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WO 2008/013432 A1
US 2012/0252077 A1
SOMA, Y., et al. "Metabolic flux redirection from a central metabolic pathway toward a synthetic pathway using a metabolic toggle switch", Metabolic Engineering. 2014 (May), vol. 23, pages 175-184

ABSTRACT

Disclosed is a microorganism of *Escherichia* sp. producing O-acetyl homoserine, and a method of producing O-acetyl homoserine in high yield using the microorganism.

MICROORGANISM PRODUCING *O*-ACETYL HOMOSERINE AND THE METHOD OF PRODUCING *O*-ACETYL HOMOSERINE USING THE SAME

TECHNICAL FIELD

The present invention relates to a microorganism of *Escherichia* sp. producing *O*-acetyl homoserine, and a method of producing *O*-acetyl homoserine in high yield using the microorganism.

BACKGROUND ART

O-Acetyl homoserine acts as a precursor of methionine, which is one of the essential amino acids in the body. Methionine has been widely used a component of medical infusion solutions and raw materials for medicinal products as well as an animal feed and food additive.

Methionine can be biologically or chemically synthesized. Recently, a two-step process, in which an L-methionine precursor produced by fermentation is converted to L-methionine by an enzyme reaction, was disclosed (International Publication No. WO 2008/013432). In the above two-step process, *O*-succinyl homoserine and *O*-acetyl homoserine may be used as the methionine precursor, and it is important that *O*-acetyl homoserine be produced in high yield for large-scale cost-effective production of methionine.

DISCLOSURE

Technical Problem

The present inventors, while endeavoring to improve the production of *O*-acetyl homoserine, discovered that the reduction of the expression or activity of citrate synthase protein can significantly increase the production capability of *O*-acetyl homoserine, thereby completing the present invention.

Technical Solution

An aim of the present invention is to provide an *O*-acetyl homoserine-producing microorganism with improved *O*-acetyl homoserine production capability.

Another aim of the present invention is to provide a method for producing *O*-acetyl homoserine using the microorganism.

Advantageous Effects

The use of the microorganism with *O*-acetyl homoserine production capability according to the present invention can produce *O*-acetyl homoserine in a higher yield and in a more environmentally friendly manner than chemical synthesis. Additionally, the thus produced *O*-acetyl homoserine can be used as a precursor for the synthesis of methionine and acetic acid by *O*-acetyl homoserine sulfhydrylase, thereby enabling bioconversion of L-methionine, and the thus converted L-methionine can be widely used in the production of foods or food additives for humans as well as animal feeds or animal feed additives.

DESCRIPTION OF DRAWINGS

FIG. 1 is an expression cassette design for the construction of a microorganism with an attenuated activity of citrate synthase.

FIG. 2 is a restriction map of *pBAD24*-citrate synthase antisense RNA (asRNA) vector.

BEST MODE

In an aspect, the present invention provides an isolated microorganism of *Escherichia sp.* producing *O*-acetyl homoserine, in which the activity of endogenous citrate synthase protein is attenuated or inactivated.

In another aspect, the present invention provides a method for producing *O*-acetyl homoserine, comprising: (a) culturing an isolated microorganism of *Escherichia sp.* producing *O*-acetyl homoserine, in which the activity of endogenous citrate synthase protein is attenuated or inactivated; and (b) recovering *O*-acetyl homoserine produced during the cultivation of the microorganism.

In a further aspect, the present invention provides a method for producing methionine, comprising: (a) culturing an isolated microorganism of *Escherichia sp.* producing *O*-acetyl homoserine, in which the activity of endogenous citrate synthase protein is attenuated or inactivated; (b) recovering *O*-acetyl homoserine produced during the cultivation of the microorganism, and (c) reacting the recovered *O*-acetyl homoserine and methyl mercaptan by an enzyme reaction using an enzyme having an *O*-acetyl homoserine sulfhydrylase activity or a microorganism possessing the enzyme.

As used herein, the term "*O*-acetyl homoserine", being a specific intermediate

material in a methionine biosynthesis pathway of a microorganism, refers to an acetyl-derivative of L-homoserine. *O*-acetyl homoserine can be produced by an enzyme activity of transferring an acetyl group from acetyl-CoA to homoserine using homoserine and acetyl-CoA as substrates.

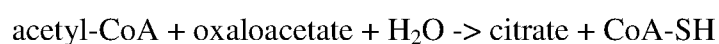
As used herein, the term “a microorganism producing *O*-acetyl homoserine” includes a microorganism, which, being a eukaryotic or prokaryotic microorganism producing *O*-acetyl homoserine within a living organism, is provided with *O*-acetyl homoserine-producing capability to its parent microorganism without *O*-acetyl homoserine-producing capability, or a microorganism which is endogenously provided with the *O*-acetyl homoserine-producing capability.

O-Acetyl homoserine-producing capability may be provided or promoted by improvement of species. The microorganisms having the *O*-acetyl homoserine-producing capability may include microorganism belonging to *Escherichia* sp., *Erwinia* sp., *Serratia* sp., *Providencia* sp., *Corynebacteria* sp., *Pseudomonas* sp., *Leptospira* sp., *Salmonella* sp., *Brevibacteria* sp., *Hypomononas* sp., *Chromobacterium* sp., and *Norcardia* sp., or fungi, or yeasts; specifically, microorganism belonging to *Escherichia* sp., *Corynebacteria* sp., *Leptospira* sp., and yeasts; and more specifically, microorganism belonging to *Escherichia* sp., as a specific example, *Escherichia coli*. The microorganisms having the *O*-acetyl homoserine-producing

[Text continued on page 3]

capability may be microorganisms producing L-lysine, L-threonine, L-isoleucine, or L-methionine, or derivatives thereof, but are not limited thereto.

As used herein, the term “citrate synthase (E.C. 2.3.3.1)” refers to an enzyme in the first step of the TCA cycle that mediates the reaction between oxaloacetate and acetyl-CoA. Specifically, citrate synthase mediates the condensation reaction between an acetate residue having two carbon atoms, which is in acetyl-CoA, and oxaloacetate having four carbon atoms, thereby generating a citrate having six carbon atoms. In *Escherichia coli*, citrate synthase is designated GltA, and citrate synthase and GltA are interchangeably used in the present invention.



Specifically, citrate synthase may be one derived from *Escherichia* sp., and more specifically, GltA derived from *Escherichia coli*. Citrate synthase may be a protein comprising an amino acid sequence represented by SEQ ID NO: 4 or those having a homology of 70% or higher with amino acid sequence of SEQ ID NO: 4, specifically 80% or higher, or more specifically, 90% or higher. Additionally, as a sequence having a homology, if the amino acid sequence is one having the same or corresponding activity of citrate synthase with that of SEQ ID NO: 4, it is obvious that amino acid sequences with a deletion, a modification, a substitution, or an addition, in part of the sequences should also be included in the scope of the present invention. Additionally, based on the genetic code degeneracy, polynucleotide sequences encoding the same amino acid sequence and variants thereof should also be included in the scope of the present invention.

As used herein, the term “endogenous” activity refers to a natural state of a protein in a microorganism or an activity state of the corresponding protein provided in the microorganism before modification.

The “the attenuation or inactivation of a protein activity compared to its endogenous activity” refers to a reduction or elimination of the protein activity when compared with that possessed in its natural state. The attenuation is a concept referring to a case when the activity of a protein is reduced compared with that originally possessed by the microorganism due to a modification in the protein-encoding gene, a case when the level of overall protein expression is lower than that of the natural type strain of the microorganism, or a combination thereof, but is not limited thereto. The inactivation includes a case when the gene encoding the protein is not expressed at all compared to that of the natural type strain, and a case when the gene is expressed but exhibits no activity.

The attenuation or inactivation of a protein activity may be achieved by various methods well known in the art. Examples of the methods may include a method of replacing the gene encoding the protein on the chromosome with a gene modified so that the enzyme activity can be reduced including the case when the protein activity is removed; a method of introducing a modification on the expression-regulating sequence of the gene encoding the protein on the chromosome; a method of replacing the expression-regulating sequence of the gene encoding the protein with a sequence having a weak activity or no activity; a method of deleting a part of or the entire gene encoding the protein on the chromosome; a method of introducing an antisense oligonucleotide (e.g., antisense RNA), which inhibits the translation from the mRNA into a protein via a complementary binding to the transcript of the gene on the chromosome; a method of making the attachment of ribosome impossible by forming a secondary structure by artificially adding a Shine-Dalgarno (SD) sequence and its complementary sequence on the front end of the SD sequence of the gene encoding the protein; a method of reverse transcription engineering (RTE), which adds a promoter so as to be reversely transcribed on the 3' terminus of the open reading frame (ORF) of the corresponding sequence, etc., and also include a combination thereof, but are not limited thereto.

Specifically, the method of deleting a part of or the entire gene encoding a protein may be performed by replacing the polynucleotide, encoding the endogenous target protein within the chromosome via a vector for inserting chromosome into a microorganism, with a polynucleotide or a marker where part of the polynucleotide sequence is deleted. For example, a method of gene deletion via homologous recombination may be used, but is not limited thereto. Additionally, as used herein the term "part", although it may vary depending on the kinds of polynucleotide, may specifically refer to 1 nucleotide to 300 nucleotides, more specifically 1 nucleotide to 100 nucleotides, and even more specifically 1 nucleotide to 50 nucleotides, but is not limited thereto.

Additionally, the method of modifying the expression regulation sequence may be performed by inducing a variation on the expression regulation sequence of the polynucleotide sequence via deletion, insertion, conservative substitution, non-conservative substitution, or a combination thereof so as to further attenuate the activity of the expression regulation sequence; or by replacing the polynucleotide sequence with a polynucleotide sequence with a weaker activity. The polynucleotide sequence may include a promoter, an operator sequence, a sequence encoding a ribosome-binding domain, and a sequence for regulating termination of transcription and translation, but is not limited thereto.

Additionally, the method of modifying the gene sequence on the chromosome may be

performed by inducing a variation in the sequence via deletion, insertion, conservative substitution, non-conservative substitution, or a combination thereof so as to further attenuate the activity of the expression regulation sequence; or by replacing the sequence with a gene sequence improved to have a weaker activity or a gene sequence improved to have no activity, but is not limited thereto.

Specifically, for the attenuation of the activity of citrate synthase protein, part of an amino acid(s) in the amino acid sequence of the citrate synthase protein may be substituted with other amino acid(s). More specifically, a citrate synthase having an amino acid sequence, in which the 145th amino acid or the 167th amino acid in the amino acid sequence of the citrate synthase protein is substituted from tyrosine (Y) or lysine (K) to other amino acid(s) may be included. Even more specifically, the citrate synthase may be one having a gene sequence encoding a modified polypeptide, in which the 145th amino acid in the amino acid sequence of the citrate synthase protein is substituted from tyrosine (Y) to alanine (A), and the 167th amino acid is substituted from lysine (K) to alanine (A). In particular, the amino acid residue number was determined in sequential order after setting the amino acid positioned next to the methionine, which is encoded by the initiation codon, as the 1st amino acid. The polypeptide may respectively have an amino acid sequence represented by SEQ ID NO: 1 or 2. Additionally, if the activity of the citrate synthase is weaker than that of a wild-type, the citrate synthase may include amino acid sequences having a homology of 80% or higher with the amino acid sequence of SEQ ID NO: 1 or 2, specifically 90% or higher, more specifically 95% or higher, and even more specifically 97% or higher. As a sequence having a homology, if the amino acid sequence is one which has substantially the same or corresponding biological activity of a protein of SEQ ID NO: 1 or 2, it is obvious that amino acid sequences with a deletion, a modification, a substitution, or an addition in part of the sequences should also be included in the scope of the present invention.

As used herein, the term “homology” refers to a percentage in identity between two polynucleotides or polypeptide moieties. The homology between sequences from a moiety to another moiety may be determined by a technology known in the art. For example, the homology may be determined by directly arranging the sequence information between two different polynucleotide molecules or two different polypeptides using a computer program arranging and easily obtaining the sequence information. The computer program may include BLAST (NCBI), CLC Main Workbench (CLC bio), MegAlign™ (DNASTAR Inc), etc. Additionally, the homology between polynucleotides may be determined by hybridizing the

polynucleotides under the condition of forming a stable double strand between homologous regions, decomposing with a single-strand-specific nuclease, and determining the decomposed fragments.

As used herein, the term “homology” refers to a relationship between proteins having “common evolutionary origin” including homologous proteins derived from superfamily proteins in all grammatical forms or with spelling variations, and those derived from different species. These proteins (and the genes encoding the same) have sequence homologies reflected by high levels of sequence similarities. However, the term “homology”, for its general use and the use in the present invention, would refer to a sequence similarity when modified by an adjective such as “very high”, instead of referring to common evolutionary origin.

In an exemplary embodiment of the present invention, the microorganism may be one in which the activity of cystathionine gamma synthase (EC 2.5.1.48), homoserine kinase (EC 2.7.1.39), or the activities of both are weaker than their endogenous activities, or inactivated.

As used herein, the term “cystathionine gamma synthase” refers to an enzyme which can synthesize cystathionine by a chemical reaction described below, using O-succinyl homoserine and L-cysteine as substrates. In the present invention, the cystathionine gamma synthase from *E. coli*, is designated as “MetB”.

O-succinyl-L-homoserine + L-cysteine → L-cystathionine + succinate

Specifically, the cystathionine gamma synthase from *E. coli*, although not particularly limited thereto, may be a protein comprising an amino acid sequence represented by SEQ ID NO: 9 or those having a homology of 70% or higher with the amino acid sequence of SEQ ID NO: 9, specifically 80% or higher, and more specifically 90% or higher. Additionally, as a sequence having a homology, if the amino acid sequence is one having the same or corresponding activity of homoserine kinase with the amino acid sequence of SEQ ID NO: 9, it is obvious that amino acid sequences with a deletion, a modification, a substitution, or an addition in part of the sequences should also be included in the scope of the present invention. Additionally, based on the genetic code degeneracy, polynucleotide sequences encoding the same amino acid sequence and variants thereof should also be included in the scope of the present invention.

The method for attenuation and inactivation of the cystathionine gamma synthase activity may be performed according to the method described above.

As used herein, the term “homoserine kinase” refers to an enzyme causing the phosphorylation of homoserine, which performs the chemical reaction described below. In the present invention, the homoserine kinase from *E. coli*, is designated as “ThrB”.



Specifically, homoserine kinase from *Escherichia* sp., although not particularly limited thereto, may be a protein comprising an amino acid sequence represented by SEQ ID NO: 11 or those having a homology of 70% or higher with the amino sequence of SEQ ID NO: 11, specifically 80% or higher, or more specifically, 90% or higher. Additionally, as a sequence having a homology, if the amino acid sequence is one having the same or corresponding activity of homoserine kinase with the amino sequence of SEQ ID NO: 11, it is obvious that amino acid sequences with a deletion, a modification, a substitution, or an addition in part of the sequences should also be included in the scope of the present invention. Additionally, based on the genetic code degeneracy, polynucleotide sequences encoding the same amino acid sequence and variants thereof should also be included in the scope of the present invention.

The method for attenuation and inactivation of the homoserine kinase activity may be performed according to the method described above.

In a specific aspect of the present invention, the microorganism may be one, in which the activity of homoserine O-acetyltransferase is further introduced or enhanced, or the endogenous homoserine O-succinyltransferase is further modified to have the activity of homoserine O-acetyltransferase.

As used herein, the term “homoserine O-acetyltransferase (EC 2.3.1.31)” refers to an enzyme having an activity of transferring an acetyl group from acetyl-CoA to homoserine.

Specifically, the microorganism according to the present invention may be introduced with the activity of homoserine O-acetyltransferase. The homoserine O-acetyltransferase may be derived from various microorganism species, for example, from a microorganism selected from *Corynebacteria* sp., *Leptospira* sp., *Deinococcus* sp., *Deinococcus* sp., *Pseudomonas* sp., and *Mycobacterium* sp. Specifically, the homoserine O-acetyltransferase may be those which include the amino acid sequences represented by SEQ ID NO: 13 (*Leptospira meyeri*), SEQ ID NO: 14 (*Corynebacterium glutamicum*), or SEQ ID NO: 15 (*Deinococcus radiodurans*), but is

not limited thereto. Additionally, the homoserine O-acetyltransferase may be a protein comprising the above amino acid sequences or those having a homology of 70% or higher with the above amino acid sequences, specifically 80% or higher, or more specifically, 90% or higher. Additionally, based on the genetic code degeneracy, polynucleotide sequences encoding the same amino acid sequence and variants thereof should also be included in the scope of the present invention.

Examples of the homoserine O-acetyltransferase to be used in the present invention are disclosed in Korean Patent Application Publication No. 10-2011-0023703 and European Patent Application Publication No. EP 2290051, and the entire specifications of these patent documents may be included as references in the present invention.

Additionally, the protein, in which the endogenous homoserine O-succinyltransferase (EC 2.3.1.46) is modified to have the activity of homoserine O-acetyltransferase, refers to a polypeptide, in which the substrate specificity of the polypeptide having the homoserine O-succinyltransferase activity is changed from succinyl-CoA to acetyl-CoA. Additionally, the modified protein, although not particularly limited thereto, may be a polypeptide having homoserine O-acetyltransferase activity, unlike its wild-type, by replacing part of the amino acid sequence of the polypeptide having homoserine O-succinyltransferase activity.

Examples of the homoserine O-succinyltransferase may be a polypeptide from *Enterobacteria* sp., *Salmonella* sp., *Pseudomonas* sp., *Bacillus* sp., or *Escherichia* sp., specifically, a polypeptide from *Escherichia* sp. having the homoserine O-succinyltransferase activity, for example, a polypeptide having homoserine O-succinyltransferase activity from *E. coli*. More specifically, the homoserine O-succinyltransferase from *E. coli* may have the amino acid sequence represented SEQ ID NO: 16, but is not limited thereto. The homoserine O-succinyltransferase from *E. coli* is designated as "MetA".

The modified homoserine O-succinyltransferase may be a variant polypeptide, in which the 111th amino acid of the polypeptide represented by SEQ ID NO: 16 or polypeptides having a homology of 95% or above with the polynucleotide sequence of SEQ ID NO: 16 is substituted with glutamic acid, and additionally, the 112nd amino acid is substituted with threonine or histidine. Specifically, the variant polypeptide may be a polypeptide having the amino acid sequence of any of SEQ ID NOS: 17 to 19. Additionally, the variant polypeptide may be a protein comprising an amino acid sequence having a homology of 70% or higher with the above amino acid sequence, specifically 80% or higher, or more specifically 90% or higher. Additionally, based on the genetic code degeneracy, polynucleotide sequences encoding the

same amino acid sequence and variants thereof should also be included in the scope of the present invention. The information on the modified homoserine O-succinyltransferase can be obtained from Korean Patent Application Publication No. 10-2012-0070531 or International Publication No. WO2012/087039, and the entire specifications of these patent documents are included as references to the present invention.

As used herein, the term “introduction or enhancement of activity” refers to providing the activity of a particular protein to a microorganism which does not possess the protein; or enhancement of the intracellular activity of the protein in the microorganism which possesses the protein, and the like, and refers to the increase of the intracellular activity of the protein compared to the endogenous activity of the protein.

As used herein, the term “introduction or enhancement of protein activity” refers to not only the drawing of a higher effect than the original function due to the increase in the activity of the protein itself, but also the increase in the activity of the protein due to the increase in endogenous gene activity, endogenous gene amplification by the internal or external factors, the increase in copy number, gene introduction from outside, increase in enzyme activity due to the substitution, modification, or mutation, etc., but is not limited thereto.

In the above, the increase in gene copy number, although not particularly limited thereto, may be performed in a state operably connected to a vector, or by being inserted into the chromosome within a host cell. Specifically, the method may be executed by introducing a vector, which a polynucleotide encoding the protein of the present invention is operably connected to and can be replicated and function irrespective of a host, into a cell of the host; or introducing a vector, to which the polynucleotide is operably connected, inserting the polynucleotide into the chromosome of the host cell, into the host cell, thereby increasing the number of gene copies within the chromosome of the host cell.

The vector is a DNA construct including the polynucleotide sequence of the polynucleotide encoding the target protein, which is operably connected to a suitable regulation sequence so that the target protein can be expressed in an appropriate host, wherein the regulation sequence includes a promoter initiating transcription, a random operator sequence for regulation of the transcription, a sequence encoding a suitable mRNA ribosome-binding domain, and a sequence for regulation of transcription and translation. The vector, after being transformed into a suitable host cell, may be replicated or function irrespective of the host genome, or may be integrated into the host genome itself.

The vector used in the present invention may not be specifically limited as long as the

vector is replicable in the host cell, and any vector known in the art may be used. Examples of the vector may include natural or recombinant plasmids, cosmids, viruses, and bacteriophages. For example, as a phage vector or cosmid vector, pWE15, M13, λ MBL3, λ MBL4, λ IXII, λ ASHII, λ APII, λ I10, λ I11, Charon4A, Charon21A, etc., may be used; and as a plasmid vector, pBR-based, pUC-based, pBluescriptII-based, pGEM-based, pTZ-based, pCL-based, pET-based, etc., may be used. Specifically, pDZ, pACYC177, pACYC184, pCL, pECCG117, pUC19, pBR322, pMW118, pCC1BAC vectors, etc., may be used.

Additionally, a polynucleotide encoding an endogenous target protein may be substituted with a modified polynucleotide using a vector for inserting into the chromosome of a microorganism. The insertion of the polynucleotide into the chromosome may be performed using a known method in the art, for example, by homologous recombination. Since the vector of the present invention can be inserted into the chromosome via homologous recombination, a selection marker for confirmation of the insertion into the chromosome may be additionally included. The selection marker is used for selection of a transformed cell, i.e., in order to confirm whether the target polynucleotide has been inserted, and markers providing selectable phenotypes such as drug resistance, nutrient requirement, resistance to cytotoxic agents, and expression of surface proteins may be used, but are not limited thereto. Under the circumstances where selective agents are treated, only the cells expressing the selection markers can survive or express other phenotypic traits, and thus the transformed cells can be easily selected.

As used herein, the term “transformation” refers to a process of introducing a vector including a polynucleotide encoding a target protein into a host cell thereby enabling the expression of the polynucleotide encoded by the protein in the host cell. For the transformed polynucleotide, it does not matter whether it is inserted into the chromosome of a host cell and located therein and located outside the chromosome, as long as it can be expressed in the host cell. Additionally, the polynucleotide includes DNA and RNA which encode the target protein. The polynucleotide may be inserted in any form insofar as it can be introduced into a host cell and expressed therein. For example, the polynucleotide may be introduced into a host cell in the form of an expression cassette, which is a genetic construct including all essential elements required for self-expression. The expression cassette may conventionally include a promoter operably connected to the polynucleotide, a transcription termination signal, a ribosome-binding domain, and a translation termination signal, and may be in the form of an expression vector capable of self-replication. Additionally, the polynucleotide may be introduced into a host cell as it is, and operably connected to a sequence essential for its expression in the host cell.

Additionally, as used herein, the term “operably connected” refers to a functional connection between a promoter sequence, which initiates and mediates the transcription of the polynucleotide encoding the target protein, and the gene sequence.

Then, the modification of the expression regulation sequence for increasing the expression of the polynucleotide, although not particularly limited thereto, may be performed by inducing a variation in the polynucleotide sequence via deletion, insertion, conservative substitution, non-conservative substitution, or a combination thereof so as to further enhance the activity of the expression regulation sequence; or by replacing the polynucleotide sequence with a polynucleotide sequence with a stronger activity. The expression regulation sequence, although not particularly limited thereto, may include a promoter, an operator sequence, a sequence encoding a ribosome-binding domain, and a sequence for regulating termination of transcription and translation, etc. Additionally, a strong exogenous promoter, instead of the original promoter, may be connected to the upper end of the expression unit of the polynucleotide.

Furthermore, the modification of the polynucleotide sequence on the chromosome, although not particularly limited thereto, may be performed by inducing a variation on the expression regulation sequence of the polynucleotide sequence via deletion, insertion, conservative substitution, non-conservative substitution, or a combination thereof so as to further enhance the activity of the polynucleotide sequence; or by replacing the polynucleotide sequence with an enhanced polynucleotide sequence with a stronger activity.

Generally, the introduction and enhancement of the protein activity may increase the activity or concentration of the corresponding protein relative to the activity or concentration of a wild-type protein or in a microorganism from at least 1%, 10%, 25%, 50%, 75%, 100%, 150%, 200%, 300%, 400%, or 500%, to a maximum of 1000% or 2000%.

Additionally, the microorganism may be one in which the activity of the endogenous homoserine O-succinyltransferase was attenuated or inactivated compared to that of the endogenous activity, in order to enhance the biosynthesis pathway of O-acetyl homoserine by blocking the pathway of biosynthesizing O-succinyl homoserine from homoserine.

The attenuation and inactivation of the homoserine O-succinyltransferase activity may be performed according to the method explained above.

In an exemplary embodiment of the present invention, the O-acetyl homoserine-producing microorganism may be one in which the activity of an enzyme involved

in the biosynthesis pathway from phosphoenolpyruvate to homoserine is additionally introduced or enhanced, in order to further increase the amount of homoserine, a substrate for the biosynthesis of O-acetyl homoserine.

Specifically, the above microorganism may be one, in which the activity of at least one protein selected from the group consisting of phosphoenolpyruvate carboxylase (ppc, EC 4.1.1.31), aspartate aminotransferase (aspC, EC 2.6.1.1), and aspartate semialdehyde dehydrogenase (asd, EC 1.2.1.11) is further introduced or enhanced.

For example, ppc gene encoding phosphoenolpyruvate carboxylate including an amino acid sequence represented by SEQ ID NO: 20, aspC gene encoding aspartate aminotransferase including an amino acid sequence represented by SEQ ID NO: 21, and asd gene encoding aspartate semialdehyde dehydrogenase including an amino acid represented by SEQ ID NO: 22 may be introduced into a microorganism. For example, the activities of the three different enzymes may be introduced and enhanced by making all the genes encoding the three different enzymes present in the chromosome of a host cell with a copy number of at least 2, but is not limited thereto. The introduction and enhancement of the activities may be performed according to the method described above.

In an exemplary embodiment of the present invention, the activity of citrate synthase protein was attenuated or inactivated by various methods, which includes deleting the citrate synthase gene in a microorganism of *E. coli* producing O-acetyl homoserine; introducing the gene encoding the modified citrate synthase protein, whose activity was attenuated compared to that of a wild-type, into the position of the citrate synthase gene; and introducing an expression vector for citrate synthase gene antisense RNA. As a result, the thus-constructed O-acetyl homoserine-producing microorganism, in which the activity of citrate synthase protein was attenuated or inactivated, showed an improved O-acetyl homoserine production capability, compared to that of the parent microorganism (Examples 1 to 4).

In another aspect, the present invention provides a method for producing O-acetyl homoserine using an O-acetyl homoserine-producing microorganism with an improved production capability of O-acetyl homoserine. Specifically, the present invention provides a method for producing O-acetyl homoserine including (a) culturing the microorganism; and (b) recovering O-acetyl homoserine produced during the cultivation of the microorganism.

The method of cultivation of the *E. coli* having O-acetyl homoserine production

capability according to the present invention may be performed according to the suitable media and culture conditions known in the art. The cultivation process may be easily adjusted by a skilled person in the art depending on the microorganism to be selected. In particular, since the microorganism of the present invention is a microorganism where the activity of the citrate synthase, which is an enzyme mediating the first step of the TCA cycle, is attenuated or inactivated, the cultivation medium may include glutamate, but is not specifically limited thereto.

Examples of the culture methods may include a batch culture, a continuous culture, and a fed-batch culture, but are not limited thereto. These various methods are, for example, disclosed in "Biochemical Engineering" by James M. Lee, Prentice-Hall International Editions, pp 138-176.

The medium used in the cultivation may appropriately meet the requirement of a specific microorganism. Specifically, examples of the microorganism culture media are disclosed in "Manual of Methods for General Bacteriology" by the American Society for Bacteriology, Washington, DC, 1981. The culture media may be those which include an appropriate carbon source, phosphorous source, inorganic compound, amino acid, and/or vitamins, etc, and cultivation may be performed in aerobic conditions while adjusting temperature, pH, etc.

Examples of the carbon source may include carbohydrates such as glucose, lactose, sucrose, lactic acid, fructose, maltose, starch, and cellulose; fats such as soybean oil, sunflower oil, castor oil, berber oil, and coconut oil; fatty acids such as palmitic acid, stearic acid, and linoleic acid; alcohols such as glycerol and ethanol; and organic acids such as acetic acid. These carbon sources may be used alone or in combination, but are not limited thereto.

Examples of the nitrogen source may include organic nitrogen sources such as peptone, yeast extract, gravy, malt extract, corn steep liquor (CSL), and bean flour; and inorganic nitrogen sources such as urea, ammonium sulfate, ammonium chloride, ammonium phosphate, ammonium carbonate, and ammonium nitrate. These nitrogen sources may be used alone or in combination, but are not limited thereto. The culture media may further include, as a phosphorous source, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, and corresponding sodium-containing salts. The culture media may include metals such as magnesium sulfate and iron sulfate. Additionally, amino acids, vitamins and appropriate precursors may be included. These culture media or precursors may be added to the culture in the form of a batch culture or continuous culture, but are not limited thereto.

Additionally, the pH of the culture may be adjusted by adding a compound such as ammonium hydroxide, potassium hydroxide, ammonia, phosphoric acid, and sulfuric acid during

cultivation in an appropriate manner. Additionally, bubble formation may be prevented during the cultivation using an antifoaming agent such as fatty acid polyglycol ester. Additionally, in order to maintain aerobic conditions in a culture liquid, an oxygen gas or a gas (e.g., air) containing an oxygen gas may be added to a culture. The culture temperature may be from 20°C to 45°C, and specifically from 25°C to 40°C, but is not limited thereto. The cultivation may be continued until the production of O-acetyl homoserine reaches the intended level, and specifically for 10 hours to 160 hours, but is not limited thereto.

The method of producing O-acetyl homoserine of the present invention may further include recovering O-acetyl homoserine from the cultured microorganism or a cultured product thereof. The recovery of the intended O-acetyl homoserine may be performed by a microorganism cultivation method according to the present invention, for example, an appropriate method known in the art such as a batch culture, a continuous culture, and a fed-batch culture.

The recovery may include a purification step.

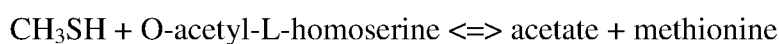
The thus-recovered O-acetyl homoserine can produce methionine by a two-step process (Korean Patent No. 10-0905381).

The two-step process includes a process of producing L-methionine and an organic acid by an enzyme reaction using an enzyme having the O-acetyl homoserine sulfhydrylase activity or a microorganism possessing the enzyme, while using the O-acetyl homoserine, which was produced by the L-methionine precursor-producing microorganism, and methyl mercaptan as substrates.

More specifically, the present invention provides a method for producing L-methionine by an enzyme reaction of O-acetyl homoserine sulfhydrylase, etc., using O-acetyl homoserine, which was accumulated by the above method, as a substrate.

In the second step, when O-acetyl homoserine is used as an L-methionine precursor, O-acetyl homoserine sulfhydrylase derived from a microorganism, specifically belonging to *Leptospira* sp., *Chromobacterium* sp., and *Hyphomonas* sp., and more specifically belonging to *Leptospira meyeri*, *Pseudomonas aurogenosa*, *Hyphomonas Neptunium*, and *Chromobacterium Violaceum* may be used.

The reaction is the same as shown below.



The additional process for producing methionine is disclosed in Korean Patent No. 10-0905381, and the entire specification of the patent may be included as a reference in the present invention.

MODE FOR INVENTION

Hereinafter, the present invention will be described in more detail with reference to the following Examples. However, these Examples are for illustrative purposes only, and the invention is not intended to be limited by these Examples.

Reference Example : Construction of O-acetyl homoserine-producing microorganism

<1-1> Deletion of metB gene derived from wild-type E. coli (International Publication No. WO 2008/013432)

An O-acetyl homoserine-producing microorganism was constructed using E. coli, a representative microorganism among Escherichia sp. To this end, wild-type E. coli K12 W3110 (ATCC27325) obtained from American Type Culture Collection (ATCC) was used. First, in order to block the synthesis pathway from O-succinyl-L-homoserine to cystathionine, cystathionine synthase-encoding metB gene (SEQ ID NO: 10) was deleted. Specifically, the cystathionine synthase-encoding metB gene was deleted via an FRT-one-step PCR deletion method (PNAS (2000) vol 97: P6640-6645).

Specifically, the metB deletion cassette was constructed via a PCR reaction based on the pKD3 vector (PNAS (2000) vol 97: P6640-6645) as a template using primers of SEQ ID NOS: 30 and 31 as follows: 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute. The resulting PCR product was electrophoresed on a 1.0% agarose gel, and a 1.2 kb DNA band obtained therefrom was purified. The recovered DNA fragment was electroporated into E. coli (K12) W3110, which was already transformed with the pKD46 vector (PNAS (2000) vol 97: P6640-6645). For electroporation, the W3110 strain transformed with the pKD46 was cultured in an LB medium containing 100 µg/L ampicillin and 5 mM arabinose (L-arabinose) at 30°C until OD₆₀₀=0.6, and used after washing twice with sterile distilled water and once with 10% glycerol. Electroporation was performed at 2500 V. The recovered strain was streaked on an LB plate containing 25 µg/L chloramphenicol, cultured at 37°C overnight, and the strain showing resistance was selected. The selected strain was subjected to a PCR reaction under the same conditions based on the strain as a template

using the same primers, and the deletion of metB gene was confirmed by observing the gene size of 1.2 kb on a 1.0% agarose gel. The thus-confirmed strain was cultured in an LB medium after transforming again with the pCP20 vector (PNAS (2000) vol 97: P6640-6645), and the final metB gene-deleted strain, where the gene size was reduced to 150 bp on a 1.0% agarose gel through PCR performed under the same conditions, was constructed and the removal of the chloramphenicol marker was confirmed. The thus-constructed strain was designated as “W3-B”.

<1-2> Deletion of thrB gene (International Publication No. WO 2008/013432)

In an effort to increase the amount of O-succinylhomoserine synthesis from homoserine, thrB gene, which is a homoserine kinase-encoding gene, was deleted. For deletion of the thrB gene from the W3-B strain constructed in Example 1, the FRT one step PCR deletion method used in the deletion of metB gene was used.

A thrB deletion cassette was constructed via PCR based on the pKD4 vector (PNAS (2000) vol 97: P6640-6645) as a template using primers of SEQ ID NOS: 32 and 33 as follows: 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute.

The resulting PCR product was electrophoresed on a 1.0% agarose gel, and a 1.6 kb DNA band obtained therefrom was purified. The recovered DNA fragment was electroporated into W3-B strain, which was already transformed with the pKD46 vector. The recovered strain was streaked on an LB plate containing 50 µg/L kanamycin, cultured at 37°C overnight, and the strain showing resistance was selected.

The selected strain was subjected to a PCR reaction under the same conditions directly based on the strain as a template using the same primers of SEQ ID NOS: 32 and 33, and confirmed the deletion of thrB gene by selecting the strain having the gene size of 1.6 kb on a 1.0% agarose gel. The thus-confirmed strain was cultured in an LB medium after transforming again with the pCP20 vector, and the final thrB gene-deleted strain, where the gene size was reduced to 150 bp on a 1.0% agarose gel through PCR performed under the same conditions, was constructed and the removal of the kanamycin marker was confirmed. The thus-constructed strain was designated as “W3-BT”.

<1-3> A variant metA with homoserine acetyltransferase activity (International Publication No. WO 2012/087039)

In order to strengthen the homoserine acetyltransferase activity in the strain obtained in

Reference Example <1-2>, it was intended to introduce mutant type metA gene (SEQ ID NOS: 24 and 26) encoding homoserine acetyltransferase.

First, in order to construct the variant of metA gene with a strengthened activity, a PCR reaction was performed based on the chromosome of a wild-type strain W3110 as a template using the primers of SEQ ID NOS: 34 and 35, and the metA gene encoding homoserine O-succinyltransferase was amplified.

The primers used in the PCR reaction were prepared based on the polynucleotide sequence of the E. coli chromosome, NC_000913, registered in the NIH Gene Bank, and the primers of SEQ ID NOS: 34 and 35 have the EcoRV and HindIII restriction sites, respectively. The thus-obtained PCR product and the pCL1920 plasmid including Pcj1 were respectively treated with EcoRV and HindIII, and the PCR product was cloned into the pCL1920 plasmid. E. coli DH5 α was transformed using the cloned plasmid, and the transformed E. coli DH5 α was selected on LB plates containing 50 μ g/mL spectinomycin, and the plasmid was obtained therefrom. The thus-obtained plasmid was designated as “pCL_Pcj1_metA”.

Then, the 111th amino acid, glycine (Gly), of O-succinyltransferase was substituted with glutamic acid (Glu) (G111E) based on the above-constructed pCL_Pcj1_metA plasmid as a template using a site directed mutagenesis kit (Stratagene, USA). The thus-constructed plasmid including the variant of G111E metA gene was designated as “pCL_Pcj1_metA (EL)”.

Additionally, in order to substitute the 111th amino acid of O-succinyltransferase from glycine to glutamic acid, and the 112nd amino acid from leucine to histidine, primers of SEQ ID NOS: 38 and 39 were used. The plasmid including metA gene, in which the 111th amino acid was substituted from glycine to glutamic acid, and the 112nd amino acid was substituted from leucine to histidine was designated as “pCL_Pcj1_metA (EH)”.

Then, a replacement cassette, for the replacement with metA (EH) into a strain, was constructed via PCR using pKD3 vector as a template along with primers of SEQ ID NOS: 40 and 41 as follows: 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 2 minutes. The respective PCR product was obtained using pCL-Pcj1-metA (EH) as a template for the metA (EH) part of the replacement cassette along with primers of SEQ ID NOS: 42 and 43, and primers of SEQ ID NOS: 42 and 45 for the wild-type metA part. metA (EH) replacement cassettes including the chloramphenicol marker part were constructed using the three different PCR products along with the primers of SEQ ID NOS: 42 and 45, and electroporated into W3-BT strain, which was already transformed with the pKD46 vector, constructed in Reference Example <1-2>. The thus-confirmed strain was cultured in an LB medium after transforming again with the pCP20 vector, and the strain, where the

chloramphenicol marker was removed, and metA gene was substituted with metA (EH) was designated as “W3-BTA”.

<1-4> Construction of a strain with 2 copies of ppc, aspC, and asd genes (European Patent Application Publication No. EP 2290051)

In order to increase the O-acetyl homoserine-producing capability of W3-BTA strain constructed in Reference Example <1-3>, the biosynthetic pathway was enhanced by citing the prior filed patent EP 2290051. In the same manner as in the above EP patent, a strain having 2 amplified copies each of the genes, i.e., the ppc gene encoding phosphoenolpyruvate carboxylase using primers of SEQ ID NOS: 46, 47, 48, and 49; the aspC gene encoding aspartate aminotransferase using primers of SEQ ID NOS: 50 and 51; and the asd gene encoding aspartate semialdehyde dehydrogenase using primers of SEQ ID NOS: 52, 53, 54, and 55, was constructed. In particular, the above strain with an enhanced biosynthetic pathway while producing O-acetyl homoserine was designated as “W3-BTA2PCD” (also called “WCJM”).

<1-5> Flask-culture Experiments

The amount of O-acetyl homoserine production by the strain constructed in Reference Examples <1-3> and <1-4> was tested by an Erlenmeyer flask-culture.

Specifically, W3110, W3-BTA, and WCJM strains were inoculated into LB media, and cultured at 33°C overnight. Then, a single colony thereof was inoculated into 3 mL of LB medium, cultured at 33°C for 5 hours, diluted 200-fold in a 250 mL Erlenmeyer flask containing 25 mL of an O-acetyl homoserine-producing medium, cultured at 33°C at a rate of 200 rpm for 30 hours, and the amount of O-acetyl homoserine production was examined via HPLC analysis. The media compositions used are shown in Table 1 below, and the amount of O-acetyl homoserine production examined is shown in Table 2 below.

[Table 1] Composition of O-acetyl homoserine-producing flask-culture

Composition	Conc. (per liter)
glucose	40 g
(NH ₄) ₂ SO ₄	17 g
KH ₂ PO ₄	1.0 g
MgSO ₄ · 7H ₂ O	0.5 g
FeSO ₄ · 7H ₂ O	5 mg

MnSO ₄ · 8H ₂ O	5 mg
ZnSO ₄	5 mg
CaCO ₃	30 g
yeast extract	2 g
methionine	0.15 g
threonine	0.15 g

[Table 2]

	OD (562nm)	Glucose consumption (g/L)	O-Acetyl homoserine (g/L)
W3110	14.2	40	0
W3-BTA	8.4	36	0.9
WCJM	9.6	35	1.2

The result revealed that the wild-type W3110 did not produce O-acetyl homoserine at all, whereas the W3-BTA strain produced 0.9 g/L of O-acetyl homoserine and the WCJM strain, which was strengthened with the biosynthesis pathway, produced 1.2 g/L of O-acetyl homoserine.

Example 1: Deletion of citrate synthase activity

<1-1> Construction of a citrate synthase gene-deleted microorganism in an O-acetyl homoserine-producing microorganism

Citrate synthase (GltA) is the enzyme in the first step of the TCA cycle, and starts with the reaction between oxaloacetate and acetyl-CoA. The growth inhibition by decrease in the TCA cycle is well known (Meded Rijksuniv Gent Fak Landbouwkde Toegep Biol Wet. 2001; 66(3a): 333-6). However, in order to increase the amount of acetyl-CoA used as a substrate for O-acetyl homoserine, W3-BTA and WCJM strains where citrate synthase activity is deleted were to be produced.

Specifically, the citrate synthase gene in W3-BTA and WCJM strains was deleted via PCR based on pKD4 vector as a template using primers of SEQ ID NOS: 56 and 57 as follows: 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 2 minutes. The resulting PCR product was electrophoresed on a 1.0% agarose gel,

and the size of the gene was confirmed to be 1.6 kb, and its DNA was purified. The recovered DNA fragments were electroporated into W3-BTA and WCJM strains, which were already transformed with the pKD46 vector. For electroporation, W3-BTA and WCJM strains, transformed with the pKD46 vector, were cultured in an LB medium containing 100 µg/L ampicillin and 5 mM arabinose at 30°C until OD₆₀₀=0.6, and washed twice with distilled water and once with 10% glycerol to be used. Electroporation was performed at 2500 V. The thus-recovered strains were streaked on LB plates containing 50 µg/L kanamycin, cultured at 37°C, and the strains showing resistance were selected.

The selected strains were subjected to PCR using the primers of SEQ ID NOS: 58 and 59 under the same conditions, electrophoresed on a 1.0% agarose gel, and the size of the gene was observed to be 2.5 kb, thereby confirming that a deletion cassette was inserted into the citrate synthase gene portion on the chromosome. The thus-confirmed strains were transformed again with the pCP20 vector, cultured in LB media, and strains having a deletion of the citrate synthase gene, whose size was reduced to 1.1 kb on a 1.0% agarose gel, were constructed by PCR, and it was confirmed that kanamycin markers were removed. The thus-constructed strains were designated as “W3-BTA-AD” and “WCJM-AD”, respectively.

<1-2> Evaluation of a citrate synthase gene-deleted microorganism in an O-acetyl homoserine-producing microorganism

W3-BTA-AD and WCJM-AD strains can grow in an LB medium, but due to the deletion of the citrate synthase gene, they could not grow in an O-acetyl homoserine-containing medium. In order to test the amount of O-acetyl homoserine production, an Erlenmeyer flask-culture was performed under the condition (Table 3 – a composition adding glutamate in the medium) of adding 3 g/L of glutamate into the existing composition of the culture medium.

Specifically, W3-BTA-AD and WCJM-AD strains were inoculated into LB media and cultured at 33°C overnight. Then, a single colony thereof was inoculated into 3 mL of LB medium, cultured at 33°C for 5 hours, diluted 200-fold in a 250 mL Erlenmeyer flask containing 25 mL of an O-acetyl homoserine-producing medium (with glutamate added), cultured at 33°C at a rate of 200 rpm for 30 hours, and the amount of O-acetyl homoserine production was examined via HPLC analysis. The amount of O-acetyl homoserine production examined is shown in Table 4 below.

[Table 3] Composition of a medium with glutamate added to a basal medium

Composition	Conc. (per liter)
glucose	40 g
(NH ₄) ₂ SO ₄	17 g
KH ₂ PO ₄	1.0 g
MgSO ₄ · 7H ₂ O	0.5 g
FeSO ₄ · 7H ₂ O	5 mg
MnSO ₄ · 8H ₂ O	5 mg
ZnSO ₄	5 mg
CaCO ₃	30 g
yeast extract	2 g
methionine	0.15 g
threonine	0.15 g
glutamate	3 g

[Table 4] Production of O-acetyl homoserine via flask-culture

	OD (562 nm)	Glucose consumption (g/L)	O-acetyl homoserine (g/L)	Glutamate (g/L)
W3-BTA	9.9	38	0.9	3.2
W3-BTA-AD	6.1	34	1.4	2.3
WCJM	9.2	37	1.3	3.5
WCJM-AD	5.6	33	2.1	1.7

The result of the O-acetyl homoserine production via flask-culture revealed that the W3-BTA strain produced 0.9 g/L of O-acetyl homoserine, and W3-BTA-AD produced 1.4 g/L of O-acetyl homoserine, which is a 55.6% increase, although it showed a decrease in its glucose consumption. The WCJM strain produced 1.3 g/L of O-acetyl homoserine while the WCJM-AD strain produced 2.1 g/L of O-acetyl homoserine, thus confirming that the O-acetyl homoserine production capability was improved by 61.5% due to the deletion of the citrate synthase gene.

Example 2: Attenuation of the activity of citrate synthase protein

<2-1> Kinds of citrate synthase gene modifications

The WCJM-AD strain constructed in Example <1-1> showed a low culture rate, and three different types of variants, which showed an attenuated activity and a reduced binding ability to acetyl-CoA according to various modificationss of citrate synthase known in numerous references (The Journal of Biological Chemistry, 2003, 278, 35435-35443), were selected. The information on the three different types of variants is shown in Table 5, which shows modified genes in which the 145th amino acid, tyrosine (Y), was substituted with alanine (A), and the 167th amino acid, lysine(K), was substituted with alanine (A), and the 204th amino acid, threonine (T), was substituted with alanine (A).

[Table 5]

Evaluation on citrate synthase (gltA) variants

	KM VALUE [mM]	
	Acetyl-CoA	OAA
WT	0.12	0.026
Y145A	0.23	0.051
K167A	0.22	0.037
T204A	0.21	0.004

<2-2> Construction of a microorganism with attenuated citrate synthase protein activity in an O-acetyl homoserine-producing microorganism

The present inventors intended to increase the production capability by introducing the variants, in which the activity of citrate synthase protein was attenuated as explained in Example <2-1>, into the O-acetyl homoserine-producing microorganism.

In order to introduce the three different types of citrate synthase gene variants into the WCJM-AD strain, a modification replacement cassette was designed as shown in FIG. 1. Each variant was synthesized by substituting a primer with a nucleotide, and each cassette was constructed through 3 PCR products. For the citrate synthase gene portion, the W3110 strain was used as a template, and the modification on the 145th amino acid produced PCR reactions were performed using the primers of SEQ ID NOS: 60 and 63 and SEQ ID NOS: 62 and 61, respectively, and obtained PCR products with a size of 514 bp and 1,112 bp.

Likewise, the modification on the 167th amino acid produced PCR products with a size of 580 bp and 1,046 bp using the primers of SEQ ID NOS: 60 and 65, and the primers of SEQ ID

NOS: 64 and 61, and the modification on the 204th amino acid produced PCR products with a size of 688 bp and 936 bp using the primers of SEQ ID NOS: 60 and 67 and SEQ ID NOS: 66 and 61. For the common kanamycin portion, PCR reactions were performed based on the pKD4 vector as a template using the primers of SEQ ID NOS: 68 and 69. In particular, for the insertion into the position of the citrate synthase gene, the cassette was constructed so as to include the polynucleotide sequence downstream of the citrate synthase gene in the SEQ ID NO: 69, and a PCR product with a size of 1,571 bp was obtained via electrophoresis. A sewing PCR reaction was performed based on the kanamycin DNA fragment, which is the common part with each of the two DNA fragments collected according to the modifications, respectively, using the primers of SEQ ID NOS: 60 and 69, as follows: 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 4 minutes. Each of the final PCR products was confirmed on a 1.0% agarose gel, and the DNA fragments with a size of 3,115 bp for the three different kinds of cassettes of citrate synthase gene modifications. The collected DNA fragments were electroporated into the WCJM-AD strain, which was already transformed with the pKD46 vector. For electroporation, the W3110 strain transformed with the pKD46 was cultured in an LB medium containing 100 µg/L ampicillin and 5 mM arabinose at 30°C until OD₆₀₀=0.6, and used after washing twice with sterile distilled water and once with 10% glycerol. Electroporation was performed at 2500 V. The recovered strain was streaked on an LB plate containing 25 µg/L kanamycin, cultured at 37°C overnight, and the strain showing resistance was selected. The selected strain was subjected to a PCR reaction under the same conditions based on the strain as a template using the same primers of SEQ ID NOS: 58 and 59, and confirmed the deletion of metB gene by observing the gene size of 3.7 kb on a 1.0% agarose gel, thereby confirming that a modification cassette, in which the amino acid of the citrate synthase gene was substituted, was inserted. The thus-confirmed strain was cultured in an LB medium after transforming again with the pCP20 vector, and the three variant strains regarding the citrate synthase activity, where the gene size was reduced to 2.5 kb on a 1.0% agarose gel through PCR performed under the same conditions, were constructed and the removal of the kanamycin marker was confirmed. The thus-constructed strains were designated as “WCJM-A145”, “WCJM-A167”, and “WCJM-A204”, and the sequence information of the citrate synthase gene introduced with modifications are shown in SEQ ID NOS: 1 to 3 (amino acid sequences) and SEQ ID NOS: 5 to 7 (nucleotide sequences), respectively.

<2-3> Evaluation of microorganisms with attenuated citrate synthase activity in O-acetyl homoserine-producing microorganisms

An Erlenmeyer flask-culture was performed in order to examine the amount of O-acetyl homoserine production by three different strains of WCJM-A145, WCJM-A167, and WCJM-A204, in which the activity of the citrate synthase gene was attenuated. Four kinds of strains, i.e., WCJM-A145, WCJM-A167, and WCJM-A204 strains including the WCJM strain, were inoculated into LB media, and cultured at 33°C overnight. Then, a single colony thereof was inoculated into 3 mL of LB medium, cultured at 33°C for 5 hours, diluted 200-fold in a 250 mL Erlenmeyer flask containing 25 mL of an O-acetyl homoserine-producing medium, cultured at 33°C at a rate of 200 rpm for 30 hours, and the amount of O-acetyl homoserine production was examined via HPLC analysis. The results are shown in Table 6 below.

[Table 6] Production of O-acetyl homoserine via flask-culture

	OD (562 nm)	Glucose consumption (g/L)	O-acetyl homoserine (g/L)	Glutamate (g/L)
WCJM	8.9	35	1.3	1.3
WCJM-A145	7.4	35	2.0	0
WCJM-A167	6.3	29	1.9	0
WCJM-A204	9.1	40	1.1	1.8

The result of O-acetyl homoserine production via flask-culture revealed that the WCJM strain produced 1.3 g/L of O-acetyl homoserine, and the two strains, WCJM-A145 and WCJM-A167, produced 2.0 g/L and 1.9 g/L of O-acetyl homoserine, respectively, while the amount of their glucose consumption decreased along with the decrease in their absorbance (OD). Considering the specific decrease of glutamate from 1.3 g/L to 0 g/L, it was confirmed that the result is due to the decrease in TCA cycle flow caused by the attenuation in the citrate synthase activity. However, the WCJM-A204 strain showed an increase in glutamate while showing a decrease in the amount of O-acetyl homoserine production to 0.2 g/L, thus confirming the modification is one with a strengthened activity.

Example 3: Attenuation in expression of citrate synthase protein

<3-1> Construction of expression vector for citrate synthase gene antisense RNA (asRNA)

The present inventors made an effort to apply an antisense RNA (asRNA) technology in

order to attenuate the expression of citrate synthase protein. The antisense RNA technology is a method for reducing protein expression by neutralizing the binding between citrate synthase mRNA and ribosome, via overexpression of the complementary binding portion to the citrate synthase mRNA of the target gene. This method has the advantage in that it can regulate the level of inhibition by controlling the binding force with the mRNA of the citrate synthase gene, and this method is also useful for the construction of a recombinant microorganism because this method can effectively construct and reduce gene expression via antisense RNA controlling gene expression, not necessitating the conventional process of gene deletion.

The vector construction was performed referring to a reference (Methods Mol Biol. 2012; 815:307-19. doi: 10.1007/978-1-61779-424-7_23.), and for overexpression, the antisense RNA region of the synthase gene was to be introduced into the pBAD24 plasmid capable of induction. The pBAD24-citrate synthase asRNA vector map is shown in FIG. 2. The region where the antisense RNA of the citrate synthase gene was expressed has a size of 100 bp including the 52 bp region of the promoter region and the 48 bp region from the initiation codon of the citrate synthase, and a 38 bp paired termini (PT) structure, which reduces the instability of the antisense RNA (asRNA), is connected to both flanking regions. The antisense RNA region of the citrate synthase gene was obtained using the primers of SEQ ID NOS: 70 and 71, and the NcoI and HindIII restriction sites were included to be cloned into a vector.

The thus-obtained PCR product had a size of 194 bp, and the PCR product was cloned into the pBAD24 plasmid after treating them with EcoRV and HindIII, respectively. The thus-cloned plasmid was used to transform *E. coli* DH5 α , and the transformed *E. coli* DH5 α was selected from the LB plates containing 100 μ g/mL of ampicillin, and obtained the plasmid therefrom. The thus-obtained plasmid was designated as “pBAD24-gltA asRNA”.

<3-2> Introduction of an expression vector of antisense RNA of citrate synthase gene into an O-acetyl homoserine-producing microorganism and evaluation thereof

The pBAD24-gltA-asRNA, an expression vector of antisense RNA of citrate synthase gene, was transformed into the WCJM strain, which is an O-acetyl homoserine-producing microorganism. Here, the transformed strain was designated as “WCJM/A-asRNA”. In particular, it was attempted to control the expression amount of the citrate synthase protein by controlling the expression amount of the antisense RNA of the citrate synthase, and here, the expression amount of the antisense RNA can be controlled according to the concentration of arabinose.

As a result, it was confirmed that the amount of the O-acetyl homoserine production

increased when the activity of the citrate synthase was attenuated as in Example 2.

Additionally, an Erlenmeyer flask-culture was performed to examine whether the amount of O-acetyl homoserine production increases as the expression amount of the citrate synthase decreases.

Specifically, WCJM and WCJM/A-asRNA strains were inoculated into LB media, and cultured at 33°C overnight. Then, a single colony thereof was inoculated into 3 mL of LB medium, cultured at 33°C for 5 hours, diluted 200-fold in a 250 mL Erlenmeyer flask containing 25 mL of an O-acetyl homoserine-producing medium. In particular, in order to control the expression amount of antisense RNA of the citrate synthase, arabinose was added at concentrations of 0 mM, 2 mM, and 5 mM, and cultured at 33°C at a rate of 200 rpm for 15 hours and 30 hours. The amount of O-acetyl homoserine production was examined via HPLC analysis, and the results are shown in Tables 7 and 8 below.

[Table 7]

15 Hours	Arabinose	OD (562nm)	Glucose consumption (g/L)	O-acetyl homoserine (g/L)
WCJM	0 mM	4.2	9.7	0.5
WCJM	2 mM	4.5	8.9	0.6
WCJM	5 mM	4.7	8.9	0.5
WCJM/ A-asRNA	0 mM	4.5	10.1	0.6
WCJM/ A-asRNA	2 mM	4.2	8.8	0.6
WCJM/ A-asRNA	5 mM	3.4	6.9	0.5

[Table 8]

30 Hours	Arabinose	OD (562nm)	Glucose consumption (g/L)	O-acetyl homoserine (g/L)
WCJM	0 mM	8.9	32	1.4
WCJM	2 mM	9.1	34	1.3

WCJM	5 mM	8.9	33	1.3
WCJM/ A-asRNA	0 mM	9.2	33	1.3
WCJM/ A-asRNA	2 mM	8.8	32	1.6
WCJM/ A-asRNA	5 mM	7.1	29	1.7

As a result, it was confirmed that, when cultured for 15 hours, the WCJM/A-asRNA strain showed a decrease in OD by about 1 according to the concentration of arabinose, whereas the concentration of O-acetyl homoserine was similar. However, when cultured for 30 hours, the WCJM strain, which is a control strain, showed the same OD and O-acetyl homoserine concentration even when the concentration of arabinose increased, whereas the WCJM/A-asRNA strain, which is a strain introduced with the expression vector for citrate synthase RNA antisense, showed a marked difference as the concentration of arabinose increased. The OD was 9.2 when the arabinose concentration was 0 mM, whereas the OD was 7.1 at 5 mM of the arabinose concentration, a decrease of 5.1, and the amount of O-acetyl homoserine increased by 30.8% although the glucose consumption was small. From these results, it was confirmed that not only the attenuation in citrate synthase activity but also in the attenuation in protein expression exhibit the same results.

Example 4: Attenuation and inactivation of citrate synthase activity in a microorganism with high production yield of O-acetyl homoserine

<4-1> Construction of a microorganism with high O-acetyl homoserine production yield with inactivated citrate synthase activity and evaluation thereof

International Publication No. WO 2012/087039 discloses in detail a method for constructing an O-acetyl homoserine-producing microorganism from a threonine-producing microorganism derived from a wild-type W3110 strain, due to NTG mutation. In particular, the constructed strain producing O-acetyl homoserine with high yield was deposited at Korean Culture Center of Microorganisms (KCCM) under the Accession No of KCCM 11146P.

The KCCM11146P strain can consume 40 g/L of glucose during a flask-culture and produces about 15 g/L to 16 g/L of O-acetyl homoserine and is thus regarded as having high

O-acetyl homoserine production capability. Accordingly, in order to examine whether the strain produces a higher yield of O-acetyl homoserine when the citrate synthase activity is deleted, the same was applied to the KCCM11146P strain. The construction method was the same as in Example <1-1>, and by this method, the KCCM11146P strain, where the citrate synthase activity was deleted, was constructed and designated as “KCCM11146P-AD”.

The amount of O-acetyl homoserine production by the KCCM11146P strain, where the citrate synthase activity was deleted, was tested by an Erlenmeyer flask-culture. The KCCM11146P or KCCM11146P-AD strain was inoculated into an LB medium and cultured at 33°C overnight. Then, a single colony thereof was inoculated into 3 mL of LB medium, cultured at 33°C for 5 hours, diluted 200-fold in a 250 mL Erlenmeyer flask containing 25 mL of an O-acetyl homoserine-producing medium (with glutamate added), and cultured at 33°C at a rate of 200 rpm for 30 hours. The amount of O-acetyl homoserine production was examined via HPLC analysis, and the results are shown in Table 9 below.

[Table 9] Production of O-acetyl homoserine via flask-culture

	OD (562nm)	Glucose consumption (g/L)	O-acetyl homoserine (g/L)	Glutamate (g/L)
KCCM11146P	18.3	60	14.2	4.6
KCCM11146P-AD	14.6	60	16.7	1.8

The result of O-acetyl homoserine production via flask-culture revealed that the KCCM11146P strain produced 14.2 g/L of O-acetyl homoserine, and the KCCM11146P-AD strain produced 16.7 g/L of O-acetyl homoserine, a 17.6% increase, although it showed a decrease in absorbance (OD).

<4-2> Construction of a microorganism with high O-acetyl homoserine production yield with attenuated citrate synthase activity and evaluation thereof

In order to examine whether the KCCM11146P strain, which is a strain with high production yield of O-acetyl homoserine, produces a higher yield of O-acetyl homoserine even when the citrate synthase activity is attenuated, the modification on the 145th amino acid (from tyrosine (Y) to alanine (A)) and the modification on the 167th amino acid (from lysine (K) to alanine (A)), which showed the highest O-acetyl homoserine-producing capabilities among the three variant types attenuating the protein activities explained in Example <2-1>, were applied to

the KCCM11146P strain.

The construction method was the same as in Example <2-2>, and by the method, two KCCM11146P strains, where the citrate synthase activity was attenuated, were constructed and designated as “KCCM11146P-A145” and “KCCM11146P-A167”, respectively.

The amount of O-acetyl homoserine production by the two strains of KCCM11146P-A145 and KCCM11146P-A167, where the citrate synthase activity was attenuated, was tested by an Erlenmeyer flask-culture. The three strains, i.e., KCCM11146P-A145 and KCCM11146P-A167 strains and the KCCM11146P strain, were inoculated into LB media, and cultured at 33°C overnight. Then, a single colony thereof was inoculated into 3 mL of LB medium, cultured at 33°C for 5 hours, diluted 200-fold in a 250 mL Erlenmeyer flask containing 25 mL of an O-acetyl homoserine-producing medium, and cultured at 33°C at a rate of 200 rpm for 30 hours. The amount of O-acetyl homoserine production was examined via HPLC analysis, and the results are shown in Table 10 below.

[Table 10] Production of O-acetyl homoserine via flask-culture

	OD (562nm)	Glucose consumption (g/L)	O-acetyl homoserine (g/L)	Glutamate (g/L)
KCCM11146P	16.3	60	15.0	1.6
KCCM11146P-A145	14.6	60	17.5	0
KCCM11146P-A167	14.2	60	17.3	0

The result of O-acetyl homoserine production via flask-culture revealed that the KCCM11146P strain produced 15.0 g/L of O-acetyl homoserine, and the two strains of KCCM11146P-A145 and KCCM11146P-A167 showed similar results as in Example <2-3>. The two strains respectively produced 17.5 g/L and 17.3 g/L of O-acetyl homoserine, an increase of about 16.7%, although they both showed a decrease in absorbance (OD).

The strain with high production yield of O-acetyl homoserine also showed a decrease in glutamate from 1.6 g/L to 0 g/L, according to the decrease in TCA cycle flow caused by the attenuation in the citrate synthase activity.

These results demonstrate that citrate synthase activity enables production of O-acetyl

homoserine by applying the attenuated modification. Additionally, they also indicate that when a conversion reaction is conducted based on the *O*-acetyl homoserine, which was produced according to the International Publication No. WO2008/013432, as a template, and using a conversion enzyme, which additionally has the activities of cystathionine gamma synthase, *O*-succinylhomoserine sulfhydrylase, and *O*-acetyl homoserine sulfhydrylase, it is possible to simultaneously synthesize L-methionine and acetate.

The present inventors confirmed that the *KCCM11146P* strain, the variant on the 167th amino acid of the citrate synthase, has an improved production of *O*-acetyl homoserine, designated the *KCCM11146P-A167* strain as “CA05-4007”, and deposited it at the Korean Culture Center of Microorganisms (KCCM), an international depositary authority under the Budapest Treaty, on Nov. 22, 2013 (Accession No: *KCCM 11483P*).

From the foregoing, a skilled person in the art to which the present invention pertains will be able to understand that the present invention may be embodied in other specific forms without modifying the technical concepts or essential characteristics of the present invention. In this regard, the exemplary embodiments disclosed herein are only for illustrative purposes and should not be construed as limiting the scope of the present invention. On the contrary, the present invention is intended to cover not only the exemplary embodiments but also various alternatives, modifications, equivalents and other embodiments that may be included within the spirit and scope of the present invention as defined by the appended claims.

Throughout the specification and claims, unless the context requires otherwise, the word “comprise” or variations such as “comprises” or “comprising”, will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.



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

INTERNATIONAL FORM

To. CJ CheilJedang Corporation
CJ CHEILJEDANG CENTER,
330, DONGHO-RO,
JUNG-GU, SEOUL 100-400,
REPUBLIC OF KOREA

RECEIPT IN THE CASE OF AN ORIGINAL
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR : <i>Escherichia coli</i> CA05-4007	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM11483P
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on November. 22. 2013. (date of the original deposit) ¹	
IV. INTERNATIONAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : Yurim B/D 45, Hongjenae-2ga-gil Seodaemun-gu SEOUL 120-861 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s) : Date: November. 22. 2013.

¹ Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired; where a deposit made outside the Budapest Treaty after the acquisition of the status of international depositary authority is converted into a deposit under the Budapest Treaty, such date is the date on which the microorganism was received by the international depositary authority.

Form BP/4

Sole page

한국미생물보존센터

120-027 서울시 서대문구 홍제동 361-22 유원빌딩 Tel: 02-381-0260, 386-3950 Fax: 02-381-0855 E-mail: kccm@kccm.or.kr

KOREAN CULTURE CENTER OF MICROORGANISMS

Yurim Bldg. 361-22 Hongje-dong, Seodaemun-gu, Seoul, 120-0131, Korea Tel: 82-2-381-0260, 386-3950 Fax: 82-2-381-0855 E-mail: kccm@kccm.or.kr

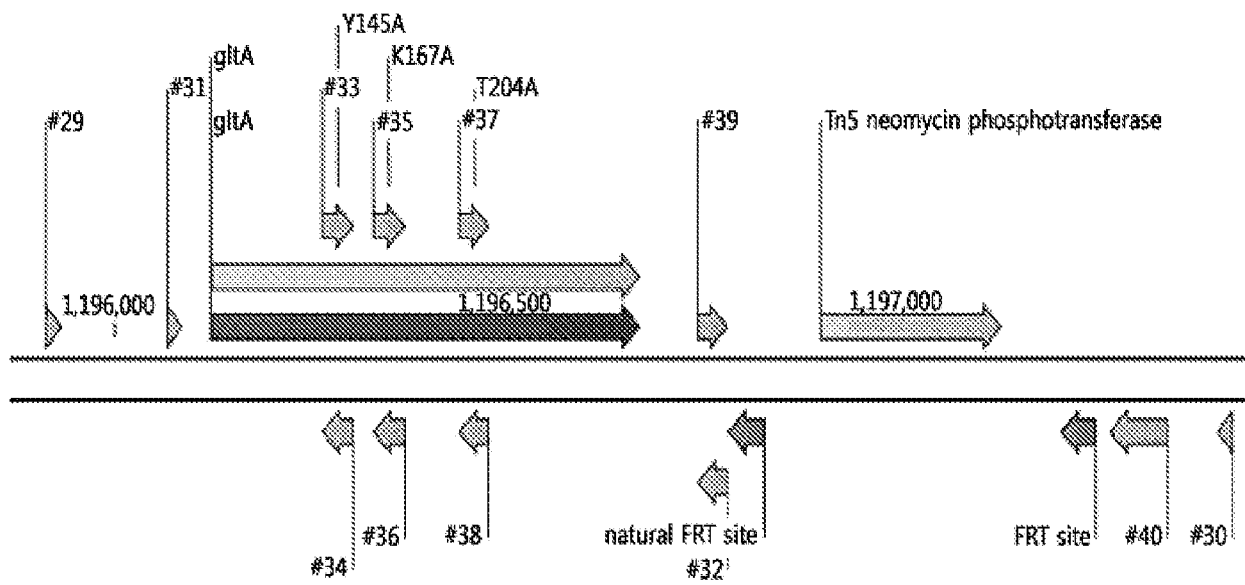
CLAIMS

1. An isolated microorganism of *Escherichia* sp. producing *O*-acetyl homoserine, wherein the endogenous activity of citrate synthase is attenuated or inactivated.
2. The isolated microorganism of *Escherichia* sp. according to claim 1, wherein the microorganism with the attenuated endogenous activity of citrate synthase has an amino acid sequence represented by SEQ ID NO: 1 or SEQ ID NO: 2.
3. The isolated microorganism of *Escherichia* sp. according to claim 1, wherein the activity of cystathionine gamma synthase, homoserine kinase, or both is further attenuated or inactivated compared to their endogenous activities.
4. The isolated microorganism of *Escherichia* sp. according to claim 1, wherein the activity of homoserine *O*-acetyltransferase is further introduced or enhanced, or the endogenous homoserine *O*-succinyltransferase is further modified to have the activity of homoserine *O*-acetyltransferase.
5. The isolated microorganism of *Escherichia* sp. according to claim 1, wherein the activity of at least one protein selected from the group consisting of phosphoenolpyruvate carboxylase, aspartate aminotransferase, and aspartate semialdehyde dehydrogenase is further introduced or enhanced.
6. The isolated microorganism of *Escherichia* sp. according to claim 1, wherein the microorganism is *Escherichia coli*.
7. A method for producing *O*-acetyl homoserine, comprising:
 - (a) culturing the isolated microorganism of any of claims 1 to 6; and
 - (b) recovering *O*-acetyl homoserine produced during the cultivation of the microorganism.
8. A method for producing methionine, comprising:
 - (a) culturing the isolated microorganism of any one of claims 1 to 6;
 - (b) recovering *O*-acetyl homoserine produced during the cultivation of the microorganism, and

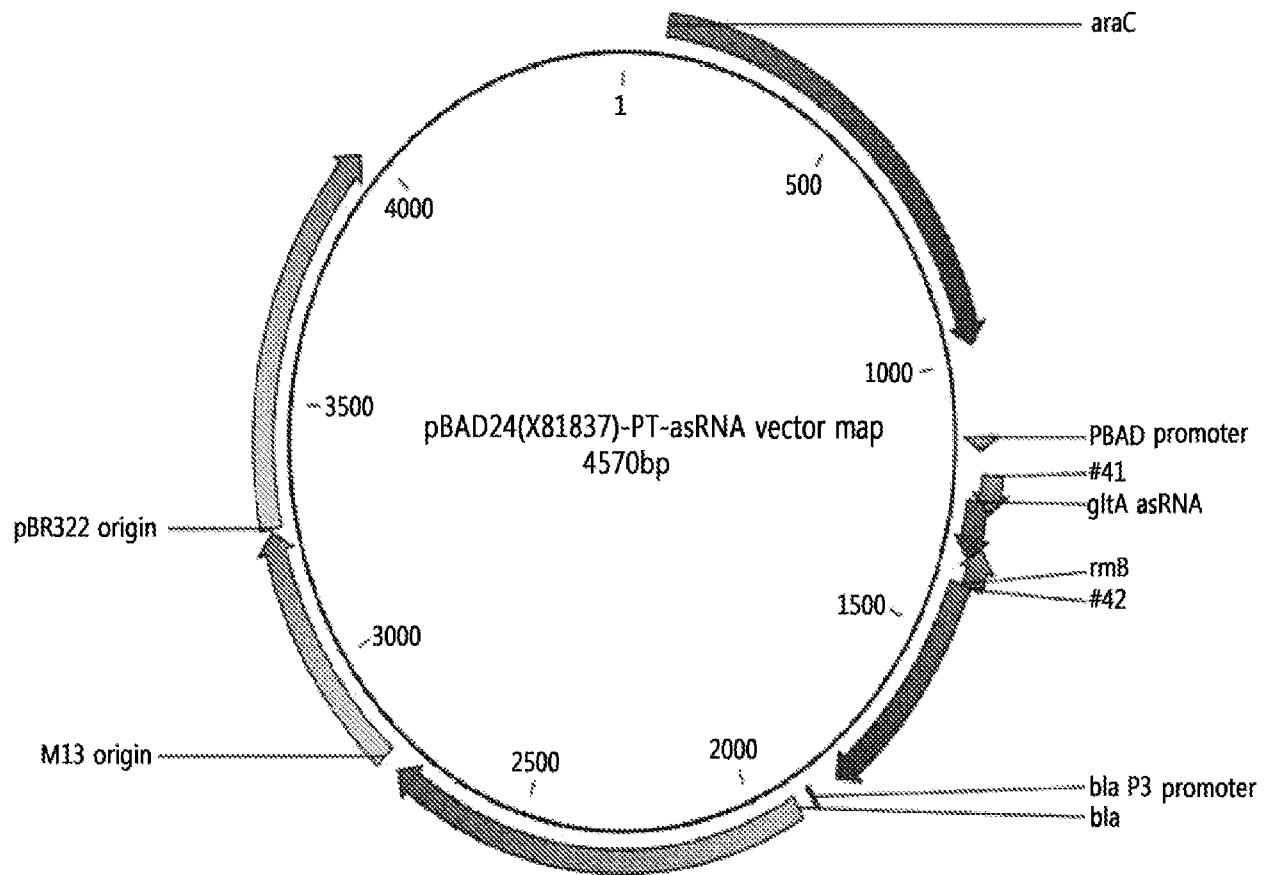
(c) reacting the recovered O-acetyl homoserine and methyl mercaptan by an enzyme reaction using an enzyme having an O-acetyl homoserine sulfhydrylase activity or a microorganism possessing the enzyme.

DRAWINGS

[FIG. 1]



[FIG. 2]



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 of
 producing O-acetyl homoserine using the same
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Ile	Ala	Ser	Leu	Trp	Gly	Pro	Ala	His	Gly	Gly	Ala	Asn	Glu	Ala	Ala	
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		275					280					285				
Val	Arg	Arg	Ala	Lys	Asp	Lys	Asn	Asp	Ser	Phe	Arg	Leu	Met	Gly	Phe	
	290					295					300					

Gly	His	Arg	Val	Tyr	Lys	Asn	Tyr	Asp	Pro	Arg	Ala	Thr	Val	Met	Arg	305		310		315		320
Glu	Thr	Cys	His	Glu	Val	Leu	Lys	Glu	Leu	Gly	Thr	Lys	Asp	Asp	Leu		325		330		335	
Leu	Glu	Val	Ala	Met	Glu	Leu	Glu	Asn	Ile	Ala	Leu	Asn	Asp	Pro	Tyr		340		345		350	
Phe	Ile	Glu	Lys	Lys	Leu	Tyr	Pro	Asn	Val	Asp	Phe	Tyr	Ser	Gly	Ile		355		360		365	
Ile	Leu	Lys	Ala	Met	Gly	Ile	Pro	Ser	Ser	Met	Phe	Thr	Val	Ile	Phe	370		375		380		
Ala	Met	Ala	Arg	Thr	Val	Gly	Trp	Ile	Ala	His	Trp	Ser	Glu	Met	His	385		390		395		400
Ser	Asp	Gly	Met	Lys	Ile	Ala	Arg	Pro	Arg	Gln	Leu	Tyr	Thr	Gly	Tyr		405		410		415	
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 120

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 180

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 240

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300

tatgacgaat ttaaaactac ggtgacccgt cataccatga tccacgagca gattacccgt
360

ctgttccatg ctttccgtcg cgactcgcat ccaatggcag tcatgtgtgg tattaccggc
420

gcgctggcgg cgttcgcgca cgactcgctg gatgttaaca atcctcgtca ccgtgaaatt
480

gccgcgttcc gcctgctgtc gaaaatgccg accatggccg cgatgtgtta caagtattcc
540

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600

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660

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720

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840

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960

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1080

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1140

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1200

agtgacggta tgaagattgc ccgtccgcgt cagctgtata caggatatga aaaacgcgac
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120

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180

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240

tctaactacc tggaagtttg ttacatcctg ctgaatgggtg aaaaaccgac tcaggaacag
300

tatgacgaat ttaaaaactac ggtgaccctg cataccatga tccacgagca gattaccctg
360

ctgttccatg ctttccgtcg cgactcgcat ccaatggcag tcatgtgtgg tattaccggc
420

gcgctggcgg cgttctatca cgactcgctg gatgttaaca atcctcgtca ccgtgaaatt
480

gccgcgttcc gcctgctgtc ggcgatgccg accatggccg cgatgtgtta caagtattcc
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attggtcagc catttgttta cccgcgcaac gatctctcct acgccggtaa cttcctgaat
 600

atgatgttct ccacgccgtg cgaaccgtat gaagttaatc cgattctgga acgtgctatg
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gaccgtattc tgatcctgca cgctgaccat gaacagaacg cctctacctc caccgtgcgt
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 780

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 960

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 1020

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 1080

aacgtcgatt tctactctgg tatcatcctg aaagcgatgg gtattccgtc ttccatgttc
 1140

accgtcattt tcgcaatggc acgtaccgtt ggctggatcg cccactggag cgaaatgcac
 1200

agtgacggta tgaagattgc ccgtccgcgt cagctgtata caggatatga aaaacgcgac
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120

ttcacctttg acccaggctt cacttcaacc gcatcctgcg aatctaaaat tactttttatt
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gatggtgatg aaggtatttt gctgcaccgc ggtttcccga tcgatcagct ggcgaccgat
240

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gccgcgttcc gcctgctgtc gaaaatgccg accatggccg cgatgtgtta caagtattcc
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960

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1020

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aacgtcgatt tctactctgg tatcatcctg aaagcgatgg gtattccgtc ttccatgttc
1140

accgtcattt tcgcaatggc acgtaccggt ggctggatcg cccactggag cgaaatgcac
1200

agtgacggta tgaagattgc ccgtccgcgt cagctgtata caggatatga aaaacgcgac
1260

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1284

<210> 8
<211> 1284
<212> DNA
<213> Escherichia coli

<220>
<221> gene
<222> (1) .. (1284)
<223> GltA

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ttcacctttg acccaggctt cacttcaacc gcatcctgcg aatctaaaat tactttttatt
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gatggatgatg aaggatatttt gctgcaccgc gggtttcccga tcgatcagct ggcgaccgat
240

tctaactacc tggaagtttg ttacatcctg ctgaatgggtg aaaaaccgac tcaggaacag
300

tatgacgaat ttaaaaactac ggtgaccctg cataccatga tccacgagca gattaccctg
360

ctgttccatg ctttccgtcg cgactcgcat ccaatggcag tcatgtgtgg tattaccggc
420

gcgctggcgg cgttctatca cgactcgctg gatgttaaca atcctcgta cctgaaatt
480

gccgcgttcc gcctgctgtc gaaaatgccg accatggccg cgatgtgtta caagtattcc
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720

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780

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840

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900

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960

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Lys	Arg	Gly	Cys	Tyr	Arg	Val	Leu	Phe	Val	Asp	Gln	Gly	Asp	Glu	Gln	
		115					120					125				
Ala	Leu	Arg	Ala	Ala	Leu	Ala	Glu	Lys	Pro	Lys	Leu	Val	Leu	Val	Glu	
	130					135					140					
Ser	Pro	Ser	Asn	Pro	Leu	Leu	Arg	Val	Val	Asp	Ile	Ala	Lys	Ile	Cys	
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His	Leu	Ala	Arg	Glu	Val	Gly	Ala	Val	Ser	Val	Val	Asp	Asn	Thr	Phe	
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Arg	Asn	Ala	Gln	Ala	Ile	Val	Lys	Tyr	Leu	Gln	Thr	Gln	Pro	Leu	Val	
			260					265					270			
Lys	Lys	Leu	Tyr	His	Pro	Ser	Leu	Pro	Glu	Asn	Gln	Gly	His	Glu	Ile	
		275					280					285				
Ala	Ala	Arg	Gln	Gln	Lys	Gly	Phe	Gly	Ala	Met	Leu	Ser	Phe	Glu	Leu	
	290					295					300					
Asp	Gly	Asp	Glu	Gln	Thr	Leu	Arg	Arg	Phe	Leu	Gly	Gly	Leu	Ser	Leu	
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Phe	Thr	Leu	Ala	Glu	Ser	Leu	Gly	Gly	Val	Glu	Ser	Leu	Ile	Ser	His	

	325		330		335										
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			340					345					350		
Ala	Gly	Ile	Ser	Glu	Thr	Leu	Leu	Arg	Ile	Ser	Thr	Gly	Ile	Glu	Asp
		355					360					365			
Gly	Glu	Asp	Leu	Ile	Ala	Asp	Leu	Glu	Asn	Gly	Phe	Arg	Ala	Ala	Asn
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 <222> (1) .. (1161)
 <223> metB

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 120
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 420

gtactggtag aaagcccaag taatccattg ttacgcgtcg tggatattgc gaaaatctgc
 480

catctggcaa gggaagtcgg ggcggtgagc gtggtggata acaccttctt aagcccggca
 540

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 600

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 660

gaactggcct ggtgggcaaa caatatggc gtgacgggcg gcgcgtttga cagctatctg
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ctgctacgtg ggttgcgaaac gctggtgccg cgtatggagc tggcgcagcg caacgcgcag
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cgtatctcca ccggtattga agatggcgaa gatttaattg ccgacctgga aaatggcttc
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<212> PRT
<213> Escherichia coli

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<222> (1)..(310)
<223> thrB

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

Val	Tyr	Gln	Cys	Trp	Glu	Arg	Phe	Cys	Gln	Glu	Leu	Gly	Lys	Gln	Ile
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Pro	Val	Ala	Met	Thr	Leu	Glu	Lys	Asn	Met	Pro	Ile	Gly	Ser	Gly	Leu
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Gly	Ser	Ser	Ala	Cys	Ser	Val	Val	Ala	Ala	Leu	Met	Ala	Met	Asn	Glu
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His	Cys	Gly	Lys	Pro	Leu	Asn	Asp	Thr	Arg	Leu	Leu	Ala	Leu	Met	Gly
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Glu	Leu	Glu	Gly	Arg	Ile	Ser	Gly	Ser	Ile	His	Tyr	Asp	Asn	Val	Ala
	130					135					140				

Pro	Cys	Phe	Leu	Gly	Gly	Met	Gln	Leu	Met	Ile	Glu	Glu	Asn	Asp	Ile
145					150					155					160

Ile	Ser	Gln	Gln	Val	Pro	Gly	Phe	Asp	Glu	Trp	Leu	Trp	Val	Leu	Ala
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Tyr	Pro	Gly	Ile	Lys	Val	Ser	Thr	Ala	Glu	Ala	Arg	Ala	Ile	Leu	Pro
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195 200 205

Gly Phe Ile His Ala Cys Tyr Ser Arg Gln Pro Glu Leu Ala Ala Lys
210 215 220

Leu Met Lys Asp Val Ile Ala Glu Pro Tyr Arg Glu Arg Leu Leu Pro
225 230 235 240

Gly Phe Arg Gln Ala Arg Gln Ala Val Ala Glu Ile Gly Ala Val Ala
245 250 255

Ser Gly Ile Ser Gly Ser Gly Pro Thr Leu Phe Ala Leu Cys Asp Lys
260 265 270

Pro Glu Thr Ala Gln Arg Val Ala Asp Trp Leu Gly Lys Asn Tyr Leu
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<223> thrB

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120

gcagagacat tcagtctcaa caacctcgga cgctttgccg ataagctgcc gtcagaacca
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240

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840

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<210> 13

<211> 379

<212> PRT

<213> Leptospira meyeri

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 <221> PEPTIDE
 <222> (1) .. (379)
 <223> homoserine O-acetyltransferase

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Glu	Thr	Ile	Thr	Pro	Leu	Glu	Ile	Ala	Tyr	Glu	Thr	Tyr	Gly	Thr	Leu
		35					40					45			
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Trp	Asp	Tyr	Tyr	Ile	Gly	Pro	Gly	Lys	Ser	Phe	Asp	Thr	Asn	Arg	Tyr
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Phe	Ile	Ile	Ser	Ser	Asn	Val	Ile	Gly	Gly	Cys	Lys	Gly	Ser	Ser	Gly
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		115					120					125			
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Lys	Asn	Cys	Ile	Val	Met	Ala	Ser	Ser	Ser	Glu	His	Ser	Ala	Gln	Gln
			180					185					190		
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Trp	Asn	Gln	Gly	Leu	Tyr	Thr	Gln	Glu	Asn	Arg	Pro	Ser	Lys	Gly	Leu	
210					215					220						
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225					230					235					240	
Met	Arg	Glu	Lys	Phe	Gly	Arg	Lys	Pro	Pro	Lys	Gly	Asn	Ile	Gln	Ser	
245					250					255						
Thr	Asp	Phe	Ala	Val	Gly	Ser	Tyr	Leu	Ile	Tyr	Gln	Gly	Glu	Ser	Phe	
260					265					270						
Val	Asp	Arg	Phe	Asp	Ala	Asn	Ser	Tyr	Ile	Tyr	Val	Thr	Lys	Ala	Leu	
275					280					285						
Asp	His	Phe	Ser	Leu	Gly	Thr	Gly	Lys	Glu	Leu	Thr	Lys	Val	Leu	Ala	
290					295					300						
Lys	Val	Arg	Cys	Arg	Phe	Leu	Val	Val	Ala	Tyr	Thr	Ser	Asp	Trp	Leu	
305					310					315					320	
Tyr	Pro	Pro	Tyr	Gln	Ser	Glu	Glu	Ile	Val	Lys	Ser	Leu	Glu	Val	Asn	
325					330					335						
Ala	Val	Pro	Val	Ser	Phe	Val	Glu	Leu	Asn	Asn	Pro	Ala	Gly	Arg	His	
340					345					350						
Asp	Ser	Phe	Leu	Leu	Pro	Ser	Glu	Gln	Gln	Asp	Ser	Ile	Leu	Arg	Asp	
355					360					365						
Phe	Leu	Ser	Ser	Thr	Asp	Glu	Gly	Val	Phe	Leu						
370					375											

<210> 14
 <211> 379
 <212> PRT
 <213> Corynebacterium glutamicum

 <220>
 <221> PEPTIDE
 <222> (1) .. (379)
 <223> homoserine O-acetyltransferase

<400> 14

Met Pro Thr Leu Ala Pro Ser Gly Gln Leu Glu Ile Gln Ala Ile Gly
1 5 10 15

Asp Val Ser Thr Glu Ala Gly Ala Ile Ile Thr Asn Ala Glu Ile Ala
20 25 30

Tyr His Arg Trp Gly Glu Tyr Arg Val Asp Lys Glu Gly Arg Ser Asn
35 40 45

Val Val Leu Ile Glu His Ala Leu Thr Gly Asp Ser Asn Ala Ala Asp
50 55 60

Trp Trp Ala Asp Leu Leu Gly Pro Gly Lys Ala Ile Asn Thr Asp Ile
65 70 75 80

Tyr Cys Val Ile Cys Thr Asn Val Ile Gly Gly Cys Asn Gly Ser Thr
85 90 95

Gly Pro Gly Ser Met His Pro Asp Gly Asn Phe Trp Gly Asn Arg Phe
100 105 110

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

Asp Ala Leu Gly Ile Thr Thr Val Ala Ala Val Val Leu Leu Gly Gly
130 135 140

Ser Met Gly Gly Ala Arg Thr Leu Glu Trp Ala Ala Met Tyr Pro Glu
145 150 155 160

Thr Val Gly Ala Ala Ala Val Leu Ala Val Ser Ala Arg Ala Ser Ala
165 170 175

Trp Gln Ile Gly Ile Gln Ser Ala Gln Ile Lys Ala Ile Glu Asn Asp
180 185 190

His His Trp His Glu Gly Asn Tyr Tyr Glu Ser Gly Cys Asn Pro Ala
195 200 205

Thr Gly Leu Gly Ala Ala Arg Arg Ile Ala His Leu Thr Tyr Arg Gly
210 215 220

Glu	Leu	Glu	Ile	Asp	Glu	Arg	Phe	Gly	Thr	Lys	Ala	Gln	Lys	Asn	Glu	225	230	235	240
Asn	Pro	Leu	Gly	Pro	Tyr	Arg	Lys	Pro	Asp	Gln	Arg	Phe	Ala	Val	Glu	245	250	255	
Ser	Tyr	Leu	Asp	Tyr	Gln	Ala	Asp	Lys	Leu	Val	Gln	Arg	Phe	Asp	Ala	260	265	270	
Gly	Ser	Tyr	Val	Leu	Leu	Thr	Asp	Ala	Leu	Asn	Arg	His	Asp	Ile	Gly	275	280	285	
Arg	Asp	Arg	Gly	Gly	Leu	Asn	Lys	Ala	Leu	Glu	Ser	Ile	Lys	Val	Pro	290	295	300	
Val	Leu	Val	Ala	Gly	Val	Asp	Thr	Asp	Ile	Leu	Tyr	Pro	Tyr	His	Gln	305	310	315	320
Gln	Glu	His	Leu	Ser	Arg	Asn	Leu	Gly	Asn	Leu	Leu	Ala	Met	Ala	Lys	325	330	335	
Ile	Val	Ser	Pro	Val	Gly	His	Asp	Ala	Phe	Leu	Thr	Glu	Ser	Arg	Gln	340	345	350	
Met	Asp	Arg	Ile	Val	Arg	Asn	Phe	Phe	Ser	Leu	Ile	Ser	Pro	Asp	Glu	355	360	365	
Asp	Asn	Pro	Ser	Thr	Tyr	Ile	Glu	Phe	Tyr	Ile						370	375		

<210> 15
 <211> 334
 <212> PRT
 <213> Deinococcus radiodurans

<220>
 <221> PEPTIDE
 <222> (1) .. (334)
 <223> homoserine O-acetyltransferase

<400>	15														
Met	Thr	Ala	Val	Leu	Ala	Gly	His	Ala	Ser	Ala	Leu	Leu	Leu	Thr	Glu
1				5					10					15	

Glu	Pro	Asp	Cys	Ser	Gly	Pro	Gln	Thr	Val	Val	Leu	Phe	Arg	Arg	Glu	
			20					25					30			
Pro	Leu	Leu	Leu	Asp	Cys	Gly	Arg	Ala	Leu	Ser	Asp	Val	Arg	Val	Ala	
		35					40					45				
Phe	His	Thr	Tyr	Gly	Thr	Pro	Arg	Ala	Asp	Ala	Thr	Leu	Val	Leu	His	
	50					55					60					
Ala	Leu	Thr	Gly	Asp	Ser	Ala	Val	His	Glu	Trp	Trp	Pro	Asp	Phe	Leu	
	65				70					75					80	
Gly	Ala	Gly	Arg	Pro	Leu	Asp	Pro	Ala	Asp	Asp	Tyr	Val	Val	Cys	Ala	
				85					90					95		
Asn	Val	Leu	Gly	Gly	Cys	Ala	Gly	Thr	Thr	Ser	Ala	Ala	Glu	Leu	Ala	
			100					105					110			
Ala	Thr	Cys	Ser	Gly	Pro	Val	Pro	Leu	Ser	Leu	Arg	Asp	Met	Ala	Arg	
		115					120					125				
Val	Gly	Arg	Ala	Leu	Leu	Asp	Ser	Leu	Gly	Val	Arg	Arg	Val	Arg	Val	
	130					135					140					
Ile	Gly	Ala	Ser	Met	Gly	Gly	Met	Leu	Ala	Tyr	Ala	Trp	Leu	Leu	Glu	
145					150					155					160	
Cys	Pro	Asp	Leu	Val	Glu	Lys	Ala	Val	Ile	Ile	Gly	Ala	Pro	Ala	Arg	
			165						170					175		
His	Ser	Pro	Trp	Ala	Ile	Gly	Leu	Asn	Thr	Ala	Ala	Arg	Ser	Ala	Ile	
			180					185					190			
Ala	Leu	Ala	Pro	Gly	Gly	Glu	Gly	Leu	Lys	Val	Ala	Arg	Gln	Ile	Ala	
		195					200					205				
Met	Leu	Ser	Tyr	Arg	Ser	Pro	Glu	Ser	Leu	Ser	Arg	Thr	Gln	Ala	Gly	
	210					215					220					
Gln	Arg	Val	Pro	Gly	Val	Pro	Ala	Val	Thr	Ser	Tyr	Leu	His	Tyr	Gln	
225					230					235					240	
Gly	Glu	Lys	Leu	Ala	Ala	Arg	Phe	Asp	Glu	Gln	Thr	Tyr	Cys	Ala	Leu	
			245						250					255		

Thr Trp Ala Met Asp Ala Phe Gln Pro Ser Ser Ala Asp Leu Lys Ala
260 265 270

Val Arg Ala Pro Val Leu Val Val Gly Ile Ser Ser Asp Leu Leu Tyr
275 280 285

Pro Ala Ala Glu Val Arg Ala Cys Ala Ala Glu Leu Pro His Ala Asp
290 295 300

Tyr Trp Glu Leu Gly Ser Ile His Gly His Asp Ala Phe Leu Met Asp
305 310 315 320

Pro Gln Asp Leu Pro Glu Arg Val Gly Ala Phe Leu Arg Ser
325 330

<210> 16
<211> 309
<212> PRT
<213> Escherichia coli

<220>
<221> PEPTIDE
<222> (1)..(309)
<223> homoserine O-succinyl transferase

<400> 16
Met Pro Ile Arg Val Pro Asp Glu Leu Pro Ala Val Asn Phe Leu Arg
1 5 10 15

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

Ile Arg Pro Leu Lys Val Leu Ile Leu Asn Leu Met Pro Lys Lys Ile
35 40 45

Glu Thr Glu Asn Gln Phe Leu Arg Leu Leu Ser Asn Ser Pro Leu Gln
50 55 60

Val Asp Ile Gln Leu Leu Arg Ile Asp Ser Arg Glu Ser Arg Asn Thr
65 70 75 80

Pro Ala Glu His Leu Asn Asn Phe Tyr Cys Asn Phe Glu Asp Ile Gln

				85				90				95			
Asp	Gln	Asn	Phe	Asp	Gly	Leu	Ile	Val	Thr	Gly	Ala	Pro	Leu	Gly	Leu
100				105				110							
Val	Glu	Phe	Asn	Asp	Val	Ala	Tyr	Trp	Pro	Gln	Ile	Lys	Gln	Val	Leu
115				120				125							
Glu	Trp	Ser	Lys	Asp	His	Val	Thr	Ser	Thr	Leu	Phe	Val	Cys	Trp	Ala
130				135				140							
Val	Gln	Ala	Ala	Leu	Asn	Ile	Leu	Tyr	Gly	Ile	Pro	Lys	Gln	Thr	Arg
145				150				155				160			
Thr	Glu	Lys	Leu	Ser	Gly	Val	Tyr	Glu	His	His	Ile	Leu	His	Pro	His
165				170				175							
Ala	Leu	Leu	Thr	Arg	Gly	Phe	Asp	Asp	Ser	Phe	Leu	Ala	Pro	His	Ser
180				185				190							
Arg	Tyr	Ala	Asp	Phe	Pro	Ala	Ala	Leu	Ile	Arg	Asp	Tyr	Thr	Asp	Leu
195				200				205							
Glu	Ile	Leu	Ala	Glu	Thr	Glu	Glu	Gly	Asp	Ala	Tyr	Leu	Phe	Ala	Ser
210				215				220							
Lys	Asp	Lys	Arg	Ile	Ala	Phe	Val	Thr	Gly	His	Pro	Glu	Tyr	Asp	Ala
225				230				235				240			
Gln	Thr	Leu	Ala	Gln	Glu	Phe	Phe	Arg	Asp	Val	Glu	Ala	Gly	Leu	Asp
245				250				255							
Pro	Asp	Val	Pro	Tyr	Asn	Tyr	Phe	Pro	His	Asn	Asp	Pro	Gln	Asn	Thr
260				265				270							
Pro	Arg	Ala	Ser	Trp	Arg	Ser	His	Gly	Asn	Leu	Leu	Phe	Thr	Asn	Trp
275				280				285							
Leu	Asn	Tyr	Tyr	Val	Tyr	Gln	Ile	Thr	Pro	Tyr	Asp	Leu	Arg	His	Met
290				295				300							
Asn	Pro	Thr	Leu	Asp											
305															

<210> 17
 <211> 309
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant polypeptide having homoserine O-acetyl transferase activity, metA EL

<400> 17

Met Pro Ile Arg Val Pro Asp Glu Leu Pro Ala Val Asn Phe Leu Arg
 1 5 10 15

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

Ile Arg Pro Leu Lys Val Leu Ile Leu Asn Leu Met Pro Lys Lys Ile
 35 40 45

Glu Thr Glu Asn Gln Phe Leu Arg Leu Leu Ser Asn Ser Pro Leu Gln
 50 55 60

Val Asp Ile Gln Leu Leu Arg Ile Asp Ser Arg Glu Ser Arg Asn Thr
 65 70 75 80

Pro Ala Glu His Leu Asn Asn Phe Tyr Cys Asn Phe Glu Asp Ile Gln
 85 90 95

Asp Gln Asn Phe Asp Gly Leu Ile Val Thr Gly Ala Pro Leu Glu Leu
 100 105 110

Val Glu Phe Asn Asp Val Ala Tyr Trp Pro Gln Ile Lys Gln Val Leu
 115 120 125

Glu Trp Ser Lys Asp His Val Thr Ser Thr Leu Phe Val Cys Trp Ala
 130 135 140

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

Thr Glu Lys Leu Ser Gly Val Tyr Glu His His Ile Leu His Pro His
 165 170 175

Ala Leu Leu Thr Arg Gly Phe Asp Asp Ser Phe Leu Ala Pro His Ser

180						185						190					
Arg	Tyr	Ala	Asp	Phe	Pro	Ala	Ala	Leu	Ile	Arg	Asp	Tyr	Thr	Asp	Leu		
195						200						205					
Glu	Ile	Leu	Ala	Glu	Thr	Glu	Glu	Gly	Asp	Ala	Tyr	Leu	Phe	Ala	Ser		
210						215						220					
Lys	Asp	Lys	Arg	Ile	Ala	Phe	Val	Thr	Gly	His	Pro	Glu	Tyr	Asp	Ala		
225						230						235					
Gln	Thr	Leu	Ala	Gln	Glu	Phe	Phe	Arg	Asp	Val	Glu	Ala	Gly	Leu	Asp		
245						250						255					
Pro	Asp	Val	Pro	Tyr	Asn	Tyr	Phe	Pro	His	Asn	Asp	Pro	Gln	Asn	Thr		
260						265						270					
Pro	Arg	Ala	Ser	Trp	Arg	Ser	His	Gly	Asn	Leu	Leu	Phe	Thr	Asn	Trp		
275						280						285					
Leu	Asn	Tyr	Tyr	Val	Tyr	Gln	Ile	Thr	Pro	Tyr	Asp	Leu	Arg	His	Met		
290						295						300					
Asn Pro Thr Leu Asp																	
305																	

<210> 18
 <211> 309
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Variant polypeptide having homoserine O-acetyl transferase activity, metA ET

<400> 18															
Met	Pro	Ile	Arg	Val	Pro	Asp	Glu	Leu	Pro	Ala	Val	Asn	Phe	Leu	Arg
1				5					10					15	
Glu	Glu	Asn	Val	Phe	Val	Met	Thr	Thr	Ser	Arg	Ala	Ser	Gly	Gln	Glu
			20					25					30		
Ile Arg Pro Leu Lys Val Leu Ile Leu Asn Leu Met Pro Lys Lys Ile															

35					40					45						
Glu	Thr	Glu	Asn	Gln	Phe	Leu	Arg	Leu	Leu	Ser	Asn	Ser	Pro	Leu	Gln	
50					55					60						
Val	Asp	Ile	Gln	Leu	Leu	Arg	Ile	Asp	Ser	Arg	Glu	Ser	Arg	Asn	Thr	
65					70					75					80	
Pro	Ala	Glu	His	Leu	Asn	Asn	Phe	Tyr	Cys	Asn	Phe	Glu	Asp	Ile	Gln	
85					90					95						
Asp	Gln	Asn	Phe	Asp	Gly	Leu	Ile	Val	Thr	Gly	Ala	Pro	Leu	Glu	Thr	
100					105					110						
Val	Glu	Phe	Asn	Asp	Val	Ala	Tyr	Trp	Pro	Gln	Ile	Lys	Gln	Val	Leu	
115					120					125						
Glu	Trp	Ser	Lys	Asp	His	Val	Thr	Ser	Thr	Leu	Phe	Val	Cys	Trp	Ala	
130					135					140						
Val	Gln	Ala	Ala	Leu	Asn	Ile	Leu	Tyr	Gly	Ile	Pro	Lys	Gln	Thr	Arg	
145					150					155					160	
Thr	Glu	Lys	Leu	Ser	Gly	Val	Tyr	Glu	His	His	Ile	Leu	His	Pro	His	
165					170					175						
Ala	Leu	Leu	Thr	Arg	Gly	Phe	Asp	Asp	Ser	Phe	Leu	Ala	Pro	His	Ser	
180					185					190						
Arg	Tyr	Ala	Asp	Phe	Pro	Ala	Ala	Leu	Ile	Arg	Asp	Tyr	Thr	Asp	Leu	
195					200					205						
Glu	Ile	Leu	Ala	Glu	Thr	Glu	Glu	Gly	Asp	Ala	Tyr	Leu	Phe	Ala	Ser	
210					215					220						
Lys	Asp	Lys	Arg	Ile	Ala	Phe	Val	Thr	Gly	His	Pro	Glu	Tyr	Asp	Ala	
225					230					235					240	
Gln	Thr	Leu	Ala	Gln	Glu	Phe	Phe	Arg	Asp	Val	Glu	Ala	Gly	Leu	Asp	
245					250					255						
Pro	Asp	Val	Pro	Tyr	Asn	Tyr	Phe	Pro	His	Asn	Asp	Pro	Gln	Asn	Thr	
260					265					270						
Pro	Arg	Ala	Ser	Trp	Arg	Ser	His	Gly	Asn	Leu	Leu	Phe	Thr	Asn	Trp	

275

280

285

Leu Asn Tyr Tyr Val Tyr Gln Ile Thr Pro Tyr Asp Leu Arg His Met
 290 295 300

Asn Pro Thr Leu Asp
 305

<210> 19

<211> 309

<212> PRT

<213> Artificial Sequence

<220>

<223> Variant polypeptide having homoserine O-acetyl transferase activity, metA EH

<400> 19

Met Pro Ile Arg Val Pro Asp Glu Leu Pro Ala Val Asn Phe Leu Arg
 1 5 10 15

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

Ile Arg Pro Leu Lys Val Leu Ile Leu Asn Leu Met Pro Lys Lys Ile
 35 40 45

Glu Thr Glu Asn Gln Phe Leu Arg Leu Leu Ser Asn Ser Pro Leu Gln
 50 55 60

Val Asp Ile Gln Leu Leu Arg Ile Asp Ser Arg Glu Ser Arg Asn Thr
 65 70 75 80

Pro Ala Glu His Leu Asn Asn Phe Tyr Cys Asn Phe Glu Asp Ile Gln
 85 90 95

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

Val Glu Phe Asn Asp Val Ala Tyr Trp Pro Gln Ile Lys Gln Val Leu
 115 120 125

Glu Trp Ser Lys Asp His Val Thr Ser Thr Leu Phe Val Cys Trp Ala

130		135		140											
Val	Gln	Ala	Ala	Leu	Asn	Ile	Leu	Tyr	Gly	Ile	Pro	Lys	Gln	Thr	Arg
145					150					155					160
Thr	Glu	Lys	Leu	Ser	Gly	Val	Tyr	Glu	His	His	Ile	Leu	His	Pro	His
				165					170					175	
Ala	Leu	Leu	Thr	Arg	Gly	Phe	Asp	Asp	Ser	Phe	Leu	Ala	Pro	His	Ser
			180					185					190		
Arg	Tyr	Ala	Asp	Phe	Pro	Ala	Ala	Leu	Ile	Arg	Asp	Tyr	Thr	Asp	Leu
		195					200					205			
Glu	Ile	Leu	Ala	Glu	Thr	Glu	Glu	Gly	Asp	Ala	Tyr	Leu	Phe	Ala	Ser
	210						215				220				
Lys	Asp	Lys	Arg	Ile	Ala	Phe	Val	Thr	Gly	His	Pro	Glu	Tyr	Asp	Ala
225					230						235				240
Gln	Thr	Leu	Ala	Gln	Glu	Phe	Phe	Arg	Asp	Val	Glu	Ala	Gly	Leu	Asp
				245					250					255	
Pro	Asp	Val	Pro	Tyr	Asn	Tyr	Phe	Pro	His	Asn	Asp	Pro	Gln	Asn	Thr
			260					265					270		
Pro	Arg	Ala	Ser	Trp	Arg	Ser	His	Gly	Asn	Leu	Leu	Phe	Thr	Asn	Trp
		275					280					285			
Leu	Asn	Tyr	Tyr	Val	Tyr	Gln	Ile	Thr	Pro	Tyr	Asp	Leu	Arg	His	Met
	290						295				300				
Asn	Pro	Thr	Leu	Asp											
305															

<210> 20
 <211> 883
 <212> PRT
 <213> Escherichia coli

 <220>
 <221> PEPTIDE
 <222> (1) .. (883)
 <223> phosphoenolpyruvate carboxylase

<400> 20
 Met Asn Glu Gln Tyr Ser Ala Leu Arg Ser Asn Val Ser Met Leu Gly
 1 5 10 15

 Lys Val Leu Gly Glu Thr Ile Lys Asp Ala Leu Gly Glu His Ile Leu
 20 25 30

 Glu Arg Val Glu Thr Ile Arg Lys Leu Ser Lys Ser Ser Arg Ala Gly
 35 40 45

 Asn Asp Ala Asn Arg Gln Glu Leu Leu Thr Thr Leu Gln Asn Leu Ser
 50 55 60

 Asn Asp Glu Leu Leu Pro Val Ala Arg Ala Phe Ser Gln Phe Leu Asn
 65 70 75 80

 Leu Ala Asn Thr Ala Glu Gln Tyr His Ser Ile Ser Pro Lys Gly Glu
 85 90 95

 Ala Ala Ser Asn Pro Glu Val Ile Ala Arg Thr Leu Arg Lys Leu Lys
 100 105 110

 Asn Gln Pro Glu Leu Ser Glu Asp Thr Ile Lys Lys Ala Val Glu Ser
 115 120 125

 Leu Ser Leu Glu Leu Val Leu Thr Ala His Pro Thr Glu Ile Thr Arg
 130 135 140

 Arg Thr Leu Ile His Lys Met Val Glu Val Asn Ala Cys Leu Lys Gln
 145 150 155 160

 Leu Asp Asn Lys Asp Ile Ala Asp Tyr Glu His Asn Gln Leu Met Arg
 165 170 175

 Arg Leu Arg Gln Leu Ile Ala Gln Ser Trp His Thr Asp Glu Ile Arg
 180 185 190

 Lys Leu Arg Pro Ser Pro Val Asp Glu Ala Lys Trp Gly Phe Ala Val
 195 200 205

 Val Glu Asn Ser Leu Trp Gln Gly Val Pro Asn Tyr Leu Arg Glu Leu
 210 215 220

Asn	Glu	Gln	Leu	Glu	Glu	Asn	Leu	Gly	Tyr	Lys	Leu	Pro	Val	Glu	Phe	225	230	235	240
Val	Pro	Val	Arg	Phe	Thr	Ser	Trp	Met	Gly	Gly	Asp	Arg	Asp	Gly	Asn		245	250	255
Pro	Asn	Val	Thr	Ala	Asp	Ile	Thr	Arg	His	Val	Leu	Leu	Leu	Ser	Arg	260	265	270	
Trp	Lys	Ala	Thr	Asp	Leu	Phe	Leu	Lys	Asp	Ile	Gln	Val	Leu	Val	Ser	275	280	285	
Glu	Leu	Ser	Met	Val	Glu	Ala	Thr	Pro	Glu	Leu	Leu	Ala	Leu	Val	Gly	290	295	300	
Glu	Glu	Gly	Ala	Ala	Glu	Pro	Tyr	Arg	Tyr	Leu	Met	Lys	Asn	Leu	Arg	305	310	315	320
Ser	Arg	Leu	Met	Ala	Thr	Gln	Ala	Trp	Leu	Glu	Ala	Arg	Leu	Lys	Gly	325	330	335	
Glu	Glu	Leu	Pro	Lys	Pro	Glu	Gly	Leu	Leu	Thr	Gln	Asn	Glu	Glu	Leu	340	345	350	
Trp	Glu	Pro	Leu	Tyr	Ala	Cys	Tyr	Gln	Ser	Leu	Gln	Ala	Cys	Gly	Met	355	360	365	
Gly	Ile	Ile	Ala	Asn	Gly	Asp	Leu	Leu	Asp	Thr	Leu	Arg	Arg	Val	Lys	370	375	380	
Cys	Phe	Gly	Val	Pro	Leu	Val	Arg	Ile	Asp	Ile	Arg	Gln	Glu	Ser	Thr	385	390	395	400
Arg	His	Thr	Glu	Ala	Leu	Gly	Glu	Leu	Thr	Arg	Tyr	Leu	Gly	Ile	Gly	405	410	415	
Asp	Tyr	Glu	Ser	Trp	Ser	Glu	Ala	Asp	Lys	Gln	Ala	Phe	Leu	Ile	Arg	420	425	430	
Glu	Leu	Asn	Ser	Lys	Arg	Pro	Leu	Leu	Pro	Arg	Asn	Trp	Gln	Pro	Ser	435	440	445	
Ala	Glu	Thr	Arg	Glu	Val	Leu	Asp	Thr	Cys	Gln	Val	Ile	Ala	Glu	Ala	450	455	460	

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

Pro	Thr	Gly	Gly	Val	Glu	Ser	Leu	Arg	Ala	Ile	Pro	Trp	Ile	Phe	Ala	
705					710					715					720	
Trp	Thr	Gln	Asn	Arg	Leu	Met	Leu	Pro	Ala	Trp	Leu	Gly	Ala	Gly	Thr	
				725					730					735		
Ala	Leu	Gln	Lys	Val	Val	Glu	Asp	Gly	Lys	Gln	Ser	Glu	Leu	Glu	Ala	
			740					745					750			
Met	Cys	Arg	Asp	Trp	Pro	Phe	Phe	Ser	Thr	Arg	Leu	Gly	Met	Leu	Glu	
		755					760					765				
Met	Val	Phe	Ala	Lys	Ala	Asp	Leu	Trp	Leu	Ala	Glu	Tyr	Tyr	Asp	Gln	
	770					775					780					
Arg	Leu	Val	Asp	Lys	Ala	Leu	Trp	Pro	Leu	Gly	Lys	Glu	Leu	Arg	Asn	
785					790					795					800	
Leu	Gln	Glu	Glu	Asp	Ile	Lys	Val	Val	Leu	Ala	Ile	Ala	Asn	Asp	Ser	
				805					810					815		
His	Leu	Met	Ala	Asp	Leu	Pro	Trp	Ile	Ala	Glu	Ser	Ile	Gln	Leu	Arg	
			820					825					830			
Asn	Ile	Tyr	Thr	Asp	Pro	Leu	Asn	Val	Leu	Gln	Ala	Glu	Leu	Leu	His	
		835					840					845				
Arg	Ser	Arg	Gln	Ala	Glu	Lys	Glu	Gly	Gln	Glu	Pro	Asp	Pro	Arg	Val	
	850					855					860					
Glu	Gln	Ala	Leu	Met	Val	Thr	Ile	Ala	Gly	Ile	Ala	Ala	Gly	Met	Arg	
865					870					875					880	

Asn Thr Gly

<210> 21
 <211> 396
 <212> PRT
 <213> Escherichia coli

<220>
 <221> PEPTIDE
 <222> (1) .. (396)

<223> aspartate aminotransferase

<400> 21

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

Ala	Glu	Gly	Leu	Arg	Ala	Phe	Ala	Ala	Met	His	Lys	Glu	Leu	Ile	Val	225	230	235	240
Ala	Ser	Ser	Tyr	Ser	Lys	Asn	Phe	Gly	Leu	Tyr	Asn	Glu	Arg	Val	Gly	245	250	255	
Ala	Cys	Thr	Leu	Val	Ala	Ala	Asp	Ser	Glu	Thr	Val	Asp	Arg	Ala	Phe	260	265	270	
Ser	Gln	Met	Lys	Ala	Ala	Ile	Arg	Ala	Asn	Tyr	Ser	Asn	Pro	Pro	Ala	275	280	285	
His	Gly	Ala	Ser	Val	Val	Ala	Thr	Ile	Leu	Ser	Asn	Asp	Ala	Leu	Arg	290	295	300	
Ala	Ile	Trp	Glu	Gln	Glu	Leu	Thr	Asp	Met	Arg	Gln	Arg	Ile	Gln	Arg	305	310	315	320
Met	Arg	Gln	Leu	Phe	Val	Asn	Thr	Leu	Gln	Glu	Lys	Gly	Ala	Asn	Arg	325	330	335	
Asp	Phe	Ser	Phe	Ile	Ile	Lys	Gln	Asn	Gly	Met	Phe	Ser	Phe	Ser	Gly	340	345	350	
Leu	Thr	Lys	Glu	Gln	Val	Leu	Arg	Leu	Arg	Glu	Glu	Phe	Gly	Val	Tyr	355	360	365	
Ala	Val	Ala	Ser	Gly	Arg	Val	Asn	Val	Ala	Gly	Met	Thr	Pro	Asp	Asn	370	375	380	
Met	Ala	Pro	Leu	Cys	Glu	Ala	Ile	Val	Ala	Val	Leu					385	390	395	

<210> 22
 <211> 367
 <212> PRT
 <213> Escherichia coli

 <220>
 <221> PEPTIDE
 <222> (1) .. (367)
 <223> aspartate-semialdehyde dehydrogenase

<400> 22

Met Lys Asn Val Gly Phe Ile Gly Trp Arg Gly Met Val Gly Ser Val
1 5 10 15

Leu Met Gln Arg Met Val Glu Glu Arg Asp Phe Asp Ala Ile Arg Pro
20 25 30

Val Phe Phe Ser Thr Ser Gln Leu Gly Gln Ala Ala Pro Ser Phe Gly
35 40 45

Gly Thr Thr Gly Thr Leu Gln Asp Ala Phe Asp Leu Glu Ala Leu Lys
50 55 60

Ala Leu Asp Ile Ile Val Thr Cys Gln Gly Gly Asp Tyr Thr Asn Glu
65 70 75 80

Ile Tyr Pro Lys Leu Arg Glu Ser Gly Trp Gln Gly Tyr Trp Ile Asp
85 90 95

Ala Ala Ser Ser Leu Arg Met Lys Asp Asp Ala Ile Ile Ile Leu Asp
100 105 110

Pro Val Asn Gln Asp Val Ile Thr Asp Gly Leu Asn Asn Gly Ile Arg
115 120 125

Thr Phe Val Gly Gly Asn Cys Thr Val Ser Leu Met Leu Met Ser Leu
130 135 140

Gly Gly Leu Phe Ala Asn Asp Leu Val Asp Trp Val Ser Val Ala Thr
145 150 155 160

Tyr Gln Ala Ala Ser Gly Gly Gly Ala Arg His Met Arg Glu Leu Leu
165 170 175

Thr Gln Met Gly His Leu Tyr Gly His Val Ala Asp Glu Leu Ala Thr
180 185 190

Pro Ser Ser Ala Ile Leu Asp Ile Glu Arg Lys Val Thr Thr Leu Thr
195 200 205

Arg Ser Gly Glu Leu Pro Val Asp Asn Phe Gly Val Pro Leu Ala Gly
210 215 220

Ser Leu Ile Pro Trp Ile Asp Lys Gln Leu Asp Asn Gly Gln Ser Arg

225		230		235		240									
Glu	Glu	Trp	Lys	Gly	Gln	Ala	Glu	Thr	Asn	Lys	Ile	Leu	Asn	Thr	Ser
				245					250					255	
Ser	Val	Ile	Pro	Val	Asp	Gly	Leu	Cys	Val	Arg	Val	Gly	Ala	Leu	Arg
			260					265					270		
Cys	His	Ser	Gln	Ala	Phe	Thr	Ile	Lys	Leu	Lys	Lys	Asp	Val	Ser	Ile
		275					280					285			
Pro	Thr	Val	Glu	Glu	Leu	Leu	Ala	Ala	His	Asn	Pro	Trp	Ala	Lys	Val
	290					295					300				
Val	Pro	Asn	Asp	Arg	Glu	Ile	Thr	Met	Arg	Glu	Leu	Thr	Pro	Ala	Ala
305					310					315					320
Val	Thr	Gly	Thr	Leu	Thr	Thr	Pro	Val	Gly	Arg	Leu	Arg	Lys	Leu	Asn
			325						330					335	
Met	Gly	Pro	Glu	Phe	Leu	Ser	Ala	Phe	Thr	Val	Gly	Asp	Gln	Leu	Leu
		340						345					350		
Trp	Gly	Ala	Ala	Glu	Pro	Leu	Arg	Arg	Met	Leu	Arg	Gln	Leu	Ala	
	355						360					365			

<210> 23
 <211> 933
 <212> DNA
 <213> Escherichia coli

<220>
 <221> gene
 <222> (1)..(933)
 <223> homoserine O-succinyl transferase

<400> 23
 atggttaaag tttatgcccc ggcttccagt gccaatatga gcgtcggggtt tgatgtgctc
 60
 ggggcggcgg tgacacctgt tgatggtgca ttgctcggag atgtagtcac gggtgaggcg
 120

gcagagacat tcagtctcaa caacctcgga cgctttgccg ataagctgcc gtcagaacca
180

cgggaaaata tcgtttatca gtgctgggag cgtttttgcc aggaactggg taagcaaatt
240

ccagtggcga tgaccctgga aaagaatatg ccgacggtt cgggcttagg ctccagtgcc
300

tgttcgggtg tcgcggcgct gatggcgatg aatgaacact gcggcaagcc gcttaatgac
360

actcgtttgc tggctttgat gggcgagctg gaaggccgta tctccggcag cattcattac
420

gacaacgtgg caccgtgttt tctcgggtgt atgcagttga tgatcgaaga aaacgacatc
480

atcagccagc aagtgccagg gtttgatgag tggctgtggg tgctggcgta tccggggatt
540

aaagtctcga cggcagaagc cagggttatt ttaccggcgc agtatcgccg ccaggattgc
600

attgcgcacg ggcgacatct ggcaggcttc attcacgcct gctattcccg tcagcctgag
660

cttgccgcga agctgatgaa agatgttatc gctgaaccct accgtgaacg gttactgcca
720

ggcttccggc aggcgcggca ggcggtcgcg gaaatcggcg cggtagcgag cggtatctcc
780

ggctccggcc cgaccttggt cgctctgtgt gacaagccgg aaaccgcca gcgcgttgcc
840

gactggttgg gtaagaacta cctgcaaaat caggaagggt ttgttcatat ttgccggctg
900

g a t a c g g c g g g c g c a c g a g t a c t g g a a a a c t a a
933

<211> 930
<212> DNA
<213> Artificial Sequence

<220>

<223> Variant polypeptide having homoserine O-acetyl transferase activity, metA EL

<400> 24

atgccgattc gtgtgccgga cgagctaccc gccgtcaatt tcttgcgtag agaaaacgtc
60

tttgtgatga caacttctcg tgcgtctggg caggaaattc gtccacttaa ggttctgata
120

cttaacctga tgccgaagaa gattgaaact gaaaatcagt ttctgcgcct gctttcaaac
180

tcacctttgc aggtcgatat tcagctgttg cgcatacatt cccgtgaatc gcgcaaacacg
240

ccgcagagc atctgaacaa cttctactgt aactttgaag atattcagga tcagaacttt
300

gacggtttga ttgtaactgg tgcgccgctg gaactgggtg agtttaatga tgcgccttac
360

tggccgcaga tcaaacaggt gctggagtgg tcgaaagatc acgtcacctc gacgctgttt
420

gtctgctggg cggtacaggc cgcgctcaat atcctctacg gcattcctaa gcaaactcgc
480

accgaaaaac tctctggcgt ttacgagcat catattctcc atcctcatgc gcttctgacg
540

cgtggctttg atgattcatt cctggcaccg cattcgcgct atgctgactt tccggcagcg
600

ttgattcgtg attacaccga tctggaaatt ctggcagaga cggaagaagg ggatgcatat
660

ctgtttgccg gtaaagataa gcgcattgcc tttgtgacgg gccatcccga atatgatgcg
720

caaacgctgg cgcaggaatt tttccgcgat gtggaagccg gactagaccc ggatgtaccg
780

tataactatt tcccgcacaa tgatccgcaa aatacaccgc gagcgagctg gcgtagtcac
840

ggtaattttac tgttttaccaa ctggctcaac tattacgtct accagatcac gccatacgat
900

c t a c g g c a c a t g a a t c c a a c g c t g g a t t a a
930

<210> 25

<211> 930

<212> DNA

<213> Artificial Sequence

<220>

<223> Variant polypeptide having homoserine O-acetyl transferase
activity, metA ET

<400> 25

atgccgattc gtgtgccgga cgagctaccc gccgtcaatt tcttgcggtga agaaaacgtc
60

tttgtgatga caacttctcg tgcgtctggt caggaaattc gtccacttaa ggttctgatc
120

cttaacctga tgccgaagaa gattgaaact gaaaatcagt ttctgcgctt gctttcaaac
180

tcacctttgc aggtcgatat tcagctgttg cgcacgcatt cccgtgaatc gcgcaacacg
240

cccgcagagc atctgaacaa cttctactgt aactttgaag atattcagga tcagaacttt
300

gacggtttga ttgtaactgg tgcgccgctg gaaaccgtgg agtttaatga tgcgcgttac
360

tggccgcaga tcaaacaggt gctggagtgg tcgaaagatc acgtcacctc gacgctgttt
420

gtctgctggg cggtacaggc cgcgctcaat atcctctacg gcattcctaa gcaaactcgc
480

accgaaaaac tctctggcgt ttacgagcat catattctcc atcctcatgc gcttctgacg
540

cgtggctttg atgattcatt cctggcaccg cattcgcgct atgctgactt tccggcagcg
600

ttgattcgtg attacaccga tctggaaatt ctggcagaga cggaagaagg ggatgcatat
660

ctgtttgccg gtaaagataa gcgcattgcc tttgtgacgg gccatcccga atatgatgcg
720

caaacgctgg cgcaggaatt tttccgcgat gtggaagccg gactagaccc ggatgtaccg
780

tataactatt tcccgcacaa tgatccgcaa aatacaccgc gagcgagctg gcgtagtcac
840

ggtaatttac tgtttaccaa ctggctcaac tattacgtct accagatcac gccatacgat
900

c t a c g g c a c a t g a a t c c a a c g c t g g a t t a a
930

<210> 26

<211> 930

<212> DNA

<213> Artificial Sequence

<220>

<223> Variant polypeptide having homoserine O-acetyl transferase
activity, metA EH

<400> 26

atgccgattc gtgtgccgga cgagctaccc gccgtcaatt tcttgcggtga agaaaacgtc
60

tttgtgatga caacttctcg tgcgtctggt caggaaattc gtccacttaa ggttctgatc
120

cttaacctga tgccgaagaa gattgaaact gaaaatcagt ttctgcgctt gctttcaaac
180

tcacctttgc aggtcgatat tcagctgttg cgcacgatt cccgtgaatc gcgcaacacg
240

cccgcagagc atctgaacaa cttctactgt aactttgaag atattcagga tcagaacttt
300

gacggtttga ttgtaactgg tgcgccgctg gaacatgtgg agtttaatga tgtcgcttac
360

tggccgcaga tcaaacaggt gctggagtgg tcgaaagatc acgtcacctc gacgctgttt
420

gtctgctggg cggtacaggc cgcgctcaat atcctctacg gcattcctaa gcaaactcgc
480

accgaaaaac tctctggcgt ttacgagcat catattctcc atcctcatgc gcttctgacg
540

cgtggctttg atgattcatt cctggcaccg cattcgcgct atgctgactt tccggcagcg
600

ttgattcgtg attacaccga tctggaaatt ctggcagaga cggaagaagg ggatgcatat
660

ctgtttgccg gtaaagataa gcgcattgcc tttgtgacgg gccatcccga atatgatgcg
720

caaacgctgg cgcaggaatt tttccgcgat gtggaagccg gactagaccc ggatgtaccg
780

tataactatt tcccgcacaa tgatccgcaa aatacaccgc gagcgagctg gcgtagtcac
840

ggtaattttac tgtttaccaa ctgggtcaac tattacgtct accagatcac gccatacgat
900

c t a c g g c a c a t g a a t c c a a c g c t g g a t t a a
930

<211> 2652
<212> DNA
<213> Escherichia coli

<220>
<221> gene
<222> (1)..(2652)
<223> phosphoenolpyruvate carboxylase

<400> 27
atgaacgaac aatattccgc attgcgtagt aatgtcagta tgctcggcaa agtgctggga
60

gaaaccatca aggatgcggtt gggagaacac attcttgaac gcgtagaaac tatccgtaag
120

ttgtcgaaat cttcacgcgc tggcaatgat gctaaccgcc aggagttgct caccacctta
180

caaaatttgt cgaacgacga gctgctgccc gttgcgcgtg cgtttagtca gttcctgaac
240

ctggccaaca ccgccgagca ataccacagc atttcgccga aaggcgaagc tgccagcaac
300

ccggaagtga tcgcccgcac cctgcgtaaa ctgaaaaacc agccggaact gagcgaagac
360

accatcaaaa aagcagtgga atcgctgtcg ctggaactgg tcctcacggc tcacccaacc
420

gaaattaccc gtcgtacact gatccacaaa atggtggaag tgaacgcctg tttaaaacag
480

ctcgataaca aagatatcgc tgactacgaa cacaaccagc tgatgcgtcg cctgcgccag
540

ttgatcgccc agtcatggca taccgatgaa atccgtaagc tgcgtccaag cccggtagat
600

gaagccaaat ggggctttgc cgtagtgga aacagcctgt ggcaaggcgt accaaattac
660

ctgcgcgaac tgaacgaaca actggaagag aacctcggct acaaactgcc cgtcgaattt
720

gttcggtcc gttttacttc gtggatgggc ggcgaccgcg acggcaaccc gaacgtcact
780

gccgatatca cccgccacgt cctgctactc agccgctgga aagccaccga tttgttcctg
840

aaagatattc aggtgctggc ttctgaactg tcgatgggtg aagcgacccc tgaactgctg
900

gcgctgggtg gcgaagaagg tgccgcagaa ccgtatcgct atctgatgaa aaacctgcgt
960

tctcgctga tggcgacaca ggcattggctg gaagcgcgcc tgaaaggcga agaactgcc
1020

aaaccagaag gcctgctgac acaaaacgaa gaactgtggg aaccgctcta cgcttgctac
1080

cagtcacttc aggcgtgtgg catgggtatt atcgccaacg gcgatctgct cgacaccctg
1140

cgccgcgtga aatgtttcgg cgtaccgctg gtccgtattg atatccgtca ggagagcacg
1200

cgtcataccg aagcgctggg cgagctgacc cgctacctcg gtatcggcga ctacgaaagc
1260

tggtcagagg ccgacaaaca ggcgttcctg atccgcgaac tgaactccaa acgtccgctt
1320

ctgccgcgca actggcaacc aagcgccgaa acgcgcgaag tgctcgatac ctgccagggtg
1380

attgccgaag caccgcaagg ctccattgcc gcctacgtga tctcgatggc gaaaacgccg
1440

tccgacgtac tggctgtcca cctgctgctg aaagaagcgg gtatcgggtt tgcgatgccg
1500

gttgctccgc tgtttgaaac cctcgatgat ctgaacaacg ccaacgatgt catgaccag
1560

ctgctcaata ttgactggta tcgtggcctg attcagggca aacagatggg gatgattggc
1620

tattccgact cagcaaaaga tgcgggagtg atggcagctt cctggggcgca atatcaggca
1680

caggatgcat taatcaaaac ctgcgaaaaa gcgggtattg agctgacgtt gttccacggt
1740

cgcgggcggtt ccattggtcg cggcggcgca cctgctcatg cggcgctgct gtcacaaccg
1800

ccaggaagcc tgaaaggcgg cctgcgcgta accgaacagg gcgagatgat ccgctttaaa
1860

tatggtctgc cagaaatcac cgtcagcagc ctgtcgcttt ataccggggc gattctggaa
1920

gccaacctgc tgccaccgcc ggagccgaaa gagagctggc gtcgcattat ggatgaactg
1980

tcagtcatct cctgcgatgt ctaccgcggc tacgtacgtg aaaacaaaga ttttgtgcct
2040

tacttccgct ccgctacgcc ggaacaagaa ctgggcaaac tgccgttggg ttcacgtccg
2100

gcgaaacgtc gcccaaccgg cggcgtcgag tcactacgcg ccattccgtg gatcttcgcc
2160

tggacgcaaa accgtctgat gctccccgcc tggctgggtg caggtaaggc gctgcaaaaa
2220

gtggtcgaag acggcaaaca gagcgagctg gaggtatgt gccgcgattg gccattcttc
2280

tcgacgcgtc tcggcatgct ggagatggtc ttcgccaaag cagacctgtg gctggcgga
2340

tactatgacc aacgcctggt agacaaagca ctgtggccgt taggtaaaga gttacgcaac
2400

ctgcaagaag aagacatcaa agtggtgctg gcgattgcca acgattccca tctgatggcc
2460

gatctgccgt ggattgcaga gtctattcag ctacggaata ttacaccga cccgctgaac
2520

gtattgcagg ccgagttgct gcaccgctcc cgccaggcag aaaaagaagg ccaggaaccg
2580

gacacctcgcg tcgaacaagc gttaatggtc actattgccg ggattgcggc aggtatgcgt
2640

a a t a c c g g c t a a
2652

<210> 28
<211> 1191
<212> DNA
<213> Escherichia coli

<220>
<221> gene
<222> (1)..(1191)
<223> aspartate aminotransferase

<400> 28
atgtttgaga acattaccgc cgctcctgcc gacccgattc tgggcctggc cgatctgttt
60

cgtgccgatg aacgtcccgg caaaattaac ctcgggattg gtgtctataa agatgagacg
120

ggcaaaaccc cggtactgac cagcgtgaaa aaggctgaac agtatctgct cgaaaatgaa
180

accaccaaaa attacctcgg cattgacggc atccctgaat ttggtcgctg cactcaggaa
240

ctgctgtttg gttaaaggtag cgccctgatc aatgacaaac gtgctcgcac ggcacagact
300

ccgggggggca ctggcgccact acgcgtggct gccgatttcc tggcaaaaaa taccagcgtt
360

aagcgtgtgt gggtgagcaa cccaagctgg ccgaaccata agagcgtctt taactctgca
420

ggtctggaag ttcgtgaata cgcttattat gatgcggaaa atcacactct tgacttcgat
480

gcactgatta acagcctgaa tgaagctcag gctggcgacg tagtgctggt ccatggctgc
540

tgccataacc caaccggtat cgaccctacg ctggaacaat ggcaaacact ggcacaactc
600

tccgttgaga aaggctgggt accgctgttt gacttcgctt accagggttt tgcccgtggt
660

ctggaagaag atgctgaagg actgcgcgct ttcgcggcta tgcataaaga gctgattggt
720

gccagttcct actctaaaaa ctttggcctg tacaacgagc gtgttggcgc ttgtactctg
780

gttgctgccg acagtgaaac cgttgatcgc gcattcagcc aaatgaaagc ggcgattcgc
840

gctaactact ctaaccacc agcacacggc gcttctgttg ttgccaccat cctgagcaac
900

gatgcgttac gtgcgatttg ggaacaagag ctgactgata tgcgccagcg tattcagcgt
960

atgcgtcagt tgttcgtcaa tacgctgcag gaaaaaggcg caaaccgcga cttcagcttt
1020

atcatcaaac agaacggcat gttctccttc agtggcctga caaaagaaca agtgctgcgt
1080

ctgcgcgaag agtttggcgt atatgcggtt gcttctggtc gcgtaaagt ggccgggatg
1140

acaccagata acatggctcc gctgtgcgaa gcgattgtgg cagtgctgta a
1191

<210> 29

<211> 1104

<212> DNA

<213> Escherichia coli

<220>

<221> gene

<222> (1) .. (1104)
<223> aspartate-semialdehyde dehydrogenase

<400> 29

atgaaaaatg ttgggttttat cggctggcgc ggtatggtcg gctccgttct catgcaacgc
60

atggttgaag agcgcgactt cgacgccatt cgccctgtct tcttttctac ttctcagctt
120

ggccaggctg cgccgtcttt tggcggaacc actggcacac ttcaggatgc ctttgatctg
180

gaggcgctaa aggccctcga tatcattgtg acctgtcagg gcggcgatta taccaacgaa
240

atctatccaa agcttcgtga aagcggatgg caaggttact ggattgacgc agcatcgtct
300

ctgcgcatga aagatgacgc catcatcatt cttgaccccg tcaatcagga cgtcattacc
360

gacggattaa ataatggcat caggactttt gttggcggtg actgtaccgt aagcctgatg
420

ttgatgtcgt tgggtgggtt attcgccaat gatcttggtg attgggtgtc cgttgcaacc
480

taccaggccg cttccggcgg tgggtgcgcga catatgcgtg agttattaac ccagatgggc
540

catctgtatg gccatgtggc agatgaactc gcgaccccg tctctgctat tctcgatatc
600

gaacgcaaag tcacaacctt aaccgtagc ggtgagctgc cgggtggataa ctttggcgtg
660

ccgctggcgg gtagcctgat tccgtggatc gacaaacagc tcgataacgg tcagagccgc
720

gaagagtgga aagggcaggc ggaaaccaac aagatcctca acacatcttc cgtaattccg
780

gtagatgggt tatgtgtgcg tgtcggggca ttgcgctgcc acagccaggc attcactatt
840

aaattgaaaa aagatgtgtc tattccgacc gtggaagaac tgctggctgc gcacaatccg
900

tgggcgaaag tcgttccgaa cgatcgggaa atcactatgc gtgagctaac cccagctgcc
960

gttaccggca cgctgaccac gccggtaggc cgcctgcgta agctgaatat gggaccagag
1020

ttcctgtcag cctttaccgt gggcgaccag ctgctgtggg gggccgcgga gccgctgcgt
1080

c g g a t g c t t c g t c a a c t g g c g t a a
1104

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<220>
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<400> 30
ttactctggg gcctgacatt tcaccgacaa agcccaggga acttcatcac gtgtaggctg
60

g a g c t g c t t c
70

<210> 31
<211> 70
<212> DNA
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<220>
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<400> 31
ttacccttgg tttgcagccc ggaagccatt ttccaggctg gcaattaaat catatgaata
60

t c c t c c t t a g
70

<210> 32
<211> 70
<212> DNA
<213> Artificial Sequence

<220>
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<400> 32
aaagaatatg ccgatcgggtt cgggcttagg ctccagtgcc tgttcgggtgg gtgtaggctg
60

g a g c t g c t t c
70

<210> 33
<211> 70
<212> DNA
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<220>
<223> Primer

<400> 33
agacaaccga catcgctttc aacattggcg accggagccg ggaaggcaaa catatgaata
60

t c c t c c t t a g
70

<210> 34
<211> 29
<212> DNA
<213> Artificial Sequence

<220>

<223> Primer

<400> 34

a a t t g a t a t c a t g c c g a t t c g t g t g c c g g
29

<210> 35

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 35

a a t t a a g c t t t t a a t c c a g c g t t g g a t t c a t g t g
34

<210> 36

<211> 44

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 36

t t g t a a c t g g t g c g c c g c t g g a a c t g g t g g g g t t t a a t g a t g t c
44

<210> 37

<211> 44

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 37
gacatcatta aacccccacca gttccagcgg cgcaccagtt acaa
44

<210> 38
<211> 44
<212> DNA
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<220>
<223> Primer

<400> 38
tgtaactggt gcgccgctgg aacatgtggg gtttaatgat gtcg
44

<210> 39
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 39
cgacatcatt aaacccccaca tgttccagcg gcgcaccagt taca
44

<210> 40
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 40
tttccgaaac gtacctcagc aggtgtaggc tggagctgct tc
42

<210> 41
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 41
gaataaaatt tattcacctg ctgcatatga atatacctcct tag
43

<210> 42
<211> 29
<212> DNA
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<220>
<223> Primer

<400> 42
aattgatatc atgccgattc gtgtgccgg
29

<210> 43
<211> 27
<212> DNA
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<220>
<223> Primer

<400> 43
aattaaagcct gctgaggta c gtttcgg
27

<210> 44

<211> 23
<212> DNA
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<220>
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<400> 44
c a g c a g g t g a a t a a a t t t t a t t c
23

<210> 45
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<212> DNA
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<220>
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<400> 45
c g c g a a t g g a a g c t g t t t c c
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<210> 46
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<220>
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<400> 46
g c c g g a a t t c t g t c g g a t g c g a t a c t t g c g c
31

<210> 47
<211> 30
<212> DNA
<213> Artificial Sequence

<220>

<223> Primer

<400> 47

g a a g g a g c t c
30

a g a a a a c c c t

c g c g c a a a a g

<210> 48

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 48

g c c g g a g c t c
31

t g t c g g a t g c

g a t a c t t g c g

c

<210> 49

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 49

g a a g g g t a c c
30

a g a a a a c c c t

c g c g c a a a a g

<210> 50

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 50
t c c g a g c t c a t a a g c g t a g c g c a t c a g g c a
30

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<220>
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<400> 51
t c c g a g c t c g t c c a c c t a t g t t g a c t a c a t
30

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<220>
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<400> 52
c c g g a a t t c c c a g g a g a g c a a t a a g c a
27

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<220>
<223> Primer

<400> 53

c t a g t c t a g a
28

t g c t c t a t t t

a a c t c c c g

<210> 54
<211> 28
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<220>
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<400> 54
c t a g t c t a g a
28

c c a g g a g a g c

a a t a a g c a

<210> 55
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 55
c c g g a a t t c t
27

g c t c t a t t t a

a c t c c c g

<210> 56
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<220>
<223> Primer

<400> 56
aggcgctaag gagaccttaa atggctgata caaaagcaaa actcaccctc tgtaggctgg
60

a g c t g c t t c g
70

<210> 57
<211> 70
<212> DNA
<213> Artificial Sequence

<220>
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<400> 57
gggttaaaat atttacaact tagcaatcaa ccattaacgc ttgatatcgc atgggaatta
60

g c c a t g g t c c
70

<210> 58
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<212> DNA
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<220>
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<400> 58
c c t t t c t a t a a c t g c g c g t c a t
22

<210> 59
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 59

a g g g g t a t a g

21

c t a c g c c a g a

a

<210> 60

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 60

g a a g g c a a a t

20

t t a a g t t c c g

<210> 61

<211> 40

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 61

cgaagcagct

ccagcctaca

ggtatagata

gacgtcattt

40

<210> 62

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 62

cgggcgcgctg

gcggcggttcg

cgcacgactc

gctggatggtt

aa

42

<210> 63
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 63
ttaacatcca gcgagtcgtg cgcgaaacgcc gccagcgcgc cg
42

<210> 64
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 64
cgcgttccgc ctgctgtcgg cgatgccgac catggccgcg at
42

<210> 65
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 65
atcgcggccca tggtcggcat cgccgacagc aggcggaacg cg
42

<210> 66
<211> 40

<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 66
tcctgaatat gatgttctcc gcgccgtgcg aaccgtatga
40

<210> 67
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 67
tcatacgggtt cgcacggcgc ggagaacatc atattcagga
40

<210> 68
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 68
aaatgacgtc tatctatacc tgtaggctgg agctgcttcg
40

<210> 69
<211> 76
<212> DNA
<213> Artificial Sequence

<220>

<223> Primer

<400> 69

gcaaaaactaa attattggta tcatgaattt gttgtatgat gaataaaata taggggatgg
60

g a a t t a g c c a

76

t g g t c c

<210> 70

<211> 67

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 70

ccatggagga ggaattaacc atgcagtggg ggtgggtggg gtgcgataac agctgtatcc
60

c c g t t g a
67

<210> 71

<211> 67

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 71

aagcttagga ggaattaacc atgcagtggg ggtgggtggg gtgcgatgca aatttaagtt
60

c c g g c a g
67