Thr Asp Lys Pro Gln Ala Val Asn Ala Leu Leu Lys Glu Ala	225	230	240
Glu Asn Arg Asn Leu Thr Leu Leu Gln Ala Arg Leu Ser Gln Asp Leu	245	250	255
Ala Arg Glu Gln Ile Arg Gln Ala Gln Asp Gly His Leu Pro Thr Leu	260	265	270
Asp Leu Thr Ala Ser Thr Gly Val Ser Asp Thr Ser Tyr Ser Gly Ser	275	280	285
Lys Thr His Asn Ser Thr Gln Tyr Asp Asp Ser Asn Met Gly Gln Asn	290	295	300
Lys Ile Gly Leu Ser Phe Ser Leu Pro Leu Tyr Gln Gly Gly Met Val	305	310	320
Asn Ser Gln Val Lys Gln Ala Gln Tyr Asn Phe Val Gly Ala Ser Glu	325	330	335
Gln Leu Glu Ser Ala His Arg Ser Val Val Gln Thr Val Arg Ser Ser	340	345	350
Phe Asn Asn Ile Asn Ala Ser Ile Ser Ser Ile Asn Ala Tyr Lys Gln	355	360	365
Ala Val Val Ser Ala Gln Ser Ser Leu Asp Ala Asn Glu Ala Gly Tyr	370	375	380
Ser Val Gly Thr Arg Thr Ile Val Asp Val Leu Asp Ala Thr Thr Ala	385	390	400
Leu Tyr Glu Ala Lys Gln Gln Leu Ala Asn Ala Arg Tyr Asn Tyr Leu	405	410	415
Ile Asn Gln Leu Asn Ile Lys Asn Ala Leu Gly Thr Leu Asn Glu Gln	420	425	430
Asp Leu Val Ala Leu Asn Asn Ala Leu Gly Lys Pro Ile Ser Thr Ser	435	440	445
Pro Asp Asn Val Ala Pro Glu Thr Pro Gln Gln Asp Ala Ala Ala Asp	450	455	460
Gly Tyr Asn Ala Ser Thr Val Gln Pro Ala Ser Ala Arg Ser Thr Ser	465	470	480
Ser Asn Gly Asn Asn Pro Phe Arg Asn	485		

-continued

<210> SEQ ID NO 19  
 <211> LENGTH: 1479  
 <212> TYPE: DNA  
 <213> ORGANISM: *Klebsiella pneumoniae*  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (1)..(1479)

<400> SEQUENCE: 19

atg caa atg aag aaa ttg ctc ccc att ctt atc ggc ctg agc ctg acc	48
Met Gln Met Lys Lys Leu Leu Pro Ile Leu Ile Gly Leu Ser Leu Thr	
1 5 10 15	
ggg ttc agc gcc atg agc cag gcg gaa aac ctg ctt cag gtt tac cag	96
Gly Phe Ser Ala Met Ser Gln Ala Glu Asn Leu Leu Gln Val Tyr Gln	
20 25 30	
cag gca cgc atc agc aac ccc gat ctg cgt aaa tcg gca gcc gat cgt	144
Gln Ala Arg Ile Ser Asn Pro Asp Leu Arg Lys Ser Ala Ala Asp Arg	
35 40 45	
gac gcc gcg ttc gaa aag atc aac gaa gcg cgc agt cca tta ctg cct	192
Asp Ala Ala Phe Glu Lys Ile Asn Glu Ala Arg Ser Pro Leu Leu Pro	
50 55 60	
cag ctt ggg ctg gga gcg gat tat acc tat aac aat ggc tat cgc gac	240
Gln Leu Gly Leu Gly Ala Asp Tyr Thr Tyr Asn Asn Gly Tyr Arg Asp	
65 70 75 80	
agc aac ggc atc aat tca aac gtc acc agc ggc tcg ctg cag tta acg	288
Ser Asn Gly Ile Asn Ser Asn Val Thr Ser Gly Ser Leu Gln Leu Thr	
85 90 95	
cag gtt ctg ttt gat atg tcg aaa tgg cgc gcc ctg acg ctg cag gaa	336
Gln Val Leu Phe Asp Met Ser Lys Trp Arg Ala Leu Thr Leu Gln Glu	
100 105 110	
aaa acg gca ggg att cag gat gtc acg tat cag acc gat cag caa aca	384
Lys Thr Ala Gly Ile Gln Asp Val Thr Tyr Gln Thr Asp Gln Gln Thr	
115 120 125	
ctg att ctg aat acc gcg acg gcc tat ttt aaa gtg ctg gcc gcc atc	432
Leu Ile Leu Asn Thr Ala Thr Ala Tyr Phe Lys Val Leu Ala Ala Ile	
130 135 140	
gac acg ctt tcc tat acc gaa gcg cag aaa cag gct att tac cgc cag	480
Asp Thr Leu Ser Tyr Thr Glu Ala Gln Lys Gln Ala Ile Tyr Arg Gln	
145 150 155 160	
ttg gat caa acc acg cag cgc ttt aac gta ggc ctg gtg gcg atc acc	528
Leu Asp Gln Thr Thr Gln Arg Phe Asn Val Gly Leu Val Ala Ile Thr	
165 170 175	
gac gtg cag aac gcc cgt tca caa tac gat gcc gtg ctg gcg aac gaa	576
Asp Val Gln Asn Ala Arg Ser Gln Tyr Asp Ala Val Leu Ala Asn Glu	
180 185 190	
gtc acc gcg cgt aac gat ctc gac aac gcc gtc gaa gaa ctg cgt cag	624
Val Thr Ala Arg Asn Asp Leu Asp Asn Ala Val Glu Glu Leu Arg Gln	
195 200 205	
gtc acc ggt aat tac tat ccg gag ctg gcc tcc ctg aac gtg aat ggc	672
Val Thr Gly Asn Tyr Tyr Pro Glu Leu Ala Ser Leu Asn Val Asn Gly	
210 215 220	
ttc aaa acc aac aag ccg cag gcg gtc aac gcc ctg ctg aag gaa gcg	720
Phe Lys Thr Asn Lys Pro Gln Ala Val Asn Ala Leu Leu Lys Glu Ala	
225 230 235 240	
gag aac cgc aac ctg tcg ctg ctg cag gcg cgt ctg aac cag gac ctg	768
Glu Asn Arg Asn Leu Ser Leu Leu Gln Ala Arg Leu Asn Gln Asp Leu	
245 250 255	
gct cgc gag cag att cgc cag gcg cag gac gcc cat ttg ccg acg ctc	816
Ala Arg Glu Gln Ile Arg Gln Ala Gln Asp Gly His Leu Pro Thr Leu	
260 265 270	
agc cta tcc gcg tcg agt ggg ata tcg aat act agc tac agt ggt tca	864

-continued

Ser	Leu	Ser	Ala	Ser	Ser	Gly	Ile	Ser	Asn	Thr	Ser	Tyr	Ser	Gly	Ser		
		275					280					285					
aaa	acc	cat	aat	aat	cct	cag	caa	tac	cag	gat	aac	gat	gcc	ggg	cag		912
Lys	Thr	His	Asn	Asn	Pro	Gln	Gln	Tyr	Gln	Asp	Asn	Asp	Ala	Gly	Gln		
		290				295				300							
aac	caa	atc	ggc	ctg	aac	ttc	tct	ctg	cca	ctg	tat	cag	ggc	ggc	gcg		960
Asn	Gln	Ile	Gly	Leu	Asn	Phe	Ser	Leu	Pro	Leu	Tyr	Gln	Gly	Gly	Ala		
305					310					315					320		
gtg	acc	tcg	cag	gtc	aaa	cag	gcg	caa	tac	aac	ttc	gtc	ggc	gcc	agc		1008
Val	Thr	Ser	Gln	Val	Lys	Gln	Ala	Gln	Tyr	Asn	Phe	Val	Gly	Ala	Ser		
				325					330					335			
gag	cag	ctg	gaa	agc	gcc	cac	cgc	agc	gtc	gtg	cag	act	gtg	cgt	tca		1056
Glu	Gln	Leu	Glu	Ser	Ala	His	Arg	Ser	Val	Val	Gln	Thr	Val	Arg	Ser		
			340					345						350			
tcg	ttt	aac	aac	gtg	aac	gcc	tcc	atc	agc	agc	atc	aac	gcc	tac	aaa		1104
Ser	Phe	Asn	Asn	Val	Asn	Ala	Ser	Ile	Ser	Ser	Ile	Asn	Ala	Tyr	Lys		
		355				360							365				
cag	gcg	gtg	gtc	tct	gcg	caa	agc	tcc	ctg	gat	gcc	atg	gaa	gct	ggc		1152
Gln	Ala	Val	Val	Ser	Ala	Gln	Ser	Ser	Leu	Asp	Ala	Met	Glu	Ala	Gly		
		370				375					380						
tac	tcg	gtg	ggc	acg	cgt	act	atc	ggt	gac	gtc	ctc	gac	gcc	acc	act		1200
Tyr	Ser	Val	Gly	Thr	Arg	Thr	Ile	Val	Asp	Val	Leu	Asp	Ala	Thr	Thr		
385				390						395					400		
acg	ctg	tat	aac	gct	aag	cag	cag	ctc	tcg	aat	gcg	cgc	tac	aac	tac		1248
Thr	Leu	Tyr	Asn	Ala	Lys	Gln	Gln	Leu	Ser	Asn	Ala	Arg	Tyr	Asn	Tyr		
				405					410					415			
ctg	atc	aac	gag	ctg	aac	att	aag	tcg	gcg	tta	ggc	acc	ctg	aac	gag		1296
Leu	Ile	Asn	Glu	Leu	Asn	Ile	Lys	Ser	Ala	Leu	Gly	Thr	Leu	Asn	Glu		
			420				425						430				
cag	gat	ctg	gtc	gcc	ctg	aac	aac	acg	ctg	ggt	aaa	ccc	atc	tcc	acc		1344
Gln	Asp	Leu	Val	Ala	Leu	Asn	Asn	Thr	Leu	Gly	Lys	Pro	Ile	Ser	Thr		
		435				440						445					
tcc	gca	gat	agc	gtc	gcg	ccg	gaa	aat	ccg	caa	cag	gat	gcc	acc	gct		1392
Ser	Ala	Asp	Ser	Val	Ala	Pro	Glu	Asn	Pro	Gln	Gln	Asp	Ala	Thr	Ala		
		450				455					460						
gat	ggc	tac	ggc	aac	act	acc	gcg	gcg	gtg	aag	ccg	gcg	tcc	gca	cgg		1440
Asp	Gly	Tyr	Gly	Asn	Thr	Thr	Ala	Ala	Val	Lys	Pro	Ala	Ser	Ala	Arg		
465				470						475				480			
acc	acc	cag	agc	agc	ggc	agc	aat	ccg	ttc	cgt	cag	taa					1479
Thr	Thr	Gln	Ser	Ser	Gly	Ser	Asn	Pro	Phe	Arg	Gln						
				485					490								

&lt;210&gt; SEQ ID NO 20

&lt;211&gt; LENGTH: 492

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Klebsiella pneumoniae

&lt;400&gt; SEQUENCE: 20

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





<213> ORGANISM: Enterobacter sakazakii

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1)..(1494)

<400> SEQUENCE: 21

```
atg caa atg aag aaa ctg ctc ccc atc ctt atc ggc ctg agc ctg agc ctg agc
48 Met Gln Met Lys Ile Leu Ile Leu Pro Ile Leu Ile Gly Leu Ser Leu Thr
1 5 10 15
ggc ttc agc gcc atg aac ctg agc gca aac ctg ctg cag gtt tac cag
96 Gly Phe Ser Ala Met Ser Gln Ala Gln Asn Leu Leu Gln Val Tyr Gln
20 25 30
cag gca cgt tta agt aac ccc agc ctg agc gct gct gac cgc
144 Gln Ala Arg Leu Ser Asn Pro Asp Leu Arg Ser Ser Ala Ala Asp Arg
35 40 45
gac gcc gca ttc gaa aaa att aac gaa gcc cgc agt cct tta ctt ccg
192 Asp Ala Ala Phe Lys Ile Asn Gln Ala Arg Ser Pro Leu Leu Pro
50 55 60
cag ctc ggc ctg ggt gca gat tac aac att aac agc ggt ttt cgc gat
240 Gln Leu Gly Leu Gly Ala Asp Tyr Thr Tyr Asn Ser Gly Phe Arg Asp
70 75 80
aac gac ggc gta gac agc act gcc aag agc ggc ctg cgc caa tta acg
288 Asn Asp Gly Val Asp Ser Thr Ala Lys Ser Ala Ser Leu Gln Leu Thr
85 90 95
cag acc att ttc gat atg tcc aaa tgg cgc gcc ctg agc ctg cag gaa
336 Gln Thr Ile Phe Asp Met Ser Lys Trp Arg Ala Leu Thr Leu Gln
100 105 110
aaa acc gca ggc att cag gat gty acc tac cag acc gat cag cag agc
384 Lys Thr Ala Gly Ile Gln Asp Val Thr Tyr Gln Thr Asp Gln Thr
115 120 125
ctg arg ctg aac act gcc aca gct tat ttc cag gtg ctg agc ggc atc
432 Leu Met Leu Asn Thr Ala Thr Ala Tyr Phe Gln Val Leu Ser Ala Ile
130 135 140
gac ggc ctg tcc tac gaa ggc cag aaa cag ggc atc tac cgc cag
480 Asp Ala Leu Ser Tyr Thr Gln Ala Gln Lys Gln Ala Ile Tyr Arg Gln
145 150 155
ctc gat caa acc acc cag cgt ttt aac gtg ggc ctg gta ggc atc acc
528 Leu Asp Gln Thr Thr Gln Arg Phe Asn Val Gly Leu Val Ala Ile Thr
160 165 170 175
gac gtg cag aac gcc ggc cag taa cgc cag tac gat aac gtg ctc ggc
576 Asp Val Gln Asn Ala Arg Ala Gln Tyr Asp Asn Val Leu Ala Asn Gln
180 185 190
gtg acc ggc cgt aac aac ctc gac aac ggc ctg gaa cag ctg gca cag
624 Val Thr Ala Arg Asn Asn Ala Leu Gln Asp Gln Arg Gln
195 200 205
gtg acg ggc aac tac tac ccc cag ctg cgc ctg agc gtc gat aat
672 Val Thr Gly Asn Tyr Tyr Pro Gln Leu Ala Ser Leu Asn Val Asp Asn
210 215 220
ttc aaa acc acc aaa ccc ggc gcc gct aac ggc ctg ctc gaa gag gca
720 Phe Lys Thr Thr Lys Pro Ala Ala Val Asn Ala Leu Leu Lys Gln Ala
225 230 235 240
gaa cag cgt aac ctg agc ctg ctg cag ggc cgt ctg agc cag gat ctg
768 Gln Gln Arg Asn Leu Thr Ala Arg Gln Leu Ser Gln Asp Leu
245 250 255
ggc cgt gag cag atc cgc tac gct gaa acc ggc cat atg ccc agc ctg
816 Ala Arg Gln Ile Arg Tyr Ala Gln Thr Gly His Met Pro Thr Leu
260 265 270
ggc tta acg ggc tcc agc gtc agc gtc tcc gac acc gac tac agc ggc agc
864 Gly Leu Thr Ala Ser Ser Val Ser Asp Thr Asp Tyr Ser Gly Ser
275 280 285
aaa acc agc agc ggc ggc gca agc cgt tac gct gac agc aaa atc ggc
912
```

-continued-

-continued

Lys	Thr	Ser	Gly	Ala	Ala	Ala	Ser	Arg	Tyr	Ala	Asp	Ser	Lys	Ile	Gly		
	290					295					300						
cag	aac	tcc	atc	ggc	ctg	agc	ttc	aac	ctg	ccg	ctc	tac	agc	ggc	ggc	960	
Gln	Asn	Ser	Ile	Gly	Leu	Ser	Phe	Asn	Leu	Pro	Leu	Tyr	Ser	Gly	Gly		
305				310					315					320			
tcg	gtg	aca	tca	caa	gtt	aaa	caa	gcg	cag	tac	agc	ttc	gtg	ggt	gcc	1008	
Ser	Val	Thr	Ser	Gln	Val	Lys	Gln	Ala	Gln	Tyr	Ser	Phe	Val	Gly	Ala		
				325					330					335			
agc	gaa	aaa	ctg	gaa	agc	gcg	cac	cgc	aac	gtc	gtg	cag	acc	gtg	cgt	1056	
Ser	Glu	Lys	Leu	Glu	Ser	Ala	His	Arg	Asn	Val	Val	Gln	Thr	Val	Arg		
			340					345					350				
tcg	tct	tat	aac	aac	gtt	aac	gcc	tcc	atc	agc	agc	atc	aaa	gcc	tat	1104	
Ser	Ser	Tyr	Asn	Asn	Val	Asn	Ala	Ser	Ile	Ser	Ser	Ile	Lys	Ala	Tyr		
		355				360						365					
gag	cag	gcg	gtc	gtg	tcc	gcg	caa	agc	tca	ctg	gat	gcg	atg	gaa	gcc	1152	
Glu	Gln	Ala	Val	Val	Ser	Ala	Gln	Ser	Ser	Leu	Asp	Ala	Met	Glu	Ala		
	370					375					380						
ggt	tac	tcg	gtc	ggt	acg	cgt	acc	atc	gtc	gat	gtg	ctc	gac	gcc	acc	1200	
Gly	Tyr	Ser	Val	Gly	Thr	Arg	Thr	Ile	Val	Asp	Val	Leu	Asp	Ala	Thr		
385					390					395				400			
acc	acg	ctg	tac	aac	gcc	aaa	cag	cag	ctc	tcc	agc	gcg	cgt	tat	aac	1248	
Thr	Thr	Leu	Tyr	Asn	Ala	Lys	Gln	Gln	Leu	Ser	Ser	Ala	Arg	Tyr	Asn		
				405					410					415			
tac	ctg	atc	aac	cag	ctc	aat	att	aaa	tct	gcg	ctg	ggt	acg	ctc	aac	1296	
Tyr	Leu	Ile	Asn	Gln	Leu	Asn	Ile	Lys	Ser	Ala	Leu	Gly	Thr	Leu	Asn		
			420					425					430				
gag	cag	gat	ctg	gtc	gcg	ctg	aat	aac	tcg	ctg	ggc	aaa	ccg	gtc	tct	1344	
Glu	Gln	Asp	Leu	Val	Ala	Leu	Asn	Asn	Ser	Leu	Gly	Lys	Pro	Val	Ser		
		435					440					445					
acc	gcg	cct	gaa	agc	gtc	gcc	ccg	gaa	aac	ccg	gag	cag	gac	gcc	gcc	1392	
Thr	Ala	Pro	Glu	Ser	Val	Ala	Pro	Glu	Asn	Pro	Glu	Gln	Asp	Ala	Ala		
		450				455					460						
gtg	aat	aac	atg	gcg	aac	ggc	ggc	ggc	aat	gcg	cct	gcc	atg	cag	cct	1440	
Val	Asn	Asn	Met	Ala	Asn	Gly	Gly	Gly	Asn	Ala	Pro	Ala	Met	Gln	Pro		
465					470				475					480			
gcc	gcg	gcc	acc	cgt	agc	agc	aac	agc	aac	agc	ggc	aac	ccg	ttc	cgt	1488	
Ala	Ala	Ala	Thr	Arg	Ser	Ser	Asn	Ser	Asn	Ser	Gly	Asn	Pro	Phe	Arg		
				485				490					495				
cag	taa															1494	
Gln																	

&lt;210&gt; SEQ ID NO 22

&lt;211&gt; LENGTH: 497

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Enterobacter sakazakii

&lt;400&gt; SEQUENCE: 22

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

-continued

---

Gln Thr Ile Phe Asp Met Ser Lys Trp Arg Ala Leu Thr Leu Gln Glu  
                   100  105  110

Lys Thr Ala Gly Ile Gln Asp Val Thr Tyr Gln Thr Asp Gln Gln Thr  
                   115  120  125

Leu Met Leu Asn Thr Ala Thr Ala Tyr Phe Gln Val Leu Ser Ala Ile  
                   130  135  140

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

Leu Asp Gln Thr Thr Gln Arg Phe Asn Val Gly Leu Val Ala Ile Thr  
   165  170  175

Asp Val Gln Asn Ala Arg Ala Gln Tyr Asp Asn Val Leu Ala Asn Glu  
                   180  185  190

Val Thr Ala Arg Asn Asn Leu Asp Asn Ala Leu Glu Gln Leu Arg Gln  
                   195  200  205

Val Thr Gly Asn Tyr Tyr Pro Gln Leu Ala Ser Leu Asn Val Asp Asn  
                   210  215  220

Phe Lys Thr Thr Lys Pro Ala Ala Val Asn Ala Leu Leu Lys Glu Ala  
 225  230  235  240

Glu Gln Arg Asn Leu Thr Leu Leu Gln Ala Arg Leu Ser Gln Asp Leu  
   245  250  255

Ala Arg Glu Gln Ile Arg Tyr Ala Glu Thr Gly His Met Pro Thr Leu  
                   260  265  270

Gly Leu Thr Ala Ser Ser Ser Val Ser Asp Thr Asp Tyr Ser Gly Ser  
                   275  280  285

Lys Thr Ser Gly Ala Ala Ala Ser Arg Tyr Ala Asp Ser Lys Ile Gly  
                   290  295  300

Gln Asn Ser Ile Gly Leu Ser Phe Asn Leu Pro Leu Tyr Ser Gly Gly  
 305  310  315  320

Ser Val Thr Ser Gln Val Lys Gln Ala Gln Tyr Ser Phe Val Gly Ala  
   325  330  335

Ser Glu Lys Leu Glu Ser Ala His Arg Asn Val Val Gln Thr Val Arg  
                   340  345  350

Ser Ser Tyr Asn Asn Val Asn Ala Ser Ile Ser Ser Ile Lys Ala Tyr  
                   355  360  365

Glu Gln Ala Val Val Ser Ala Gln Ser Ser Leu Asp Ala Met Glu Ala  
                   370  375  380

Gly Tyr Ser Val Gly Thr Arg Thr Ile Val Asp Val Leu Asp Ala Thr  
 385  390  395  400

Thr Thr Leu Tyr Asn Ala Lys Gln Gln Leu Ser Ser Ala Arg Tyr Asn  
   405  410  415

Tyr Leu Ile Asn Gln Leu Asn Ile Lys Ser Ala Leu Gly Thr Leu Asn  
                   420  425  430

Glu Gln Asp Leu Val Ala Leu Asn Asn Ser Leu Gly Lys Pro Val Ser  
                   435  440  445

Thr Ala Pro Glu Ser Val Ala Pro Glu Asn Pro Glu Gln Asp Ala Ala  
                   450  455  460

Val Asn Asn Met Ala Asn Gly Gly Gly Asn Ala Pro Ala Met Gln Pro  
 465  470  475  480

Ala Ala Ala Thr Arg Ser Ser Asn Ser Asn Ser Gly Asn Pro Phe Arg  
   485  490  495

Gln

&lt;210&gt; SEQ ID NO 23

&lt;211&gt; LENGTH: 1401

-continued

```

<212> TYPE: DNA
<213> ORGANISM: Erwinia carotovora
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1401)

<400> SEQUENCE: 23

atg caa atg aag aaa ttg ctc cct ctt ctt att ggt ctg agc ctg ggt      48
Met Gln Met Lys Lys Leu Leu Pro Leu Leu Ile Gly Leu Ser Leu Gly
1          5          10          15

ggc ttt agc gcc atg agt cag gcg gaa aac cta tta cag gtt tac cag      96
Gly Phe Ser Ala Met Ser Gln Ala Glu Asn Leu Leu Gln Val Tyr Gln
          20          25          30

cag gca aaa agc acc aac cct gat tta cgc agc tct gcg gca acc cgc     144
Gln Ala Lys Ser Thr Asn Pro Asp Leu Arg Ser Ser Ala Ala Thr Arg
          35          40          45

gac gcc gcg ttt gaa aaa atc aat gaa tca cgc agc ccg ctg ctg cca     192
Asp Ala Ala Phe Glu Lys Ile Asn Glu Ser Arg Ser Pro Leu Leu Pro
          50          55          60

cag ttg ggt tta ggc gct gac tat acc tac aac aga ggc tac cgt gac     240
Gln Leu Gly Leu Gly Ala Asp Tyr Thr Tyr Asn Arg Gly Tyr Arg Asp
65          70          75          80

agc aaa ggc gtc aac agc gac gtc aag ggt gct tca ctg caa ttg acc     288
Ser Lys Gly Val Asn Ser Asp Val Lys Gly Ala Ser Leu Gln Leu Thr
          85          90          95

cag acg ctg ttc gac atg tcc aaa tgg cgt gcg ctg aca ttg cag gaa     336
Gln Thr Leu Phe Asp Met Ser Lys Trp Arg Ala Leu Thr Leu Gln Glu
          100          105          110

aaa caa gcc ggt att gaa gac gta acc tat cag acc gct caa cag aac     384
Lys Gln Ala Gly Ile Glu Asp Val Thr Tyr Gln Thr Ala Gln Gln Asn
          115          120          125

ctg atg ctg aac acg gcg acc gct tat ttc aac gtg ctg cgc gct att     432
Leu Met Leu Asn Thr Ala Thr Ala Tyr Phe Asn Val Leu Arg Ala Ile
          130          135          140

gac tca ctg tcc tac atc aac gcg cag aaa cag gca att tat cgc cag     480
Asp Ser Leu Ser Tyr Ile Asn Ala Gln Lys Gln Ala Ile Tyr Arg Gln
          145          150          155          160

ttg gat caa acg aca cag cgt ttc aac gta ggt ctg gtt gcc att acc     528
Leu Asp Gln Thr Thr Gln Arg Phe Asn Val Gly Leu Val Ala Ile Thr
          165          170          175

gac gtt cag aac gct cgc gca caa tat gac agc gtg cta gcc aat gaa     576
Asp Val Gln Asn Ala Arg Ala Gln Tyr Asp Ser Val Leu Ala Asn Glu
          180          185          190

gtg ttg acg cgt aat acg cta gat aat gcg ctg gaa tca ctg cgc cag     624
Val Leu Thr Arg Asn Thr Leu Asp Asn Ala Leu Glu Ser Leu Arg Gln
          195          200          205

att acg ggc aat ttc tac ccg caa ttg gct ggt ctg aac atc gag cgt     672
Ile Thr Gly Asn Phe Tyr Pro Gln Leu Ala Gly Leu Asn Ile Glu Arg
          210          215          220

ttc tct acc cag aaa cct gaa gcc gtt aac aac ctg ctg aaa gaa gcc     720
Phe Ser Thr Gln Lys Pro Glu Ala Val Asn Asn Leu Leu Lys Glu Ala
          225          230          235          240

gaa aac cgc aac ttg aac ctg ttg tcc gca cgt ttg agc cag gat ttg     768
Glu Asn Arg Asn Leu Asn Leu Leu Ser Ala Arg Leu Ser Gln Asp Leu
          245          250          255

gca cgt gag cag att cgc tcc gcc gaa aca ggc tat atg ccg acg ctg     816
Ala Arg Glu Gln Ile Arg Ser Ala Glu Thr Gly Tyr Met Pro Thr Leu
          260          265          270

gac ctc acc gca tcg acg ggc gtg agc gat acc cgc tac tcg ggt tca     864
Asp Leu Thr Ala Ser Thr Gly Val Ser Asp Thr Arg Tyr Ser Gly Ser
          275          280          285

```

-continued

aga aca cag aac agt aac tcg ttt aac gac acc gac gca ggg caa cac	912
Arg Thr Gln Asn Ser Asn Ser Phe Asn Asp Thr Asp Ala Gly Gln His	
290 295 300	
aga gta ggc atc aac ttc act ctg ccg ctt tac agc ggt ggc gct acc	960
Arg Val Gly Ile Asn Phe Thr Leu Pro Leu Tyr Ser Gly Gly Ala Thr	
305 310 315 320	
aat tct cag gtg aag cag gca cag cac agc tat gtt agc tct agt gaa	1008
Asn Ser Gln Val Lys Gln Ala Gln His Ser Tyr Val Ser Ser Ser Glu	
325 330 335	
ctg ctg gaa agc gca cac cgt tct gtt atc cag acg gta cgt tca tcg	1056
Leu Leu Glu Ser Ala His Arg Ser Val Ile Gln Thr Val Arg Ser Ser	
340 345 350	
ttt aac aat att tct gcc tcc atc agc agc atc aac gct tac aaa cag	1104
Phe Asn Asn Ile Ser Ala Ser Ile Ser Ser Ile Asn Ala Tyr Lys Gln	
355 360 365	
gct gaa gtg tct gca caa agc tct ttg gat gca atg gaa gct ggc tat	1152
Ala Glu Val Ser Ala Gln Ser Ser Leu Asp Ala Met Glu Ala Gly Tyr	
370 375 380	
cag gta gga acg cgc acc atc gtt gac gta ctg gat gcc acc acc acg	1200
Gln Val Gly Thr Arg Thr Ile Val Asp Val Leu Asp Ala Thr Thr Thr	
385 390 395 400	
ctg tat aac gcc aaa cag cag ctc tcc agc gca cgt tat gat tac ctg	1248
Leu Tyr Asn Ala Lys Gln Gln Leu Ser Ser Ala Arg Tyr Asp Tyr Leu	
405 410 415	
atc aat cag tta aac atc aag tcc gca cag ggc acg ctg agc gaa acc	1296
Ile Asn Gln Leu Asn Ile Lys Ser Ala Gln Gly Thr Leu Ser Glu Thr	
420 425 430	
gat ctg caa gcg ctg aat gcg tca ttg ggt cag ccg gtt tcc act aca	1344
Asp Leu Gln Ala Leu Asn Ala Ser Leu Gly Gln Pro Val Ser Thr Thr	
435 440 445	
ccg acc gta acg gac aat acc gcc ccg cag gca aca acc gcc tcg gcg	1392
Pro Thr Val Thr Asp Asn Thr Ala Pro Gln Ala Thr Thr Ala Ser Ala	
450 455 460	
cag cgt taa	1401
Gln Arg	
465	

&lt;210&gt; SEQ ID NO 24

&lt;211&gt; LENGTH: 466

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Erwinia carotovora

&lt;400&gt; SEQUENCE: 24

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

-continued

---

Leu Met Leu Asn Thr Ala Thr Ala Tyr Phe Asn Val Leu Arg Ala Ile  
 130 135 140

Asp Ser Leu Ser Tyr Ile Asn Ala Gln Lys Gln Ala Ile Tyr Arg Gln  
 145 150 155 160

Leu Asp Gln Thr Thr Gln Arg Phe Asn Val Gly Leu Val Ala Ile Thr  
 165 170 175

Asp Val Gln Asn Ala Arg Ala Gln Tyr Asp Ser Val Leu Ala Asn Glu  
 180 185 190

Val Leu Thr Arg Asn Thr Leu Asp Asn Ala Leu Glu Ser Leu Arg Gln  
 195 200 205

Ile Thr Gly Asn Phe Tyr Pro Gln Leu Ala Gly Leu Asn Ile Glu Arg  
 210 215 220

Phe Ser Thr Gln Lys Pro Glu Ala Val Asn Asn Leu Leu Lys Glu Ala  
 225 230 235 240

Glu Asn Arg Asn Leu Asn Leu Leu Ser Ala Arg Leu Ser Gln Asp Leu  
 245 250 255

Ala Arg Glu Gln Ile Arg Ser Ala Glu Thr Gly Tyr Met Pro Thr Leu  
 260 265 270

Asp Leu Thr Ala Ser Thr Gly Val Ser Asp Thr Arg Tyr Ser Gly Ser  
 275 280 285

Arg Thr Gln Asn Ser Asn Ser Phe Asn Asp Thr Asp Ala Gly Gln His  
 290 295 300

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

Asn Ser Gln Val Lys Gln Ala Gln His Ser Tyr Val Ser Ser Ser Glu  
 325 330 335

Leu Leu Glu Ser Ala His Arg Ser Val Ile Gln Thr Val Arg Ser Ser  
 340 345 350

Phe Asn Asn Ile Ser Ala Ser Ile Ser Ser Ile Asn Ala Tyr Lys Gln  
 355 360 365

Ala Glu Val Ser Ala Gln Ser Ser Leu Asp Ala Met Glu Ala Gly Tyr  
 370 375 380

Gln Val Gly Thr Arg Thr Ile Val Asp Val Leu Asp Ala Thr Thr Thr  
 385 390 395 400

Leu Tyr Asn Ala Lys Gln Gln Leu Ser Ser Ala Arg Tyr Asp Tyr Leu  
 405 410 415

Ile Asn Gln Leu Asn Ile Lys Ser Ala Gln Gly Thr Leu Ser Glu Thr  
 420 425 430

Asp Leu Gln Ala Leu Asn Ala Ser Leu Gly Gln Pro Val Ser Thr Thr  
 435 440 445

Pro Thr Val Thr Asp Asn Thr Ala Pro Gln Ala Thr Thr Ala Ser Ala  
 450 455 460

Gln Arg  
 465

<210> SEQ ID NO 25  
 <211> LENGTH: 1494  
 <212> TYPE: DNA  
 <213> ORGANISM: Serratia proteamaculans  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (1)..(1494)

<400> SEQUENCE: 25

atg aag aaa ctg ctc ccc ctt ctt atc gga ctg agc ctg ggc ggc ttc  
 Met Lys Lys Leu Leu Pro Leu Leu Ile Gly Leu Ser Leu Gly Gly Phe  
 1 5 10 15

-continued

agt gca atg agc cag gca gag aac ctg ctg cag gtc tac aaa cag gcc	96
Ser Ala Met Ser Gln Ala Glu Asn Leu Leu Gln Val Tyr Lys Gln Ala	
20 25 30	
agg gaa agt aac ccg gat ctg cgc aaa acc gcc gct gac cgt gac gcc	144
Arg Glu Ser Asn Pro Asp Leu Arg Lys Thr Ala Ala Asp Arg Asp Ala	
35 40 45	
gca ttc gaa aaa atc aac gaa gca cgc agc ccg ttg ctg ccg cag ttg	192
Ala Phe Glu Lys Ile Asn Glu Ala Arg Ser Pro Leu Leu Pro Gln Leu	
50 55 60	
ggg ttg agc gcc ggt tac act tac acc aat ggc tac cgt gac agc aaa	240
Gly Leu Ser Ala Gly Tyr Thr Tyr Thr Asn Gly Tyr Arg Asp Ser Lys	
65 70 75 80	
gat gcc aac agc gat gcc acc agt ggc tcc ctg gcg ttg acc cag act	288
Asp Ala Asn Ser Asp Ala Thr Ser Gly Ser Leu Ala Leu Thr Gln Thr	
85 90 95	
atc ttc gac atg tcc aaa tgg cgt gcg ctg acg ctg cag gaa aaa acc	336
Ile Phe Asp Met Ser Lys Trp Arg Ala Leu Thr Leu Gln Glu Lys Thr	
100 105 110	
gcc ggc att tcc gac gtg act ttc caa acc tcg tca cag cag ctg atc	384
Ala Gly Ile Ser Asp Val Thr Phe Gln Thr Ser Ser Gln Gln Leu Ile	
115 120 125	
ctc gat acc gct acc gcc tat ttt aac gtg ctg agc gcc atc gat acg	432
Leu Asp Thr Ala Thr Ala Tyr Phe Asn Val Leu Ser Ala Ile Asp Thr	
130 135 140	
ctg tcc tac acc cag gcg aac aag caa gcg gtt tac cgc acc ctg gac	480
Leu Ser Tyr Thr Gln Ala Asn Lys Gln Ala Val Tyr Arg Thr Leu Asp	
145 150 155 160	
cag acc acc caa cgc ttt aac gtg ggc ctg gtc gcg atc acc gac gtg	528
Gln Thr Thr Gln Arg Phe Asn Val Gly Leu Val Ala Ile Thr Asp Val	
165 170 175	
caa aac gcc cgt tcg tcc tac gat acc gtg ctg gcg gcc gaa gtc acc	576
Gln Asn Ala Arg Ser Ser Tyr Asp Thr Val Leu Ala Ala Glu Val Thr	
180 185 190	
gcc cgt aac aac ctg gac aac gcg ctg gaa aaa ctg cgc cag gtc acc	624
Ala Arg Asn Asn Leu Asp Asn Ala Leu Glu Lys Leu Arg Gln Val Thr	
195 200 205	
ggc acc ttc tat ccg gaa ctg gcc tcg ttg aat acc gac cgt ttc aac	672
Gly Thr Phe Tyr Pro Glu Leu Ala Ser Leu Asn Thr Asp Arg Phe Asn	
210 215 220	
acc aaa cgc ccg gat gca gtc aat aat ctg ctg aaa gaa gcc gaa agc	720
Thr Lys Arg Pro Asp Ala Val Asn Asn Leu Leu Lys Glu Ala Glu Ser	
225 230 235 240	
cgt aac ctg agc ctg ttg tcc gct cgc ctg agc cag gat ctg gcc cgt	768
Arg Asn Leu Ser Leu Leu Ser Ala Arg Leu Ser Gln Asp Leu Ala Arg	
245 250 255	
gag cag atc cgt tcc gca cag acc ggt tat atg cct acc gtt gat ttc	816
Glu Gln Ile Arg Ser Ala Gln Thr Gly Tyr Met Pro Thr Val Asp Phe	
260 265 270	
agc gca tcc act gcg gtg agc aat act aat tac agc ggt tct cgc aac	864
Ser Ala Ser Thr Ala Val Ser Asn Thr Asn Tyr Ser Gly Ser Arg Asn	
275 280 285	
gtg aac aac gac gct gat att ggt cag aac aaa gtg ggc ctg agc ttt	912
Val Asn Asn Asp Ala Asp Ile Gly Gln Asn Lys Val Gly Leu Ser Phe	
290 295 300	
aac ttg ccg ttg tac agc ggc ggc cag acc aac tca cag gtg cag cag	960
Asn Leu Pro Leu Tyr Ser Gly Gly Gln Thr Asn Ser Gln Val Gln Gln	
305 310 315 320	
gcg cag tac aac ttc gtt ggc gcc agt gag caa ctg gaa agc gcc cac	1008
Ala Gln Tyr Asn Phe Val Gly Ala Ser Glu Gln Leu Glu Ser Ala His	
325 330 335	

-continued

---

```

cgc agc gta gtg cag acc gtg cgt tct tcg ttc aat aac gtg aat gcc 1056
Arg Ser Val Val Gln Thr Val Arg Ser Ser Phe Asn Asn Val Asn Ala
      340      345      350

tcg atc agc agc atc aac gcc tac caa caa gcg gta gtg tct gcc cag 1104
Ser Ile Ser Ser Ile Asn Ala Tyr Gln Gln Ala Val Val Ser Ala Gln
      355      360      365

agt tca ttg gat gcg acc gag gcc ggt tac cag gta ggt acc cgt acc 1152
Ser Ser Leu Asp Ala Thr Glu Ala Gly Tyr Gln Val Gly Thr Arg Thr
      370      375      380

atc gtc gac gtg ctg gat gcg acc agt acg ctg tat aac gcc aag caa 1200
Ile Val Asp Val Leu Asp Ala Thr Ser Thr Leu Tyr Asn Ala Lys Gln
      385      390      395      400

cag ctc tcc agc gcg cgt tat acc tac ctg atc aac caa ctg aac atc 1248
Gln Leu Ser Ser Ala Arg Tyr Thr Tyr Leu Ile Asn Gln Leu Asn Ile
      405      410      415

aag tcg gcg ctc ggt acc ctg aac gag aac gat ctg atg atg ctg aat 1296
Lys Ser Ala Leu Gly Thr Leu Asn Glu Asn Asp Leu Met Met Leu Asn
      420      425      430

ggc gca ttg ggt aaa ccg att tct act tcg caa gac gtg gta gcg cca 1344
Gly Ala Leu Gly Lys Pro Ile Ser Thr Ser Gln Asp Val Val Ala Pro
      435      440      445

ccg act acc gca cag gac gct tac gct gaa ggc tat aac ggc aac gcc 1392
Pro Thr Thr Ala Gln Asp Ala Tyr Ala Glu Gly Tyr Asn Gly Asn Ala
      450      455      460

cct gcg cca caa act gca gca ccg gtt gcc acc cgc gcc tcc gca ccg 1440
Pro Ala Pro Gln Thr Ala Ala Pro Val Ala Thr Arg Ala Ser Ala Pro
      465      470      475      480

gcg gcc acc acc agc cag cct gca cgc acc agc ggt aat cca ttc cgt 1488
Ala Ala Thr Thr Ser Gln Pro Ala Arg Thr Ser Gly Asn Pro Phe Arg
      485      490      495

aat tga 1494
Asn

<210> SEQ ID NO 26
<211> LENGTH: 497
<212> TYPE: PRT
<213> ORGANISM: Serratia proteamaculans

<400> SEQUENCE: 26
Met Lys Lys Leu Leu Pro Leu Leu Ile Gly Leu Ser Leu Gly Gly Phe
1      5      10
Ser Ala Met Ser Gln Ala Glu Asn Leu Leu Gln Val Tyr Lys Gln Ala
20     25     30
Arg Glu Ser Asn Pro Asp Leu Arg Lys Thr Ala Ala Asp Arg Asp Ala
35     40     45
Ala Phe Glu Lys Ile Asn Glu Ala Arg Ser Pro Leu Leu Pro Gln Leu
50     55     60
Gly Leu Ser Ala Gly Tyr Thr Tyr Thr Asn Gly Tyr Arg Asp Ser Lys
65     70     75     80
Asp Ala Asn Ser Asp Ala Thr Ser Gly Ser Leu Ala Leu Thr Gln Thr
85     90     95
Ile Phe Asp Met Ser Lys Trp Arg Ala Leu Thr Leu Gln Glu Lys Thr
100    105   110
Ala Gly Ile Ser Asp Val Thr Phe Gln Thr Ser Ser Gln Gln Leu Ile
115    120   125
Leu Asp Thr Ala Thr Ala Tyr Phe Asn Val Leu Ser Ala Ile Asp Thr
130    135   140
Leu Ser Tyr Thr Gln Ala Asn Lys Gln Ala Val Tyr Arg Thr Leu Asp

```





-continued

1	5	10	15	
ggc gcc cac gcc gag aac ctg ctc gat att tac caa caa gcc cag atc Gly Ala His Ala Glu Asn Leu Leu Asp Ile Tyr Gln Gln Ala Gln Ile 20 25 30				96
aag gac acc caa ctg cag gaa tcc aag gcc aag cgt gac caa gcc ttc Lys Asp Thr Gln Leu Gln Glu Ser Lys Ala Lys Arg Asp Gln Ala Phe 35 40 45				144
gag aag atc aat gaa tcc cgc gca gcc ctc ttg ccg caa atc aat ctg Glu Lys Ile Asn Glu Ser Arg Ala Ala Leu Leu Pro Gln Ile Asn Leu 50 55 60				192
gga gcc gcc ctg aac tac ctg caa aac aag ggt gat acc cag acc aac Gly Ala Gly Leu Asn Tyr Leu Gln Asn Lys Gly Asp Thr Gln Thr Asn 65 70 75 80				240
agc aac gct act gcc tcc ctc tcg ctg gat caa tct atc tat cgt cgc Ser Asn Ala Thr Gly Ser Leu Ser Leu Asp Gln Ser Ile Tyr Arg Arg 85 90 95				288
agc aac tgg gtc aac ctg gac ctg acc gag aag agc gcc acc cag tcc Ser Asn Trp Val Asn Leu Asp Leu Thr Glu Lys Ser Ala Thr Gln Ser 100 105 110				336
gat gtg gcc tac aac ctc gaa ata cag aat ctg atg ctg cgc acc gcc Asp Val Ala Tyr Asn Leu Glu Ile Gln Asn Leu Met Leu Arg Thr Ala 115 120 125				384
cag gcc tat ttc aac gtg ctc aag gca atg gac acc ctg gaa ttc gtc Gln Ala Tyr Phe Asn Val Leu Lys Ala Met Asp Thr Leu Glu Phe Val 130 135 140				432
cgc gcc aac aag gcc gcc gta gaa cgt cag ctg gaa cag acc cag cag Arg Ala Asn Lys Ala Ala Val Glu Arg Gln Leu Glu Gln Thr Gln Gln 145 150 155 160				480
cgc ttc gaa gtg gcc ctg acc gcc atc acg gac gtg cat gag gct gaa Arg Phe Glu Val Gly Leu Thr Ala Ile Thr Asp Val His Glu Ala Glu 165 170 175				528
gcc gag cgc gat cag gca ctg gcg gac gag atc aat gcc gag aac acg Ala Glu Arg Asp Gln Ala Leu Ala Asp Glu Ile Asn Ala Glu Asn Thr 180 185 190				576
ctg gac aac agc tac gag agt ctg cgc gag ctg acc gcc atc gac cac Leu Asp Asn Ser Tyr Glu Ser Leu Arg Glu Leu Thr Gly Ile Asp His 195 200 205				624
cgt cag ctg gac gta ctc aac act gag cgt ttc agc ccg cag aag acg Arg Gln Leu Asp Val Leu Asn Thr Glu Arg Phe Ser Pro Gln Lys Thr 210 215 220				672
ccg ttc aac tcc gac aaa tgg ctg gag ctg gca ctg gac aag aac ctg Pro Phe Asn Ser Asp Lys Trp Leu Glu Leu Ala Leu Asp Lys Asn Leu 225 230 235 240				720
caa ctg cac agc gcc cgc atc ggc aag gat atc gcc aag gag cag atc Gln Leu His Ser Ala Arg Ile Gly Lys Asp Ile Ala Lys Glu Gln Ile 245 250 255				768
gat ctg gcc aag acc ggt cac gag ccg acg ctg gat ctg ggt gcc ggt Asp Leu Ala Lys Thr Gly His Glu Pro Thr Leu Asp Leu Gly Ala Gly 260 265 270				816
ctc tcc agc acc tat agc gat tac aag gac gag atc cgc aac ccc gag Leu Ser Ser Thr Tyr Ser Asp Tyr Lys Asp Glu Ile Arg Asn Pro Glu 275 280 285				864
agc aac agc aat cag gcc aac ata ggc ctg aac ttc aag ctg ccg ctc Ser Asn Ser Ser Asn Gln Gly Asn Ile Gly Leu Asn Phe Lys Leu Pro Leu 290 295 300				912
tac acg ggt gcc gcg acc acc tcc cag gtc aag cag tcc cag ttc aac Tyr Thr Gly Gly Ala Thr Thr Ser Gln Val Lys Gln Ser Gln Phe Asn 305 310 315 320				960
tat gtg gcg gcc agc gag cag ctg gag cgc agc ttc cgc tct gtg cag Tyr Val Ala Ala Ser Glu Gln Leu Glu Arg Ser Phe Arg Ser Val Gln				1008

-continued

325				330				335								
agc	aca	gta	cgc	tcc	tcc	tat	aac	aac	gtg	aac	gcc	agc	ata	ggg	tcg	1056
Ser	Thr	Val	Arg	Ser	Ser	Tyr	Asn	Asn	Val	Asn	Ala	Ser	Ile	Gly	Ser	
			340					345					350			
gta	cgc	gcc	tac	ggc	cag	tcc	gtc	atc	tcc	gcc	gac	agc	gcc	ctc	aag	1104
Val	Arg	Ala	Tyr	Gly	Gln	Ser	Val	Ile	Ser	Ala	Asp	Ser	Ala	Leu	Lys	
		355					360					365				
gcc	acc	gaa	gcg	ggc	tat	gaa	gtc	ggg	acc	cgc	acc	ata	gtc	gac	gtg	1152
Ala	Thr	Glu	Ala	Gly	Tyr	Glu	Val	Gly	Thr	Arg	Thr	Ile	Val	Asp	Val	
		370				375						380				
ctg	gac	tct	acc	cgc	aag	ctc	tac	cag	gcc	aag	cag	aaa	ctc	tcc	gaa	1200
Leu	Asp	Ser	Thr	Arg	Lys	Leu	Tyr	Gln	Ala	Lys	Gln	Lys	Leu	Ser	Glu	
		385			390					395				400		
gcc	cgt	tac	aac	tac	att	ctc	agc	atc	ctc	tcc	ctc	aag	cag	gcc	gcc	1248
Ala	Arg	Tyr	Asn	Tyr	Ile	Leu	Ser	Ile	Leu	Ser	Leu	Lys	Gln	Ala	Ala	
			405					410						415		
ggc	acg	ctg	gag	cag	aaa	gat	ctg	gaa	gaa	gta	aac	cag	gga	ctg	ata	1296
Gly	Thr	Leu	Glu	Gln	Lys	Asp	Leu	Glu	Glu	Val	Asn	Gln	Gly	Leu	Ile	
			420					425					430			
cct	gcc	gct	cag	gtc	aag	aac	aag	tcc	tga							1326
Pro	Ala	Ala	Gln	Val	Lys	Asn	Lys	Ser								
		435					440									

&lt;210&gt; SEQ ID NO 28

&lt;211&gt; LENGTH: 441

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Aeromonas salmonicida

&lt;400&gt; SEQUENCE: 28

Met	Lys	Arg	Thr	Leu	Leu	Ser	Ala	Met	Val	Leu	Leu	Gly	Val	Ser	Ala	
1				5					10					15		
Gly	Ala	His	Ala	Glu	Asn	Leu	Leu	Asp	Ile	Tyr	Gln	Gln	Ala	Gln	Ile	
			20					25					30			
Lys	Asp	Thr	Gln	Leu	Gln	Glu	Ser	Lys	Ala	Lys	Arg	Asp	Gln	Ala	Phe	
		35						40				45				
Glu	Lys	Ile	Asn	Glu	Ser	Arg	Ala	Ala	Leu	Leu	Pro	Gln	Ile	Asn	Leu	
		50				55					60					
Gly	Ala	Gly	Leu	Asn	Tyr	Leu	Gln	Asn	Lys	Gly	Asp	Thr	Gln	Thr	Asn	
		65			70					75					80	
Ser	Asn	Ala	Thr	Gly	Ser	Leu	Ser	Leu	Asp	Gln	Ser	Ile	Tyr	Arg	Arg	
			85						90					95		
Ser	Asn	Trp	Val	Asn	Leu	Asp	Leu	Thr	Glu	Lys	Ser	Ala	Thr	Gln	Ser	
		100						105						110		
Asp	Val	Ala	Tyr	Asn	Leu	Glu	Ile	Gln	Asn	Leu	Met	Leu	Arg	Thr	Ala	
		115					120					125				
Gln	Ala	Tyr	Phe	Asn	Val	Leu	Lys	Ala	Met	Asp	Thr	Leu	Glu	Phe	Val	
		130				135					140					
Arg	Ala	Asn	Lys	Ala	Ala	Val	Glu	Arg	Gln	Leu	Glu	Gln	Thr	Gln	Gln	
		145			150					155					160	
Arg	Phe	Glu	Val	Gly	Leu	Thr	Ala	Ile	Thr	Asp	Val	His	Glu	Ala	Glu	
			165						170					175		
Ala	Glu	Arg	Asp	Gln	Ala	Leu	Ala	Asp	Glu	Ile	Asn	Ala	Glu	Asn	Thr	
		180						185					190			
Leu	Asp	Asn	Ser	Tyr	Glu	Ser	Leu	Arg	Glu	Leu	Thr	Gly	Ile	Asp	His	
		195					200					205				
Arg	Gln	Leu	Asp	Val	Leu	Asn	Thr	Glu	Arg	Phe	Ser	Pro	Gln	Lys	Thr	
		210				215					220					

-continued

---

Pro Phe Asn Ser Asp Lys Trp Leu Glu Leu Ala Leu Asp Lys Asn Leu  
 225 230 235 240

Gln Leu His Ser Ala Arg Ile Gly Lys Asp Ile Ala Lys Glu Gln Ile  
 245 250 255

Asp Leu Ala Lys Thr Gly His Glu Pro Thr Leu Asp Leu Gly Ala Gly  
 260 265 270

Leu Ser Ser Thr Tyr Ser Asp Tyr Lys Asp Glu Ile Arg Asn Pro Glu  
 275 280 285

Ser Asn Ser Asn Gln Gly Asn Ile Gly Leu Asn Phe Lys Leu Pro Leu  
 290 295 300

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

Tyr Val Ala Ala Ser Glu Gln Leu Glu Arg Ser Phe Arg Ser Val Gln  
 325 330 335

Ser Thr Val Arg Ser Ser Tyr Asn Asn Val Asn Ala Ser Ile Gly Ser  
 340 345 350

Val Arg Ala Tyr Gly Gln Ser Val Ile Ser Ala Asp Ser Ala Leu Lys  
 355 360 365

Ala Thr Glu Ala Gly Tyr Glu Val Gly Thr Arg Thr Ile Val Asp Val  
 370 375 380

Leu Asp Ser Thr Arg Lys Leu Tyr Gln Ala Lys Gln Lys Leu Ser Glu  
 385 390 395 400

Ala Arg Tyr Asn Tyr Ile Leu Ser Ile Leu Ser Leu Lys Gln Ala Ala  
 405 410 415

Gly Thr Leu Glu Gln Lys Asp Leu Glu Glu Val Asn Gln Gly Leu Ile  
 420 425 430

Pro Ala Ala Gln Val Lys Asn Lys Ser  
 435 440

&lt;210&gt; SEQ ID NO 29

&lt;211&gt; LENGTH: 1326

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Vibrio vulnificus

&lt;220&gt; FEATURE:

&lt;221&gt; NAME/KEY: CDS

&lt;222&gt; LOCATION: (1)..(1326)

&lt;400&gt; SEQUENCE: 29

atg aaa aaa ctg ctt cca cta ctt att ggt gca gcg cta ggt agc ctg 48  
 Met Lys Lys Leu Leu Pro Leu Leu Ile Gly Ala Ala Leu Gly Ser Leu  
 1 5 10 15

agt tct tca gtg tgg gct gat tcc ttg gca gaa atc tat gat ctg gca 96  
 Ser Ser Ser Val Trp Ala Asp Ser Leu Ala Glu Ile Tyr Asp Leu Ala  
 20 25 30

aag caa aac gat cca cag tta ttg agc gta caa gct aaa cgt gac gcc 144  
 Lys Gln Asn Asp Pro Gln Leu Leu Ser Val Gln Ala Lys Arg Asp Ala  
 35 40 45

gca ttt gaa gcg gtc act tct agc cgt agt acc tta tta ccg caa att 192  
 Ala Phe Glu Ala Val Thr Ser Ser Arg Ser Thr Leu Leu Pro Gln Ile  
 50 55 60

aat tta acc gca ggt tat aac cta aaa cgc ggt gat acg gat ctt gat 240  
 Asn Leu Thr Ala Gly Tyr Asn Leu Lys Arg Gly Asp Thr Asp Leu Asp  
 65 70 75 80

gct ggg gcg acg atc gat aat gac caa aat gca tta act gct ggg att 288  
 Ala Gly Ala Thr Ile Asp Asn Asp Gln Asn Ala Leu Thr Ala Gly Ile  
 85 90 95

aat ttc tct cag gaa ctg tat cag cgt tcc tct tgg atc acg cta gac 336  
 Asn Phe Ser Gln Glu Leu Tyr Gln Arg Ser Ser Trp Ile Thr Leu Asp  
 100 105 110

aac gca gag aaa agc gct cgt caa gca gat gca tac gca gca acg  
 384 Asn Ala Gln Lys Ser Ala Arg Gln Ala Asp Ala Tyr Ala Thr  
 115  
 caa cag ggt tct ggt atc tca aga acc gcg caa gcg tac tct gag gtc cta  
 432 Gln Gln Gly Leu Ile Leu Arg Thr Ala Gln Ala Tyr Phe Gln Val Leu  
 130  
 135  
 aaa gcg caa gac aac tca gaa ttt gtc cgt gca gaa aaa gcg ggt  
 480 Lys Ala Gln Asp Asn Leu Gln Phe Val Arg Ala Gln Lys Ala Val  
 145  
 150  
 gct cgt cag cta gag caa acc aaa caa cgt ttt gaa gtg ggt ctc tcg  
 528 Ala Arg Gln Leu Gln Thr Lys Gln Arg Phe Gln Val Gly Leu Ser  
 160  
 170  
 gcc att aca gac gtc cat gac gcc caa gcg caa tac gat ggc gta tta  
 576 Ala Ile Thr Asp Val His Asp Ala Gln Tyr Asp Gly Val Leu  
 180  
 185  
 gct gac gaa gtt ctg gcc gaa aac agc cta acc aac agt tat gaa gcg  
 624 Ala Asp Gln Val Leu Ala Gln Asn Ser Leu Thr Asn Ser Tyr Gln Ala  
 195  
 200  
 tct gct gaa atc aca ggt caa gag cat aaa aac cty aac gty tta gat  
 672 Leu Arg Gln Ile Thr Gly Gln His Lys Asn Leu Asn Val Leu Asp  
 210  
 215  
 acc aag cgt ttc tca gca agc cgc tca aat gct tca gct gaa acc tct  
 720 Thr Lys Arg Phe Ser Ala Ser Arg Ser Asn Ala Ser Ala Gln Thr Leu  
 225  
 230  
 atc gaa gaa gcg caa gag aaa aac tca agc tca cty tca gcg cgt atc  
 768 Ile Gln Gln Ala Gln Lys Asn Leu Ser Leu Leu Ser Ala Arg Ile  
 245  
 250  
 aca aaa gac atc gcc aaa gac aat att tct cta gcy agc tct ggc cac  
 816 Thr Lys Asp Ile Ala Lys Asp Asn Ile Ser Leu Ala Ser Ser Gly His  
 260  
 265  
 ctt cca tct cty act cta gac ggt ggc tac aac tac gca gac gtt agt  
 864 Leu Pro Ser Leu Thr Leu Asp Gly Tyr Asn Tyr Ala Asp Val Ser  
 275  
 280  
 aac agt gca caa agt gat ggt aca acc aat aat ttc aat gtt gtt gta  
 912 Asn Ser Ala Gln Ser Asp Gly Thr Thr Asn Asn Phe Asn Val Gly Val  
 290  
 295  
 aat ctc gtt gtt cca ctc tat acc ggt ggt aat aca acg tcy caa acc  
 960 Asn Leu Val Val Pro Leu Tyr Thr Gly Asn Thr Thr Ser Gln Thr  
 305  
 310  
 aaa caa gct gag ttt aat tac gtc tct gcy agc caa gat ctt gaa gcc  
 1008 Lys Gln Ala Gln Phe Asn Tyr Val Ser Ala Ser Gln Asp Leu Gln Ala  
 325  
 330  
 act tat cgc ggt gtc gtc gaa gaa gaa gtc cga gcg caa aac aac atc  
 1056 Thr Tyr Arg Gly Val Val Lys Gln Val Arg Ala Gln Asn Asn Ile  
 340  
 345  
 aat gcc tca atc gcc gca ctt cgt gcy tat gag caa tct gtt gtt tct  
 1104 Asn Ala Ser Ile Gly Ala Leu Arg Ala Tyr Gln Gln Ser Val Val Ser  
 355  
 360  
 gcy cgt tca gca tca gaa gca acc gaa gca ggc ttt gat gtg ggt act  
 1152 Ala Arg Ser Ala Leu Gln Ala Thr Gln Ala Gly Phe Asp Val Gly Thr  
 370  
 375  
 gtt act att gtt gat gtc ctt gat gtc acc act cgt gct gct tac gat gcc  
 1200 Arg Thr Ile Val Asp Val Leu Asp Ala Thr Arg Leu Tyr Asp Ala  
 385  
 390  
 aac aaa aac cta tcy aac tca cgc tac aac tca aac tca atc tcy agt gta cty  
 1248 Asn Lys Asn Leu Ser Asn Ala Arg Tyr Asn Tyr Ile Leu Ser Val Leu  
 405  
 410  
 caa ctt cgt cag gcc gty gtt aca cty agc gag caa gat gta cty gat  
 1296 Gln Leu Arg Gln Ala Val Gly Thr Leu Ser Gln Asp Val Leu Asp  
 420  
 430

-continued

ggt gat gct ggt ttg att gcg aaa aag taa 1326  
 Val Asp Ala Gly Leu Ile Ala Lys Lys  
           435                  440

<210> SEQ ID NO 30  
 <211> LENGTH: 441  
 <212> TYPE: PRT  
 <213> ORGANISM: *Vibrio vulnificus*

<400> SEQUENCE: 30

Met Lys Lys Leu Leu Pro Leu Leu Ile Gly Ala Ala Leu Gly Ser Leu  
 1                  5                  10                  15  
 Ser Ser Ser Val Trp Ala Asp Ser Leu Ala Glu Ile Tyr Asp Leu Ala  
           20                  25                  30  
 Lys Gln Asn Asp Pro Gln Leu Leu Ser Val Gln Ala Lys Arg Asp Ala  
           35                  40                  45  
 Ala Phe Glu Ala Val Thr Ser Ser Arg Ser Thr Leu Leu Pro Gln Ile  
           50                  55                  60  
 Asn Leu Thr Ala Gly Tyr Asn Leu Lys Arg Gly Asp Thr Asp Leu Asp  
   65                  70                  75                  80  
 Ala Gly Ala Thr Ile Asp Asn Asp Gln Asn Ala Leu Thr Ala Gly Ile  
           85                  90                  95  
 Asn Phe Ser Gln Glu Leu Tyr Gln Arg Ser Ser Trp Ile Thr Leu Asp  
           100                  105                  110  
 Asn Ala Glu Lys Ser Ala Arg Gln Ala Asp Ala Ala Tyr Ala Ala Thr  
           115                  120                  125  
 Gln Gln Gly Leu Ile Leu Arg Thr Ala Gln Ala Tyr Phe Glu Val Leu  
   130                  135                  140  
 Lys Ala Gln Asp Asn Leu Glu Phe Val Arg Ala Glu Lys Ala Ala Val  
   145                  150                  155                  160  
 Ala Arg Gln Leu Glu Gln Thr Lys Gln Arg Phe Glu Val Gly Leu Ser  
           165                  170                  175  
 Ala Ile Thr Asp Val His Asp Ala Gln Ala Gln Tyr Asp Gly Val Leu  
           180                  185                  190  
 Ala Asp Glu Val Leu Ala Glu Asn Ser Leu Thr Asn Ser Tyr Glu Ala  
           195                  200                  205  
 Leu Arg Glu Ile Thr Gly Gln Glu His Lys Asn Leu Asn Val Leu Asp  
   210                  215                  220  
 Thr Lys Arg Phe Ser Ala Ser Arg Ser Asn Ala Ser Ala Glu Thr Leu  
   225                  230                  235                  240  
 Ile Glu Glu Ala Gln Glu Lys Asn Leu Ser Leu Leu Ser Ala Arg Ile  
           245                  250                  255  
 Thr Lys Asp Ile Ala Lys Asp Asn Ile Ser Leu Ala Ser Ser Gly His  
           260                  265                  270  
 Leu Pro Ser Leu Thr Leu Asp Gly Gly Tyr Asn Tyr Ala Asp Val Ser  
           275                  280                  285  
 Asn Ser Ala Gln Ser Asp Gly Thr Thr Asn Asn Phe Asn Val Gly Val  
   290                  295                  300  
 Asn Leu Val Val Pro Leu Tyr Thr Gly Gly Asn Thr Thr Ser Gln Thr  
   305                  310                  315                  320  
 Lys Gln Ala Glu Phe Asn Tyr Val Ser Ala Ser Gln Asp Leu Glu Ala  
           325                  330                  335  
 Thr Tyr Arg Gly Val Val Lys Glu Val Arg Ala Gln Asn Asn Asn Ile  
           340                  345                  350  
 Asn Ala Ser Ile Gly Ala Leu Arg Ala Tyr Glu Gln Ser Val Val Ser

-continued

Ala	Arg	Ser	Ala	Leu	Glu	Ala	Thr	Glu	Ala	Gly	Phe	Asp	Val	Gly	Thr
370						375					380				
Arg	Thr	Ile	Val	Asp	Val	Leu	Asp	Ala	Thr	Arg	Arg	Leu	Tyr	Asp	Ala
385				390						395					400
Asn	Lys	Asn	Leu	Ser	Asn	Ala	Arg	Tyr	Asn	Tyr	Ile	Leu	Ser	Val	Leu
			405						410					415	
Gln	Leu	Arg	Gln	Ala	Val	Gly	Thr	Leu	Ser	Glu	Gln	Asp	Val	Leu	Asp
			420					425					430		
Val	Asp	Ala	Gly	Leu	Ile	Ala	Lys	Lys							
	435						440								

What is claimed is:

1. A method for producing L-cysteine, L-cystine, a derivative or precursor thereof, or a mixture thereof, which comprises culturing a bacterium belonging to the family Enterobacteriaceae in a medium and collecting L-cysteine, L-cystine, a derivative or precursor thereof, or a mixture thereof from the medium, wherein the bacterium has the ability to produce L-cysteine and has been modified so that an activity of a protein encoded by a *tolC* gene is increased as compared to a non-modified bacterium,
  - wherein the activity of the protein is increased by increasing expression amount of the *tolC* gene, increasing translation amount of the *tolC* gene, or combinations thereof.
2. The method according to claim 1, wherein the derivative of L-cysteine is a thiazolidine derivative.
3. The method according to claim 1, wherein the precursor of L-cysteine is O-acetylserine or N-acetylserine.
4. The method according to claim 1, wherein expression amount of the *tolC* gene is increased by increasing a copy number of the *tolC* gene, or by modifying an expression control sequence of the gene.
5. The method according to claim 1, wherein the protein is selected from the group consisting of:
  - (a) a protein comprising the amino acid sequence of SEQ ID NO: 2, and
  - (b) a protein comprising the amino acid sequence of SEQ ID NO: 2, but wherein one to five amino acid residues are substituted, deleted, inserted or added, wherein the increase of the activity in the bacterium improves the ability of the bacterium to produce L-cysteine.
6. The method according to claim 1, wherein the *tolC* gene is selected from a group consisting of:
  - (a) a DNA comprising the nucleotide sequence of SEQ ID NO: 1,
  - (b) a DNA which hybridizes with the nucleotide sequence of SEQ ID NO: 1, or a probe prepared from the nucleotide sequence, under stringent conditions comprising washing at 60° C. at a salt concentration of 0.1×SSC and 0.1% SDS, and codes for a protein, wherein the increase of the activity in the bacterium improves the ability of the bacterium to produce L-cysteine.
7. The method according to claim 1, wherein the bacterium contains a mutant serine acetyltransferase in which feedback inhibition by L-cysteine has been attenuated.
8. The method according to claim 1, wherein the bacterium has increased activity of the protein encoded by a *ydeD* gene.
9. The method according to claim 1, wherein the bacterium has decreased activity of a protein having cysteine desulfhydrase activity.
10. The method according to claim 7, wherein the bacterium has increased activity of the protein encoded by a *ydeD* gene.
11. The method according to claim 7, wherein the bacterium has decreased activity of a protein having cysteine desulfhydrase activity.
12. The method according to claim 8, wherein the bacterium has decreased activity of a protein having cysteine desulfhydrase activity.
13. The method according to claim 10, wherein the bacterium has decreased activity of a protein having cysteine desulfhydrase activity.
14. The method according to claim 9, wherein the protein having the cysteine desulfhydrase activity is tryptophanase.
15. The method according to claim 1, wherein the bacterium is an *Escherichia* bacterium.
16. The method according to claim 15, wherein the bacterium is *Escherichia coli*.

\* \* \* \* \*