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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 November 2007 (29.11.2007)

PCT

(10) International Publication Number
WO 2007/136133 A1

(51) International Patent Classification:
C12N 1/21 (2006.01) *C12P 13/04* (2006.01)
C12N 15/31 (2006.01) *C12P 13/08* (2006.01)
C07K 14/245 (2006.01)

(21) International Application Number: PCT/JP2007/060935

(22) International Filing Date: 23 May 2007 (23.05.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2006117420 23 May 2006 (23.05.2006) RU
60/867,151 24 November 2006 (24.11.2006) US

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

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

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*
— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2007/136133 A1

(54) Title: A METHOD FOR PRODUCING AN L-AMINO ACID USING A BACTERIUM OF THE ENTEROBACTERIACEAE FAMILY

(57) Abstract: A method is described for producing an L-amino acid, for example L-threonine, L-lysine, L-leucine, L-histidine, L-cysteine, L-phenylalanine, L-arginine, L-tryptophan, L-glutamic acid, L-valine, and L-isoleucine, by fermentation of glucose using a bacterium of the *Enterobacteriaceae* family, wherein the bacterium has been modified to enhance the activity of the high-affinity arabinose transporter coded by the *araFGH* operon.

DESCRIPTION

A METHOD FOR PRODUCING AN L-AMINO ACID USING A BACTERIUM OF THE *ENTEROBACTERIACEAE* FAMILY

Technical field

The present invention relates to a method for producing an L-amino acid such as L-threonine, L-lysine, L-leucine, L-histidine, L-cysteine, L-phenylalanine, L-arginine, L-tryptophan, L-glutamic acid, L-valine, and L-isoleucine by fermentation.

Background art

Conventionally, L-amino acids are industrially produced by fermentation methods utilizing strains of microorganisms obtained from natural sources, or mutants thereof. Typically, the microorganisms are modified to enhance production yields of L-amino acids.

Many techniques to enhance L-amino acid production yields have been reported, including transformation of microorganisms with recombinant DNA (see, for example, U.S. Patent No. 4,278,765). Other techniques for enhancing production yields include increasing the activities of enzymes involved in amino acid biosynthesis and/or desensitizing the target enzymes of the feedback inhibition by the resulting L-amino acid (see, for example, WO 95/16042 or U.S. Patent Nos. 4,346,170, 5,661,012 and 6,040,160).

Strains useful in production of L-threonine by fermentation are known, including strains with increased activities of enzymes involved in L-threonine biosynthesis (U.S. Patent Nos. 5,175,107; 5,661,012; 5,705,371; 5,939,307; EP 0219027), strains resistant to chemicals such as L-threonine and its analogs (WO 01/14525A1, EP 301572 A2, U.S. Patent No. 5,376,538), strains with target enzymes desensitized to feedback inhibition by the produced L-amino acid or its by-products (U.S. Patent Nos. 5,175,107; 5,661,012), and strains with inactivated threonine degradation enzymes (U.S. Patent Nos. 5,939,307; 6,297,031).

The known threonine-producing strain *Escherichia coli* VKPM B-3996 (U.S. Patent Nos. 5,175,107 and 5,705,371) is presently one of the best known threonine producers. To construct the VKPM B-3996 strain, several mutations and a plasmid, described below, were

introduced into the parent strain *E. coli* K-12 (VKPM B-7). A mutant *thrA* gene (mutation *thrA*442) encodes aspartokinase homoserine dehydrogenase I, which is resistant to feedback inhibition by threonine. A mutant *ilvA* gene (mutation *ilvA*442) encodes threonine deaminase which has decreased activity, and results in a decreased rate of isoleucine biosynthesis and a leaky phenotype of isoleucine starvation. In bacteria containing the *ilvA*442 mutation, transcription of the *thrABC* operon is not repressed by isoleucine; and therefore, this mutation results in very efficient threonine production. Inactivation of the *tdh* gene encoding threonine dehydrogenase results in the prevention of threonine degradation. The genetic determinant of saccharose assimilation (*scrKYABR* genes) was transferred to this strain. To increase expression of the genes controlling threonine biosynthesis, the plasmid pVIC40 containing the mutant threonine operon *thrA442BC* was introduced into the intermediate strain TDH6. The amount of L-threonine which accumulates during fermentation of the strain can be up to 85 g/l.

By optimizing the main biosynthetic pathway of a desired compound, further improvement of L-amino acid producing strains can be accomplished via supplementation of the bacterium with increasing amounts of sugars as a carbon source, for example, glucose or arabinose. Despite the efficiency of glucose transport by PTS, access to the carbon source in a highly productive strain still may be insufficient.

It is known that the active transport of sugars and other metabolites into bacterial cells is accomplished by several different transport systems.

Among these, there are two inducible transport systems for L-arabinose utilization. The low-affinity permease (K_M about 0.1 mM) is encoded by the *araE* gene at min 61.3 and the high-affinity system (K_M ; 1 to 3 μM) is specified by the *araFGH* operon at min 44.8. The *araF* gene encodes a periplasmic binding protein (306 amino acids) with chemotactic receptor function and the *araG* locus encodes at least one inner membrane protein. Both high- and low-affinity transports are under the control of the *araC* gene product and are thus part of the *ara* regulon (*Escherichia coli* and *Salmonella*, Second Edition, Editor in Chief: F.C. Neidhardt, ASM Press, Washington D.C., 1996).

The *araFGH* operon is the "high-affinity" L-arabinose transport operon. This operon encodes three proteins. The first is a 33,000 Mr protein that is the product of the promoter-proximal L-arabinose binding protein coding sequence, *araF*. A 52,000 Mr protein is encoded

by *araG* which is downstream of *araF*. A 31,000 Mr protein is encoded by *araH* which is downstream of *araG*. Both of the products of the *araG* and *araH* genes are localized in the membrane fraction of the cell, implying a role in the membrane-associated complex of the high-affinity L-arabinose transport system (Horazdovsky, B.F. and Hogg, R.W., J.Mol.Biol;197(1):27-35(1987)).

Expression plasmids containing various portions of *araFGH* operon sequences were assayed for their ability to facilitate the high-affinity L-arabinose transport process in a strain lacking the chromosomal copy of this operon. Accumulation studies demonstrated that the specific induction of all three genes was necessary to restore high-affinity L-arabinose transport. Kinetic analysis of this genetically reconstituted transport system indicated that it functions with essentially wild-type parameters. Therefore, L-arabinose-binding protein-mediated transport appears to require only two inducible membrane-associated components (*araG* and *araH*) in addition to the binding protein (*araF*) (Horazdovsky, B.F. and Hogg, R.W., J.Bacteriol; 171(6):3053-9 (1989)).

However, there have been no reports to date of using a bacterium of the *Enterobacteriaceae family* with enhanced expression of the *araFGH* operon for the purpose of increasing the production of L-amino acids by fermentation of glucose.

SUMMARY OF THE INVENTION

Objects of the present invention include enhancing the productivity of L-amino acid-producing strains and providing a method for producing L-amino acids using these strains.

The above objects were achieved by finding that enhancing the expression of the *araFGH* operon encoding the L-arabinose transporter can increase production of L-amino acids, such as L-threonine, L-lysine, L-leucine, L-histidine, L-cysteine, L-phenylalanine, L-arginine, L-tryptophan, L-glutamic acid, L-valine, and L-isoleucine, by fermentation using glucose as a carbon source. The insufficient access to the carbon source was simulated by deleting the PTS transport system (*ptsHI-crr*) in the L-amino acid producing strain.

It is an object of the present invention to provide an L-amino acid producing bacterium of the *Enterobacteriaceae* family, wherein said bacterium has been modified to enhance the expression of the *araFGH* operon.

It is a further object of the present invention to provide the bacterium described above, wherein the expression of the *araFGH* operon is enhanced by modifying an expression control sequence of the *araFGH* operon so that the gene expression is enhanced, or by increasing the copy number of the *araFGH* operon.

It is a further object of the present invention to provide the bacterium described above, wherein said bacterium is selected from the group consisting of the genera *Escherichia, Enterobacter, Erwinia, Klebsiella, Pantoea, Providencia, Salmonella, Serratia, Shigella,* and *Morganella.*

It is a further object of the present invention to provide the bacterium described above, wherein said operon encodes:

(A) a protein comprising the amino acid sequence of SEQ ID NO: 2 or a variant thereof;

(B) a protein comprising the amino acid sequence of SEQ ID NO: 4 or a variant thereof; and

(C) a protein comprising the amino acid sequence of SEQ ID NO: 6 or a variant thereof;

wherein said variants have the activity of the high-affinity L-arabinose transporter when said variants are combined together.

It is a further object of the present invention to provide the bacterium described above, wherein said operon comprises:

(A) a DNA comprising the nucleotide sequence of nucleotides 1 to 990 in SEQ ID NO: 1, or a DNA which is able to hybridize to a sequence complementary to said sequence, or a probe prepared from said sequence under stringent conditions;

(B) a DNA comprising the nucleotide sequence of nucleotides 1 to 1515 in SEQ ID NO: 3, or a DNA which is able to hybridize to a sequence complementary to said sequence, or a probe prepared from said sequence under stringent conditions; and

(C) a DNA comprising the nucleotide sequence of nucleotides 1 to 990 in SEQ ID NO: 5, or a DNA which is able to hybridize to a sequence complementary to said sequence, or a probe prepared from said sequence under stringent conditions; and

wherein, said DNAs encode proteins which have an activity of the high-affinity L-arabinose transporter when said proteins are combined together.

It is a further object of the present invention to provide the bacterium described above, wherein said stringent conditions comprise washing at 60°C at a salt concentration of 1 x SSC and 0.1 % SDS, for approximately 15 minutes.

It is a further object of the present invention to provide the bacterium described above, wherein said bacterium has been additionally modified to enhance the activity of glucokinase.

It is a further object of the present invention to provide the bacterium described above, wherein said bacterium has been additionally modified to enhance the activity of xylose isomerase.

It is a further object of the present invention to provide the bacterium described above, wherein said bacterium is an L-threonine producing bacterium.

It is a further object of the present invention to provide the bacterium described above, wherein said bacterium has been additionally modified to enhance expression of a gene selected from the group consisting of:

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

It is a further object of the present invention to provide the bacterium described above, wherein said bacterium is an L-lysine producing bacterium.

It is a further object of the present invention to provide the bacterium described above, wherein said bacterium is an L-histidine producing bacterium.

It is a further object of the present invention to provide the bacterium described above, wherein said bacterium is an L-phenylalanine producing bacterium.

It is a further object of the present invention to provide the bacterium described above, wherein said bacterium is an L-arginine producing bacterium.

It is a further object of the present invention to provide the bacterium described above, wherein said bacterium is an L-tryptophan producing bacterium.

It is a further object of the present invention to provide the bacterium described above, wherein said bacterium is an L-glutamic acid producing bacterium.

It is a further object of the present invention to provide a method for producing an L-amino acid comprising cultivating the bacterium described above in a culture medium which contains glucose as a carbon source, and isolating the L-amino acid from the culture medium.

It is a further object of the present invention to provide the method described above, wherein said L-amino acid is L-threonine.

It is a further object of the present invention to provide the method described above, wherein said L-amino acid is L-lysine.

It is a further object of the present invention to provide the method described above, wherein said L-amino acid is L-histidine.

It is a further object of the present invention to provide the method described above, wherein said L-amino acid is L-phenylalanine.

It is a further object of the present invention to provide the method described above, wherein said L-amino acid is L-arginine.

It is a further object of the present invention to provide the method described above, wherein said L-amino acid is L-tryptophan.

It is a further object of the present invention to provide the method described above, wherein said L-amino acid is L-glutamic acid.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the relative positions of primers P1 and P2 on plasmid pMW118-attL-Cm-attR.

Figure 2 shows construction of a chromosomal DNA fragment which includes the inactivated *ptsHI-crr* operon.

Figure 3 shows substitution of the native promoter region of the *araFGH* operon in *E. coli* with the hybrid $P_{L\text{-}tac}$ promoter.

Figure 4 shows the influence of $P_{L\text{-}tac}$*araFGH* on growth of the PTS^- strain. In the Figure, MG1655 means *E. coli* strain MG1655; MGΔpts means *E. coli* strain MG1655ΔptsHI-crr; and MGΔpts-P-araFGH means *E. coli* strain MG1655 ΔptsHI-crr $P_{L\text{-}tac}$*araFGH.*

Figure 5 shows the alignment of the primary sequences of the AraF from *Escherichia coli (ECO*, SEQ ID NO: 2*),Shigella dysenteriae serotype 1 (SHD*, SEQ ID NO: 19*), Shigella sonney(SHS*, SEQ ID NO: 18*), Erwinia carotovora subsp. atroseptica(ERC*, SEQ ID NO: 17*), Yersinia pestis(YPE*, SEQ ID NO: 16*), Yersinia pseudotuberculosis(YPS*, SEQ ID NO: 15*), Pseudomonas pseudomallei(PSP*, SEQ ID NO: 22*), Pseudomonas mallei(PSM*, SEQ ID NO:20*), Pseudomonas solanacearum(PSS*, SEQ ID NO:21*)*. The alignment was done by using the PIR Multiple Alignment program (http://pir.georgetown.edu). The identical amino acids are marked by asterisk (*), similar amino acids are marked by colon (:).

Figure 6 shows the alignment of the primary sequences of the AraG from *Escherichia coli (ECO*, SEQ ID NO: 4*), Shigella dysenteriae serotype 1 (SHD*, SEQ ID NO:26*), Shigella sonney(SHS*, SEQ ID NO: 27*), Erwinia carotovora subsp. atroseptica(ERC*, SEQ ID NO: 25*), Yersinia pestis(YPE*, SEQ ID NO: 23*), Yersinia pseudotuberculosis(YPS*, SEQ ID NO: 24*), Pseudomonas pseudomallei(PSP*, SEQ ID NO: 28*), Pseudomonas mallei(PSM*, SEQ ID NO: 29*), Pseudomonas solanacearum(PSS*, SEQ ID NO: 30*)*. The alignment was done by using the PIR Multiple Alignment program (http://pir.georgetown.edu). The identical amino acids are marked by asterisk (*), similar amino acids are marked by colon (:).

Figure 7 shows the alignment of the primary sequences of the AraH from *Escherichia coli (ECO*, SEQ ID NO: 6*), Shigella dysenteriae serotype 1 (SHD*, SEQ ID NO: 34*), Shigella sonney(SHS*, SEQ ID NO: 35*), Erwinia carotovora subsp. atroseptica(ERC*, SEQ ID NO: 33*), Yersinia pestis(YPE*, SEQ ID NO: 31*), Yersinia pseudotuberculosis(YPS*, SEQ ID NO: 32*), Pseudomonas pseudomallei(PSP*, SEQ ID NO: 36*), Pseudomonas mallei(PSM*, SEQ ID NO:

37*)*, *Pseudomonas solanacearum(PSS*, SEQ ID NO: 38*)*. The alignment was done by using the PIR Multiple Alignment program (http://pir.georgetown.edu). The identical amino acids are marked by asterisk (*), similar amino acids are marked by colon (:).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In the present invention, "L-amino acid-producing bacterium" means a bacterium which has an ability to cause accumulation of an L-amino acid in a medium when the bacterium is cultured in the medium. The L-amino acid-producing ability may be imparted or enhanced by breeding. The phrase "L-amino acid-producing bacterium" as used herein also means a bacterium which is able to produce and cause accumulation of an L-amino acid in a culture medium in an amount larger than a wild-type or parental strain of the bacterium, for example, *E. coli*, such as *E. coli* K-12, and preferably means that the bacterium is able to cause accumulation in a medium of an amount not less than 0.5 g/L, more preferably not less than 1.0 g/L of the target L-amino acid. The term "L-amino acids" includes L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, and L-valine. L-threonine, L-lysine, L-histidine, L-phenylalanine, L-arginine, L-tryptophan, and L-glutamic acid are particularly preferred.

The *Enterobacteriaceae* family includes bacteria belonging to the genera *Escherichia, Enterobacter, Erwinia, Klebsiella, Pantoea, Providencia, Salmonella, Serratia, Shigella, Morganella,* etc.. Specifically, those classified into the *Enterobacteriaceae* according to the taxonomy used in the NCBI (National Center for Biotechnology Information) database (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=91347) can be used. A bacterium belonging to the genus *Escherichia* or *Pantoea* is preferred.

The phrase "a bacterium belonging to the genus *Escherichia*" means that the bacterium is classified into the genus *Escherichia* according to the classification known to a person skilled in the art of microbiology. Examples of a bacterium belonging to the genus *Escherichia* as used in the present invention include, but are not limited to, *Escherichia coli* (*E. coli*).

The bacterium belonging to the genus *Escherichia* that can be used in the present invention is not particularly limited; however, for example, bacteria described by Neidhardt, F.C. et al. (*Escherichia coli* and *Salmonella typhimurium*, American Society for Microbiology, Washington D.C., 1208, Table 1) are encompassed by the present invention.

The phrase "a bacterium belonging to the genus *Pantoea*" means that the bacterium is classified into the genus *Pantoea* according to the classification known to a person skilled in the art of microbiology. Some species of *Enterobacter agglomerans* have been recently re-classified into *Pantoea agglomerans*, *Pantoea ananatis*, *Pantoea stewartii*, or the like, based on the nucleotide sequence analysis of 16S rRNA etc. (Int. J. Syst. Bacteriol., 43, 162-173 (1993)).

The bacterium of the present invention encompasses a strain of the *Enterobacteriaceae* family which has an ability to produce an L-amino acid and has been modified to enhance the expression of the *araFGH* operon. In addition, the bacterium of the present invention encompasses a strain of the *Enterobacteriaceae* family which has an ability to produce an L-amino acid and has been transformed with a DNA fragment encoding the *araFGH* operon so that components of the L-arabinose transporter encoded by the DNA fragment are expressed.

The phrase "activity of high-affinity L-arabinose transporter" means an activity of transporting sugars, such as L-arabinose and glucose, into the cell. The activity of the high-affinity L-arabinose transporter can be detected and measured by using membrane vesicles as described by Daruwalla et al (Biochem J., 200(3), 611-27 (1981)) or by complementation of high-affinity arabinose transport in an *araFGH* knockout strain (Horazdovsky, B.F. and Hogg, R.W., J. Bacteriol; 171(6):3053-9 (1989)).

The phrase "enhance the expression of the operon" means that the expression of the operon is increased compared to that of a non-modified strain, for example, a wild-type strain. Examples of such modifications include increasing the copy number of the operon(s) per cell, increasing the expression level of the operon(s), and so forth. The quantity of the copy number of the operon is measured, for example, by Southern blotting using a probe based on the operon sequence, fluorescence *in situ* hybridization (FISH), and the like. The level of operon expression can be measured by various known methods including Northern blotting, quantitative RT-PCR, and the like. Furthermore, wild-type strains that can act as a control

include, for example, *Escherichia coli* K-12 or *Pantoea ananatis* FERM BP-6614 (WO2004099426, AU2004236516A1). *Pantoea ananatis* FERM BP-6614 was deposited at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Ministry of International Trade and Industry (currently, International Patent Organism Depositary, National Institute of Advanced Industrial Science and Technology, Tsukuba Central 6, 1-1, Higashi 1-Chome, Tsukuba-shi, Ibaraki-ken, 305-8566, Japan) on February 19, 1998 and received an accession number of FERM P-16644. It was then converted to an international deposit under the provisions of Budapest Treaty on January 11, 1999 and received an accession number of FERM BP-6614. Although this strain was identified as *Enterobacter agglomerans* when it was isolated, it has been re-classified into *Pantoea ananatis* based on nucleotide sequence analysis of 16S rRNA etc. as described above. As a result of enhancing the intracellular activity of L-arabinose transporter, increased levels of various L-amino acids, for example, L-threonine, L-lysine, L-histidine, L-phenylalanine, L-tryptophan, or L-glutamic acid in a medium is observed.

The *araFGH* operon includes three genes in the following order. The *araF* gene (synonyms - *ECK1899, b1901*) encodes the L-arabinose- binding protein (synonym - B1901). The *araF* gene (nucleotides complementary to nucleotides 1,983,163 to 1,984,152 in the sequence of GenBank accession NC_000913, gi: 16129851) is located between the *yecI* and *araG* genes on the chromosome of *E. coli* K-12. The *araG* gene (synonyms - *ECK1898, b1900*) encodes the ATP-binding component of the L-arabinose transporter (synonym - B1900). The *araG* gene (nucleotides complementary to nucleotides 1,981,579 to 1,983,093 in the sequence of GenBank accession NC_000913, gi: 16129850) is located between the *araF* and *araG* genes on the chromosome of *E. coli* K-12. The *araH* gene (synonyms - *ECK1897, b4460, G8206*) encodes the L-arabinose- binding protein (synonym - B4460). The *araH* gene (nucleotides complementary to nucleotides 1,980,578 to 1,981,567 in the sequence of GenBank accession NC_000913, gi: 49176167) is located between the *araG* and *ots* genes on the chromosome of E. coli K-12. *araFGH* operons from the following microorganisms have also been elucidated: *Shigella dysenteriae serotype 1*, *Shigella sonney*, *Erwinia carotovora subsp. atroseptica*, *Yersinia pestis*, *Yersinia pseudotuberculosis*, *Pseudomonas pseudomallei*, *Pseudomonas mallei*, *Pseudomonas solanacearum*. Examples of the *araF*, *araG*, and *araH*

genes from *Escherichia coli* are represented by SEQ ID NO: 1, SEQ ID NO: 3, and SEQ ID NO: 5, respectively. The amino acid sequences encoded by the *araF*, *araG*, and *araH* genes are presented by SEQ ID NO: 2, SEQ ID NO: 4, and SEQ ID NO: 6, respectivly.

Upon being transported into the cell, glucose is phosphorylated by glucokinase, which is encoded by the *glk* gene. So, it is also desirable to modify the bacterium to have enhanced activity of glucokinase. The *glk* gene which encodes glucokinase of *Escherichia coli* has been elucidated (nucleotide numbers 2506481 to 2507446 in the sequence of GenBank accession NC_000913.1, gi:16127994). The *glk* gene is located between the *b2387* and the *b2389* ORFs on the chromosome of *E. coli* K-12.

Under appropriate conditions, xylose isomerase encoded by the *xylA* gene also efficiently catalyzes the conversion of D-glucose to D-fructose (Wovcha, M.G. et al, Appl Environ Microbiol. 45(4): 1402-4 (1983)). So, it is also desirable to modify the bacterium to have an enhanced activity of xylose isomerase. The *xylA* gene which encodes xylose isomerase of *Escherichia coli* has been elucidated (nucleotide numbers 3728788 to 3727466 in the sequence of GenBank accession NC_000913.2, gi: 49175990). The *xylA* gene is located between the *xylB* and *xylF* genes on the chromosome of *E. coli* K-12.

The *araFGH*, *glk* and *xylA* genes can be obtained by PCR (polymerase chain reaction; refer to White, T.J. *et al.*, *Trends Genet.*, *5*, 185 (1989)) utilizing primers prepared based on the known nucleotide sequences of the genes. Genes coding for L-arabinose permease from other microorganisms can be obtained in a similar manner.

The *araFGH* operon derived from *Escherichia coli* is exemplified by a DNA which encodes the following proteins:

(A) a protein comprising the amino acid sequence of SEQ ID NO: 2 or a variant thereof;

(B) a protein comprising the amino acid sequence of SEQ ID NO: 4 or a variant thereof; and

(C) a protein comprising the amino acid sequence of SEQ ID NO: 6 or a variant thereof.

The phrase "variant protein" as used in the present invention means a protein which has changes in the sequence, whether they are deletions, insertions, additions, or

substitutions of amino acids, but still maintains the desired activity at a useful level, for example, useful for the enhanced production of an L-amino acid. The number of changes in the variant protein depends on the position in the three dimensional structure of the protein or the type of amino acid residues. The number of changes may be 1 to 30, preferably 1 to 15, and more preferably 1 to 5 for the proteins shown as SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO: 6. These changes in the variants can occur in regions of the protein which are not critical for the function of the protein. This is because some amino acids have high homology to one another so the three dimensional structure or activity is not affected by such a change. These changes in the variant protein can occur in regions of the protein which are not critical for the function of the protein. Therefore, the protein variants may have a homology of not less than 70 %, preferably not less than 80 %, and more preferably not less than 90 %, and most preferably not less than 95 % with respect to the entire amino acid sequences shown in any of SEQ ID NO. 2, SEQ ID NO. 4 and SEQ ID NO. 6 as long as the activity of L-arabinose transporter is maintained when combined with the corresponding components of the high-affinity L-arabinose transporter. For example, the components of the high-affinity L-arabinose transporter may be combined as follows: a variant of the protein shown in SEQ ID NO: 2 is combined with the proteins having the amino acid sequences of SEQ ID NO: 4 and SEQ ID NO: 6, a variant of protein shown in SEQ ID NO: 4 is combined with the proteins having the amino acid sequences of SEQ ID NO: 2 and SEQ ID NO: 6, and a variant of the protein shown in SEQ ID NO: 6 is combined with proteins having the amino acid sequences of SEQ ID NO: 2 and SEQ ID NO: 4 . Homology between two amino acid sequences can be determined using the well-known methods, for example, the computer program BLAST 2.0, which calculates three parameters: score, identity and similarity.

The substitution, deletion, insertion, or addition of one or several amino acid residues should be conservative mutation(s) so that the activity is maintained. The representative conservative mutation is a conservative substitution. 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 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.

Data comparing the primary sequences of *ara FGH* from *Escherichia coli (ECO), Shigella dysenteriae serotype 1 (SHD), Shigella sonney(SHS), Erwinia carotovora subsp. atroseptica(ERC), Yersinia pestis(YPE), Yersinia pseudotuberculosis(YPS), Pseudomonas pseudomallei(PSP), Pseudomonas mallei(PSM), Pseudomonas solanacearum(PSS)* show a high level of homology among these proteins (see Figure 5, Figure 6, Figure 7). From this point of view, substitutions or deletions of the amino acid residues which are identical (marked by asterisk) in all the above-mentioned proteins are likely crucial for their function. It is possible to substitute similar (marked by colon) amino acids residues by the similar amino acid residues without deterioration of the protein activity. But modifications of other non-conserved amino acid residues may not lead to alteration of the activity of high- affinity L-arabinose transporter.

The DNAs which encode substantially the same proteins as components of L-arabinose transporter may be obtained, for example, by modifying the nucleotide sequences of DNAs encoding components of L-arabinose transporter (SEQ ID NO: 1, SEQ ID NO: 3 or SEQ ID NO: 5 respectively), for example, by means of the site-directed mutagenesis method so that one or more amino acid residues at a specified site are deleted, substituted, inserted, or added. DNAs modified as described above may be obtained by conventionally known mutation treatments. Such treatments include hydroxylamine treatment of the DNA encoding proteins of present invention, or treatment of the bacterium containing the DNA with UV irradiation or a reagent such as N-methyl-N'-nitro-N-nitrosoguanidine or nitrous acid.

DNAs encoding substantially the same proteins as components of L-arabinose transporter can be obtained by expressing DNAs having a mutation as described above in an appropriate cell, and investigating the activity of the expressed product. DNAs encoding substantially the same protein as components of L-arabinose transporter can also be obtained by isolating DNAs that are hybridizable with probes having nucleotide sequences which

contain, for example, the nucleotide sequences shown in any of SEQ ID NO: 1, SEQ ID NO: 3, and SEQ ID NO: 5 under the stringent conditions, and encode proteins having the activities of components of L-arabinose transporter. The "stringent conditions" referred to herein are conditions under which so-called specific hybrids are formed, and non-specific hybrids are not formed. For example, stringent conditions can be exemplified by conditions under which DNAs having high homology, for example, DNAs having homology of not less than 50%, preferably not less than 60%, more preferably not less than 70%, further preferably not less than 80%, and still more preferably not less than 90%, and most preferably not less than 95% are able to hybridize with each other, but DNAs having homology lower than the above are not able to hybridize with each other. Alternatively, stringent conditions may be exemplified by conditions under which DNA is able to hybridize at a salt concentration equivalent to ordinary washing conditions in Southern hybridization, i.e., 1 x SSC, 0.1% SDS, preferably 0.1 x SSC, 0.1% SDS, at 60°C. Duration of washing depends on the type of membrane used for blotting and, as a rule, what is recommended by the manufacturer. For example, recommended duration of washing, for example, for the Hybond™ N+ nylon membrane (Amersham), under stringent conditions is approximately 15 minutes. Preferably, washing is performed 2 to 3 times.

Partial sequences of the nucleotide sequences of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 can also be used as probes. Probes may be prepared by PCR using primers based on the nucleotide sequences of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 and DNA fragments containing the nucleotide sequences of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 as templates. When a DNA fragment having a length of about 300 bp is used as the probe, the hybridization conditions for washing include, for example, 50°C, 2 x SSC and 0.1% SDS.

The substitution, deletion, insertion, or addition of nucleotides as described above also may include a mutation which naturally occurs (mutant or variant), for example, due to variety in the species or genus of bacterium which contains the components of the L-arabinose transporter.

"Transformation of a bacterium with DNA encoding a protein" means introduction of the DNA into a bacterium, for example, by conventional methods. Transformation of this

DNA will result in an increase in expression of the gene encoding the protein of the present invention, and will enhance the activity of the protein in the bacterial cell. Methods of transformation include any known methods that have hitherto been reported. For example, a method of treating recipient cells with calcium chloride so as to increase permeability of the cells to DNA has been reported for *Escherichia coli* K-12 (Mandel, M. and Higa, A., *J. Mol. Biol.*, *53*, 159 (1970)) and may be used.

Methods of enhancing gene expression include increasing the gene copy number. Introducing a gene into a vector that is able to function in a bacterium of the *Enterobacteriaceae* family increases the copy number of the gene. Preferably, low copy vectors are used. Examples of low-copy vectors include but are not limited to pSC101, pMW118, pMW119, and the like. The term "low copy vector" applies to vectors which have up to 5 copies per cell.

Increasing the copy number of the *araFGH* operon can also be achieved by introducing multiple copies of the *araFGH* operon into the chromosomal DNA of the bacterium. In order to introduce multiple copies of the operon into a bacterial chromosome, homologous recombination is carried out using a sequence whose multiple copies exist as targets in the chromosomal DNA. Sequences having multiple copies in the chromosomal DNA include, but are not limited to repetitive DNA, or inverted repeats existing at the end of a transposable element. Also, as disclosed in U.S. Patent No. 5,595,889, it is possible to incorporate the *araFGH* operon into a transposon, and allow it to be transferred to introduce multiple copies of the gene into the chromosomal DNA. Introduction of multiple copies of the gene into a bacterial chromosome can be also achieved by Mu integration, or the like. For example, one act of Mu integration allows introduction of up to 3 copies of the gene into a bacterial chromosome.

Enhancing gene expression may also be achieved by placing the DNA of the present invention under the control of a potent promoter. For example, the P_{tac} promoter, the *lac* promoter, the *trp* promoter, the *trc* promoter, the P_R, or the P_L promoters of lambda phage are all known to be potent promoters. The use of a potent promoter can be combined with increasing the gene copy number.

Alternatively, the effect of a promoter can be enhanced by, for example, introducing a mutation into the promoter to increase the transcription level of a gene located downstream of the promoter. Furthermore, it is known that substitution of several nucleotides in the spacer between ribosome binding site (RBS) and the start codon, especially the sequences immediately upstream of the start codon, profoundly affect the mRNA translatability. For example, a 20-fold range in the expression levels was found, depending on the nature of the three nucleotides preceding the start codon (Gold *et al.*, Annu. Rev. Microbiol., 35, 365-403, 1981; Hui *et al.*, EMBO J., 3, 623-629, 1984). Previously, it was shown that the *rhtA23* mutation is an A-for-G substitution at the -1 position relative to the ATG start codon (ABSTRACTS of 17th International Congress of Biochemistry and Molecular Biology in conjugation with 1997 Annual Meeting of the American Society for Biochemistry and Molecular Biology, San Francisco, California August 24-29, 1997, abstract No. 457). Therefore, it may be suggested that the *rhtA23* mutation enhances the *rhtA* gene expression and, as a consequence, increases the resistance to threonine, homoserine, and some other substances transported out of cells.

Moreover, it is also possible to introduce a nucleotide substitution into the promoter region of the *araFGH* operon on the bacterial chromosome, which results in stronger promoter function. The alteration of the expression control sequence can be performed, for example, in the same manner as the gene substitution using a temperature-sensitive plasmid, as disclosed in International Patent Publication WO 00/18935 and Japanese Patent Application Laid-Open No. JP 1-215280 A.

Methods for preparation of plasmid DNA include, but are not limited to digestion and ligation of DNA, transformation, selection of an oligonucleotide as a primer and the like, or other methods well known to one skilled in the art. These methods are described, for instance, in Sambrook, J., Fritsch, E.F., and Maniatis, T., "Molecular Cloning A Laboratory Manual, Second Edition", Cold Spring Harbor Laboratory Press (1989).

The above-described techniques and guidances for enhancing an activity of arabinose transporter are similarly applied to enhancing activities of xylose isomerase and glucokinase.

The bacterium of the present invention can be obtained by the introduction of the aforementioned DNAs into a bacterium which inherently has the ability to produce L-amino

acids. Alternatively, the bacterium of the present invention can be obtained by imparting an ability to produce L-amino acids to a bacterium which already contains the DNAs.

L-amino acid-producing bacteria

As a bacterium of the present invention which is modified to enhance expression of the *araFGH* genes, bacteria which are able to produce L-amino acids may be used.

The bacterium of the present invention can be obtained by enhancing expression of the *araFGH* genes in a bacterium which inherently has the ability to produce L-amino acids. Alternatively, the bacterium of present invention can be obtained by imparting the ability to produce L-amino acids to a bacterium already having the enhanced expression of the *araFGH* genes.

L-threonine-producing bacteria

Examples of parent strains for deriving the L-threonine-producing bacteria of the present invention include, but are not limited to, strains belonging to the genus *Escherichia,* such as *E. coli* TDH-6/pVIC40 (VKPM B-3996) (U.S. Patent No. 5, 175, 107, U.S. Patent No. 5,705,371), *E. coli* 472T23/pYN7 (ATCC 98081) (U.S. Patent No.5,631,157), *E. coli* NRRL-21593 (U.S. Patent No. 5,939,307), *E. coli* FERM BP-3756 (U.S. Patent No. 5,474,918), *E. coli* FERM BP-3519 and FERM BP-3520 (U.S. Patent No. 5,376,538), *E. coli* MG442 (Gusyatiner et al., Genetika (in Russian), 14, 947-956 (1978)), *E. coli* VL643 and VL2055 (EP 1149911 A), and the like.

The strain TDH-6 is deficient in the *thrC* gene, as well as being sucrose-assimilative, and the *ilvA* gene has a leaky mutation. This strain also has a mutation in the *rhtA* gene, which imparts resistance to high concentrations of threonine or homoserine. The strain B-3996 contains the plasmid pVIC40 which was obtained by inserting a *thrA*BC* operon which includes a mutant *thrA* gene into a RSF1010-derived vector. This mutant *thrA* gene encodes aspartokinase homoserine dehydrogenase I which has substantially desensitized feedback inhibition by threonine. The strain B-3996 was deposited on November 19, 1987 in the All-Union Scientific Center of Antibiotics (Russia, 117105 Moscow, Nagatinskaya Street 3-A) under the accession number RIA 1867. The strain was also deposited in the Russian National

Collection of Industrial Microorganisms (VKPM) (Russia, 117545 Moscow, 1 Dorozhny proezd, 1) on April 7, 1987 under the accession number VKPM B-3996.

E. coli VKPM B-5318 (EP0593792B) may also be used as a parent strain for deriving L-threonine-producing bacteria of the present invention. The strain B-5318 is prototrophic with regard to isoleucine, and a temperature-sensitive lambda-phage C1 repressor and PR promoter replaces the regulatory region of the threonine operon in plasmid pVIC40. The strain VKPM B-5318 was deposited in the Russian National Collection of Industrial Microorganisms (VKPM) on May 3, 1990 under accession number of VKPM B-5318.

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

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

The *thrA* gene which encodes aspartokinase homoserine dehydrogenase I of *Escherichia coli* has been elucidated (nucleotide positions 337 to 2799, GenBank accession NC_000913.2, gi: 49175990). The *thrA* gene is located between the *thrL* and *thrB* genes on the chromosome of *E. coli* K-12. The *thrB* gene which encodes homoserine kinase of *Escherichia coli* has been elucidated (nucleotide positions 2801 to 3733, GenBank accession NC_000913.2, gi: 49175990). The *thrB* gene is located between the *thrA* and *thrC* genes on the chromosome of *E. coli* K-12. The *thrC* gene which encodes threonine synthase of *Escherichia coli* has been elucidated (nucleotide positions 3734 to 5020, GenBank accession NC_000913.2, gi: 49175990). The *thrC* gene is located between the *thrB* gene and the *yaaX* open reading frame on the chromosome of *E. coli* K-12. All three genes function as a single threonine operon. To enhance expression of the threonine operon, the attenuator region which

affects the transcription is desirably removed from the operon (WO2005/049808, WO2003/097839).

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

The *rhtA* gene exists at 18 min on the *E. coli* chromosome close to the *glnHPQ* operon, which encodes components of the glutamine transport system. The *rhtA* gene is identical to ORF1 (*ybiF* gene, nucleotide positions 764 to 1651, GenBank accession number AAA218541, gi:440181) and is located between the *pexB* and *ompX* genes. The unit expressing a protein encoded by the ORF1 has been designated the *rhtA* gene (rht: resistance to homoserine and threonine). Also, it was revealed that the *rhtA*23 mutation is an A-for-G substitution at position -1 with respect to the ATG start codon (ABSTRACTS of the 17th International Congress of Biochemistry and Molecular Biology in conjugation with Annual Meeting of the American Society for Biochemistry and Molecular Biology, San Francisco, California August 24-29, 1997, abstract No. 457, EP 1013765 A).

The *asd* gene of *E. coli* has already been elucidated (nucleotide positions 3572511 to 3571408, GenBank accession NC_000913.1, gi:16131307), and can be obtained by PCR (polymerase chain reaction; refer to White, T.J. et al., Trends Genet., 5, 185 (1989)) utilizing primers prepared based on the nucleotide sequence of the gene. The *asd* genes of other microorganisms can be obtained in a similar manner.

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

L-lysine-producing bacteria

Examples of L-lysine-producing bacteria belonging to the genus *Escherichia* include mutants having resistance to an L-lysine analogue. The L-lysine analogue inhibits growth of bacteria belonging to the genus *Escherichia*, but this inhibition is fully or partially desensitized

when L-lysine coexists in a medium. Examples of the L-lysine analogue include, but are not limited to, oxalysine, lysine hydroxamate, S-(2-aminoethyl)-L-cysteine (AEC), γ-methyllysine, α-chlorocaprolactam and so forth. Mutants having resistance to these lysine analogues can be obtained by subjecting bacteria belonging to the genus *Escherichia* to a conventional artificial mutagenesis treatment. Specific examples of bacterial strains useful for producing L-lysine include *Escherichia coli* AJ11442 (FERM BP-1543, NRRL B-12185; see U.S. Patent No. 4,346,170) and *Escherichia coli* VL611. In these microorganisms, feedback inhibition of aspartokinase by L-lysine is desensitized.

The strain WC196 may be used as an L-lysine producing bacterium of *Escherichia coli*. This bacterial strain was bred by conferring AEC resistance to the strain W3110, which was derived from *Escherichia coli* K-12. The resulting strain was designated *Escherichia coli* AJ13069 and was deposited at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology (currently National Institute of Advanced Industrial Science and Technology, International Patent Organism Depositary, Tsukuba Central 6, 1-1, Higashi 1-Chome, Tsukuba-shi, Ibaraki-ken, 305-8566, Japan) on December 6, 1994 and received an accession number of FERM P-14690. Then, it was converted to an international deposit under the provisions of the Budapest Treaty on September 29, 1995, and received an accession number of FERM BP-5252 (U.S. Patent No. 5,827,698).

Examples of parent strains for deriving L-lysine-producing bacteria of the present invention also include strains in which expression of one or more genes encoding an L-lysine biosynthetic enzyme are enhanced. Examples of such genes include, but are not limited to, genes encoding dihydrodipicolinate synthase (*dapA*), aspartokinase (*lysC*), dihydrodipicolinate reductase (*dapB*), diaminopimelate decarboxylase (*lysA*), diaminopimelate dehydrogenase (*ddh*) (U.S. Patent No. 6,040,160), phosphoenolpyrvate carboxylase (*ppc*), aspartate semialdehyde dehydrogenease (*asd*), and aspartase (*aspA*) (EP 1253195 A). In addition, the parent strains may have an increased level of expression of the gene involved in energy efficiency (*cyo*) (EP 1170376 A), the gene encoding nicotinamide nucleotide transhydrogenase (*pntAB*) (U.S. Patent No. 5,830,716), the *ybjE* gene (WO2005/073390), or combinations thereof.

Examples of parent strains for deriving L-lysine-producing bacteria of the present invention also include strains having decreased or eliminated activity of an enzyme that catalyzes a reaction for generating a compound other than L-lysine by branching off from the biosynthetic pathway of L-lysine. Examples of the enzymes that catalyze a reaction for generating a compound other than L-lysine by branching off from the biosynthetic pathway of L-lysine include homoserine dehydrogenase, lysine decarboxylase (U.S. Patent No. 5,827,698), and the malic enzyme (WO2005/010175).

L-cysteine-producing bacteria

Examples of parent strains for deriving L-cysteine-producing bacteria of the present invention include, but are not limited to, strains belonging to the genus *Escherichia,* such as *E. coli* JM15 which is transformed with different *cysE* alleles coding for feedback-resistant serine acetyltransferases (U.S. Patent No. 6,218,168, Russian patent application 2003121601); *E. coli* W3110 having over-expressed genes which encode proteins suitable for secreting substances toxic for cells (U.S. Patent No. 5,972,663); *E. coli* strains having lowered cysteine desulfohydrase activity (JP11155571A2); *E. coli* W3110 with increased activity of a positive transcriptional regulator for cysteine regulon encoded by the *cysB* gene (WO0127307A1), and the like.

L-leucine-producing bacteria

Examples of parent strains for deriving L-leucine-producing bacteria of the present invention include, but are not limited to, strains belonging to the genus *Escherichia,* such as *E. coli* strains resistant to leucine (for example, the strain 57 (VKPM B-7386, U.S. Patent No. 6,124,121)) or leucine analogs including β-2-thienylalanine, 3-hydroxyleucine, 4-azaleucine, 5,5,5-trifluoroleucine (JP 62-34397 B and JP 8-70879 A); *E. coli* strains obtained by the gene engineering method described in WO96/06926; *E. coli* H-9068 (JP 8-70879 A), and the like.

The bacterium of the present invention may be improved by enhancing the expression of one or more genes involved in L-leucine biosynthesis. Examples include genes of the *leuABCD* operon, which are preferably represented by a mutant *leuA* gene coding for isopropylmalate synthase freed from feedback inhibition by L-leucine (US Patent 6,403,342).

In addition, the bacterium of the present invention may be improved by enhancing the expression of one or more genes coding for proteins which excrete L-amino acid from the bacterial cell. Examples of such genes include the b2682 and b2683 genes (*ygaZH* genes) (EP 1239041 A2).

L-histidine-producing bacteria

Examples of parent strains for deriving L-histidine-producing bacteria of the present invention include, but are not limited to, strains belonging to the genus *Escherichia*, such as *E. coli* strain 24 (VKPM B-5945, RU2003677); *E. coli* strain 80 (VKPM B-7270, RU2119536); *E. coli* NRRL B-12116 – B12121 (U.S. Patent No. 4,388,405); *E. coli* H-9342 (FERM BP-6675) and H-9343 (FERM BP-6676) (U.S. Patent No. 6,344,347); *E. coli* H-9341 (FERM BP-6674) (EP1085087); *E. coli* AI80/pFM201 (U,S. Patent No. 6,258,554) and the like.

Examples of parent strains for deriving L-histidine-producing bacteria of the present invention also include strains in which expression of one or more genes encoding an L-histidine biosynthetic enzyme are enhanced. Examples of such genes include genes encoding ATP phosphoribosyltransferase (*hisG*), phosphoribosyl AMP cyclohydrolase (*hisI*), phosphoribosyl-ATP pyrophosphohydrolase (*hisIE*), phosphoribosylformimino-5-aminoimidazole carboxamide ribotide isomerase (*hisA*), amidotransferase (*hisH*), histidinol phosphate aminotransferase (*hisC*), histidinol phosphatase (*hisB*), histidinol dehydrogenase (*hisD*), and so forth.

It is known that the L-histidine biosynthetic enzymes encoded by *hisG* and *hisBHAFI* are inhibited by L-histidine, and therefore an L-histidine-producing ability can also be efficiently enhanced by introducing a mutation which confers resistance to the feedback inhibition into ATP phosphoribosyltransferase (Russian Patent Nos. 2003677 and 2119536).

Specific examples of strains having an L-histidine-producing ability include *E. coli* FERM-P 5038 and 5048 which have been introduced with a vector carrying a DNA encoding an L-histidine-biosynthetic enzyme (JP 56-005099 A), *E. coli* strains introduced with *rht*, a gene for an amino acid-export (EP1016710A), *E. coli* 80 strain imparted with sulfaguanidine, DL-1,2,4-triazole-3-alanine, and streptomycin-resistance (VKPM B-7270, Russian Patent No. 2119536), and so forth.

L-glutamic acid-producing bacteria

Examples of parent strains for deriving L-glutamic acid-producing bacteria of the present invention include, but are not limited to, strains belonging to the genus *Escherichia,* such as *E. coli* VL334thrC^{+} (EP 1172433). *E. coli* VL334 (VKPM B-1641) is an L-isoleucine and L-threonine auxotrophic strain having mutations in *thrC* and *ilvA* genes (U.S. Patent No. 4,278,765). A wild-type allele of the *thrC* gene was transferred by the method of general transduction using a bacteriophage P1 grown on the wild-type *E. coli* strain K12 (VKPM B-7) cells. As a result, an L-isoleucine auxotrophic strain VL334thrC^{+} (VKPM B-8961), which is able to produce L-glutamic acid, was obtained.

Examples of parent strains for deriving the L-glutamic acid-producing bacteria of the present invention include, but are not limited to, strains in which expression of one or more genes encoding an L-glutamic acid biosynthetic enzyme are enhanced. Examples of such genes include genes encoding glutamate dehydrogenase (*gdhA*), glutamine synthetase (*glnA*), glutamate synthetase (*gltAB*), isocitrate dehydrogenase (*icdA*), aconitate hydratase (*acnA, acnB*), citrate synthase (*gltA*), phosphoenolpyruvate carboxylase (*ppc*), pyruvate carboxylase (*pyc*), pyruvate dehydrogenase (*aceEF, lpdA*), pyruvate kinase (*pykA*, *pykF*), phosphoenolpyruvate synthase (*ppsA*), enolase (*eno*), phosphoglyceromutase (*pgmA, pgmI*), phosphoglycerate kinase (*pgk*), glyceraldehyde-3-phophate dehydrogenase (*gapA*), triose phosphate isomerase (*tpiA*), fructose bisphosphate aldolase (*fbp*), phosphofructokinase (*pfkA, pfkB*), and glucose phosphate isomerase (*pgi*).

Examples of strains modified so that expression of the citrate synthetase gene, the phosphoenolpyruvate carboxylase gene, and/or the glutamate dehydrogenase gene is/are enhanced include those disclosed in EP1078989A, EP955368A, and EP952221A.

Examples of parent strains for deriving the L-glutamic acid-producing bacteria of the present invention also include strains having decreased or eliminated activity of an enzyme that catalyzes synthesis of a compound other than L-glutamic acid by branching off from an L-glutamic acid biosynthesis pathway. Examples of such enzymes include isocitrate lyase (*aceA*), α-ketoglutarate dehydrogenase (*sucA*), phosphotransacetylase (*pta*), acetate kinase (*ack*), acetohydroxy acid synthase (*ilvG*), acetolactate synthase (*ilvI*), formate acetyltransferase (*pfl*),

lactate dehydrogenase (*ldh*), and glutamate decarboxylase (*gadAB*). Bacteria belonging to the genus *Escherichia* deficient in α-ketoglutarate dehydrogenase activity or having reduced α-ketoglutarate dehydrogenase activity and methods for obtaining them are described in U.S. Patent Nos. 5,378,616 and 5,573,945. Specifically, these strains include the following:

E. coli W3110sucA::Km^R

E. coli AJ12624 (FERM BP-3853)

E. coli AJ12628 (FERM BP-3854)

E. coli AJ12949 (FERM BP-4881)

E. coli W3110sucA::Km^R is a strain obtained by disrupting the α-ketoglutarate dehydrogenase gene (hereinafter referred to as "*sucA* gene") of *E. coli* W3110. This strain is completely deficient in α-ketoglutarate dehydrogenase.

Other examples of L-glutamic acid-producing bacterium include those which belong to the genus *Escherichia* and have resistance to an aspartic acid antimetabolite. These strains can also be deficient in α-ketoglutarate dehydrogenase activity and include, for example, *E. coli* AJ13199 (FERM BP-5807) (U.S. Patent No. 5,908,768), FFRM P-12379, which additionally has a low L-glutamic acid decomposing ability (U.S. Patent No. 5,393,671); AJ13138 (FERM BP-5565) (U.S. Patent No. 6,110,714), and the like.

Examples of L-glutamic acid-producing bacteria include mutant strains belonging to the genus *Pantoea* which are deficient in α-ketoglutarate dehydrogenase activity or have a decreased α-ketoglutarate dehydrogenase activity, and can be obtained as described above. Such strains include *Pantoea ananatis* AJ13356. (U.S. Patent No. 6,331,419). *Pantoea ananatis* AJ13356 was deposited at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Ministry of International Trade and Industry (currently, National Institute of Advanced Industrial Science and Technology, International Patent Organism Depositary, Central 6, 1-1, Higashi 1-Chome, Tsukuba-shi, Ibaraki-ken, 305-8566, Japan) on February 19, 1998 under an accession number of FERM P-16645. It was then converted to an international deposit under the provisions of Budapest Treaty on January 11, 1999 and received an accession number of FERM BP-6615. *Pantoea ananatis* AJ13356 is deficient in α-ketoglutarate dehydrogenase activity as a result of disruption of the αKGDH-E1 subunit gene (*sucA*). The above strain was identified as

Enterobacter agglomerans when it was isolated and deposited as the *Enterobacter agglomerans* AJ13356. However, it was recently re-classified as *Pantoea ananatis* on the basis of nucleotide sequencing of 16S rRNA and so forth. Although AJ13356 was deposited at the aforementioned depository as *Enterobacter agglomerans*, for the purposes of this specification, they are described as *Pantoea ananatis*.

L-phenylalanine-producing bacteria

Examples of parent strains for deriving L-phenylalanine-producing bacteria of the present invention include, but are not limited to, strains belonging to the genus *Escherichia*, such as *E. coli* AJ12739 (tyrA::Tn10, tyrR) (VKPM B-8197); *E. coli* HW1089 (ATCC 55371) harboring the mutant pheA34 gene (U.S. Patent No. 5,354,672); *E. coli* MWEC101-b (KR8903681); *E. coli* NRRL B-12141, NRRL B-12145, NRRL B-12146 and NRRL B-12147 (U.S. Patent No. 4,407,952). Also, as a parent strain, *E. coli* K-12 [W3110 (tyrA)/pPHAB (FERM BP-3566), *E. coli* K-12 [W3110 (tyrA)/pPHAD] (FERM BP-12659), *E. coli* K-12 [W3110 (tyrA)/pPHATerm] (FERM BP-12662) and *E. coli* K-12 [W3110 (tyrA)/pBR-aroG4, pACMAB] named as AJ 12604 (FERM BP-3579) may be used (EP 488424 B1). Furthermore, L-phenylalanine producing bacteria belonging to the genus *Escherichia* with an enhanced activity of the protein encoded by the *yedA* gene or the *yddG* gene may also be used (U.S. patent applications 2003/0148473 A1 and 2003/0157667 A1).

L-tryptophan-producing bacteria

Examples of parent strains for deriving the L-tryptophan-producing bacteria of the present invention include, but are not limited to, strains belonging to the genus *Escherichia*, such as *E. coli* JP4735/pMU3028 (DSM10122) and JP6015/pMU91 (DSM10123) are deficient in the tryptophanyl-tRNA synthetase encoded by mutant *trpS* gene (U.S. Patent No. 5,756,345); *E. coli* SV164 (pGH5) having a *serA* allele encoding phosphoglycerate dehydrogenase free from feedback inhibition by serine and a *trpE* allele encoding anthranilate synthase free from feedback inhibition by tryptophan (U.S. Patent No. 6,180,373); *E. coli* AGX17 (pGX44) (NRRL B-12263) and AGX6(pGX50)aroP (NRRL B-12264) deficient in the enzyme tryptophanase (U.S. Patent No. 4,371,614); *E. coli* AGX17/pGX50,pACKG4-pps in

which a phosphoenolpyruvate-producing ability is enhanced (WO9708333, U.S. Patent No. 6,319,696), and the like may be used. L-tryptophan-producing bacteria belonging to the genus *Escherichia* with an enhanced activity of the identified protein encoded by and the *yedA* gene or the *yddG* gene may also be used (U.S. patent applications 2003/0148473 A1 and 2003/0157667 A1).

Examples of parent strains for deriving the L-tryptophan-producing bacteria of the present invention also include strains in which one or more activities of the enzymes selected from anthranilate synthase, phosphoglycerate dehydrogenase, and tryptophan synthase are enhanced. The anthranilate synthase and phosphoglycerate dehydrogenase are both subject to feedback inhibition by L-tryptophan and L-serine, so that a mutation desensitizing the feedback inhibition may be introduced into these enzymes. Specific examples of strains having such a mutation include a *E. coli* SV164 which harbors desensitized anthranilate synthase and a transformant strain obtained by introducing into the *E. coli* SV164 the plasmid pGH5 (WO 94/08031), which contains a mutant *serA* gene encoding feedback-desensitized phosphoglycerate dehydrogenase.

Examples of parent strains for deriving the L-tryptophan-producing bacteria of the present invention also include strains into which the tryptophan operon which contains a gene encoding desensitized anthranilate synthase has been introduced (JP 57-71397 A, JP 62-244382 A, U.S. Patent No. 4,371,614). Moreover, L-tryptophan-producing ability may be imparted by enhancing expression of a gene which encodes tryptophan synthase, among tryptophan operons (*trpBA*). The tryptophan synthase consists of α and β subunits which are encoded by the *trpA* and *trpB* genes, respectively. In addition, L-tryptophan-producing ability may be improved by enhancing expression of the isocitrate lyase-malate synthase operon (WO2005/103275).

L-proline-producing bacteria

Examples of parent strains for deriving L-proline-producing bacteria of the present invention include, but are not limited to, strains belonging to the genus *Escherichia,* such as *E. coli* 702ilvA (VKPM B-8012) which is deficient in the *ilvA* gene and is able to produce L-proline (EP 1172433). The bacterium of the present invention may be improved by enhancing

the expression of one or more genes involved in L-proline biosynthesis. Examples of such genes for L-proline producing bacteria which are preferred include the *proB* gene coding for glutamate kinase of which feedback inhibition by L-proline is desensitized (DE Patent 3127361). In addition, the bacterium of the present invention may be improved by enhancing the expression of one or more genes coding for proteins excreting L-amino acid from bacterial cell. Such genes are exemplified by b2682 and b2683 genes (*ygaZH* genes) (EP1239041 A2).

Examples of bacteria belonging to the genus *Escherichia*, which have an activity to produce L-proline include the following *E. coli* strains: NRRL B-12403 and NRRL B-12404 (GB Patent 2075056), VKPM B-8012 (Russian patent application 2000124295), plasmid mutants described in DE Patent 3127361, plasmid mutants described by Bloom F.R. et al (The 15th Miami winter symposium, 1983, p. 34), and the like.

L-arginine-producing bacteria

Examples of parent strains for deriving L-arginine-producing bacteria of the present invention include, but are not limited to, strains belonging to the genus *Escherichia*, such as *E. coli* strain 237 (VKPM B-7925) (U.S. Patent Application 2002/058315 A1) and its derivative strains harboring mutant N-acetylglutamate synthase (Russian Patent Application No. 2001112869), *E. coli* strain 382 (VKPM B-7926) (EP1170358A1), an arginine-producing strain into which *argA* gene encoding N-acetylglutamate synthetase is introduced therein (EP1170361A1), and the like.

Examples of parent strains for deriving L-arginine producing bacteria of the present invention also include strains in which expression of one or more genes encoding an L-arginine biosynthetic enzyme are enhanced. Examples of such genes include genes encoding N-acetylglutamyl phosphate reductase (*argC*), ornithine acetyl transferase (*argJ*), N-acetylglutamate kinase (*argB*), acetylornithine transaminase (*argD*), ornithine carbamoyl transferase (*argF*), argininosuccinic acid synthetase (*argG*), argininosuccinic acid lyase (*argH*), and carbamoyl phosphate synthetase (*carAB*).

L-valine-producing bacteria

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

Examples of parent strains for deriving L-valine-producing bacteria of the present invention include also include mutants having a mutation of amino-acyl t-RNA synthetase (U.S. Patent No. 5,658,766). For example, *E. coli* VL1970, which has a mutation in the *ileS* gene encoding isoleucine tRNA synthetase, can be used. *E. coli* VL1970 has been deposited in the Russian National Collection of Industrial Microorganisms (VKPM) (Russia, 117545 Moscow, 1 Dorozhny Proezd, 1) on June 24, 1988 under accession number VKPM B-4411.

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

L-isoleucine-producing bacteria

Examples of parent strains for deriving L-isoleucine producing bacteria of the present invention include, but are not limited to, mutants having resistance to 6-dimethylaminopurine (JP 5-304969 A), mutants having resistance to an isoleucine analogue such as thiaisoleucine and isoleucine hydroxamate, and mutants additionally having resistance to DL-ethionine and/or arginine hydroxamate (JP 5-130882 A). In addition, recombinant strains transformed with genes encoding proteins involved in L-isoleucine biosynthesis, such as threonine deaminase and acetohydroxate synthase, can also be used as parent strains (JP 2-458 A, FR 0356739, and U.S. Patent No. 5,998,178).

2. Method of the present invention

Oxaloacetate (OAA) serves as a substrate for the reaction which results in the synthesis of Thr and Lys. OAA results from a reaction of PEP with phosphoenol pyrvate carboxlase (PEPC) functioning as a catalyst. Therefore, elevation of the PEPC concentration in a cell can be very important for fermentative production of these amino acids. When using glucose as

the carbon source in fermentation, glucose is internalized by the glucose-phosphontransferase (Glc-PTS) system. This system consumes PEP, and proteins in the PTS are encoded by *ptsG* and *ptsHIcrr*. During internalization, one molecule of PEP and one molecule of pyruvate (Pyr) are generated from one molecule of glucose.

An L-threonine-producing strain and an L-lysine-producing strain which have been modified to have an ability to utilize sucrose (Scr-PTS) have higher productivity of these amino acids when cultured in sucrose rather than glucose (EP 1149911 A2). It is believed that three molecules of PEP and one molecule of Pyr are generated from one molecule of sucrose by the Scr-PTS, increasing the ratio of PEP/Pyr, and thereby facilitating the synthesis of Thr and Lys from sucrose. Furthermore, it has been reported that Glc-PTS is subject to several expression controls (Postma P.W. et al., Microbiol Rev., 57(3), 543-94 (1993); Clark B. et al. J. Gen. Microbiol., 96(2), 191-201 (1976); Plumbridge J., Curr. Opin. Microbiol., 5(2), 187-93 (2002); Ryu S. et al., J. Biol. Chem., 270(6):2489-96 (1995)), and hence it is possible that the incorporation of glucose itself can be a rate-limiting step in amino acid fermentation.

Increasing the ratio of PEP/Pyr even more by increasing expression of the *araFGH operon* in a threonine-producing strain, a lysine-producing strain, a histidine-producing strain, a phenylalanine-producing strain, an arginine-producing strain, a tryptophan-producing strain and/or a glutamic acid-producing strain should further increase the corresponding amino acid production. Because four molecules of PEP are generated from two molecules of glucose, the ratio of PEP/Pyr is expected to be greatly improved. Due to the increased expression of the *araFGH operon*, removal of the expression control glc-PTS is expected.

The method of the present invention is a method for producing an L-amino acid by cultivating the bacterium of the present invention in a culture medium to produce and excrete the L-amino acid into the medium, and collecting the L-amino acid from the medium.

In the present invention, the cultivation, collection, and purification of an L-amino acid from the medium and the like may be performed in a manner similar to conventional fermentation methods wherein an amino acid is produced using a bacterium.

A medium used for culture may be either a synthetic or natural medium, so long as the medium includes a carbon source and a nitrogen source and minerals and, if necessary, appropriate amounts of nutrients which the bacterium requires for growth. The carbon source

may include various carbohydrates such as glucose and sucrose, and various organic acids. Depending on the mode of assimilation of the chosen microorganism, alcohol, including ethanol and glycerol, may be used. As the nitrogen source, various ammonium salts such as ammonia and ammonium sulfate, other nitrogen compounds such as amines, a natural nitrogen source such as peptone, soybean-hydrolysate, and digested fermentative microorganism can be used. As minerals, potassium monophosphate, magnesium sulfate, sodium chloride, ferrous sulfate, manganese sulfate, calcium chloride, and the like can be used. As vitamins, thiamine, yeast extract, and the like, can be used.

The cultivation is preferably performed under aerobic conditions, such as a shaking culture, and a stirring culture with aeration, at a temperature of 20 to 40 °C, preferably 30 to 38 °C. The pH of the culture is usually between 5 and 9, preferably between 6.5 and 7.2. The pH of the culture can be adjusted with ammonia, calcium carbonate, various acids, various bases, and buffers. Usually, a 1 to 5-day cultivation leads to accumulation of the target L-amino acid in the liquid medium.

After cultivation, solids such as cells can be removed from the liquid medium by centrifugation or membrane filtration, and then the L-amino acid can be collected and purified by ion-exchange, concentration, and/or crystallization methods.

Examples

The present invention will be more concretely explained below with reference to the following non-limiting Examples.

Example 1. Construction of the *E. coli* strain having a disrupted PTS transport system.

1. Deletion of the *ptsHI-crr* operon

The *ptsHI-crr* operon was deleted in a chosen strain by the method initially developed by Datsenko, K.A. and Wanner, B.L. (Proc. Natl. Acad. Sci. USA, 2000, 97(12): 6640-6645) called "Red-driven integration". The DNA fragment containing the Cm^R marker encoded by the *cat* gene was obtained by PCR, using primers P1 (SEQ ID NO: 7) and P2(SEQ ID NO: 8) and plasmid pMW118-attL-Cm-attR as a template (WO 05/010175). Primer P1 contains both a region complementary to the 36-nt region located at the 5' end of the *ptsHI-crr* operon, and a

region complementary to the 24-nt attL region. Primer P2 contains both a region complementary to the 36-nt region located at the 3' end of the *ptsHI-crr* operon, and a region complementary to the 24-nt attR region. Conditions for PCR were as follows: denaturation for 3 min at 95°C; profile for two first cycles: 1 min at 95°C, 30 sec at 50°C, 40 sec at 72°C; profile for the last 25 cycles: 30 sec at 95°C, 30 sec at 54°C, 40 sec at 72°C; final step: 5 min at 72°C.

A 1699-bp PCR product (Fig. 2) was obtained and purified in agarose gel and was used for electroporation of the *E. coli* strain MG1655 (ATCC 700926), which contains the plasmid pKD46, the replication of which is temperature-sensitive. The plasmid pKD46 (Datsenko, K.A. and Wanner, B.L., Proc. Natl. Acad. Sci. USA, 2000, 97:12:6640-45) includes a 2,154 nucleotide DNA fragment of phage λ (nucleotide positions 31088 to 33241, GenBank accession no. J02459), and contains genes of the λ Red homologous recombination system (γ, β, exo genes) under the control of the arabinose-inducible P_{araB} promoter. The plasmid pKD46 is necessary for integration of the PCR product into the chromosome of strain MG1655. MG1655 can be obtained from American Type Culture Collection. (P.O. Box 1549 Manassas, VA 20108, U.S.A.).

Electrocompetent cells were prepared as follows: *E. coli* MG1655/pKD46 was grown overnight at 30 °C in LB medium containing ampicillin (100 mg/l), and the culture was diluted 100 times with 5 ml of SOB medium (Sambrook et al, "Molecular Cloning: A Laboratory Manual, Second Edition", Cold Spring Harbor Laboratory Press, 1989) containing ampicillin and L-arabinose (1 mM). The cells were grown with aeration at 30°C to an OD_{600} of ≈0.6 and then were made electrocompetent by concentrating 100-fold and washing three times with ice-cold deionized H_2O. Electroporation was performed using 70 μl of cells and ≈100 ng of the PCR product. Cells after electroporation were incubated with 1 ml of SOC medium (Sambrook et al, "Molecular Cloning: A Laboratory Manual, Second Edition", Cold Spring Harbor Laboratory Press, 1989) at 37°C for 2.5 hours and then were plated onto L-agar containing chloramphenicol (30 μg/ml) and grown at 37°C to select Cm^R recombinants. Then, to eliminate the pKD46 plasmid, two passages on L-agar with Cm at 42°C were performed and the resulting colonies were tested for sensitivity to ampicillin.

2. Verification of the *ptsHI-crr* operon deletion by PCR

The mutants without the *ptsHI-crr* operon and having the Cm resistance gene were verified by PCR. Locus-specific primers P3 (SEQ ID NO: 9) and P4 (SEQ ID NO: 10) were used in PCR for the verification. Conditions for PCR verification were as follows: denaturation for 3 min at 94°C; profile for 30 cycles: 30 sec at 94°C, 30 sec at 54°C, 1 min at 72°C; final step: 7 min at 72°C. The PCR product obtained in the reaction using the parental ptsHI-crr $^{+}$ strain MG1655 as a template was ~3.0 kbp in length. The PCR product obtained in the reaction using the cells of the mutant strain as a template was ~2.0 kbp in length (Fig.2). The mutant strain was named MG1655 Δ ptsHI-crr::cat.

3. Elimination of Cm resistance gene (*cat* gene) from the chromosome of *E. coli* MG1655- Δ ptsHI-crr::cat strain

The Cm resistance gene (*cat* gene) was deleted from the chromosome of the *E. coli* MG1655 Δ ptsHI-crr::cat strain using the *int-xis* system. For that purpose *E. coli* strain MG1655 ΔptsHI-crr::cat was transformed with plasmid pMWts-Int/Xis (WO 05/010175). Transformant clones were selected on LB-medium containing 100 μg/ml of ampicillin. Plates were incubated overnight at 30°C. Transformant clones were cured from the *cat* gene by spreading the separate colonies at 37°C (at this temperature repressor CIts is partially inactivated and transcription of the *int/xis* genes is derepressed) followed by selection of $Cm^{S}Ap^{R}$ variants. Elimination of the *cat* gene from the chromosome of the strain was verified by PCR. Locus-specific primers P3 (SEQ ID NO: 9) and P4 (SEQ ID NO: 10) were used in PCR for the verification. Conditions for PCR verification were as described above. The PCR product obtained in reaction using cells without the *cat* gene as a template was ~0.4 kbp in length. Thus, the strain with the inactivated *ptsHI-crr* operon and missing the *cat* gene was obtained. This strain was named MG1655 Δ ptsHI-crr.

Example 2: Replacement of the native promoter region of the *araFGH* operon in *E. coli* with the hybrid $P_{L\text{-}tac}$ promoter.

To replace the native promoter region of the *araFGH* operon, a DNA fragment carrying a hybrid $P_{L\text{-}tac}$ promoter and the chloramphenicol resistance marker (Cm^{R}) encoded by the *cat* gene was integrated into the chromosome of the *E. coli* MG1655 ΔptsHI-crr in place of the

native promoter region by the method described by Datsenko K.A. and Wanner B.L. (Proc.Natl.Acad.Sci.USA, 2000, 97, 6640-6645) which is also called "Red-mediated integration" and/or "Red-driven integration", and is also described in Example 1 .

The hybrid P_{L-tac} promoter was obtained by PCR using the chromosomal DNA of *E. coli* strain B-3996P_{L-tac}xylE (PCT application WO2006043730) as the template, and primers P5 (SEQ ID NO11 and P6(SEQ ID NO: 12). PCR was conducted as described in Example 1.

The amplified DNA fragment was purified by agarose gel-electrophoresis, extracted using "GenElute Spin Columns" ("Sigma", USA) and precipitated by ethanol. The obtained DNA fragment was used for electroporation and Red-mediated integration into the bacterial chromosome of the *E. coli* MG1655 ΔptsHI-crr /pKD46 as described in Example 1.

Colonies which grew within 24 h were tested for the presence of a Cm^R marker instead of the *araFGH* operon native promoter region by PCR using primers P7 (SEQ ID NO: 13) and P8 (SEQ ID NO: 14). For this purpose, a freshly isolated colony was suspended in 20μl water and then 1μl of this suspension was used for PCR. PCR conditions were as described in Example 1. A few tested Cm^R colonies contained the desired ~2.1 kb DNA fragment, confirming the presence of the hybrid P_{L-tac} promoter and Cm^R marker DNA instead of ~0.4 kb *araFGH* operon native promoter region (see Figure 3). One of the obtained strains was cured from the thermosensitive plasmid pKD46 by culturing at 37 °C and named *E. coli* MG1655 ΔptsHI-crr P_{L-tac}*araFGH.*

The ability to grow on the minimal Adams medium with glucose (4%) as a carbon source was checked for the three *E. coli* strains MG1655, MG1655ΔptsHI-crr, and MG1655ΔptsHI-crr P_{L-tac}*araFGH.* As seen in Figure 4, *E. coli* MG1655ΔptsHI-crr did not grow well (μ~0.06) on the minimal Adams medium containing glucose. Enhancing the *araFGH* operon expression significantly enhanced the growth characteristics of the recipient strains on the minimal Adams medium containing glucose.

Example 3. Effect of enhancing the *araFGH* operon expression in the strain having a disrupted PTS transport system on L-threonine production.

To disrupt the PTS transport system in the threonine-producing *E. coli* strain VKPM B-3996, the *ptsH1-crr* operon was inactivated. For that purpose DNA fragments from the

chromosome of the above-described *E. coli* MG1655 ΔptsHI-crr::cat were transferred to the *E. coli* strain VKPM B-3996 by P1 transduction (Miller, J.H. Experiments in Molecular Genetics, Cold Spring Harbor Lab. Press, 1972, Plainview, NY) to obtain the strain B-3996-Δ ptsHI-crr::cat.

The mutants without the *ptsHI-crr* operon and having the Cm resistance gene were verified by PCR. Locus-specific primers P3 (SEQ ID NO: 9) and P4 (SEQ ID NO: 10) were used in PCR for the verification. Conditions for PCR verification were as described above. The PCR product obtained in the reaction using the parental ptsHI-crr $^{+}$ B-3996 strain as the template was ~3.0 kbp in length. The PCR product obtained in the reaction using the mutant strain B-3996 Δ ptsHI-crr::cat as the template was ~2.0 kbp in length (Fig.2).

The Cm resistance gene (*cat* gene) was deleted from the chromosome of the *E. coli* B-3996 ΔptsHI-crr::cat strain using the *int-xis* system. For that purpose, *E. coli* strain B-3996 ΔptsHI-crr::cat was transformed with plasmid pMWts-Int/Xis (WO 2005 010175). Transformant clones were selected on the LB-medium containing 100 μg/ml of ampicillin. Plates were incubated overnight at 30°C. Transformant clones were cured from the *cat* gene by spreading the separate colonies at 37°C (at this temperature repressor CIts is partially inactivated and transcription of the *int/xis* genes is derepressed) followed by selection of $Cm^{S}Ap^{R}$ variants. Elimination of the *cat* gene from the chromosome of the strain was verified by PCR. Locus-specific primers P3 (SEQ ID NO: 9) and P4 (SEQ ID NO: 10) were used in PCR for the verification. Conditions for PCR verification were as described above. The PCR product obtained in reaction using cells without the *cat* gene as a template was ~0.4 kbp in length. Thus, the threonine-producing strain with the inactivated *ptsHI-crr* operon and missing the *cat* gene was obtained. This strain was named B-3996ΔptsHI-crr.

For the purpose of enhancing the expression of the *araFGH* operon in *E. coli* B-3996ΔptsHI-crr, the native promoter of the *araFGH* operon was replaced with a hybrid $P_{L\text{-}tac}$ promoter. For that purpose, DNA fragments from the chromosome of the above-described *E. coli* MG1655ΔptsHI-crr $P_{L\text{-}tac}$*araFGH* were transferred to the *E. coli* strain B-3996ΔptsHI-crr by P1 transduction (Miller, J.H. Experiments in Molecular Genetics, Cold Spring Harbor Lab. Press, 1972, Plainview, NY) to obtain the *E. coli* strain B-3996-Δ ptsHI-crr $P_{L\text{-}tac}$*araFGH*.

The deletion of the *ptsHI-crr* operon in the *E. coli* strain B-3996-ΔptsHI-crr P_{L-tac}*araFGH* was verified by PCR. Locus-specific primers P3 (SEQ ID NO: 9) and P4 (SEQ ID NO: 10) were used in PCR for the verification. Conditions for PCR verification were as described above. The PCR product obtained in the reaction using the strain B-3996-ΔptsHI-crr P_{L-tac}araFGH as the template was ~0.4 kbp in length.

The substitution of the native promoter of the *araFGH* operon with hybrid P_{L-tac} promoter and Cm^R marker DNA in the *E. coli* strain B-3996-ΔptsHI-crr P_{L-tac}*araFGH* were verified by PCR. Locus-specific primers P7 (SEQ ID NO: 13) and P8 (SEQ ID NO: 14) were used in PCR for the verification. Conditions for PCR verification were as described above. The PCR product obtained in the reaction with the strain B-3996-ΔptsHI-crr P_{L-tac}*araFGH* as the template was ~2.1 kbp in length.

Then, *E. coli* strains B-3996, B-3996-ΔptsHI-crr, and B-3996-ΔptsHI-crr P_{L-tac}*araFGH* were each cultivated at 37 °C for 18 hours in a nutrient broth, and 0.3 ml of each of the obtained cultures was inoculated into 3 ml of fermentation medium having the following composition in a 20x200 mm test tube and cultivated at 37 °C for 72 hours with a rotary shaker.

After cultivation, the accumulated amount of L-threonine in the medium was determined by paper chromatography using the following mobile phase: butanol : acetic acid : water = 4 : 1 : 1 (v/v). A solution (2%) of ninhydrin in acetone was used as a visualizing reagent. The spot containing L-threonine was cut off, L-threonine was eluted in 0.5 % water solution of $CdCl_2$, and the amount of L-threonine was estimated spectrophotometrically at 540 nm. The results of five tubes of fermentations are shown in Table 1.

The composition of the fermentation medium (g/l) was as follows:

Glucose	40.0
$(NH_4)_2SO_4$	16.0
K_2HPO_4	0.7
$MgSO_4 \cdot 7H_2O$	1.0
$MnSO_4 \cdot 5H_2O$	0.01
$FeSO_4 \cdot 7H_2O$	0.01
Thiamine hydrochloride	0.002

Yeast extract 2.0

L-isolucine 0.01

$CaCO_3$ 33.0

$MgSO_4 \cdot 7H_2O$ and $CaCO_3$ were each sterilized separately.

Table 1

Strain	OD_{540}	Thr, g/l
B-3996	18.2±0.7	18.9±0.8
B-3996ΔptsHI-crr	0.85±0.01	0.2±0.01
B-3996ΔptsHI-crr P_{L-tac}*araFGH*	17.6±0.6	19.5±0.8

It can be seen from Table 1 that B-3996-ΔptsHI-crr P_{L-tac}*araFGH* caused the accumulation of a higher amount of L-threonine as compared with B-3996.

Example 4. Production of L-lysine by *E. coli* AJ11442- P_{L-tac}*araFGH*

To test the effect of enhancing the *araFGH* operon on L-lysine production, DNA fragments coding for the arabinose transporter from the chromosome of the above-described *E. coli* MG1655-ΔptsHI-crr P_{L-tac}*araFGH* strain can be transferred to the lysine-producing *E. coli* strain AJ11442 by P1 transduction (Miller, J.H. Experiments in Molecular Genetics, Cold Spring Harbor Lab. Press, 1972, Plainview, NY) to obtain strain AJ11442- P_{L-tac}*araFGH*. The strain AJ14442 was deposited at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology (currently National Institute of Advanced Industrial Science and Technology, International Patent Organism Depositary, Tsukuba Central 6, 1-1, Higashi 1-Chome, Tsukuba-shi, Ibaraki-ken, 305-8566, Japan) on May 1, 1981 and received an accession number of FERM P-5084. Then, it was converted to an international deposit under the provisions of the Budapest Treaty on October 29, 1987, and received an accession number of FERM BP-1543.

Both *E. coli* strains, AJ11442 and AJ11442-P_{L-tac}*araFGH*, can each be cultured in L-medium at 37°C, and 0.3 ml of each of the obtained cultures can be inoculated into 20 ml of the fermentation medium containing the required drugs in a 500-ml flask. The cultivation can

be carried out at 37°C for 16 h by using a reciprocal shaker at the agitation speed of 115 rpm. After the cultivation, the amounts of L-lysine and residual glucose in the medium can be measured by a known method (Biotech-analyzer AS210 manufactured by Sakura Seiki Co.). Then, the yield of L-lysine can be calculated relative to consumed glucose for each of the strains.

The composition of the fermentation medium (g/l) is as follows:

Glucose	40
$(NH_4)_2SO_4$	24
K_2HPO_4	1.0
$MgSO_4 \cdot 7H_2O$	1.0
$FeSO_4 \cdot 7H_2O$	0.01
$MnSO_4 \cdot 5H_2O$	0.01
Yeast extract	2.0

The pH is adjusted to 7.0 by KOH and the medium is autoclaved at 115°C for 10 min. Glucose and $MgSO_4$ $7H_2O$ are sterilized separately. $CaCO_3$ is dry-heat sterilized at 180°C for 2 hours and added to the medium for a final concentration of 30 g/l.

Example 5. Production of L-cysteine by *E. coli* JM15-$P_{L\text{-}tac}$*araFGH*

To test the effect of enhancing the *araFGH* operon on L-cysteine production, DNA fragments coding for the arabinose transporter from the chromosome of the above-described *E. coli* MG1655-ΔptsHI-crr $P_{L\text{-}tac}$*araFGH* strain can be transferred to the *E. coli* L-cysteine-producing strain JM15(ydeD) by P1 transduction (Miller, J.H. Experiments in Molecular Genetics, Cold Spring Harbor Lab. Press, 1972, Plainview, NY) to obtain the strain JM15(ydeD)-$P_{L\text{-}tac}$*araFGH.*

E. coli strain JM15(ydeD) is a derivative of *E. coli* strain JM15 (US Patent No. 6,218,168) which can be transformed with DNA having the *ydeD* gene, which codes for a membrane protein, and is not involved in a biosynthetic pathway of any L-amino acid (U.S. Patent No. 5,972,663). The strain JM15 (CGSC# 5042) can be obtained from The Coli Genetic Stock Collection at the *E. coli* Genetic Resource Center, MCD Biology Department, Yale University (http://cgsc.biology.yale.edu/).

Fermentation conditions for evaluation of L-cysteine production were described in detail in Example 6 of US Patent No. 6,218,168.

Example 6. Production of L-leucine by *E. coli* 57- $P_{L\text{-}tac}$*araFGH*

To test the effect of enhancing the *araFGH* operon on L- leucine production, DNA fragments coding for the arabinose transporter from the chromosome of the above-described *E. coli* MG1655-ΔptsHI-crr $P_{L\text{-}tac}$*araFGH* strain can be transferred to the *E. coli* L-leucine-producing strain 57 (VKPM B-7386, US Patent No. 6,124,121) by P1 transduction (Miller, J.H. Experiments in Molecular Genetics, Cold Spring Harbor Lab. Press, 1972, Plainview, NY) to obtain the strain 57-$P_{L\text{-}tac}$*araFGH*. The strain 57 has been deposited in the Russian National Collection of Industrial Microorganisms (VKPM) (Russia, 117545 Moscow, 1 Dorozhny proezd, 1) on May 19, 1997under accession number VKPM B-7386.

Both *E. coli* strains, 57 and 57-$P_{L\text{-}tac}$*araFGH*, can each be cultured for 18-24 hours at 37°C on L-agar plates. To obtain a seed culture, the strains can be grown on a rotary shaker (250 rpm) at 32°C for 18 hours in 20x200-mm test tubes containing 2 ml of L-broth supplemented with 4% sucrose. Then, the fermentation medium can be inoculated with 0.21 ml of seed material (10%). The fermentation can be performed in 2 ml of a minimal fermentation medium in 20x200-mm test tubes. Cells can be grown for 48-72 hours at 32°C with shaking at 250 rpm. The amount of L-leucine can be measured by paper chromatography (liquid phase composition: butanol - acetic acid - water = 4:1:1).

The composition of the fermentation medium (g/l) (pH 7.2) is as follows:

Glucose	60.0
$(NH_4)_2SO_4$	25.0
K_2HPO_4	2.0
$MgSO_4 \cdot 7H_2O$	1.0
Thiamine	0.01
$CaCO_3$	25.0

Glucose and $CaCO_3$ are sterilized separately.

Example 7. Production of L-histidine by *E. coli* strain 80-$P_{L\text{-}tac}$*araFGH*

To test the effect of enhancing the *araFGH* operon on L-histidine production, DNA fragments coding for the arabinose transporter from the chromosome of the above-described *E. coli* MG1655-ΔptsHI-crr $P_{L\text{-}tac}$*araFGH* strain can be transferred to the histidine-producing *E. coli* strain 80 by P1 transduction (Miller, J.H. Experiments in Molecular Genetics, Cold Spring Harbor Lab. Press, 1972, Plainview, NY) to obtain the strain 80-$P_{L\text{-}tac}$*araFGH*. The strain 80 was described in Russian patent 2119536 and deposited in the Russian National Collection of Industrial Microorganisms (Russia, 117545 Moscow, 1 Dorozhny proezd, 1) on October 15, 1999 under accession number VKPM B-7270 and then converted to a deposit under the Budapest Treaty on July 12, 2004.

Both *E. coli* strains, 80 and 80-$P_{L\text{-}tac}$*araFGH*, can each be cultured in L-broth for 6 h at 29°C. Then, 0.1 ml of each of the obtained cultures can be inoculated into 2 ml of fermentation medium in a 20x200-mm test tube and cultivated for 65 hours at 29°C with shaking on a rotary shaker (350 rpm). After cultivation, the amount of histidine which accumulates in the medium can be determined by paper chromatography. The paper can be developed with a mobile phase consisting of n-butanol : acetic acid : water = 4 : 1 : 1 (v/v). A solution of ninhydrin (0.5%) in acetone can be used as a visualizing reagent.

The composition of the fermentation medium (g/l) is as follows (pH 6.0):

Glucose	100.0
Mameno (soybean hydrolysate)	0.2 of as total nitrogen
L-proline	1.0
$(NH_4)_2SO_4$	25.0
KH_2PO_4	2.0
$MgSO_4 \cdot 7H_20$	1.0
$FeSO_4 \cdot 7H_20$	0.01
$MnSO_4$	0.01
Thiamine	0.001
Betaine	2.0
$CaCO_3$	60.0

Glucose, proline, betaine and $CaCO_3$ are sterilized separately. The pH is adjusted to 6.0 before sterilization.

Example 8. Production of L-glutamate by *E. coli* strain VL334thrC^{+}-$P_{L\text{-}tac}$*araFGH*

To test the effect of enhancing the *araFGH* operon on L-glutamate production, DNA fragments coding for the arabinose transporter from the chromosome of the above-described *E. coli* MG1655-ΔptsHI-crr $P_{L\text{-}tac}$*araFGH* strain can be transferred to the *E. coli* L-glutamate-producing strain VL334thrC^{+} (EP 1172433) by P1 transduction (Miller, J.H. Experiments in Molecular Genetics, Cold Spring Harbor Lab. Press, 1972, Plainview, NY) to obtain the strain VL334thrC^{+}-$P_{L\text{-}tac}$*araFGH.* The strain VL334thrC^{+} has been deposited in the Russian National Collection of Industrial Microorganisms (VKPM) (Russia, 117545 Moscow, 1 Dorozhny proezd, 1) on December 6, 2004 under the accession number VKPM B-8961 and then converted to a deposit under the Budapest Treaty on December 8, 2004.

Both strains, VL334thrC^{+} and VL334thrC^{+}- $P_{L\text{-}tac}$*araFGH*, can each be grown for 18-24 hours at 37°C on L-agar plates. Then, one loop of the cells can be transferred into test tubes containing 2ml of fermentation medium. The fermentation medium contains glucose (60g/l), ammonium sulfate (25 g/l), KH_2PO_4 (2g/l), $MgSO_4$ (1 g/l), thiamine (0.1 mg/ml), L-isoleucine (70 μg/ml), and $CaCO_3$ (25 g/l). The pH is adjusted to 7.2. Glucose and $CaCO_3$ are sterilized separately. Cultivation can be carried out at 30°C for 3 days with shaking. After the cultivation, the amount of L-glutamic acid produced can be determined by paper chromatography (liquid phase composition of butanol-acetic acid-water=4:1:1) with subsequent staining by ninhydrin (1% solution in acetone) and further elution of the compounds in 50% ethanol with 0.5% $CdCl_2$.

Example 9. Production of L-phenylalanine by *E. coli* strain AJ12739-$P_{L\text{-}tac}$*araFGH*

To test the effect of enhancing the *araFGH* operon on L-phenylalanine production, DNA fragments coding for the arabinose transporter from the chromosome of the above-described *E. coli* MG1655-ΔptsHI-crr $P_{L\text{-}tac}$*araFGH* strain can be transferred to the phenylalanine-producing *E. coli* strain AJ12739 by P1 transduction (Miller, J.H. Experiments in Molecular Genetics, Cold Spring Harbor Lab. Press, 1972, Plainview, NY) to obtain the strain AJ12739-$P_{L\text{-}tac}$*araFGH.* The strain AJ12739 has been deposited in the Russian National Collection of Industrial Microorganisms (VKPM) (Russia, 117545 Moscow, 1 Dorozhny

proezd, 1) on November 6, 2001 under accession no. VKPM B-8197 and then converted to a deposit under the Budapest Treaty on August 23, 2002.

Both strains, AJ12739- $P_{L\text{-}tac}$*araFGH* and AJ12739, can each be cultivated at 37°C for 18 hours in a nutrient broth, and 0.3 ml of each of the obtained cultures can each be inoculated into 3 ml of a fermentation medium in a 20x200-mm test tube and cultivated at 37°C for 48 hours with shaking on a rotary shaker. After cultivation, the amount of phenylalanine which accumulates in the medium can be determined by TLC. The 10x15-cm TLC plates coated with 0.11-mm layers of Sorbfil silica gel containing no fluorescent indicator (Stock Company Sorbpolymer, Krasnodar, Russia) can be used. The Sorbfil plates can be developed with a mobile phase consisting of propan-2-ol : ethylacetate : 25% aqueous ammonia : water = 40 : 40 : 7 : 16 (v/v). A solution of ninhydrin (2%) in acetone can be used as a visualizing reagent.

The composition of the fermentation medium (g/l) is as follows:

Glucose	40.0
$(NH_4)_2SO_4$	16.0
K_2HPO_4	0.1
$MgSO_4 \cdot 7H_2O$	1.0
$FeSO_4 \cdot 7H_2O$	0.01
$MnSO_4 \cdot 5H_2O$	0.01
Thiamine HCl	0.0002
Yeast extract	2.0
Tyrosine	0.125
$CaCO_3$	20.0

Glucose and magnesium sulfate are sterilized separately. $CaCO_3$ is dry-heat sterilized at 180° for 2 hours. The pH is adjusted to 7.0.

Example 10. Production of L- tryptophan by *E. coli* strain SV164 (pGH5)-$P_{L\text{-}tac}$*araFGH*

To test the effect of enhancing the *araFGH* operon on L-tryptophan production, DNA fragments coding for the arabinose transporter from the chromosome of the above-described *E. coli* MG1655-ΔptsHI-crr $P_{L\text{-}tac}$*araFGH* strain can be transferred to the tryptophan-producing *E. coli* strain SV164 (pGH5) by P1 transduction (Miller, J.H. Experiments in Molecular Genetics,

Cold Spring Harbor Lab. Press, 1972, Plainview, NY) to obtain the strain SV164(pGH5)-P_{L-tac}*araFGH*. The strain SV164 has the *trpE* allele, which encodes anthranilate synthase and is is not subject to feedback inhibition by tryptophan. The plasmid pGH5 harbors a mutant *serA* gene, which encodes phosphoglycerate dehydrogenase and is not subject to feedback inhibition by serine. The strain SV164 (pGH5) was described in detail in US patent No. 6,180,373 and European patent 0662143.

Both strains, SV164(pGH5)-P_{L-tac}*araFGH* and SV164(pGH5), can each be cultivated with shaking at 32°C for 18 hours in 3 ml of nutrient broth supplemented with tetracycline (10 mg/l, marker of pGH5 plasmid). The obtained cultures (0.3 ml each) can each be inoculated into 3 ml of a fermentation medium containing tetracycline (10 mg/l) in 20 x 200-mm test tubes, and cultivated at 32°C for 72 hours with a rotary shaker at 250 rpm. After cultivation, the amount of tryptophan which accumulates in the medium can be determined by TLC as described in Example 9.

The fermentation medium components are listed in Table 2, but should be sterilized in separate groups (A, B, C, D, E, F, and G), as shown, to avoid adverse interactions during sterilization.

Table 2

Groups	Component	Final concentration, g/l
A	KH_2PO_4	1.5
	NaCl	0.5
	$(NH_4)_2SO_4$	1.5
	L-Methionine	0.05
	L-Phenylalanine	0.1
	L-Tyrosine	0.1
	Mameno (total N)	0,07
B	Glucose	40.0
	$MgSO_4 \cdot 7H_2O$	0.3
C	$CaCl_2$	0.011
D	$FeSO_4 \cdot 7H_2O$	0.075
	Sodium citrate	1.0
E	$Na_2MoO_4 \cdot 2H_2O$	0.00015
	H_3BO_3	0.0025
	$CoCl_2 \cdot 6H_2O$	0.00007
	$CuSO_4 \cdot 5H_2O$	0.00025
	$MnCl_2 \cdot 4H_2O$	0.0016
	$ZnSO_4 \cdot 7\ H_2O$	0.0003
F	Thiamine HCl	0.005
G	$CaCO_3$	30.0
H	Pyridoxine	0.03

Group A had pH of 7.1, adjusted by NH_4OH. Each group was sterilized separately.

Example 11. Production of L-proline by *E. coli* strain 702ilvA-$P_{L\text{-}tac}$*araFGH*

To test the effect of enhancing the *araFGH* operon on L-proline production, DNA fragments coding for the arabinose transporter from the chromosome of the above-described *E. coli* MG1655-ΔptsHI-crr $P_{L\text{-}tac}$*araFGH* strain can be transferred to the proline-producing *E. coli* strain 702ilvA by P1 transduction (Miller, J.H. Experiments in Molecular Genetics, Cold Spring Harbor Lab. Press, 1972, Plainview, NY) to obtain the strain 702ilvA -$P_{L\text{-}tac}$*araFGH*. The strain 702ilvA has been deposited in the Russian National Collection of Industrial Microorganisms (VKPM) (Russia, 117545 Moscow, 1 Dorozhny proezd, 1) on July 18, 2000 under accession number VKPM B-8012 and then converted to a deposit under the Budapest Treaty on May 18, 2001.

Both *E. coli* strains, 702ilvA and 702ilvA -$P_{L\text{-}tac}$*araFGH*, can each be grown for 18-24 hours at 37°C on L-agar plates. Then, these strains can be cultivated under the same conditions as in Example 8.

Example 12. Production of L-arginine by *E. coli* strain 382-$P_{L\text{-}tac}$*araFGH*

To test the effect of enhancing the *araFGH* operon on L-arginine production, DNA fragments coding for the arabinose transporter from the chromosome of the above-described *E. coli* MG1655-ΔptsHI-crr $P_{L\text{-}tac}$*araFGH* strain can be transferred to the arginine-producing *E. coli* strain 382 by P1 transduction (Miller, J.H. Experiments in Molecular Genetics, Cold Spring Harbor Lab. Press, 1972, Plainview, NY) to obtain the strain 382 -$P_{L\text{-}tac}$*araFGH*. The strain 382 has been deposited in the Russian National Collection of Industrial Microorganisms (VKPM) (Russia, 117545 Moscow, 1 Dorozhny proezd, 1) on April 10, 2000 under accession number VKPM B-7926 and then converted to a deposit under the Budapest Treaty on May 18, 2001.

Both strains, 382-$P_{L\text{-}tac}$*araFGH* and 382, can each be cultivated with shaking at 37°C for 18 hours in 3 ml of nutrient broth, and 0.3 ml of each of the obtained cultures were inoculated into 2 ml of a fermentation medium in 20 x 200-mm test tubes and cultivated at 32°C for 48 hours on a rotary shaker.

After the cultivation, the amount of L-arginine which had accumulated in the medium can be determined by paper chromatography using the following mobile phase: butanol : acetic acid : water = 4 : 1 : 1 (v/v). A solution of ninhydrin (2%) in acetone can be used as a visualizing reagent. A spot containing L-arginine can be cut out, L-arginine can be eluted with 0.5% water solution of $CdCl_2$, and the amount of L-arginine can be estimated spectrophotometrically at 540 nm.

The composition of the fermentation medium (g/l) is as follows:

Glucose	48.0
$(NH4)_2SO_4$	35.0
KH_2PO_4	2.0
$MgSO_4 \cdot 7H_2O$	1.0
Thiamine HCl	0.0002

Yeast extract	1.0
L-isoleucine	0.1
CaCO3	5.0

Glucose and magnesium sulfate are sterilized separately. $CaCO_3$ is dry-heat sterilized at 180°C for 2 hours. The pH is adjusted to 7.0.

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. All the cited references herein are incorporated as a part of this application by reference.

Industrial Applicability

According to the present invention, production of L-amino acids by a bacterium of the *Enterobacteriaceae* family can be enhanced.

CLAIMS

1. An L-amino acid producing bacterium of the *Enterobacteriaceae* family, wherein said bacterium has been modified to enhance the expression of the *araFGH* operon.

2. The bacterium according to claim 1, wherein the expression of the *araFGH* operon is enhanced by modifying an expression control sequence of the *araFGH* operon so that the gene expression is enhanced or by increasing the copy number of the *araFGH* operon.

3. The bacterium according to claim 1 or 2, wherein said bacterium is selected from the group consisting of the genera *Escherichia, Enterobacter, Erwinia, Klebsiella, Pantoea, Providencia, Salmonella, Serratia, Shigella,* and *Morganella.*

4. The bacterium according to any one of claims 1 to 3, wherein said operon encodes:

(A) a protein comprising the amino acid sequence of SEQ ID NO: 2 or a variant thereof;

(B) a protein comprising the amino acid sequence of SEQ ID NO: 4 or a variant thereof; and

(C) a protein comprising the amino acid sequence of SEQ ID NO: 6 or a variant thereof;

wherein said variants have the activity of the high-affinity L-arabinose transporter when said variants are combined together.

5. The bacterium according to any one of claims 1 to 4, wherein said operon comprises:

(A) a DNA comprising the nucleotide sequence of nucleotides 1 to 990 in SEQ ID NO: 1, or a DNA which is able to hybridize to a sequence complementary to said sequence, or a probe prepared from said sequence under stringent conditions;

(B) a DNA comprising the nucleotide sequence of nucleotides 1 to 1515 in SEQ ID NO: 3, or a DNA which is able to hybridize to a sequence complementary to said sequence, or a probe prepared from said sequence under stringent conditions; and

(C) a DNA comprising the nucleotide sequence of nucleotides 1 to 990 in SEQ ID NO: 5, or a DNA which is able to hybridize to a sequence complementary to said sequence, or a probe prepared from said sequence under stringent conditions; and

wherein, said DNAs encode proteins which have an activity of the high-affinity L-arabinose transporter when said proteins are combined together.

6. The bacterium according to claim 5, wherein said stringent conditions comprise washing at 60°C at a salt concentration of 1 x SSC and 0.1 % SDS, for approximately 15 minutes.

7. The bacterium according to any one of claims 1 to 6, wherein said bacterium has been additionally modified to enhance the activity of glucokinase.

8. The bacterium according to any one of claims 1 to 7, wherein said bacterium has been additionally modified to enhance the activity of xylose isomerase.

9. The bacterium according to any one of claims 1 to 8, wherein said bacterium is an L-threonine producing bacterium.

10. The bacterium according to claim 9, wherein said bacterium has been additionally modified to enhance expression of a gene selected from the group consisting of:

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

11. The bacterium according to claim 10, wherein said bacterium has been modified to increase expression of said mutant *thrA* gene, said *thrB* gene, said *thrC* gene, and said *rhtA* gene.

12. The bacterium according to any one of claims 1 to 8, wherein said bacterium is an L-lysine producing bacterium.

13. The bacterium according to any one of claims 1 to 8, wherein said bacterium is an L-histidine producing bacterium.

14. The bacterium according to any one of claims 1 to 8, wherein said bacterium is an L-phenylalanine producing bacterium.

15. The bacterium according to any one of claims 1 to 8, wherein said bacterium is an L-arginine producing bacterium.

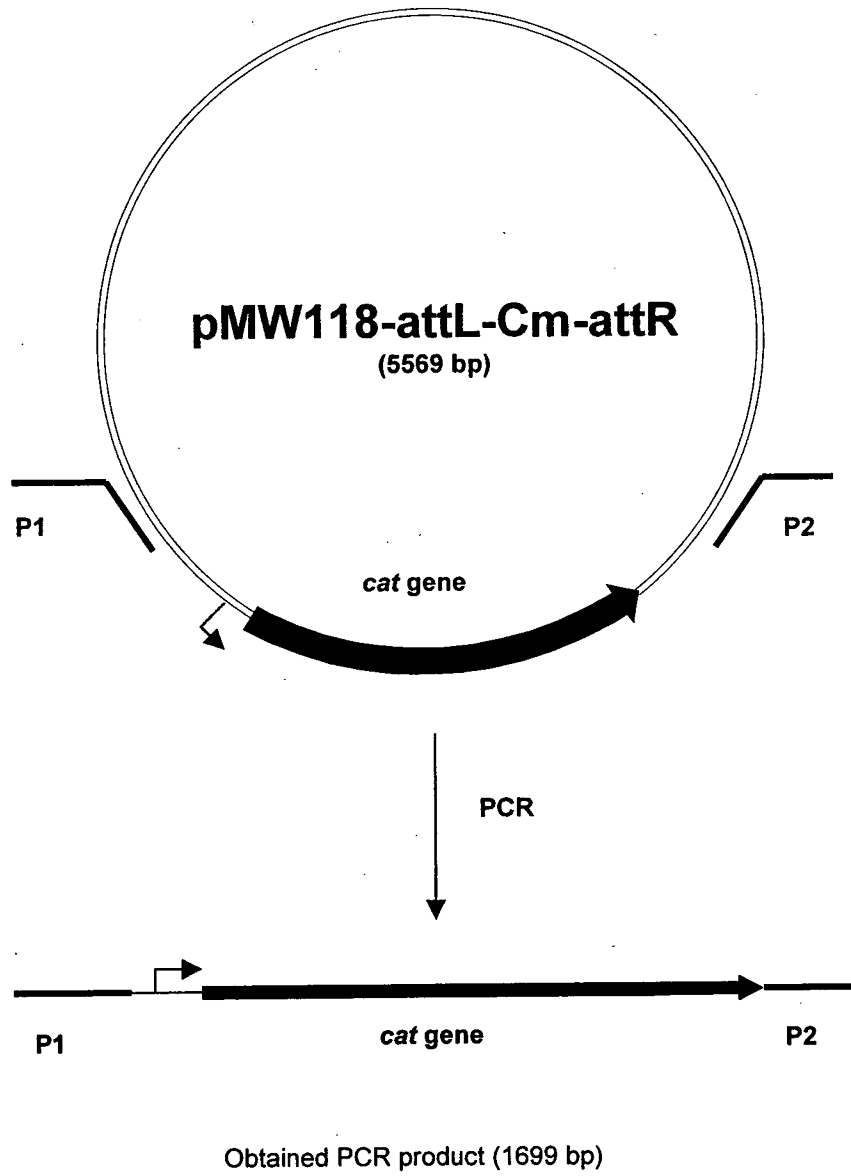
16. The bacterium according to any one of claims 1 to 8, wherein said bacterium is an L-tryptophan producing bacterium.

17. The bacterium according to any one of claims 1 to 8, wherein said bacterium is an L-glutamic acid producing bacterium.

18. A method for producing an L- amino acid comprising cultivating the bacterium according to any one of claims 1 to 17 in a culture medium which contains glucose as a carbon source, and isolating the L-amino acid from the culture medium.

19. The method according to claim 18, wherein said L-amino acid is selected from the group consisting of L-threonine, L-lysine, L-histidine, L-phenylalanine, L-arginine, L-tryptophan, and L-glutamic acid.

Fig. 1



Obtained PCR product (1699 bp)

Fig. 2

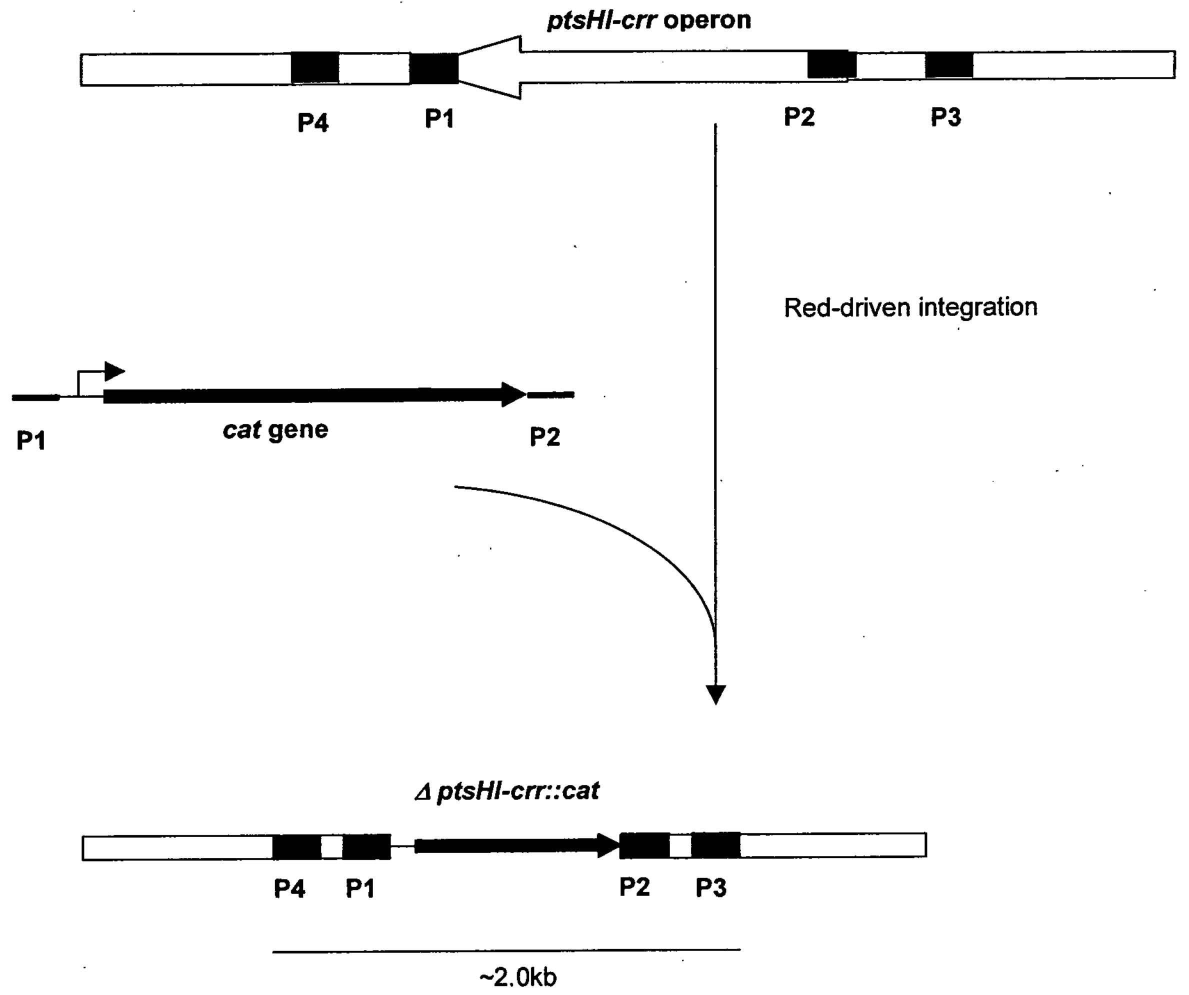


Fig. 3

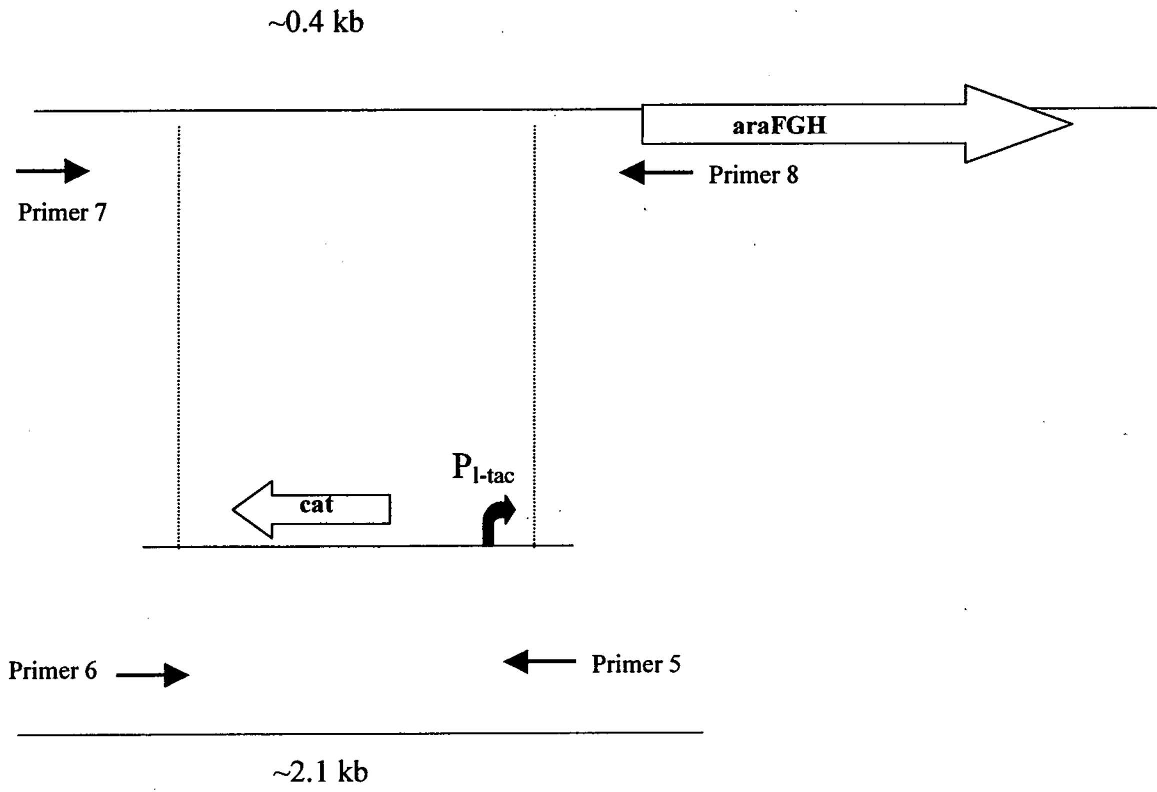


Fig. 4

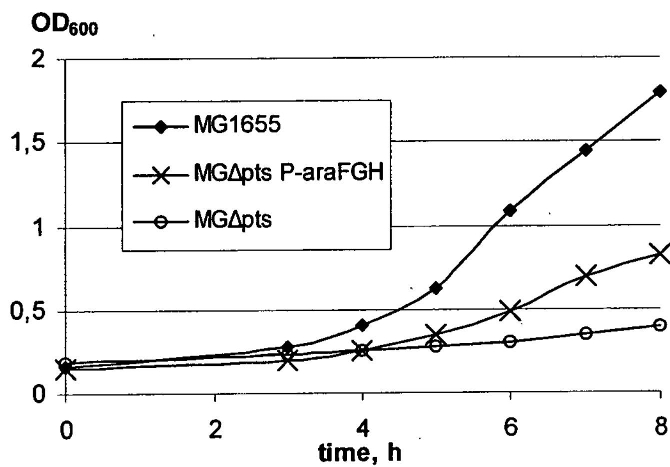


Fig. 5(A)

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YPS         -------MHKLTKALAVVGLAAVMSHSAIAESMKLGFLVKQPEEPWFQTEWKFADKAGKD
YPE         -------MHKLTKALAVVGLAAVMSHSAIAESMKLGFLVKQPEEPWFQTEWKFADKAGKD
ERC         -------MHKFTKALAAIGLAAVMSQSAMAENIKLGFLVKQPEEPWFQTEWKFADKAGKD
ECO         -------MHKFTKALAAIGLAAVMSQSAMAENLKLGFLVKQPEEPWFQTEWKFADKAGKD
SHS         -------MHKFTKALAAIGLAAVMSQSAMAENLKLGFLVKQPEEPWFQTEWKFADKAGKD
SHD         -------MHKFTKALAAIGLAAVMSQSAMAENLKLGFLVKQPEEPWFQTEWKFADKAGKD
PSM         -----MKRRTFITLAAAAAVAAAGLPAQAAEPVKIGFLVKQPEEPWFQDEWKFAELAAKD
PSS         --MNQQRRGTFRAIAAATVFAIAGGFVHAAEEVKIGFLVKQPEEPWFQDEWRFADQAAKE
PSP         MGLRWPQAALVCASLAAGLSAAAPAHAQGAAPVKIGFVVKQPDDPWFQDEWRFAEQAAKD
                      .    *.    *.      *  :*:**:****::**** **:**: *.*:

YPS         LGFEVIKIAVPDGEKTLNAIDSLAASGAKGFVICTPDPKLGPAIEAKARSYNLKVIAVDD
YPE         LGFEVIKIAVPDGEKTLNAIDSLAASGAQGFVICTPDPKLGPAIEAKARSYNLKVIAVDD
ERC         LGFDVIKIAVPDGEKTLNAIDSLAASGAKGFVICTPDPKLGPAIIAKARSYNLKVIAVDD
ECO         LGFEVIKIAVPDGEKTLNAIDSLAASGAKGFVICTPDPKLGSAIVAKARGYDMKVIAVDD
SHS         LGFEVIKIAVPDGEKTLNAIDSLAASGAKGFVICTPDPKLGSAIVAKARGYDMKVIAVDD
SHD         LGFEVIKIAVPDGEKTLNAIDSLAASGAKGFVICTPDPKLGSAIVAKARGYDMKVIAVDD
PSM         KGFTLVKIGAPSGEKVMSAIDNLAAQKAQGFIICTPDVKLGPGIVAKAKSHGLKMMTVDD
PSS         KGFKLVKIGVPSGGEVLTAIDNLGAQHAQGFVICVPDVKLGPAVVAKARQNNLKLMTVDD
PSP         KHFTLVKIAAPSGEKVSTALDSLAAQKAQGVIICAPDVKLGPGIAAKARRYGMKLMSVDD
              * ::**..*.* :. .*:*.*.*. *:*.:**.** ***..: ***:  .:*:::***

YPS         QFVNAKGKPMESVPLVMMAATKIGERQGQELWKEMNKRGWQPAETAVMAITSDELDTARR
YPE         QFVNAKGKPMESVPLVMMAATKIGERQGQELWKEMNKRGWQPAETAVMAITSDELDTARR
ERC         QFVNAKGQPMDTVPLVMMAATKIGERQGQELYKEMNKRGWKVDETGVMAITADELDTARR
ECO         QFVNAKGKPMDTVPLVMMAATKIGERQGQELYKEMQKRGWDVKESAVMAITANELDTARR
SHS         QFVNAKGKPMDTVPLVMMAATKIGERQGQELYKEMQKRGWDVKESAVMAITANELDTARR
SHD         QFVNAKGKPMDTVPLVMMAATKIGERQGQELYKEMQKRGWDVKESAVMAITSNELDTARR
PSM         RLVDGAGKPIESVPHMGISAYDIGKQVGGGIAAEIKRRGWNMNEVGAIDITYEQLPTAHD
PSS         RLVDGGGKPIEAVPHMGISATRIGEQVGEAIAQEMKKRGWNPSEVGAIRIAYDQLPTARE
PSP         QLVDGRGAPLADVPHMGISAYRIGRQVGDAIAAEAKRRGWNPAEVGVLRLAYDQLPTARE
            ::*:. * *:  ** : ::*  **.: *  :  * ::***.  * ..: :: ::* **:

YPS         RTGGSMAALQASGFPEKQIYKVPTKSNDIPGAFDAANSMLVQHPEVKNWLIVGMNDNTVL
YPE         RTGGSMAALQASGFPEKQIYKVPTKSNDIPGAFDAANSMLVQHPEVKNWLIVGMNDNTVL
ERC         RTAGSMDALKAAGFPEKQIYRVPTKSNDIPGAFDAANSMLVQHPGVKNWLIIGMNDNTVL
ECO         RTTGSMDALKAAGFPEKQIYQVPTKSNDIPGAFDAANSMLVQHPEVKHWLIVGMNDSTVL
SHS         RTTGSMDALKAAGFPEKQIYQVPTKSNDIPGAFDAANSMLVQHPEVKHWLIVGMNDSTVL
SHD         RTTGSMDALKAAGFPEKQIYQVPTKSNDIPGAFDAANSMLVQHPEVKHWLIVGMNDSTVL
PSM         RTTGATDALVAAGFPKANVIAAPQAKTDTENAFNAANIALTKNPKFKHWVAYGLNDEAVL
PSS         RTDGAVAALAKAGFPAANVLTSPQAKTDTEAAFNAADITLTKNPRFKHWIAFGLNDEAVL
PSP         RTTGAVDALKAAGFAAANVVDAPEMTADTEGAFNAANIAFTKHRNFKHWVAFGSNDDTTV
            ** *:  **  :**.  ::   *  . *   **:**:  :.::  .*:*:  * **.:.:

YPS         GGVRATEGQGFKAPNVIGIGINGVD-AVSELSKGQATGFYGSLLPSPDIHGYKSIQMLHD
YPE         GGVRATEGQGFKAPNVIGIGINGVD-AVSELSKGQATGFYGSLLPSPDIHGYKSIQMLHD
ERC         GGVRATEGQGFKAANVIGIGINGVD-AVSELSKGQATGFFGSLLPSPDIHGYKSIQMLND
ECO         GGVRATEGQGFKAADIIGIGINGVD-AVSELSKAQATGFYGSLLPSPDVHGYKSSEMLYN
SHS         GGVRATEGQGFKAADIIGIGINGVD-ALSELSKAQATGFYGSLLPSPDVHGYKSSEMLYN
SHD         GGVRATEGQGFKAADIIGIGINGVD-AVSELSKAQATGFYGSLLPSPDVHGYKSSEMLYN
PSM         GAVRAAEGRGFKAADMIGIGIGGSDSALSEFKKPQPTGFFGTVIISPKRHGEETSELMYA
PSS         GAVRAAEGHGIKAADIIGVGIGGSQSALNEFAKPEKTGFFGTVLISPKRHGYETSMNMYS
PSP         GAVRAGEGRGIGADDMIAVGINGSQVALNEFAKPKPTGFFGSILLNPRLHGYDTSVNMYD
            *.*** **:*: * ::*.:**.* : *:.*: * : ***:*::: .*  ** .:   :
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Fig. 5(B)

```
YPS          WVTKDVEPAKFTEVTDVVLITRDNFKAELEKKGLL--
YPE          WVTKDVEPAKFTEVTDVVLITRDNFKAELEKKGLL--
ERC          WVTKGVEPEKFTEVTDVVLITRDNFKVELEKKGLM--
ECO          WVAKDVEPPKFTEVTDVVLITRDNFKEELEKKGLGGK
SHS          WVAKDVEPPKFTEVTDVVLITRDNFKEELEKKGLGGK
SHD          WVAKGVEPTKFTEVTDVVLITRDNFKEELEKKGLGGK
PSM          WITQGKAPPPLTLTTG-MLATRENVAQVRETMGLAAK
PSS          WITASKAPEPLILTSG-RLMTRENEKAVRQEMGL---
PSP          WITQNRAPPPVVLTSG-TLITRANEKTARAQLGL---
             *:: .  *  .  .:.  * ** *        **
```

Fig. 6(A)

```
YPE    MQPKLYKEAIMSAPHSALQAELDAAQSPYLAFRGIGKSFPGVLALDDISFTCQAGQIHAL
YPS    ---------------------MDAAQSPYLAFRGIGKSFPGVLALDDISFTCQAGQIHAL
ERC    ----------------------MTAQSPYLSFHGIGKEFPGVKALSDISFSCHAGQIHAL
ECO    ----------------------MQQSTPYLSFRGIGKTFPGVKALTDISFDCYAGQVHAL
SHD    ----------------------MQQSTPYLSFRGIGKTFPGVKALTDISFDCYAGQVHAL
SHS    ----------------------MQQSTPYLSFRGIGKTFPGVKALTDISFDCYAGQVHAL
PSP    ------------------MAGNGGDVAAALRFDNIGKVFPGVRALDGISFDVQAGQVHGL
PSM    -------------------------MAAALRFDNIGKVFPGVRALDGISFDVQAGQVHGL
PSS    -------------------------MSAFLEFRGISKVFPGVRALSEVSFGIECGRVHGL
                                 :. * * .*.* **** **  :**   .*::*.*

YPE    MGENGAGKSTLLKILSGNYTPTQGEIHIKGKAVNFTNTTDALDAGVAIIYQELHLVPEMT
YPS    MGENGAGKSTLLKILSGNYTPTQGEIHIKGKAVNFTNTTDALDAGVAIIYQELHLVPEMT
ERC    MGENGAGKSTLLKILSGNYSPSAGEIHIQGKPVQFSNTMDALNAGVAIIYQELHLVPEMT
ECO    MGENGAGKSTLLKILSGNYAPTTGSVVINGQEMSFSDTTAALNAGVAIIYQELHLVPEMT
SHD    MGENGAGKSTLLKILSGNYAPTTGSVVINGQEMSFSDTTAALNAGVAIIYQELHLVPEMT
SHS    MGENGAGKSTLLKILSGNYAPTTGSVVINGQEMSFSDTTAALNAGVAIIYQELHLVPEMT
PSP    MGENGAGKSTLLKILGGEYQPDSGSVLVDGRAMRFPSAAASIAAGVAVIHQELQYVPDLT
PSM    MGENGAGKSTLLKILGGEYQLDSGSVLVDGRAMRFPSAAASIAAGVAVIHQELQYVPDLT
PSS    LGENGAGKSTLLKILGGDYQPDGGQIAVEGRPVAFPNARAALAAGIAVIHQELQTVPELT
       :**************.*:*    *.: :.*: : *..:  :: **:*:*:***: **::*

YPE    VAENIYLGQLPTKMGMVDRKLLRYESRIQLSHLGLDIDPDTPLKYLSIGQWQMVEIAKAL
YPS    VAENIYLGQLPTKMGMVDRKLLRYESRIQLSHLGLDIDPDTPLKYLSIGQWQMVEIAKAL
ERC    VAENIYLGQLPHKYGMVNYSLLRYEAKLQLQHLGLDIDPDTPLKYLSIGQWQMVEIAKAL
ECO    VAENIYLGQLPHKGGIVNRSLLNYEAGLQLKHLGMDIDPDTPLKYLSIGQWQMVEIAKAL
SHD    VAENIYLGQLPHKGGIVNRSLLNYEAGLQLKHLGMDIDPDTPLKYLSIGQWQMVEIAKAL
SHS    VAENIYLGQLPHKGGIVNRSLLNYEAGLQLKHLGMDIDPDTPLKYLSIGQWQMVEIAKAL
PSP    VAENLLLGRLPSALGWVRKRDAQRFVRERLAAMGVDLDAQAKLRRLSIAQRQMVEICKAL
PSM    VAENLLLGRLPSALGWVRKRDAQRFVRERLAAMGVDLDAQAKLRRLSIAQRQMVEICKAL
PSS    VMDNLLLGHLPSRGGFIRQGEAMAWTRAQLARIGVDLDPKARLKHLSIGQRQMVEICKAI
       * :*: **:**   * :            :*  :*:*:*..: *: ***.* *****.**:

YPE    ARNAKIIAFDEPTSSLSAREIEQLFRVIRELRAEGRVILYVSHRMEEIFALSDAITVFKD
YPS    ARNAKIIAFDEPTSSLSAREIEQLFRVIRELRAEGRVILYVSHRMEEIFALSDAITVFKD
ERC    ARNAKIIAFDEPTSSLSAREIEQLFRVITELRSEGRIILYVSHRMEEIFALSDAITVFKD
ECO    ARNAKIIAFDEPTSSLSAREIDNLFRVIRELRKEGRVILYVSHRMEEIFALSDAITVFKD
SHD    ARNAKIIAFDEPTSSLSAREIDNLFRVIRELRKEGRVILYVSHRMEEIFALSDAITVFKD
SHS    ARNAKIIAFDEPTSSLSAREIDNLFRVIRELRKEGRVILYVSHRMEEIFALSDAITVFKD
PSP    LRNARVIALDEPTSSLSHRETEVLFKLVDDLRRDGRALIYISHRMDEIYRLCDACTIFRD
PSM    LRNARVIALDEPTSSLSHRETEVLFKLVDDLRRDGRALIYISHRMDEIYRLCDACTIFRD
PSS    LRDARVIALDEPTSSLSVRETDILFRLVKDLRAQGRALIYISHRLDEIFALCDGCTIFRD
        *:*::**:******** ** : **::: :** :** ::*:***::**: *.*. *:*:*

YPE   GRYVRTFDDMTQVNNASLVQAMVGRNLGDIYGYQPREIGSERLTLQAVKAIGVASPISLT
YPS    GRYVRTFDDMTQVNNASLVQAMVGRNLGDIYGYQPREIGSERLTLQAVKAIGVASPISLT
ERC    GRYVCTFDDMQQVNHESLVQAMVGRNLGNIYGYAPRPHGEDRLTLKDVKAPGVKSTISLN
ECO    GRYVKTFTDMQQVDHDALVQAMVGRDIGDIYGWQPRSYGEERLRLDAVKAPGVRTPISLA
SHD    GRYVRTFTDMQQVDHDALVQAMVGRDIGDIYGWQPRSYGEERLRLDAVKAPGVRTPISLA
SHS    GRYVKTFTDMQQVDHDALVQAMVGRDIGDIYGWQPRSYGEERLRLDAVKAPGVRTPISLA
PSP    GRQVASHASLANVPRETLVRQMVGREISDIYHYAPRALGDVRLSARALEGDALRAGASFD
PSM    GRQVASHASLANVPRETLVRQMVGREISDIYHYAPRALGDVRLSARALEGDALRAGASFD
PSS    GRKVADFRSMADVTREQLVAQMVGRQIDDIFGYRPRAPGDVRLRVEGLMGPKLAEPASFA
       ** *   . .: :* .  **  ****::.:*: : **  *. **     : .  :    *:
```

Fig. 6(B)

```
YPE         VHQGEIVGLFGLVGAGRSELLKGLFGDTKLTSGKLLLDGQPLTIRSPIDAISAGIMLCPE
YPS         VHQGEIVGLFGLVGAGRSELLKGLFGDTKLTSGKLLLDGQPLTIRSPIDAISAGIMLCPE
ERC         VKQGEIVGLFGLVGAGRSELMKGLFGATKITSGQVLLDGKPLVVNSPIDAIRQGVMLCPE
ECO         VRSGEIVGLFGLVGAGRSELMKGMFGGTQITAGQVYIDQQPIDIRKPSHAIAAGMMLCPE
SHD         VRSGEIVGLFGLVGAGRSELMKGMFGGTQITAGQVYIDQQPIDIRKPSHAIAAGMMLCPE
SHS         VRSGEIVGLFGLVGAGRSELMKGLFGGTQITAGQVYIDQQPIDIRKPSHAIAAGMMLCPE
PSP         VRAGEIVGFFGLVGAGRSELMRVIYGAQRRTGGALTLDGEPLDIRSTRDAIRRGIVLCPE
PSM         VRAGEIVGFFGLVGAGRSELMRVIYGAQRRTGGALMLDGEPLDIRSTRDAIRRGIVLCPE
PSS         VRRGEIVGLFGLVGAGRSELARLVYGADRKTAGTVVLDGEPIRIRAVADAIRQGIVLCPE
            *: *****:*********** : ::*  : *.* : :* :*: :.   .**  *::****

YPE         DRKADGIIPVHSVQDNINISARRKTLTAG----CLINNRWEADNALLRIQSLNIKTPGPQ
YPS         DRKADGIIPVHSVQDNINISARRKTLTAG----CLINNRWEADNALLRIQSLNIKTPGPQ
ERC         DRKADGIIPVHSVRDNINISARRKSLKAG----FIINNQWEADNAAQRIDALNIKTPSDE
ECO         DRKAEGIIPVHSVRDNINISARRKHVLGG----CVINNGWEENNADHHIRSLNIKTPGAE
SHD         DRKAEGIIPVHSVRDNINISARRKHVLGG----CVINNGWEENNADHHIRSLNIKTPGAE
SHS         DRKAEGIIPVHSVRDNINISARRKHVLGG----CVINNGWEENNADQHIRSLNIKTPGAE
PSP         DRKEEGIVAHASVAENINISCRRHGLRAG----LFLDRKREAETADRFIKLLKIKTPNRR
PSM         DRKEEGIVAHASVAENINISCRRHGLRAG----LFLDRKREAETADRFIKLLKIKTPNRR
PSS         DRKEEGIIGCRSVSENINISCRRNRRPGWGGFNLFVDDRQEAKTADHYIARLRIKTPHRD
            *** :**:   ** :*****.**:   .      .::   * ..*   *  *.****

YPE         QLIMNLSGGNQQKAILGRWLSED-MKVILLDEPTRGIDVGAKHEIYNVIYQLAKQGIAVL
YPS         QLIMNLSGGNQQKAILGRWLSED-MKVILLDEPTRGIDVGAKHEIYNVIYQLAKQGIAVL
ERC         QLIMNLSGGNQQKVILGRWLSEE-MKVILLDEPTRGIDVGAKHEIYHVIYELANQGIAVL
ECO         QLIMNLSGGNQQKAILGRWLSEE-MKVILLDEPTRGIDVGAKHEIYNVIYALAAQGVAVL
SHD         QLIMNLSGGNQQKAILGRWLSEE-MKVILLDEPTRGIDVGAKHEIYNVIYALAAQGVAVL
SHS         QLIMNLSGGNQQKAILGRWLSEE-MKVILLDEPTRSIDVGAKHEIYNVIYALAAQGVAVL
PSP         QKIRFLSGGNQQKAILARWLAEPDLKVVILDEPTRGIDVGAKHEIYGVIYELAKRGCAIV
PSM         QKIRFLSGGNQQKAILARWLAEPDLKVVILDEPTRGIDVGAKHEIYGVIYELAKRGCAIV
PSS         QPIRLLSGGNQQKAILARWLAEDGMRVLIIDEPTRGIDVGAKNEIYQVLYELAERGVAVL
            * *  ********.**.***:*  ::*:::*****.******:***  *:* ** :* *::

YPE         FASSDLPEVLGLADRIVVMREGAISGELDHEYATEEQALSLAMLRTPNIATNTASAVA-
YPS         FASSDLPEVLGLADRIVVMREGAISGELDHEYATEEQALSLAMLRTPNIATNTASAVA-
ERC         FASSDLPEVLGLADRIIVMREGAVSGELLHADATEQKVLSLAMLRIPDIES----AVA-
ECO         FASSDLPEVLGVADRIVVMREGEIAGELLHEQADERQALSLAMPKVS-------QAVA-
SHD         FASSDLPEVLGVADRIVVMREGEIAGELLHEQADERQALSLAMPKVS-------QAVA-
SHS         FASSDLPEVLGVADRIVVMREGEIAGELLHEQADERQALSLAMPKVS-------QAVA-
PSP         MVSSELPEVLGVSDRIVVMSEGRIAGELARGEANEEAVLNLALPQG-------ATAHAA
PSM         MVSSELPEVLGVSDRIVVMREGRIAGELARGEANEEAVLNLALPQG-------ATAHAA
PSS         MISSELPEILGVADRVLVMSEGRIAGELPRAQATEHAVLNLALRPRRDPC---VAAQAA
            : **:***:**::**::** ** ::*** :   * *. .*.**:             * *
```

Fig. 7(A)

```
YPE   MKELIMSSVTLSSDKKNPVSTESNGGIPQPQQPQNAPTKSGLGLSRIWDSYGMLVVFAVV
YPS   -----MSSVTLSSDKKNPVSTESKGGIPQPQQPQNAPTKSGLGLSRIWDSYGMLVVFAVV
ERC   -----MSTVTSATSEKK--------------------KNGMGLSRIWDNYGMLVVFAVL
ECO   ----MMSSVSTSGSGAP--------------------KSSFSFGRIWDQYGMLVVFAVL
SHD   ----MMSSVSTSGSGAP--------------------KSSFSFGRIWDQYGMLVVFAVL
SHS   ----MMSSVSTSGSGAP--------------------KSSFSFGRIWDQYGMLVVFAVL
PSP   ----MQARENLPPAAAHAAAVP----------------TEDRQRWRQHAADYSLVAIFAAM
PSM   ----MQARENLPPAAAHAAAVP----------------TEDRQRWRQHAADYSLVAIFAAM
PSS   ----MSQSQPLQRADGFAASARS---------------AMNNTRLLRRLDDFSLPLIFAIL
                                                .      :    .:.:  :** :

YPE   FIGCVIFVPNFGSFINMKGLGLAISMSGMVACGMLFCLASGDFDLSVASVIACAGVTTAV
YPS   FIGCVIFVPNFGSFINMKGLGLAISMSGMVACGMLFCLASGDFDLSVASVIACAGVTTAV
ERC   FLGCAIFVPNFASFINMKGLGLAISMSGMVACGMLFCLASGDFDLSVASIIACSGVATAV
ECO   FIACAIFVPNFATFINMKGLGLAISMSGMVACGMLFCLASGDFDLSVASVIACAGVTTAV
SHD   FIACAIFVPNFATFINMKGLGLAISMSGMGACGMLFCLASGDFDLSVASVIACAGVTTAV
SHS   FIACAIFVPNFATFINMKGLGLAISMSGMVACGMLFCLASGDFDLSVASVIACAGVTTAV
PSP   FVAMSLTVDHFFSIDNMLGLALSISQIGMVACTMMFCLASRDFDLSIGSTVAFAGVLCAM
PSM   FVAMSLTVDHFFSIDNMLGLALSISQIGMVACTMMFCLASRDFDLSIGSTVAFAGVLCAM
PSS   FAALSLSVEYFFSWQNMVGLALSVSQIGMVACTMMFCLASRDFDLSIGSTVAFAGVLCAT
      * .   : *   * :   ** **.*::*   ** ** *:***** *****:.* :* :**  *.

YPE   VINMTESLWIGVGAGLLLGAACGLINGFVIARLKINALITTLATMQIVRGLAYIISDGKA
YPS   VINMTESLWIGVGAGLLLGAACGLINGFVIARLKINALITTLATMQIVRGLAYIISDGKA
ERC   VINISESLWIGVGAGLLLGVAFGLLNGFVIARLKINALITTLATMQIARGLAYIISDGKA
ECO   VINLTESLWIGVAAGLLLGVLCGLVNGFVIAKLKINALITTLATMQIVRGLAYIISDGKA
SHD   VINLTESLWIGVAAGLLLGVLCGLVNGFVIAKLKINALITTLATMQIVRGLAYIISDGKA
SHS   VINLTESLWIGVAAGLLLGILCGLVNGFVIAKLKINALITTLATMQIVRGLAYIISDGKA
PSP   VLNATDNTFVAIAAAVAAGAVIGFVNGAVIAYLRINALITTLATMEIVRGLGFIVSKGQA
PSM   VLNATDNTFVAIAAAVAAGAVIGFVNGAVIAYLRINALITTLATMEIVRGLGFIVSKGQA
PSS   VINATGSIALGIGASLLAGAVIGGINGFVIARLKINALITTLATMEIVRGLAFIASHGQA
      *:* :  .  :.:.*.:   *    * :** *** *:***********:*.***.:* *.*:*

YPE   VGIEDERFFALGYTNWFGLPAPIWITVACLVLFGFLLNKTTFGRNTLAIGGNEDAARLAG
YPS   VGIEDERFFALGYTNWFGLPAPIWITVACLVLFGFLLNKTTFGRNTLAIGGNEDAARLAG
ERC   VGIEDERFFALGYANWLGLPAPIWITIGCMILFGLLLNKTTFGRNTLAIGGNEEAARLAG
ECO   VGIEDESFFALGYANWFGLPAPIWLTVACLIIFGLLLNKTTFGRNTLAIGGNEEAARLAG
SHD   VGIEDESFFALGYANWFGLPAPIWLTVACLIIFGLLLNKTTFGRNTLAIGGNEEAARLAG
SHS   VGIEDESFFALGYANWFGLPAPIWLTVACLIIFGLLLNKTTFGRNTLAIGGNEEAARLAG
PSP   VGVSSETFIALGGLTFFGVSLPIWVTLACFVVFGVLLNQTVYGRNTLAIGGNPEASRLAG
PSM   VGVSSETFIALGGLTFFGVSLPIWVTLACFVVFGVLLNQTVYGRNTLAIGGNPEASRLAG
PSS   VGVSEMAFFDLGNTIVLGVPTPVWVAALCFVAFGVLLNKTVYGRNTLAIGGNPEAARLAG
      **:..  *: **   :*:. *:*::  *:: **.***:*.:********** :*:****

YPE   VPVVRTKIIIFVLSGLVSAAAGIILASRMTSGQPMTSIGYELIVISACILGGVSLKGGIG
YPS   VPVVRTKIIIFVLSGLVSAAAGIILASRMTSGQPMTSIGYELIVISACVLGGVSLKGGIG
ERC   VPVVRTKIIIFALSGLVSAAAGIILASRMTSGQPMTSIGYELIVISACVLGGVSLKGGIG
ECO   VPVVRTKIIIFVLSGLVSAIAGIILASRMTSGQPMTSIGYELIVISACVLGGVSLKGGIG
SHD   VPVVRTKIIIFVLSGLVSAIAGIILASRMTSGQPMTSIGYELIVISACVLGGVSLKGGIG
SHS   VPVVRTKIIIFVLSGLVSAIAGIILASRMTSGQPMTSIGYELIVISACVLGGVSLKGGIG
PSP   INVERTRVYIFLIQGAVTALAGVILASRITSGQPNAAQGFELNVISACVLGGVSLAGGRA
PSM   INVERTRVYIFLIQGAVTALAGVILASRITSGQPNAAQGFELNVISACVLGGVSLAGGRA
PSS   VNVNLTRIVIFLVQGVIAALAGVILAARITSGQPNAAQGFELNVISACVLGGVSLMGGRA
      : *  *:: ** :.* ::* **:***:*:***** :: *:** *****:****** ** .
```

Fig. 7(B)

```
YPE            KISYVIAGILILGTVENAMNLLNISPFSQYVVRGLILLAAVIFDRYKQLAKRTI
YPS            KISYVIAGILILGTVENAMNLLNISPFSQYVVRGLILLAAVIFDRYKQLAKRTI
ERC            KISYVVAGVLILGTVENAMNLLNISPFSQYVVRGLILLAAVIFDRYKQLAKKTV
ECO            KISYVVAGILILGTVENAMNLLNISPFAQYVVRGLILLAAVIFDRYKQKAKRTV
SHD            KISYVVAGILILGTVENAMNLLNISPFAQYVVRGLILLAAVIFDRYKQKAKPTV
SHS            KISYVVAGILILGTVENAMNLLNISPFAQYVVRGLILLAAVIFDRYKQKAKRIV
PSP            SISGVVIGVLIMGTVENVMNLLNIDAFYQYLVRGAILLAAVLLDQLKNRGARD-
PSM            SISGVVIGVLIMGTVENVMNLLNIDAFYQYLVRGAILLAAVLLDQLKNRGARD-
PSS            SISGVLVGVLIMGTVQNAMNLLNIDAFYQYLVRGGILLAAVLVDQIKHRGGRD-
               .** *: *:**:***:*.******..* **:*** ******:.*: *:  .
```

INTERNATIONAL SEARCH REPORT

International application No
PCT/JP2007/060935

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N1/21 C12N15/31 C07K14/245 C12P13/04 C12P13/08

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N C07K C12P

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

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

EPO-Internal, Sequence Search, WPI Data, BIOSIS, FSTA, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HORAZDOVSKY B F ET AL: "GENETIC RECONSTITUTION OF THE HIGH-AFFINITY L ARABINOSE TRANSPORT SYSTEM" JOURNAL OF BACTERIOLOGY, vol. 171, no. 6, 1989, pages 3053-3059, XP002449109 ISSN: 0021-9193 cited in the application page 3056 -----	1-17
A	SCRIPTURE J B ET AL: "HIGH-AFFINITY L ARABINOSE TRANSPORT OPERON NUCLEOTIDE SEQUENCE AND ANALYSIS OF GENE PRODUCTS" JOURNAL OF MOLECULAR BIOLOGY, vol. 197, no. 1, 1987, pages 37-46, XP008083022 ISSN: 0022-2836 the whole document -----	1-19
	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 September 2007	17/09/2007

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INTERNATIONAL SEARCH REPORT

International application No
PCT/JP2007/060935

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 02/101027 A (KYOWA HAKKO KOGYO KK [JP]; MORI HIDEO [JP]; FUJIO TATSURO [JP]; NISHIH) 19 December 2002 (2002-12-19) the whole document	1-19
A	US 5 175 107 A (DEBABOV VLADIMIR G [SU] ET AL) 29 December 1992 (1992-12-29) cited in the application the whole document	1-19
P,X	WO 2006/068273 A (AJINOMOTO KK [JP]; RYBAK KONSTANTIN VYACHESLAVOVI [RU]; SLIVINSKAYA EK) 29 June 2006 (2006-06-29) the whole document	1-19

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/JP2007/060935

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