



(22) Date de dépôt/Filing Date: 1999/04/14

(41) Mise à la disp. pub./Open to Public Insp.: 1999/10/21

(45) Date de délivrance/Issue Date: 2011/07/26

(62) Demande originale/Original Application: 2 325 798

(30) Priorités/Priorities: 1998/04/14 (JP10/103101);
1998/08/05 (JP10/221910); 1999/02/15 (JP11/35739)

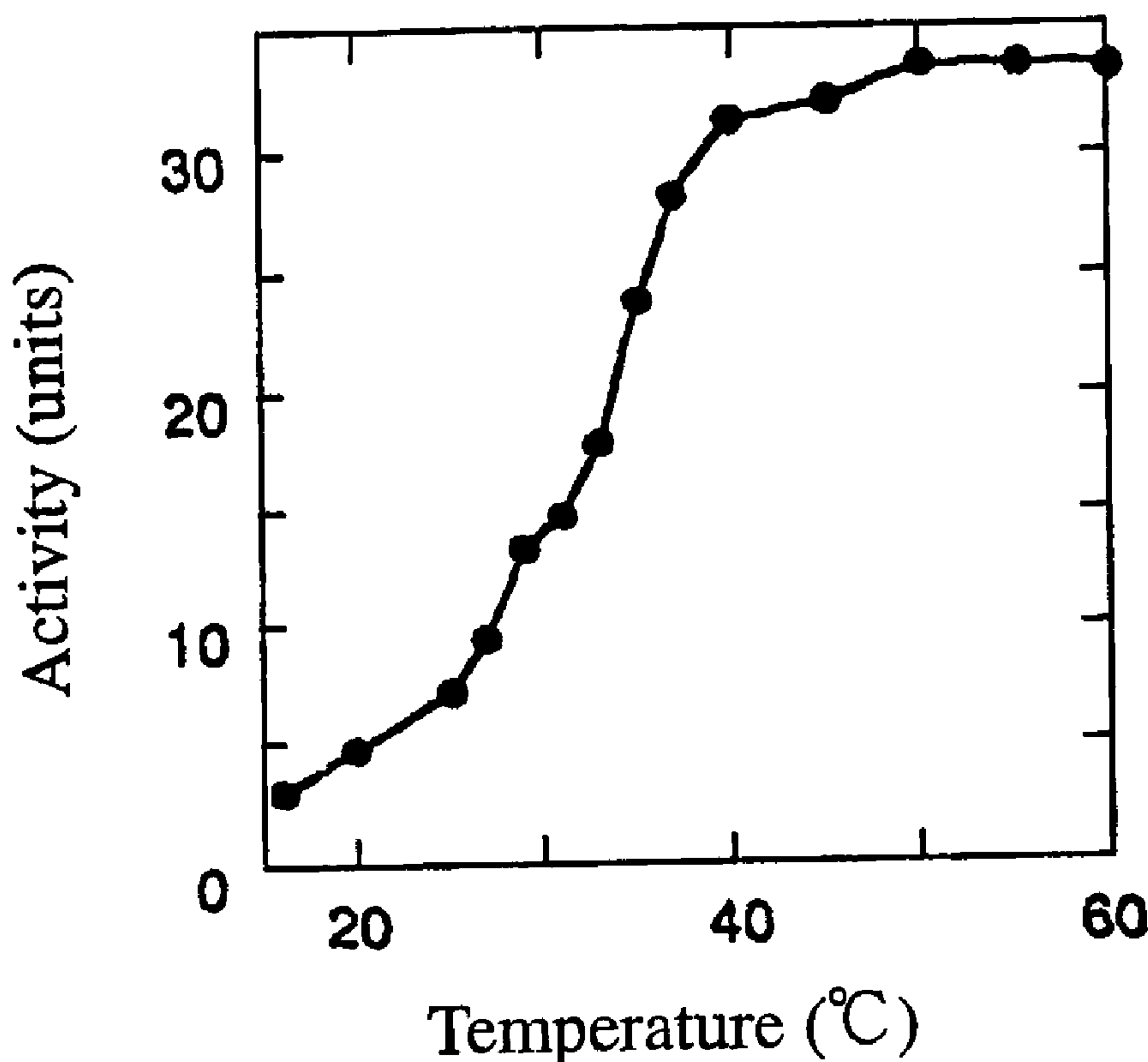
(51) Cl.Int./Int.Cl. *C12N 15/00* (2006.01),
C12N 15/63 (2006.01), *C12P 23/00* (2006.01),
C12P 7/66 (2006.01), *C12N 9/00* (2006.01),
C12N 15/52 (2006.01)

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(54) Titre : PROCESSUS DE PRODUCTION DE COMPOSES ISOPRENOIDES PAR DES MICRO-ORGANISMES

(54) Title: PROCESS FOR PRODUCING ISOPRENOID COMPOUNDS BY MICROORGANISMS



(57) Abrégé/Abstract:

Disclosed is a process for producing an isoprenoid compound by culturing a prokaryote host cell transformed with a vector containing a DNA which encodes a protein having an activity to catalyze a reaction to produce 2-C-methyl-D-erythritol 4-phosphate from 1-deoxy-D-xylulose 5-phosphate, and recovering the isoprenoid compound from the culture. The isoprenoid compound may be useful as a pharmaceutically active ingredient.

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ABSTRACT OF DISCLOSURE

Disclosed is a process for producing an isoprenoid compound by culturing a prokaryote host cell transformed with a vector containing a DNA which encodes a protein
5 having an activity to catalyze a reaction to produce 2-C-methyl-D-erythritol 4-phosphate from 1-deoxy-D-xylulose 5-phosphate, and recovering the isoprenoid compound from the culture. The isoprenoid compound may be useful as a pharmaceutically active ingredient.

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SPECIFICATION

PROCESS FOR PRODUCING ISOPRENOID COMPOUNDS BY MICROORGANISMS

Related Application

This is a divisional application of Canadian
5 Patent Application No. 2,325,798 filed April 14, 1999. The
subject matter of the parent application was restricted to a
certain embodiment and the subject matter of this divisional
application is restricted to another embodiment. The
expression "the present invention" encompasses the subject
10 matters of both the parent and divisional applications.

Technical Field

The present invention relates to a method for
producing isoprenoid compounds using a transformant derived
from a prokaryote; and a method for screening substances
15 having antibiotic or weeding activity involved in a non-
mevalonate pathway.

Background Art

Isoprenoid is a general term for compounds having
isoprene unit consisting of 5 carbon atoms as a backbone
20 structure. Isoprenoid is biosynthesized by polymerization
of isopentenyl pyrophosphate (IPP). Various kinds of
isoprenoid compounds are present in nature and many of them
are useful for humans.

For example, ubiquinone plays an important role
25 *in vivo* as an essential component of the electron transport
system. The demand for ubiquinone is increasing not only as
a pharmaceutical effective against cardiac diseases, but
also as a health food in Western countries.

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Vitamin K, an important vitamin involved in the blood coagulation system, is utilized as a hemostatic agent. Recently it has been suggested that vitamin K is involved in osteo-metabolism, and is expected to be applied to the
5 treatment of osteoporosis. Phylloquinone and menaquinone have been approved as pharmaceuticals.

In addition, ubiquinone and vitamin K are effective in inhibiting barnacles from clinging to objects, and so would make an excellent additive to paint products to
10 prevent barnacles from clinging.

Further, compounds called carotenoids having an isoprene backbone consisting of 40 carbon atoms have antioxidant effect. Carotenoids such as β -carotene, astaxanthin, and cryptoxanthin are expected to possess
15 cancer preventing and immunopotentiating activity.

As described above, isoprenoid compounds include many effective substances. Establishment of an economical process for producing these substances will be a huge benefit to the medical world and

society.

The process for producing isoprenoid compounds through fermentation has already been examined, and examination of culture conditions, strain breeding by mutagenesis, and improvement of yield by genetic engineering techniques have been tested. However, the practical results are limited to individual types of compounds, and there is no known method effective for the isoprenoid compounds in general.

Isopentenyl pyrophosphate (IPP), a backbone unit of isoprenoid compounds, has been proved to be biosynthesized from acetyl-CoA via mevalonic acid (mevalonate pathway) in eukaryotes, such as an animal and yeast.

3-Hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase is considered to be a rate-limiting enzyme in the mevalonate pathway [Mol. Biol. Cell, 5, 655 (1994)]. A test in yeast to improve the yield of carotenoids by overexpression of HMG-CoA reductase has been conducted [Misawa, et al., Summaries of Lectures on Carotenoids, 1997].

There is no knowledge which proves the presence of the mevalonate pathway in prokaryotes. In many prokaryotes, another pathway, the non-mevalonate pathway, has been found in which IPP is biosynthesized via 1-deoxy-D-xylulose 5-phosphate produced by condensation of pyruvic acid and glyceraldehyde 3-phosphate [Biochem. J., 295, 517 (1993)]. It is suggested that 1-deoxy-D-xylulose 5-phosphate is converted to IPP via 2-C-methyl-D-erythritol 4-phosphate in an experiment using ¹³C-labelled substrate [Tetrahedron Lett. 38, 4769 (1997)].

In *Escherichia coli*, a gene encoding an enzyme, 1-deoxy-D-xylulose 5-phosphate synthase (DXS) which allows biosynthesis of 1-deoxy-D-xylulose 5-phosphate by condensation of pyruvic acid and glyceraldehyde 3-phosphate, is identified [Proc. Natl. Acad. Sci. USA, 94, 12857 (1997)]. This gene is contained in an operon consisting of four ORFs that include ispA encoding farnesyl pyrophosphate synthase.

Further in *Escherichia coli*, the presence of the activity to convert 1-deoxy-D-xylulose 5-phosphate to 2-C-methyl-D-erythritol 4-phosphate is known [Tetrahedron Lett. 39, 4509 (1998)].

At present there are no known description nor suggestion to improve yield of an isoprenoid compound by genetically engineering these genes contained in the operon.

Although knowledge about the non-mevalonate pathway in prokaryotes has gradually increased,

most enzymes involved therein and genes encoding these enzymes still remain unknown.

In photosynthetic bacteria, there is a known process for effectively producing ubiquinone-10 by introducing a gene for an enzyme *ubiC* (*uviC* gene), which converts chorismate into 4-hydroxybenzoate, and a gene for *p*-hydroxybenzoate transferase (*ubiA*) (Japanese Unexamined Patent Publication 107789/96). However, there is no example which improved the productivity of isoprenoid compounds by genetically engineering genes for enzymes involved in the non-mevalonate pathway.

Moreover, there is no knowledge about how prokaryotes will be influenced when the reaction on the non-mevalonate pathway is inhibited by mutagenesis or treating with drugs.

Disclosure of the Invention

An object of this invention is to provide a process for producing isoprenoid compounds comprising integrating DNA into a vector wherein the DNA contains one or more DNA involved in biosynthesis of isoprenoid compounds useful in pharmaceuticals for cardiac diseases, osteoporosis, homeostasis, prevention of cancer, and immunopotentialization, health food and anti-fouling paint products against barnacles, introducing the resultant recombinant DNA into a host cell derived from prokaryotes, culturing the obtained transformant in a medium, allowing the transformant to produce and accumulate isoprenoid compounds in the culture, and recovering the isoprenoid compounds from the culture; a process for producing proteins comprising integrating DNA into a vector wherein the DNA contains one or more DNA encoding a protein having activity to improve efficiency in the biosynthesis of isoprenoid compounds, introducing the resultant recombinant DNA into a host cell, culturing the obtained transformant in a medium, allowing the transformant to produce and accumulate the protein in the culture, and recovering the protein from the culture; the protein; and DNA encoding the protein. A further object of this invention is to provide a method of screening a substance having antibiotic and/or weeding activities, which comprises screening the substance inhibiting enzymatic reaction on the non-mevalonic acid pathway.

The inventors have completed the invention by finding that the productivity of isoprenoid can be improved by screening DNA capable of improving the productivity for isoprenoid in prokaryotes, and introducing the obtained DNA into prokaryotes.

That is, a first aspect of the present invention is a process for producing isoprenoid

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compounds comprising integrating DNA into a vector wherein the DNA contains one or more DNA selected from the following (a), (b), (c), (d), (e) and (f):

- (a) a DNA encoding a protein having activity to catalyze a reaction to produce 1-deoxy-D-xylulose 5-phosphate from pyruvic acid and glyceraldehyde 3-phosphate,
- (b) a DNA encoding farnesyl pyrophosphate synthase,
- (c) a DNA encoding a protein that has an amino acid sequence of SEQ ID NO:3, or a protein that has an amino acid sequence wherein one to several amino acid residues are deleted, substituted or added in the amino acid sequence of SEQ ID NO: 3 and has activity to improve efficiency in the biosynthesis of isoprenoid compounds,
- (d) a DNA encoding a protein that has an amino acid sequence of SEQ ID NO:4, or a protein that has an amino acid sequence wherein one to several amino acid residues are deleted, substituted or added in the amino acid sequence of SEQ ID NO: 4 and has activity to improve efficiency in the biosynthesis of isoprenoid compounds,
- (e) a DNA encoding a protein having activity to catalyze a reaction to produce 2-C-methyl-D-erythritol 4-phosphate from 1-deoxy-D-xylulose 5-phosphate, and
- (f) a DNA encoding a protein that can hybridize under stringent conditions with DNA selected from (a), (b), (c), (d) and (e), and has activity substantially identical with that of the protein encoded by the selected DNA;

introducing the resultant recombinant vector into a host cell derived from prokaryotes, culturing the obtained transformant in a medium; allowing the transformant to produce and accumulate isoprenoid compounds in the culture; and recovering the isoprenoid compounds from the culture.

Deletions, substitutions or additions of amino acid residues in this specification can be carried out by site-directed mutagenesis, which is a technique well-known prior to the filing of this application. Further, the phrase "one to several amino acid residues" means the number of amino acid residues, which can be deleted, substituted, or added by site-directed mutagenesis, for example, 1 to 5 amino acid residues.

The protein consisting of an amino acid sequence, which has deletion, substitution or addition of one to several amino acid residues, can be prepared according to the methods described in *Molecular Cloning: A Laboratory Manual, Second Edition*, ed. Sambrook, Fritsch, and Maniatis, Cold Spring

Harbor Laboratory Press, 1989 (hereinafter referred to as Molecular Cloning, Second Edition), Current Protocols in Molecular Biology, John Wiley & Sons (1987-1997), Nucleic Acids Research, 10, 6487 (1982), Proc. Natl. Acad. Sci., USA, 79, 6409 (1982), Gene, 34, 315 (1985), Nucleic Acids Research, 13, 4431 (1985), and Proc. Natl. Acad. Sci USA, 82, 488 (1985), etc.

The above-mentioned DNA encoding a protein, which catalyzes a reaction to produce 1-deoxy-D-xylulose 5-phosphate from pyruvic acid and glyceraldehyde 3-phosphate, is for example, a DNA encoding a protein, which has an amino acid sequence of SEQ ID NO:1, 26 or 28, or a DNA encoding a protein which has an amino acid sequence wherein one to several amino acid residues are deleted, substituted or added in the amino acid sequence of SEQ ID NO: 1, 26, or 28 and has activity to catalyze a reaction to produce 1-deoxy-D-xylulose 5-phosphate from pyruvic acid and glyceraldehyde 3-phosphate.

Examples of such a DNA include a DNA having a nucleotide sequence of SEQ ID NO:6 or a DNA having a nucleotide sequence of SEQ ID NO:27 or 29.

Examples of a DNA encoding farnesyl pyrophosphate synthase include a DNA encoding a protein having an amino acid sequence of SEQ ID NO:2 or a DNA encoding a protein, which has an amino acid sequence wherein one to several amino acid residues are deleted, substituted or added in the amino acid sequence of SEQ ID NO: 2 and has enzymatic activity to produce farnesyl pyrophosphate. A specific example is a DNA having a nucleotide sequence of SEQ ID NO:7.

A specific example of the DNA encoding a protein having an amino acid sequence of SEQ ID NO:3 is a DNA having a nucleotide sequence of SEQ ID NO:8.

Further a specific example of the DNA encoding a protein having an amino acid sequence of SEQ ID NO:4 is a DNA having a nucleotide sequence of SEQ ID NO:9.

Examples of the DNA encoding a protein having activity to catalyze a reaction to produce 2-C-methyl-D-erythritol 4-phosphate from 1-deoxy-D-xylulose 5-phosphate include a DNA encoding a protein, which has an amino acid sequence of SEQ ID NO:5 or 30, or a DNA encoding a protein, which has an amino acid sequence wherein one to several amino acid residues are deleted, substituted or added in the amino acid sequence of SEQ ID NO: 5 or 30 and has activity to catalyze the reaction to produce 2-C-methyl-D-erythritol 4-phosphate from 1-deoxy-D-xylulose 5-phosphate.

Specifically, such a DNA is one having a nucleotide sequence of SEQ ID NO:10 or 31.

The above phrase "DNA...that can hybridize under stringent conditions" means a DNA that can be obtained by colony hybridization, plaque hybridization, Southern Blotting or the like using the above DNA or fragments of the DNA as a probe. Such a DNA can be identified by performing hybridization using a filter with colony- or plaque-derived DNA, or fragments of the DNA immobilized thereon, in the presence of 0.7 to 1.0 mol/l NaCl at 65°C, followed by washing the filter using about 0.1 to 2-fold SSC solution (the composition of SSC solution at 1-fold concentration is consisted of 150mol/l sodium chloride, 15mol/l sodium citrate) at 65°C.

Hybridization can be carried out according to the methods described in *Molecular Cloning*, Second Edition. Examples of DNA capable of hybridizing include a DNA that shares at least 70% or more homology, preferably, 90% or more homology with a nucleotide sequence selected from SEQ ID NOS:1, 2, 3, 4, and 5.

Examples of isoprenoid compounds include ubiquinone, vitamin K₂, and carotenoids.

A second aspect of this invention is a protein having activity to improve efficiency in the biosynthesis of isoprenoid compounds and selected from the following (a), (b) and (c):

- (a) a protein having an amino acid sequence of SEQ ID NO:3, or a protein having an amino acid sequence wherein one to several amino acid residues are deleted, substituted or added in the amino acid sequence of SEQ ID NO: 3
- (b) a protein having an amino acid sequence of SEQ ID NO:4, or a protein having an amino acid sequence wherein one to several amino acid residues are deleted, substituted or added in the amino acid sequence of SEQ ID NO: 4, and
- (c) a protein having an amino acid sequence of SEQ ID NO:5, or a protein having an amino acid sequence wherein one to several amino acid residues are deleted, substituted or added in the amino acid sequence of SEQ ID NO: 5.

A third aspect of this invention is a process for producing a protein having activity to improve efficiency in the biosynthesis of isoprenoid compounds comprising integrating DNA encoding the protein described in the second aspect above into a vector, introducing the resultant recombinant DNA into a host cell, culturing the obtained transformant in a medium, allowing the transformant to produce and accumulate the protein in the culture, and recovering the protein from the culture.

The transformants above include microorganisms belonging to the genus *Escherichia*,

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Rhodobacter or *Erwinia*.

A fourth aspect of this invention is a DNA encoding a protein having activity to improve efficiency in the biosynthesis of isoprenoid compounds selected from the following (a), (b), (c), (d), (e), (f) and (g):

- (a) a DNA encoding a protein having an amino acid sequence of SEQ ID NO:3,
- (b) a DNA encoding a protein having an amino acid sequence of SEQ ID NO:4,
- (c) a DNA encoding a protein having an amino acid sequence of SEQ ID NO:5,
- (d) a DNA having a nucleotide sequence of SEQ ID NO:8,
- (e) a DNA having a nucleotide sequence of SEQ ID NO:9,
- (f) a DNA having a nucleotide sequence of SEQ ID NO:10, and
- (g) a DNA that can hybridize with any one of DNA described in (a) to (f) under stringent conditions.

A fifth aspect of this invention is a method for screening a substance having antibiotic activity comprising screening a substance that inhibits the reaction of a protein having activity of an enzyme selected from those present on the non-mevalonate pathway in which 1-deoxy-D-xylulose 5-phosphate biosynthesized from pyruvic acid and glyceraldehyde 3-phosphate is converted to 2-C-methyl-D-erythritol 4-phosphate from which isopentenyl pyrophosphate is biosynthesized.

A sixth aspect of this invention is a method for screening a substance having weeding activity comprising screening a substance that inhibits the reaction of a protein having activity of an enzyme selected from those present on the non-mevalonate pathway in which 1-deoxy-D-xylulose 5-phosphate biosynthesized from pyruvic acid and glyceraldehyde 3-phosphate is converted to 2-C-methyl-D-erythritol 4-phosphate from which isopentenyl pyrophosphate is biosynthesized.

Examples of the proteins in the fifth and sixth aspects above include a protein of the following (a) or (b):

- (a) a protein having activity to catalyze a reaction to produce 1-deoxy-D-xylulose 5-phosphate from pyruvic acid and glyceraldehyde 3-phosphate, or
- (b) a protein having activity to catalyze a reaction to produce 2-C-methyl-D-erythritol 4-phosphate from 1-deoxy-D-xylulose 5-phosphate.

Examples of the proteins catalyzing the reaction to produce 1-deoxy-D-xylulose 5-phosphate from pyruvic acid and glyceraldehyde 3-phosphate include a protein having an amino acid sequence of

SEQ ID NO:1, or a protein having an amino acid sequence wherein one to several amino acid residues are deleted, substituted or added in the amino acid sequence of SEQ ID NO: 1, and having activity to catalyze 1-deoxy-D-xylulose 5-phosphate from pyruvic acid and glyceraldehyde 3-phosphate.

Examples of the proteins having activity to catalyze the reaction to produce 2-C-methyl-D-erythritol 4-phosphate from 1-deoxy-D-xylulose 5-phosphate include a protein having an amino acid sequence of SEQ ID NO:5, or a protein having an amino acid sequence wherein one to several amino acid residues are deleted, substituted or added in the amino acid sequence of SEQ ID NO: 5, and having activity to catalyze the reaction to produce 2-C-methyl-D-erythritol 4-phosphate from 1-deoxy-D-xylulose 5-phosphate.

A seventh aspect of this invention is a substance, which has antibiotic activity and is obtained by the screening method in the fifth invention above. Known substances obtained by the above screening method are not included in this invention.

The inventors have focused on structural similarity of fosmidomycin [3-(N-formyl-N-hydroxyamino)propylphosphonic acid] to 2-C-methyl-D-erythritol 4-phosphate, a reaction product from 1-deoxy-D-xylulose 5-phosphate reductoisomerase reaction, or a reaction intermediate assumed to be produced in this enzymatic reaction.

Based on the assumption that fosmidomycin has activity to inhibit 1-deoxy-D-xylulose 5-phosphate reductoisomerase and antibiotic activity, the inventors have conducted experiments on the screening method of the fifth aspect and also described in the following Example 10. As a result, the inventors found that fosmidomycin is a substance having the activity to inhibit 1-deoxy-D-xylulose 5-phosphate reductoisomerase and antibiotic activity, and in addition, verified the adequacy of the screening method of the fifth aspect above. However, known compound fosmidomycin is excluded from this invention.

An eighth aspect of this invention is a substance, which has weeding activity and obtained through the screening method of the sixth invention above. As described above, any substance that is obtained from the screening method and already known is excluded from this invention.

Hereinafter a more detailed explanation of this aspect will be given.

I. Cloning of DNA Encoding a Protein Involved in Biosynthesis of Isoprenoid Compounds

(1) Cloning of DNA Encoding a Protein Involved in Biosynthesis of Isoprenoid Compounds using a

Nucleotide Sequence of DNA (DXS gene) Encoding DXS

Using information on previously-determined nucleotide sequences of *E.coli* chromosome and DXS gene [Proc. Natl. Acad. Sci. USA., 94, 12857 (1997)], a DNA region containing DXS gene or genes neighboring DXS gene is obtained by cloning with PCR method from *E.coli* [Science, 230, 1350 (1985)].

An example of information on a nucleotide sequence containing DXS gene is the nucleotide sequence of SEQ ID NO:11.

A concrete example of methods for cloning the DNA region containing DXS gene is as follows.

Escherichia coli, such as an *E.coli* XL1-Blue strain (available from TOYOBO CO., LTD.), is cultured in a suitable medium for *Escherichia coli*, for example, LB liquid medium [containing 10g of Bactotrypton (manufactured by Difco Laboratories), 5g of Yeast extracts (manufactured by Difco Laboratories), 5g of NaCl per liter of water, and adjusted to pH 7.2] according to standard techniques.

After culturing, cells were recovered from the culture by centrifugation.

Chromosomal DNA is isolated from the obtained cells according to a known method, described in, for example, Molecular Cloning, Second Edition.

Using information on a nucleotide sequence of SEQ ID NO:11, a sense primer and an antisense primer, which contain DXS gene or a nucleotide sequence corresponding to the DNA region of genes neighboring DXS gene, are synthesized with a DNA synthesizer.

To introduce the amplified DNA fragments into a plasmid after amplification with PCR, it is preferable to add recognition sites appropriate for restriction enzymes, e.g., BamHI, and EcoRI to the 5' ends of sense and antisense primers.

Examples of a combination of the sense and antisense primers include a DNA having a combination of nucleotide sequences: SEQ ID NOS: 12 and 13, SEQ ID NOS: 14 and 15, SEQ ID NOS: 12 and 16, SEQ ID NOS: 17 and 18, SEQ ID NOS: 19 and 13, or SEQ ID NOS: 22 and 23.

Using the chromosomal DNA as a template, PCR is carried out with DNA Thermal Cycler (manufactured by Perkin Elmer Instruments, Inc. Japan) using the primers; TaKaRa LA-PCR™ Kit Ver. 2 (manufactured by TAKARA SHUZO CO., LTD.) or Expand™ High-Fidelity PCR System (manufactured by Boehringer Mannheim K.K.)

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In a reaction condition for PCR, PCR is carried out by 30 cycles, in the case of amplifying a DNA fragment of 2kb or less, one cycle consisting of reaction at 94°C for 30 seconds, 55°C for 30 seconds to 1 minute, and 72°C for 2 minutes; in the case of amplifying a DNA fragment of more than 2kb, one cycle consisting of reaction at 98°C for 20 seconds, and 68°C for 3 minutes; then followed by the reaction at 72°C for 7 minutes. The amplified DNA fragments are cut at sites the same as the restriction enzyme sites added to the above primers, and are fractionated and collected by using agarose gel electrophoresis, sucrose density-gradient centrifugation and the like.

For cloning the amplified DNA obtained above, an appropriate cloning vector is digested with restriction enzymes creating the cohesive ends which are able to ligate with the amplified DNA fragment. Using a recombinant DNA obtained by ligating the above amplified DNA with the cloning vector, *Escherichia coli*, e.g., *E. coli* DH5 α (available from TOYOBO CO., LTD) is transformed.

The cloning vector for use for cloning the amplified DNA, any cloning vectors including phage vectors and plasmid vectors, which can autonomously replicate in *E. coli* K12, can be used.

Expression vectors for *E. coli* can be used as cloning vectors. Concrete examples of the cloning vectors include ZAP Express [manufactured by Stratagene, Strategies, 5, 58 (1992)], pBluescript II SK(+) [Nucleic Acids Research, 17, 9494 (1989)], Lambda ZAP II (manufactured by Stratagene), λ gt10, λ gt11 (DNA Cloning, A Practical Approach, 1 49 (1985)), λ TriplEx (manufactured by Clonetec), λ ExCell (manufactured by Pharmacia), pT7T318U (manufactured by Pharmacia), pcD2 [H. Okayama and P. Berg; Mol. Cell. Biol., 3, 280 (1983)], pMW218

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(manufactured by WAKO PURE CHEMICAL INDUSTRIES., LTD), pUC118
(manufactured by TAKARA SHUZO CO., LTD.), pEG400 [J. Bac., 172,
2392 (1990)], and pQE-30 (manufactured by Qiagen. Inc).

A plasmid DNA containing a DNA of interest can be
5 obtained from the resultant transformant according to standard
techniques, such as those described in Molecular Cloning,
Second Edition, Current Protocols in Molecular Biology,
Supplement 1 to 38, John Wiley & Sons (1987 - 1997), DNA
Cloning

1: Core Techniques, A Practical Approach, Second Edition, Oxford University Press (1995).

A plasmid DNA containing a DNA encoding a protein having activity to catalyze the reaction to produce 1-deoxy-D-xylulose 5-phosphate from pyruvic acid and glyceraldehyde 3-phosphate, a DNA encoding farnesyl pyrophosphate synthase, a DNA encoding a protein having an amino acid sequence of SEQ ID NO:3, a DNA encoding a protein having an amino acid sequence of SEQ ID NO:4 or the like; and a plasmid DNA containing one or more DNAs above, can be obtained by the above methods.

Such plasmids include plasmid pADO-1 that contains all of the DNA above, plasmid pDXS-1 or pQEDXS-1 that contains a DNA having a nucleotide sequence of SEQ ID NO:6, plasmid pISP-1 that contains a DNA having a nucleotide sequence of SEQ ID NO:7, plasmid pXSE-1 that contains a DNA having a nucleotide sequence of SEQ ID NO:8, and plasmid pTFE-1 that contains a DNA having a nucleotide sequence of SEQ ID NO:9.

Using the nucleotide sequences of DNA fragments derived from *E.coli*, which have been inserted into these plasmids, homologues of the DNA can be obtained from other prokaryotes, such as microorganisms belonging to the genus *Rhodobacter*, in the same manner as described above.

(2) Cloning of DNA Encoding a Protein Having Activity to Complement methylerythritol-requiring mutant of *E.coli* (Gene Complementing Methylerythritol-requiring Mutant)

① Construction of *E.coli* methylerythritol-requiring mutant

Escherichia coli, such as *E.coli* W3110 (ATCC14948), is cultured according to standard techniques.

After culturing, cells are recovered from the obtained culture by centrifugation.

The obtained cells are washed with an appropriate buffer agent, such as 0.05mol/l Tris-maleate buffer (pH 6.0). Then the cells are suspended in the same buffer such that the cell density is 10^4 to 10^{10} cells/ml.

Mutagenesis is carried out by standard techniques using the suspension. In such a standard technique, for example, NTG is added to the suspension to a final concentration of 600mg/l, and then the mixture is maintained for 20 minutes at room temperature.

This suspension after mutagenesis is spread on minimal agar medium supplemented with 0.05 to 0.5% methylerythritol and cultured.

An example of minimal agar medium is M9 medium (Molecular Cloning, Second Edition)

supplemented with agar.

Methylerythritol that is chemically synthesized according to the method described in Tetrahedron Letters, 38, 35, 6184 (1997) may be used.

Colonies grown after culturing are replicated on minimal agar media and minimal agar media each containing 0.05 to 0.5% methylerythritol. The mutant of interest, which requires methylerythritol to grow, is selected. That is, a strain capable of growing on minimal agar media containing methylerythritol but not on minimal agar media lacking methylerythritol is selected.

Strain ME 7 is an example of the resultant methylerythritol-requiring mutant obtained by the above manipulations.

② Cloning of the Gene Complementing Methylerythritol-requiring nature

Escherichia coli, such as *E.coli* W3110 (ATCC14948), is inoculated into culture media, e.g., LB liquid medium, then cultured to the logarithmic growth phase by standard techniques.

Cells are collected from the resultant culture by centrifugation.

Chromosomal DNA is isolated and purified from the obtained cells according to standard techniques, such as those described in Molecular Cloning, Second Edition. The chromosomal DNA obtained by the method described in (1) above can be used as isolated and purified chromosomal DNA.

An appropriate amount of the chromosomal DNA is partially digested with an appropriate restriction enzyme, such as Sau 3 A I. The digested DNA fragments are fractionated by according to standard techniques, such as sucrose density-gradient centrifugation (26,000 rpm, 20°C, 20 hr).

The DNA fragments obtained by the above fractionation, 4 to 6 kb each, are ligated to a vector, e.g., pMW118 (Nippon Gene), which has been digested with an appropriate restriction enzyme to construct a chromosomal DNA library.

The methylerythritol-requiring mutant isolated in ① above, such as the strain ME 7, is transformed using the ligated DNA according to standard techniques, e.g., those described in Molecular Cloning, Second Edition.

The resulting transformants are spread on minimal agar media supplemented with a drug corresponding to a drug-resistant gene carried by the vector, such as M9 agar medium containing 100 μ g/l of ampicillin, then cultured overnight at 37°C.

Thus, transformants that have recovered their methylerythritol requirement can be selected by the

method above.

Plasmids are extracted from the resultant transformants by standard techniques. Examples of a plasmid that can allow the transformants to recover their methylerythritol requirement are pMEW73 and pQEDXR.

The nucleotide sequence of the DNA integrated into the plasmid is sequenced.

An example of such a nucleotide sequence is a sequence containing a nucleotide sequence for yaeM gene of SEQ ID NO:10. Using the information on the nucleotide sequence for yaeM gene, homologues of yaeM gene can be obtained from other prokaryotes or plants in the same manner as described above.

II. Production of Proteins having Activity to improve efficiency in the biosynthesis of isoprenoid compounds.

To express the resulting DNA in a host cell, the DNA fragment of interest is digested with restriction enzymes or deoxyribonucleases into one with a proper length containing the gene. Next the fragment is inserted into a downstream of a promoter region in an expression vector. Then the expression vector is introduced into a host cell appropriate for the expression vector.

Any host cell that can express the gene of interest can be used. Examples of the host cell include bacteria belonging to the genera *Escherichia*, *Serratia*, *Corynebacterium*, *Brevibacterium*, *Pseudomonas*, *Bacillus*, *Microbacterium* and the like, yeasts belonging to the genera *Kluyveromyces*, *Saccharomyces*, *Schizosaccharomyces*, *Trichosporon*, *Schwanniomyces*, and the like, animal cells, and insect cells.

Expression vectors used herein can autonomously replicate in the host cell above or be integrated into a chromosomal DNA, and contain a promoter at the position to which the DNA of interest as described above can be transcribed.

When a bacterium is used as a host cell, a preferable expression vector for expression of the DNA above can autonomously replicate in the bacterium and is a recombinant vector comprising a promoter, ribosome binding sequence, the DNA above and a transcription termination sequence. The expression vector may contain a gene to regulate a promoter.

Examples of the expression vector include pBTrp2, pBTac1, pBTac2 (all of them are available from Boehringer Mannheim K.K.), pKK233-2 (Pharmacia), pSE280 (Invitrogen), pGEMEX-1 (Promega), pQE-8 (Qiagen. Inc), pQE-30 (Qiagen. Inc), pKYP10 (Japanese Patent Laid Open

Publication No. 58-110600), pKYP200 (Agricultural Biological Chemistry, 48, 669, 1984), pLSA1 (Agric. Biol. Chem., 53, 277, 1989), pGEL1 (Proc. Natl. Acad. Sci. USA, 82, 4306, 1985), pBluescriptII SK+, pBluescriptII SK (-) (Stratagene), pTrS30 (FERM BP-5407), pTrS32 (FERM BP-5408), pGEX (Pharmacia), pET-3 (Novagen), pTerm2 (US4686191, US4939094, US5160735), pSupex, pUB110, pTP5, pC194, pUC18 (gene, 33, 103, 1985), pUC19 (Gene, 33, 103, 1985), pSTV28 (TAKARA SHUZO CO., LTD.), pSTV29 (TAKARA SHUZO CO., LTD.), pUC118 (TAKARA SHUZO CO., LTD.), pPA1 (Japanese Patent Laid Open Publication No. 63-233798), pEG400 (J. Bacteriol., 172, 2392, 1990), and pQE-30 (Qiagen. Inc).

Any promoter that can function in a host cell may be used. Examples of such a promoter include promoters derived from *Escherichia coli* or phages, such as *trp* promoter (P *trp*), *lac* promoter (P *lac*), P_L promoter, P_R promoter, P_{SE} promoter, SP01 promoter, SP02 promoter, and penP promoter. Furthermore, P *trp* x 2 promoter that is formed by joining two P *trp* in series, and *tac* promoter, *letI* promoter, and *lacT7* promoter, those artificially designed and modified, can be used.

Any ribosome binding sequence that can function in a host cell can be used. A preferable plasmid has a distance between Shine-Dalgarno sequence and a starting codon appropriately adjusted, of for example 6 to 18 bases long.

A transcription termination sequence is not always required for expression of the DNA of interest. Preferably, a transcription termination sequence is arranged immediately followed by a structural gene.

Examples of the host cell used herein include microorganisms belonging to the genera *Escherichia*, *Corynebacterium*, *Brevibacterium*, *Bacillus*, *Microbacterium*, *Serratia*, *Pseudomonas*, *Agrobacterium*, *Alicyclobacillus*, *Anabaena*, *Anacystis*, *Arthrobacter*, *Azobacter*, *Chromatium*, *Erwinia*, *Methylobacterium*, *Phormidium*, *Rhodobacter*, *Rhodopseudomonas*, *Rhodospirillum*, *Scenedesmun*, *Streptomyces*, *Synnecoccus*, and *Zymomonas*. Preferable host cells include microorganisms belonging to the genera *Escherichia*, *Corynebacterium*, *Brevibacterium*, *Bacillus*, *Pseudomonas*, *Agrobacterium*, *Alicyclobacillus*, *Anabaena*, *Anacystis*, *Arthrobacter*, *Azobacter*, *Chromatium*, *Erwinia*, *Methylobacterium*, *Phormidium*, *Rhodobacter*, *Rhodopseudomonas*, *Rhodospirillum*, *Scenedesmun*, *Streptomyces*, *Synnecoccus* and *Zymomonas*.

More specific examples of the host cell include *Escherichia coli* XL1-Blue, *Escherichia coli* XL2-Blue, *Escherichia coli* DH1, *Escherichia coli* DH5 α , *Escherichia coli* MC1000, *Escherichia coli*

KY3276, *Escherichia coli* W1485, *Escherichia coli* JM109, *Escherichia coli* HB101, *Escherichia coli* No. 49, *Escherichia coli* W3110, *Escherichia coli* NY49, *Escherichia coli* MP347, *Escherichia coli* NM522, *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Brevibacterium ammoniagenes*, *Brevibacterium immariophilum* ATCC14068, *Brevibacterium saccharolyticum* ATCC14066, *Brevibacterium flavum* ATCC14067, *Brevibacterium lactofermentum* ATCC13869, *Corynebacterium glutamicum* ATCC13032, *Corynebacterium glutamicum* ATCC14297, *Corynebacterium acetoacidophilum* ATCC13870, *Microbacterium ammoniophilum* ATCC15354, *Serratia ficaria*, *Serratia fonticola*, *Serratia liquefaciens*, *Serratia marcescens*, *Pseudomonas* sp. D-0110, *Agrobacterium radiobacter*, *Agrobacterium rhizogenes*, *Agrobacterium rubi*, *Anabaena cylindrica*, *Anabaena doliolum*, *Anabaena flos-aquae*, *Arthrobacter aurescens*, *Arthrobacter citreus*, *Arthrobacter globiformis*, *Arthrobacter hydrocarboglutamicus*, *Arthrobacter mysorens*, *Arthrobacter nicotianae*, *Arthrobacter paraffineus*, *Arthrobacter protophormiae*, *Arthrobacter roseoparaffinus*, *Arthrobacter sulfureus*, *Arthrobacter ureafaciens*, *Chromatium buderii*, *Chromatium tepidum*, *Chromatium vinosum*, *Chromatium warmingii*, *Chromatium fluviatile*, *Erwinia uredovora*, *Erwinia carotovora*, *Erwinia ananas*, *Erwinia herbicola*, *Erwinia punctata*, *Erwinia terreus*, *Methylobacterium rhodesianum*, *Methylobacterium extorquens*, *Phomidium* sp. ATCC29409, *Rhodobacter capsulatus*, *Rhodobacter sphaeroides*, *Rhodopseudomonas blastica*, *Rhodopseudomonas marina*, *Rhodopseudomonas palustris*, *Rhodospirillum rubrum*, *Rhodospirillum salexigens*, *Rhodospirillum salinarum*, *Streptomyces ambofaciens*, *Streptomyces aureofaciens*, *Streptomyces aureus*, *Streptomyces fungicidicus*, *Streptomyces griseochromogenes*, *Streptomyces griseus*, *Streptomyces lividans*, *Streptomyces olivogriseus*, *Streptomyces rameus*, *Streptomyces tanashiensis*, *Streptomyces vinaceus*, and *Zymomonas mobilis*.

Any method to introduce a recombinant vector into the host cell as described above may be used. Examples of such a method include a method using calcium ions (Proc. Natl. Acad. Sci. USA, 69, 2110, 1972), protoplast method (Japanese Patent Laid Open Publication No. 63-2483942), or methods described in Gene, 17, 107 (1982) or Molecular & General Genetics, 168, 111 (1979).

When yeast is used as a host cell, expression vectors are, for example, YEp13 (ATCC37115), YEp24 (ATCC37051), YCp50 (ATCC37419), pHS19, and pHS15.

Any promoter that can function in yeast can be used. Examples of such a promoter include PH05 promoter, PGK promoter, GAP promoter, ADH promoter, gal 1 promoter, gal 10 promoter, heat shock

protein promoter, MF α 1 promoter, and CUP1 promoter.

Host cells used herein include *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces lactis*, *Trichosporon pullulans*, and *Schwanniomyces alluvius*.

Any method to introduce a recombinant vector, that is, to introduce DNA into yeast may be used. Examples of such methods include Electroporation (Methods. Enzymol., 194, 182, 1990), Spheroplast method (Proc. Natl. Acad. Sci. USA, 75, 1929 (1978)), lithium acetate method (J. Bacteriol., 153, 163 (1983)), and methods described in Proc. Natl. Acad. Sci. USA, 75, 1929 (1978).

When an animal cell is used as a host cell, expression vectors are, for example, pcDNAI, pcDM8 (Funakoshi Co., Ltd), pAGE107 [Japanese Patent Laid Open Publication No. 3-22979; Cytotechnology, 3, 133 (1990)], pAS3-3 [Japanese Patent Laid Open Publication No. 2-227075, pCDM8 (Nature, 329, 840 (1987)), pcDNAI/Amp (Invitrogen), pREP4 (Invitrogen), pAGE103 [J. Biochem., 101, 1307 (1987)], and pAGE210.

Any promoter that can function in an animal cell may be used. Examples of such promoters include a promoter for IE (immediate early) gene of cytomegalovirus (human CMV), SV40 initial promoter, retrovirus promoter, metallothionein promoter, heat shock promoter, and SR α promoter. Moreover, an enhancer of human CMV IE gene may be used together with a promoter.

Host cells used herein are, for example, Namalwa cells, HBT5637 (Japanese Patent Laid Open Publication No. 63-299), COS1 cells, COS7 cells, and CHO cells.

Any method to introduce a recombinant vector into an animal cell, that is, to introduce DNA into an animal cell may be used. Examples of such methods include Electroporation [Cytotechnology, 3, 133 (1990)], calcium phosphate method (Japanese Patent Laid Open Publication No. 2-227075), lipofection [Proc. Natl. Acad. Sci., USA, 84, 7413 (1987)], and methods described in Virology, 52, 456 (1973). Recovery and culture of the transformant can be carried out according to methods described in Japanese Patent Laid Open Publication No. 2-227075 and Japanese Patent Laid Open Publication No. 2-257891.

When an insect cell is used as a host cell, proteins can be expressed according to methods described in, such as Baculovirus Expression Vectors, A Laboratory Manual, Current Protocols in Molecular Biology Supplement 1-38 (1987-1997), and Bio/Technology, 6, 47 (1988).

That is, a vector for introducing a recombinant gene and Baculovirus are co-transduced into an

insect cell to obtain a recombinant virus in the culture supernatant of the insect cell. Then an insect cell is infected with the recombinant virus, resulting in expression of the protein of interest.

Examples of the vectors to transfer genes include pVL1392, pVL1393, pBlueBacIII (all of which are manufactured by Invitrogen).

Baculoviruses used herein are, for example, Autographa californica nuclear polyhedrosis virus that infects *Barathra* insects.

Examples of the insect cells include ovarian cells of *Spodoptera frugiperda*, Sf9, and Sf21 (Baculovirus Expression Vectors, A Laboratory Manual (W. H. Freeman and Company, New York, 1992), and of *Trichoplusia ni*, High 5 (Invitrogen).

Methods of co-transduction of the vector for transferring the recombinant gene and the Baculovirus into an insect cell to prepare a recombinant virus include calcium phosphate transfection (Japanese Patent Laid Open Publication No. 2-227075) and, lipofection [Proc. Natl. Acad. Sci. USA, 84, 7413 (1987)].

Methods for expressing genes include secretory production, and fusion protein expression according to the techniques shown in Molecular Cloning, Second Edition, in addition to direct expression.

When the gene is expressed in yeasts, animal cells, or insect cells, a protein to which sugar or a sugar chain is added, can be obtained.

Proteins having activity to improve efficiency in the biosynthesis of isoprenoid compounds can be produced by culturing a transformant containing a recombinant DNA to which the above DNA has been introduced in a medium, allowing the transformant to produce and accumulate proteins having activity to improve efficiency in the biosynthesis of isoprenoid compounds in the culture, then collecting the proteins from the culture.

The transformants for producing proteins with activity to improve efficiency in the biosynthesis of isoprenoid compounds of the present invention, can be cultured by standard techniques to culture a host cell.

When the transformant of this invention is prokaryote such as *Escherichia coli* or eukaryote such as yeast, a medium for culturing such transformants contains a carbon source, a nitrogen source, and inorganic salts, which the microorganisms can assimilate, and allows the transformant to grow efficiently.

Either natural media or synthetic media can be used if they satisfy the above conditions.

Any carbon source assimilable by the microorganisms may be used. Such carbon sources include glucose, fructose, sucrose, and molasses containing them, carbohydrates e.g., starch or hydrolysates of starch, organic acids e.g., acetic acid and propionic acid, and alcohols e.g., ethanol and propanol.

Examples of nitrogen sources include ammonia, salts of inorganic acids or organic acids, e.g., ammonium chloride, ammonium sulfate, ammonium acetate, and ammonium phosphate, other nitrogen-containing compounds, peptone, meat extract, yeast extract, corn steep liquor, casein hydrolysates, soybean meal and soybean meal hydrolysate, various fermentation microorganic cells or their digests.

Examples of inorganic salts include potassium primary phosphate, potassium secondary phosphate, magnesium phosphate, magnesium sulfate, sodium chloride, ferrous sulfate, manganese sulfate, copper sulfate, and calcium carbonate.

Culturing is carried out by shaking culture or submerged aeration-agitation culture are carried out under aerobic conditions. The preferable culture temperature ranges from 15 to 40°C. The preferable culture period ranges from 16 hours to 7 days. The pH is kept within a range from 3.0 to 9.0 while culturing. The pH is adjusted using inorganic or organic acid, alkaline solutions, urea, calcium carbonate, ammonia or the like.

If necessary, an antibiotics e.g., ampicillin or tetracycline may be added to the media while culturing.

When microorganisms transformed with the expression vectors using inducible promoters are cultured, inducers may be added to the media if necessary. For example, isopropyl- β -D-thiogalactopyranoside (IPTG) or the like may be added to the media when microorganisms transformed with the expression vectors containing *lac* promoter are cultured; indoleacrylic acid (IAA) or the like may be added when microorganisms transformed with the expression vectors containing *trp* promoter are cultured.

The media for culturing a transformant obtained by using an animal cell as a host cell include a generally used RPMI1640 medium [The Journal of the American Medical Association, 199, 519 (1967)], Eagle's MEM medium [Science, 122, 501 (1952)], DMEM medium [Virology, 8, 396 (1959)],

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199 medium [Proceeding of the Society for the Biological Medicine, 73, 1 (1950)] or those to which fetal calf serum or the like is added.

Normally, the transformant is cultured in the presence of 5% CO₂ for 1 to 7 days at pH 6 to 8 and 30 to 40°C.

If necessary, antibiotics e.g., kanamycin and penicillin may be added to the medium while culturing.

Examples of media to culture a transformant obtained by using an insect cell as a host cell include a generally used TNM-FH medium (Pharmingen), Sf-900 II SFM medium (GIBCO BRL), ExCell400, ExCell405 (both manufactured by JRH Biosciences), Grace's Insect Medium (Grace, T.C.C., Nature, 195, 788 (1962)).

The transformant is generally cultured for 1 to 5 days at pH 6 to 7 and at 25°C to 30°C.

If necessary, antibiotics e.g., gentamycin may be added to the medium while culturing.

Proteins having activity to improve efficiency in the biosynthesis of isoprenoid compounds of this invention can be isolated and purified from the culture of the transformant of this invention by standard isolation and purification techniques for a enzyme.

For example, when the protein of this invention is expressed in a soluble form within the cell, after the culture is completed the cells are recovered by centrifugation, suspended in aqueous buffer, then disrupted using an ultrasonicator, french press, Manton Gaulin homogenizer, Dyno-Mill, or the like, thereby obtaining cell-free extracts. The cell-free extract is separated by centrifugation to obtain the supernatant. The purified sample can be obtained from the supernatant by one of or a combination of standard techniques for isolating and purifying enzymes. Such techniques include a solvent extracting technique, salting out technique using ammonium sulfate, desalting technique, precipitation technique using organic solvents, anion exchange chromatography using resins such as diethylaminoethyl (DEAE)-Sepharose*, and DIAION*HPA-75 (Mitsubishi Chemical Corp.), cation exchange chromatography using resins e.g., S-Sepharose*FF (Pharmacia), hydrophobic chromatography using resins e.g., butylsepharose, phenylsepharose, gel filtration using molecular sieve, affinity chromatography, chromatofocusing, and electrophoresis such as isoelectric focusing.

When the proteins that form inclusion bodies are expressed in the cells, the cells are recovered, disrupted, and separated by centrifugation, thereby obtaining precipitated fractions. From

the resulting precipitated fractions, the protein is recovered by standard techniques, and then the insoluble protein is solubilized using a protein denaturing agent. The solubilized solution is diluted or dialyzed to an extent that the solution contains no protein denaturing agent or that the concentration of protein denaturing agent does not denature protein, thereby allowing the protein to form a normal three-dimensional structure. Then the purified sample can be obtained by the same techniques for isolation and purification as described above.

When the protein of this invention or its derivative, such as a sugar-modified protein, is secreted outside the cell, the protein or its derivative, such as a sugar chain adduct, can be recovered from the culture supernatant. That is, the culture is treated by centrifugation and the like as described above so as to obtain soluble fractions. From the soluble fractions, the purified sample can be obtained using the techniques for isolation and purification as described above.

The resulting protein as described above is, for example a protein having an amino acid sequence selected from amino acid sequences of SEQ ID NOS: 1 to 5.

Moreover, the protein expressed by the method above can be chemically synthesized by techniques including Fmoc method (fluorenylmethyloxycarbonyl method), tBoc method (t-butyloxycarbonyl method). Further, the protein can be synthesized by using a peptide synthesizer of Souwa Boeki K.K. (Advanced ChemTech, U.S.A.), Perkin-Elmer Japan (Perkin-Elmer, U.S.A), Pharmacia BioTech (Pharmacia BioTech, Sweden), ALOKA CO., LTD. (Protein Technology Instrument), KURABO INDUSTRIES LTD. (Synthecell-Vega, U.S.A), PerSeptive Limited., Japan (PerSeptive, U.S.A), or SHIMADZU CORP.

III. Production of Isoprenoid Compound

Isoprenoid compounds can be produced by culturing the transformants obtained as described in II above according to the method of II above, allowing the transformants to produce and accumulate isoprenoid compounds in the culture, then recovering the isoprenoid compounds from the culture.

The above culture can yield isoprenoid compounds, such as ubiquinone, vitamin K₂, and carotenoids. Specific examples of isoprenoid compounds include ubiquinone-8 and menaquinone-8 produced using microorganisms belonging to the genus *Escherichia* as a transformant, ubiquinone-10 produced using those belonging to the genus *Rhodobacter*, vitamin K₂ produced using those belonging to the genus *Arthrobacter* as a transformant, astaxanthin produced using those belonging to the genus

Agrobacterium as a transformant, and lycopene, β -carotene, and zeaxanthin produced using those belonging to the genus *Erwinia* as a transformant.

After the culture is completed, in order to isolate and purify isoprenoid compounds, isoprenoid compounds are extracted by adding an appropriate solvent to the culture, the precipitate is removed by e.g., centrifugation, and then the product is subjected to various chromatography.

IV. Screening a Substance inhibiting Enzymatic Activity on Non-Mevalonate Pathway

(1) Determination of Enzymatic Activity on Non-Mevalonate Pathway

The enzymatic activity on non-mevalonate pathway can be determined according to normal methods for determining enzymatic activity.

The pH of the buffer used as a reaction solution to determine activity should be within a range that does not inhibit the enzymatic activity of interest. A preferable pH range includes the optimal pH.

For example, a buffer at pH 5 to 10, preferably 6 to 9 is used for 1-deoxy-D-xylulose 5-phosphate reductoisomerase.

Any buffer can be used herein so far as it does not inhibit the enzymatic activity and can be adjusted to the pH above. Examples of such a buffer include Tris-hydrochloric acid buffer, phosphate buffer, borate buffer, HEPES buffer, MOPS buffer, and bicarbonate buffer. For example, Tris-hydrochloric acid buffer can preferably be used for 1-deoxy-D-xylulose 5-phosphate reductoisomerase.

A buffer of any concentration may be employed so far as it does not inhibit the enzymatic activity. The preferable concentration ranges from 1mol/l to 1mol/l.

When the enzyme of interest requires a coenzyme, a coenzyme is added to the reaction solution. For example, NADPH, NADH or other electron donors can be used as a coenzyme for 1-deoxy-D-xylulose 5-phosphate reductoisomerase. A preferable coenzyme is NADPH.

Any concentration of the coenzyme to be added can be employed so far as it does not inhibit reaction. Such a concentration preferably ranges from 0.01 mol/l to 100 mol/l, more preferably, 0.1 mol/l to 10 mol/l.

Metal ions may be added to a reaction solution if necessary. Any metal ion can be added so far as it does not inhibit reaction. Preferable metal ions include Co^{2+} , Mg^{2+} , and Mn^{2+} .

Metal ions may be added as metallic salts. For example, a chloride, a sulfate, a carbonate, and a phosphate can be added.

Any concentration of the metal ion to be added can be employed so far as it does not inhibit reaction. A preferable concentration ranges from 0 mol/l to 100 mol/l, more preferably, 0.1 mol/l to 10 mol/l.

The substrate of the enzyme of interest is added to the reaction solution. For example, 1-deoxy-D-xylulose 5-phosphate is added for 1-deoxy-D-xylulose 5-phosphate reductoisomerase.

Any concentration of the substrate may be employed so far as it does not inhibit reaction. The preferable concentration ranges from 0.01 mol/l to 0.2 mol/l in the reaction solution.

The enzyme concentration used in reaction is not specifically limited. Normally, the concentration ranges from 0.01 mg/ml to 100 mg/ml.

An enzyme used herein is not necessarily purified into a single substance. It may contain contaminative proteins. In the search as described in (2) below, cellular extracts containing 1-deoxy-D-xylulose 5-phosphate reductoisomerase activity or cells having the same activity can be used.

Any reaction temperature may be employed so far as it does not inhibit enzymatic activity. A preferable temperature range includes the optimal temperature. That is, the reaction temperature ranges from 10°C to 60°C, more preferably, 30°C to 40°C.

Activity can be detected by a method for measuring a decrease in substrates accompanying the reaction or an increase in reaction products as the reaction proceeds.

Such a method is a method wherein the substance of interest is separated and quantitatively determined by e.g, high performance liquid chromatography (HPLC) if necessary. When NADH or NADPH increases or decreases as the reaction proceeds, activity can directly be determined by measuring the absorbance at 340 nm of the reaction solution. For example, the activity of 1-deoxy-D-xylulose 5-phosphate reductoisomerase can be detected by measuring a decrease in the absorbance at 340 nm using a spectrophotometer to determine NADPH quantity that decreases as the reaction proceeds.

(2) Screening a Substance Inhibiting Enzymatic Activity on the Non-mevalonate pathway

A substance inhibiting enzymatic activity on the non-mevalonate pathway can be screened for by adding the substance to be screened for to the enzymatic activity measurement system as described in (1) above, allowing the mixture to react similarly, and then screening a substance that suppresses the amount of the substrates decreased in comparison to a case when no such substance is added; or a substance that

suppresses the yield of the reaction product.

Screening methods include a method wherein the decrease in the amount of substrates or the increase in the amount of reaction products is traced with time; or a method where after the reaction has proceeded for a certain period the decrease in the amount of substrates or the increase in the amount of reaction products is measured.

In the method wherein the decrease in the amount of substrates or the increase in the amount of reaction products is traced with time, the amount is measured preferably at 15 seconds to 20 minutes intervals, more preferably at 1 to 3 minutes intervals during reaction.

To measure the decrease in the amount of substrates or the increase in the amount of reaction products after reaction has proceeded for a certain period, the reaction period is preferably 10 minutes to 1 day, more preferably, 30 minutes to 2 hours.

A substance inhibiting the enzymatic activity on the non-mevalonate pathway inhibits the growth of microorganisms and plants that possess the non-mevalonate pathway. The inventors have first found the fact that this substance inhibits the growth of the microorganisms and plants.

The non-mevalonate pathway is present in microorganisms and plants, but absent in animals and humans. Therefore, the substance inhibiting the enzymatic activity on the non-mevalonate pathway but not affecting human and animals can be obtained by the above described screening method.

This substance can be an effective antibiotic or herbicide.

Brief Description of Drawings

Figure 1 shows the effect of reaction temperature on 1-deoxy-D-xylulose 5-phosphate reductoisomerase activity.

Figure 2 shows the effect of the pH of the reaction solution on 1-deoxy-D-xylulose 5-phosphate reductoisomerase activity. Enzymatic activity measured at various pH in 100 mol/l Tris-hydrochloric acid buffer are shown. Activity is shown as a relative activity when activity at pH 8.0 is considered as

100 %.

Figure 3 shows a method for disrupting *yaeM* gene on a chromosome using homologous recombination.

Figure 4 shows the effect of fosmidomycin on 1-deoxy-D-xylulose 5-phosphate reductoisomerase.

Best Mode for Carrying Out the Invention

The invention will now be described by way of examples, but shall not be limited thereto. Unless otherwise specified, gene recombination shown in the examples was carried out according to techniques described in *Molecular Cloning, Second Edition* (hereinafter referred to as the standard techniques).

Example 1 Cloning of DNA encoding proteins involved in the biosynthesis of isoprenoid compounds

(1) Cloning of DNA encoding proteins involved in the biosynthesis of isoprenoid compounds using the nucleotide sequence of *E.coli* DXS gene

One platinum loop of *E.coli* XL1-Blue (purchased from TOYOBO) was inoculated into 10 ml of LB liquid medium, then cultured overnight at 37°C.

After culturing, cells were collected by centrifugation from the resultant culture.

Chromosomal DNA was isolated and purified from the cells according to the standard techniques.

Sense and antisense primers, each having BamH I and EcoR I restriction enzyme sites at their 5'-ends and consisting of nucleotide sequence pairs of SEQ ID NOS:12 and 13, 14 and 15, 12 and 16, 17 and 18, and 19 and 13; and sense and antisense primers, each having BamH I restriction enzyme site at their 5'-ends and consisting of a nucleotide sequence pair of SEQ ID NO:22 and 23; were synthesized using a DNA synthesizer.

PCR was carried out with a DNA Thermal Cycler (Perkin Elmer Instruments, Inc. Japan) using these primers, chromosomal DNA as a template, and a TaKaRa La-PCR™ Kit Ver. 2 (TAKARA SHUZO CO., LTD.), Expand™ High-Fidelity PCR System (Boehringer Mannheim K.K.) or a Taq DNA polymerase (Boehringer).

PCR was carried out for 30 cycles. In the case of amplifying a DNA fragment of 2kb or less, one cycle consisting of reaction at 94°C for 30 seconds, 55°C for 30 seconds to 1 minute, and 72°C for

2 minutes; in the case of amplifying a DNA fragment of more than 2kb, one cycle consisting of reaction at 98°C for 20 seconds, and 68°C for 3 minutes; then followed by the reaction at 72°C for 7 minutes.

Among the DNA fragments amplified by PCR, DNA fragments amplified using sense and antisense primers, each having BamH I and EcoR I restriction enzyme sites at their 5'-ends, were digested with restriction enzymes BamH I and EcoR I; DNA fragments amplified using sense and antisense primers, each having BamH I restriction enzyme site at their 5'-ends, were digested with restriction enzyme BamH I.

After the digestion, these DNA fragments treated with the restriction enzymes were subjected to agarose gel electrophoresis and recovered BamH I and EcoR I-treated DNA fragments and BamH I-treated DNA fragments.

A broad host range vector pEG 400 containing lac promoter [J. Bac., 172, 2392 (1990)] was digested with restriction enzymes BamH I and EcoR I, subjected to agarose gel electrophoresis and recovered BamH I and EcoR I-treated pEG 400 fragments.

pUC118 (TAKARA SHUZO CO., LTD.) was digested with a restriction enzyme BamH I, then subjected to agarose gel electrophoresis and recovered Bam H I-treated pUC 118 fragments.

Each of the resultant BamH I and EcoR I-treated DNA fragments was mixed with BamH I and EcoR I-treated pEG 400 fragments, then the mixture was allowed to precipitate with ethanol. The obtained DNA precipitate was dissolved in 5 μ l of distilled water for ligation reaction to occur, thereby obtaining each recombinant DNA.

Using the resultant recombinant DNA, *E.coli* (purchased from TOYOBO) DH5 α was transformed according to the standard techniques. Then the transformant was spread on LB agar medium containing 100 μ g/ml of spectinomycin, then cultured overnight at 37°C.

Some colonies of the transformant resistant to spectinomycin were cultured in 10 ml of LB liquid medium containing 100 μ g/ml of spectinomycin with shaking for 16 hours at 37°C.

The resulting culture was centrifuged, so that cells were collected.

Plasmids were isolated from the cells according to the standard techniques.

To confirm that the isolated plasmids contained the DNA fragment of interest, the plasmids were cleaved with various restriction enzymes to examine their structures and their nucleotide sequences were sequenced.

A plasmid containing a DNA with a nucleotide sequence of SEQ ID NO:6, DNA with a nucleotide sequence of SEQ ID NO:7, DNA with a nucleotide sequence of SEQ ID NO: 8, and DNA with a nucleotide sequence of SEQ ID NO: 9 was named pADO-1. A plasmid containing a DNA with a nucleotide sequence of SEQ ID NO: 6 was named pDXS-1. A plasmid containing a DNA with a nucleotide sequence of SEQ ID NO: 7 was named pISP-1. A plasmid containing a DNA with a nucleotide sequence of SEQ ID NO: 9 was named pTFE-1.

The above BamH I-treated DNA fragments and BamH I-treated pUC118 fragments were mixed, then the mixture was allowed to precipitate with ethanol. The resulting DNA precipitate was dissolved in 5 μ l of distilled water for ligation reaction to occur to obtain recombinant DNA. *Escherichia coli* was transformed using the recombinant DNA in the same manner as described above, then plasmids were isolated from the transformants.

To confirm the isolated plasmids contain the DNA fragments of interest, the plasmids were cleaved with various restriction enzymes to examine their structures and their nucleotide sequences were sequenced in the same manner as described above.

These plasmids were digested with BamH I. The DNA fragments of interest were recovered in the same manner as described above, then sub-cloned into an expression vector pQE30 (Qiagen. Inc).

The plasmid obtained by the sub-cloning above and having a nucleotide sequence of SEQ ID NO:6 was named pQEDXS-1.

(2) Cloning of the Gene Complementing Methylerythritol-requiring nature

① Selection of Methylerythritol-requiring Mutant of *Escherichia coli*

E.coli W3110 (ATCC14948) was inoculated into LB liquid medium and cultured to its logarithmic growth phase.

After culturing, cells were recovered from the resulting culture by centrifugation.

The cells were washed with 0.05 mol/l Tris-maleate buffer (pH 6.0), then suspended in the same buffer to the cell density of 10^9 cells/ml.

Mutation was induced by adding NTG to the suspension to a final concentration of 600mg/l, and then the mixture was maintained for 20 minutes at room temperature.

These NTG treated cells were spread on M9 minimal agar medium containing 0.1 % methylerythritol (Molecular Cloning, Second Edition) plate and cultured.

Methylerythritol was chemically synthesized according to the method described in Tetrahedron Letters, 38, 35, 6184 (1997).

Colonies grown on M9 minimal agar medium containing 0.1 % methylerythritol were replicated on M9 minimal agar medium and on M9 minimal agar medium containing 0.1 % methylerythritol. The mutant of interest, a strain requiring methylerythritol to grow, was selected. That is, a strain capable of growing on a minimal agar medium containing 0.1 % methylerythritol but not on the same lacking methylerythritol was selected.

The thus obtained methylerythritol-requiring mutant ME7 was used in the following experiments.

②Cloning of the Gene Complementing Methylerythritol-requiring nature

Escherichia coli W3110 (ATCC14948) was inoculated into LB liquid medium, then cultured to its logarithmic growth phase. Then cells were collected from the resultant culture by centrifugation.

Chromosomal DNA was isolated and purified from the obtained cells according to the standard techniques.

200 μ g of the chromosomal DNA was partially digested with a restriction enzyme, Sau 3AI. The resulting DNA fragments were fractionated by sucrose density-gradient centrifugation (26,000 rpm, 20°C, 20 hr).

The DNA fragments obtained by the above fractionation, 4 to 6 kb each, were ligated to pMW118 vector (Nippon Gene), which had been digested with a restriction enzyme BamH I, constructing a genomic DNA library.

Using this genomic DNA library, the strain ME7 isolated in ① above was transformed according to the standard techniques.

The resulting transformants were spread on LB agar medium supplemented with 100 μ g/l of ampicillin, then cultured overnight at 37°C.

Plasmids were extracted from each colony that grew on the agar medium and then the nucleotide sequences were determined.

The plasmids determined its nucleotides sequence had contained the nucleotide sequence of SEQ ID NO:10. These plasmids were named pMEW41 and pMEW73.

A plasmid extracted from one strain of the clones having the sequence was named pMEW73.

The pMEW73 was double-digested with Hind III and Sac I. The resultant Hind III and Sac I-

treated DNA fragment having a nucleotide sequence of SEQ ID NO:10 was ligated to multi-cloning sites of broad host range vector pEG400 [J. Bac., 172, 2392 (1990)], constructing pEGYM1.

The Hind III - Sac I-treated DNA fragment was ligated to the Hind III – Sac I site of vector pUC19 (Gene, 33, 103 (1985)), constructing pUCYM-1.

According to the information on the nucleotide sequence of chromosomal DNA of *Escherichia coli* based on Genbank data base, the DNA fragment that had been inserted into the vector was confirmed to contain yaeM gene.

A recombinant vector, which can express yaeM gene sufficiently, was constructed by following method with PCR [Science, 230, 1350 (1985)].

A sense primer having a sequence of SEQ ID NO:20 and an antisense primer having a sequence of SEQ ID NO:21 were synthesized using a DNA synthesizer.

A Bam H I restriction enzyme recognition site was added to each 5'-end of the sense and antisense primers.

yaeM gene was amplified by PCR with DNA Thermal Cycler (Perkin Elmer Instruments, Inc. Japan) using chromosomal DNA of *E.coli* as a template, these primers and Taq DNA polymerase (Boelinger).

PCR was carried out by 30 cycles, one cycle consisting of reaction at 94°C for 30 seconds, reaction at 55°C for 30 seconds, and reaction at 72°C for 2 minutes followed by reaction at 72°C for 7 minutes.

After the amplified DNA fragments and pUC118 (TAKARA SHUZO CO., LTD.) were digested with a restriction enzyme BamH I, each of the DNA fragments were purified by agarose gel electrophoresis.

Both of these fragments were mixed, then the mixture was allowed to precipitate with ethanol. The resultant DNA precipitate was dissolved in 5 μ l of distilled water for ligation reaction to occur, thereby obtaining recombinant DNA.

The recombinant DNA was confirmed to be yaeM gene by determining the nucleotide sequences, then sub-cloned to expression vector pQE30 (Qiagen, Inc).

The resulting recombinant DNA was named pQEYM1.

The strain ME7 was transformed using pQEYM1 by standard techniques. The transformant

was spread on LB agar medium containing 100 μ g/ml of ampicillin, then cultured overnight at 37°C.

The transformants were confirmed to form colonies at the same growth rate as wild-type strain, suggesting that *yaeM* gene complemented mutation in the strain ME7.

Example 2 Production of Ubiquinone-8 (CoQ8) using Recombinant *Escherichia coli*

(1) *E.coli* DH5 α were transformed using the plasmids pADO-1, pDXS-1, and pXSE-1, those obtained in Example 1 above, and pEG400 as a control, respectively, then *E.coli* DH5 α /pADO-1, *E.coli* DH5 α /pDXS-1, *E.coli* DH5 α /pXSE-1 and *E.coli* DH5 α /pEG400 that showed resistance to spectinomycin at a concentration of 100 μ g/ml were obtained

These transformants were inoculated into a test tube containing 10 ml of LB medium supplemented with thiamine and vitamin B₆, 100 mg/l each, 50 mg/l of p-hydroxybenzoic acid, and 100 μ g/ml of spectinomycin. Then the transformants were cultured with shaking for 72 hours at 30°C.

After the culture was completed, each culture was concentrated 10 - fold.

To each 300 μ l of concentrated culture, 300 μ l 2-butanol and 300 μ l glass beads were added. Isoprenoid compounds were extracted with the solvent while disrupting the cells by Multi Beads Shocker MB-200 (YASUI KIKAI) for 5 minutes. Then the 2-butanol layer was collected by centrifugation.

The amount of CoQ8 produced by the transformants was calculated by Quantitative analysis of the CoQ8 in the butanol layer using high performance liquid chromatography (LC-10A, SHIMADZU CORP.).

HPLC was carried out using Develosil ODS-HG-5 (NOMURA CHEMICAL K.K.) as a column, and methanol:n-hexane = 8:2 solution as a mobile phase at 1ml/min of the flow rate and 275 nm of the measuring wavelength.

Table 1 shows the results.

Table 1

CoQ8 Production by transformant of *Escherichia coli*

Transformant	Cell Amount (OD660)	Amount of CoQ8 Produced (mg/L)	Intracellular Content * ¹
<i>E.coli</i> DH5 α /pEG400	5.8	0.63	1.1
<i>E.coli</i> DH5 α /pADO-1	5.5	0.98	1.8
<i>E.coli</i> DH5 α /pDXS-1	5.2	0.85	1.6
<i>E.coli</i> DH5 α /pXSE-1	5.6	0.67	1.2

*1: Intracellular content is shown with a value obtained by dividing a 10-fold CoQ8 production (mg/L) by a cell amount (OD660).

The amount of CoQ8 produced was significantly higher in DH5 α /pADO-1, DH5 α /pDXS-1 and DH5 α /pXSE-1 than in the control strain DH5 α /pEG400. In particular, the highest productivity was shown by DH5 α /pADO-1 to which all DNA obtained in Example 1 were introduced.

(2) *E.coli* DH5 α /pDXS-1 or *E.coli* DH5 α /pEG400, as obtained in (1) above, was inoculated into a test tube containing 10 ml of a M9 medium, and then cultured with shaking for 72 hours at 30°C.

After the culture was completed, the amount of CoQ8 produced by the transformants was calculated in the same manner as in (1) above.

Table 2 shows the results.

Table 2

CoQ8 Production by transformant of *Escherichia coli*

Transformant	Cell amount (OD660)	Amount of CoQ8 Produced (mg/L)	Intracellular Content * ¹
<i>E.coli</i> DH5 α /pEG400	3.1	0.49	1.6
<i>E.coli</i> DH5 α /pDXS-1	2.5	1.02	4.1

*1: Intracellular content is shown with a value obtained by dividing a 10-fold CoQ8 production (mg/L) by a cell amount (OD660).

The amount of CoQ8 produced was significantly higher in DH5 α /pDXS-1 than in the control strain DH5 α /pEG400.

(3) Production of CoQ8 using Recombinant *Escherichia coli*

The plasmid pEGYM1 obtained in Example 1 or pEG400 as a control was introduced into *E.coli* DH5 α and *E.coli* DH5 α /pEGYM1 and *E.coli* DH5 α /pEG400 that show resistance to spectinomycin at a concentration of 100 μ g/ml were obtained.

These transformants were inoculated into a test tube containing 10 ml of LB medium supplemented with 1% glucose, 100 mg/l of vitamin B₁, 100 mg/l of vitamin B₆, 50 mg/l of p-hydroxybenzoic acid. Then the transformants were cultured with shaking for 72 hours at 30°C.

After the culture was completed, the amount of CoQ8 produced by the transformants was calculated in the same manner as in (1) above.

Table 3 shows the results.

Table 3

CoQ8 Production by transformants of *Escherichia coli*

Transformant	Cell amount (OD660)	Amount of CoQ8 Produced (mg/L)	Intracellular Content *1
<i>E.coli</i> DH5 α /pEG400	14.44	0.83	0.57
<i>E.coli</i> DH5 α /pEGYM1	13.12	0.94	0.71

*1: Intracellular content is shown with a value obtained by dividing a 10-fold CoQ8 production (mg/L) by a cell amount (OD660).

The amount of CoQ8 produced was significantly higher in DH5 α /pEGYM1 than in the control strain DH5 α /pEG400.

Example 3 Production of Menaquinone-8 (MK-8) by Recombinant *Escherichia coli*

(1) The *E.coli* DH5 α /pADO-1 or *E.coli* DH5 α /pEG400, obtained in Example 2 (1), was inoculated into a test tube containing 10 ml of TB medium supplemented with 100 μ g/ml of spectinomycin, and then cultured with shaking for 72 hours at 30°C. The TB medium had been prepared by dissolving 12 g of bacto-trypton (Difco), 24 g of yeast extract (Difco), and 5 g of glycerol into 900 ml of water followed by the addition of 100ml of aqueous solution containing 0.17mol/l KH₂PO₄ and 0.72mol/l K₂HPO₄.

After the culture was completed, MK-8 was quantified in the same quantifying method for CoQ8 as in Example 2 (1), then the amount of MK-8 produced by the transformants was calculated.

Table 4 shows the results.

Table 4

MK-8 Production by transformants of *Escherichia coli*

Transformant	Cell amount (OD660)	Amount of MK-8 Produced (mg/L)	Intracellular Content * ¹
<i>E.coli</i> DH5 α /pEG400	23.2	1.1	0.46
<i>E.coli</i> DH5 α /pADO-1	23.5	1.8	0.75

*1: Intracellular content is shown with a value obtained by dividing a 10-fold CoQ8 production amount (mg/L) by a cell amount (OD660).

The amount of MK-8 produced was significantly higher in DH5 α /pADO-1 than in the control DH5 α /pEG400.

(2) *E.coli* DH5 α /pDXS-1 or *E.coli* DH5 α /pEG400, obtained in Example 2 (1), was cultured in the same manner in (1) above, then the amount of MK-8 produced by the transformants was calculated.

Table 5 shows the results.

Table 5

Production of MK-8 by transformants of *Escherichia coli*

Transformant	Cell amount (OD660)	Amount of MK-8 Produced (mg/L)	Intracellular Content * ¹
<i>E.coli</i> DH5 α /pEG400	42.8	2.41	0.56
<i>E.coli</i> DH5 α /pDXS-1	44.0	2.96	0.67

*1: Intracellular content is shown with a value obtained by dividing a 10-fold CoQ8 production (mg/L) by a cell amount (OD660).

The amount of MK-8 produced was significantly higher in DH5 α /pDXS-1 than in the control strain DH5 α /pEG400.

Example 4 Production of CoQ8 by recombinant *Erwinia carotovora*

A plasmid pDXS-1 obtained in Example 1 or pEG400 as a control, was introduced into *Erwinia carotovora* IFO-3380, thereby obtaining transformants IFO-3380/pDXS-1 and IFO-3380/pEG400, both of which were resistant to spectinomycin at a concentration of 100 μ g/ml.

These transformants were inoculated into a test tube containing 10 ml of LB medium supplemented with 100 μ g/ml of spectinomycin, and then cultured with shaking for 72 hours at 30°C.

After the culture was completed, the amount of CoQ8 produced by the transformants was calculated in the same manner as in Example 2 (1).

Table 6 shows the results.

Table 6

CoQ8 Production by transformants of *Erwinia carotovora*

Transformant	Cell amount (OD660)	Amount of CoQ8 Produced (mg/L)	Intracellular Content *1
IFO-3380/pEG400	1.68	0.26	1.5
IFO-3380/pDXS-1	2.48	0.45	1.8

*1: Intracellular content is shown with a value obtained by dividing a 10-fold CoQ8 production (mg/L) by a cell amount (OD660).

The amount of CoQ8 produced was significantly higher in IFO-3380/pDXS-1 than in the control strain IFO-3380/pEG400.

Example 5 Production of ubiquinone and carotenoids by recombinant *Erwinia uredovora*

The plasmids pUCYM-1, pQEDXS-1, pQEYM-1, obtained in Example 1, or pUC19 and pQE30 as controls were introduced into *Erwinia uredovora* DSM-30080 by electroporation, and then the transformants, *E. uredovora* DSM-30080/pUCYM-1, *E. uredovora* DSM-30080/pQEDXS-1, *E. uredovora* DSM-30080/pQEYM-1, *E. uredovora* DSM-30080/pUC19 and *E. uredovora* DSM-30080/pQE30, which showed resistant to ampicillin at a concentration of 100 μ g/ml were obtained.

These transformants were inoculated into a test tube containing 10 ml of LB medium supplemented with 100 μ g/ml of ampicillin, 1 % glucose, vitamin B₁ and vitamin B₆, 100 mg/l each, and 50 mg/l of p-hydroxybenzoic acid. Then the transformants were cultured by shaking for 72 hours at 30°C.

After the culture was completed, the amount of CoQ8 produced by the transformants was calculated in the same manner as in Example 2 (1).

The produced amount of carotenoid pigments was calculated by detecting the absorbance at 450 nm for the 2-butanol layer using a spectrophotometer in the same manner as in Example 2 (1).

Table 7 shows the results.

Table 7

Production of CoQ8 and Carotenoids by transformants of *E. uredovora*

Transformants	Cell amount	CoQ8		Carotenoids	
	OD660	Production mg/L	Intracellular content ratio Relative value	Production Relative value	Intracellular content ratio Relative value
DSM-30080/pUC19	2.00	1.15	1.0	1.0	1.0
DSM-30080/pUCYM-1	1.88	1.39	1.3	1.5	1.6
DSM-30080/pQE30	2.52	1.29	1.0	1.0	1.0
DSM-30080/pQEYM-1	1.92	1.36	1.4	1.7	2.2
DSM-30080/pQEDXS-1	2.12	3.21	3.0	5.6	6.7

Both CoQ8 production and carotenoid pigment production were significantly higher in DSM-30080/pUCYM-1 than in the control strain DSM-30080/pUC19.

Similarly, both CoQ8 production and carotenoid pigment production were significantly higher in DSM-30080/pQEYM-1 and DSM-30080/pQEDXS-1 than in the control strain DSM-30080/pQE30.

Example 6 Cloning of the DNA Encoding Proteins Involved in the Biosynthesis of Isoprenoid Compounds from a Photosynthetic Bacterium *Rhodobacter sphaeroides*

(1) Cloning of DXS Gene from *R. sphaeroides*

The Genbank database was searched for DXS homologue conserved in other species using the DXS nucleotide sequence found in *E.coli*. As a result, DXS homologues were found in *Haemophilus influenzae* (P45205), *Rhodobacter capsulatus* (P26242), *Bacillus subtilis* (P54523), *Synechocystis* sp. PCC6803 (P73067) and *Mycobacterium tuberculosis* (007184) and the like. Highly conserved amino acid sequences were selected by comparison of these sequences. A nucleotide sequence corresponding to such a conserved amino acid sequence was designed in consideration of the codon usage in *R.sphaeroides*. A DNA fragment having a nucleotide sequence of SEQ ID NO:32 and of SEQ ID NO:33, and a DNA fragment having a nucleotide sequence of SEQ ID NO:34 were synthesized by DNA synthesizer.

PCR was carried out with DNA Thermal Cycler (Perkin Elmer Instruments, Inc. Japan) using chromosomal DNA of *R.sphaeroides* KY4113 (FERM-P4675) as a template, the primers above, and an Expand™ High-Fidelity PCR System (Boehringer Mannheim K.K.).

PCR was carried out by 30 cycles, one cycle consisting of reaction at 94°C for 40 seconds, reaction at 60°C for 40 seconds, reaction at 72°C for 1 minute, followed by reaction at 72°C for 1

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minute, thereby obtaining the DNA fragment of interest. The DNA fragments were DIG-labeled using DIG DNA Labeling Kit (Boehringer Mannheim K.K.).

To obtain the full length DXS gene of *R. sphaeroides*, a genomic DNA library of a strain KY4113 was constructed. The strain KY4113 was cultured overnight in LB medium, extracting the chromosomal DNA. The chromosomal DNA was partially digested with a restriction enzyme Sau3AI, and then 4 to 6 kb DNA fragments were purified by sucrose density-gradient centrifugation. The DNA fragments were ligated with BamH I-digested vector pUC19 using a Ligation Pack (Nippon gene), and *E. coli* DH5 α was transformed using the ligated DNA. The transformants were spread on LB agar medium containing 100 μ g/ml of ampicillin, thus obtaining about 10,000 colonies. As a result of screening by colony hybridization using the DIG-labeled DNA fragment as a probe, which had been obtained by the above method, two types of DNA fragments were detected. As a result of sequencing, ORF sharing high degrees of sequence homology with known DXS gene of other species was found from each DNA fragment. An amino acid sequence of SEQ ID NO:26 was named DXS1 and that of SEQ ID NO:27 was named DXS2.

(2) Confirmation of Complementarity using *E. coli* DXS Gene-deleted mutant

① Selection of *E. coli* DXS gene-deleted strain

E. coli W3110 (ATCC14948) was inoculated into LB liquid medium, and then cultured to its logarithmic growth phase. After culturing, cells were collected from the culture by centrifugation.

The cells were washed with 0.05 mol/l Tris-maleate buffer (pH 6.0) and suspended in the same buffer to a cell density of 10^9 cells/ml.

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NTG was added to the suspension to a final concentration of 600 mg/l, then the mixture was maintained for 20 minutes at room temperature to induce mutation.

The resultant NTG-treated cells were spread on a M9
5 minimum agar medium (Molecular Cloning, Second Edition) plate containing 0.1% 1-deoxyxylulose, then cultured. 1-Deoxyxylulose had been chemically synthesized according to the method described in J.C.S. Perkin Trans I, 2131-2137 (1982).

Colonies grew on M9 minimum agar medium containing
10 0.1% 1-deoxyxylulose were replicated on M9 minimal agar medium and on M9 minimal agar medium containing 0.1 % 1-deoxyxylulose. The mutant of interest, a strain requiring 1-deoxyxylulose to grow, was selected. That is, a strain capable of growing on minimal agar medium containing 1-deoxyxylulose but not on the
15 same medium lacking 1-deoxyxylulose was selected.

The thus selected and obtained mutant was named ME1.

When pDXS-1 was introduced into the strain ME1, deficiency in 1-deoxyxylulose of the strain ME1 was complemented. Therefore the strain ME1 was confirmed to be a strain from which DXS gene was deleted.

(3) Complementation Studies on DXS1 and DXS2

DNA fragment encoding DXS1 of SEQ ID NO:27 or a DNA fragment encoding DXS2 of SEQ ID NO:29, respectively, both derived from the strain KY4113, was ligated to downstream of the lac promoter of a vector pUC19 respectively to construct recombinant plasmids.

When the constructed plasmids were introduced into the strain ME1, both DXS1 and DXS 2 each complemented the 1-deoxyxylulose-deficiency in the strain ME 1.

Therefore, *R. sphaeroides* was shown to have two genes, DXS1 and DXS2, having activity to catalyze the reaction to produce 1-deoxy-D-xylulose 5-phosphate from pyruvic acid and glyceraldehyde 3-phosphate.

(4) Cloning of Gene Complementing Methylerythritol-requiring nature derived from *R. sphaeroides*

The *E.coli* Methylerythritol-requiring mutant ME7 obtained in Example 1 (2) ① was inoculated into LB liquid medium containing 0.1% methylerythritol, cultured to its logarithmic growth phase, then centrifuged to collect cells.

The cells were washed twice with 1mol/l HEPES aqueous solution containing 10 % glycerol so as to remove the medium components as far as possible.

Plasmids were extracted from the genomic library of *R. sphaeroides* KY4113 constructed in Example 6 (1). Then the plasmids were introduced into the washed cells by electroporation according to standard techniques.

Next, the cells were spread on LB agar medium containing 100 μ g /l of ampicillin, then cultured overnight at 37°C.

After picking up the colonies grown on the medium, the colonies were inoculated into LB liquid medium to culture, then plasmids were extracted from the cells cultured.

When the plasmids extracted were introduced again into the strain ME 7, the transformants could grow in a medium lacking methylerythritol. Therefore it was confirmed that the plasmid contained a DNA fragment complementing methylerythritol-requiring nature derived from *R. sphaeroides*.

As a result of sequencing of the nucleotide sequence of the DNA fragment, the DNA sequence of

SEQ ID NO:31 encoding an amino acid sequence that shares high homology with *E.coli* yaeM was found.

Example 7 Production of Ubiquinone-10 (CoQ10) by Recombinant Photosynthetic Bacteria

A *glnB* promoter derived from the strain KY4113 was ligated upstream of the DNA fragment DXS1 of SEQ ID NO:27 and DXS2 of SEQ ID NO:29, both obtained in Example 6. Then the product was inserted into a broad host range vector pEG400, thus constructing plasmids. These plasmids were named pRSDX-1 and pRSDX-2, respectively. In addition, yaeM and DXS1 were joined in tandem, then the product was ligated downstream of *glnB* promoter, thereby constructing a plasmid. The plasmid was named pRSYMDX1. These plasmids were introduced into *R. sphaeroides* KY4113, respectively, by electroporation (Bio-Rad Laboratories).

Then the cells were spread on LB agar medium containing spectinomycin at a concentration of 100 μ g/ml, then cultured for 3 days at 30°C.

Next, colonies that grew on the medium were inoculated into LB medium containing spectinomycin at a concentration of 100 μ g/ml, cultured overnight. Then, the cultured cells were collected by centrifugation.

It was confirmed that the cells of each strain contained the introduced plasmid by extracting the plasmids from the cells (Qiagen, Inc). Thus obtained transformants were named KY4113/pRSDX-1, KY4113/pRSDX-2, KY4113/pRSYMDX1 and KY4113/pEG400.

A platinum loop of each transformant was inoculated into a test tube containing 5 ml of seed medium (2 % glucose, 1 % peptone, 1 % yeast extract, 0.5 % NaCl, pH 7.2 adjusted with NaOH) and then cultured for 24 hours at 30°C.

0.5ml of the resultant culture was inoculated into a test tube containing 5 ml of ubiquinone-10 production medium, then cultured by shaking for 5 days at 30°C.

The ubiquinone-10 production medium consisted of 4 % blackstrap molasses, 2.7 % glucose, 4 % corn steep liquor, 0.8 % ammonium sulfate, 0.05 % potassium primary phosphate, 0.05 % potassium secondary phosphate, 0.025 % magnesium sulfate heptahydrate, 3 mg/l of ferrous sulfate heptahydrate, 8 mg/l of thiamine, 8 mg/l of nicotinic acid, and 1ml/l of trace element, had previously been adjusted to pH 9, supplemented with 1 % calcium carbonate, then autoclaved.

Then the amount of CoQ10 produced by the transformants was calculated in the same

manner as in quantification of CoQ8 in Example 2 (1). Table 8 shows the results.

Table 8

	Cell amount [OD660]	Amount of CoQ10 Accumulated [mg/l]
KY4113/pEG400	23.7	65.2
KY4113/pRSDX-1	23	81
KY4113/pRSDX-2	24.4	81.9
KY4113/pRSYMDX1	25.8	117.9

The amount of CoQ10 produced was significantly higher in KY4113/pRSDX-1, KY4113/pRSDX-2 and KY4113/pRSYMDX1 than in the control strain KY4113/pEG400.

Example 8 Determination of the Activity of the Enzyme Encoded by *yaeM* Gene

(1) Overexpression of *yaeM* Gene

A recombinant plasmid that can express *yaeM* gene sufficiently was constructed using PCR [Science, 230, 1350 (1985)], as follows.

A sense primer having a nucleotide sequence of SEQ ID NO:24 and an antisense primer having a nucleotide sequence of SEQ ID NO:25 were synthesized using a DNA synthesizer.

A restriction enzyme BamH I site was added to each of 5'-ends of the sense and antisense primers.

yaeM gene was amplified by PCR using chromosomal DNA of *E.coli* as a template, these primers, Tag DNA polymerase (Boehringer), and DNA Thermal cycler (Perkin Elmer Japan).

PCR was carried out by 30 cycles, one cycle consisting of reaction at 94°C for 30 seconds, reaction at 55°C for 30 seconds, and reaction at 72°C for 2 minutes followed by reaction at 72°C for 7 minutes.

The amplified DNA fragments and pUC118 (TAKARA SHUZO Co., Ltd.) were digested with a restriction enzyme BamH I, then each DNA fragment was purified by agarose gel electrophoresis.

Both purified fragments were mixed together, then treated with ethanol, allowing DNA to precipitate. The resultant DNA precipitate was dissolved in 5 μ l of distilled water for ligation reaction to occur, thereby obtaining recombinant DNA.

The recombinant DNA was confirmed to be *yaeM* gene by determining its DNA sequence.

Plasmids were extracted from the microorganism having the recombinant DNA, digested with a restriction enzyme BamH I, and subjected to agarose gel electrophoresis, thereby obtaining DNA

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fragments containing BamH I-treated *yaem* gene.

pQE30 (Qiagen, Inc) was digested with a restriction enzyme BamH I, then subjected to agarose gel electrophoresis, thereby obtaining BamH I-treated pQE30 fragments.

The resultant DNA fragments containing BamH I-treated *yaem* gene were mixed with BamH I-digested pQE30 fragments, and treated with ethanol for DNA to precipitate. The DNA precipitate was dissolved in 5 μ l of distilled water for ligation reaction to occur, thereby obtaining recombinant DNA.

E. coli JM109 was transformed using the recombinant DNA by standard techniques. Then the transformants were spread on LB agar medium containing 100 μ g/ml of ampicillin, then cultured overnight at 37°C.

Plasmids were isolated from the *E.coli* in the same manner as described above.

Similarly, the isolated plasmid was cleaved with various restriction enzymes to examine the structure, then the nucleotide sequence was determined, thereby confirming the plasmids contained the DNA fragments of interest. The plasmid was named pQEDXR.

(2) Determination of Activity of *yaem* Gene Product

① Purification of *yaem* gene product

The pQEDXR constructed in (1) was introduced into *E.coli* M15 (Qiagen, Inc) having pREP4 by standard techniques, and a strain M15/pREP4+pQEDXR resistant to 200 μ g/ml of ampicillin and 25 μ g/ml of kanamycin was obtained.

The strain M15/pREP4+pQEDXR was cultured at 37°C in 100 ml of LB liquid medium containing 200 μ g/ml of ampicillin and 25 μ g/ml of kanamycin. When the turbidity at 660nm reached 0.8, isopropyl thiogalactoside was added to a final concentration of 0.2 mol/l. Subsequently, the strain was cultured for 5 hours at 37°C, then the supernatant of the culture was removed by centrifugation (3000 rpm, 10 minutes). The cells were suspended in 6 ml of 100 mol/l Tris-hydrochloric acid buffer (pH 8.0), then disrupted using a ultrasonicator (SONIFIER, BRANSON) while cooling with ice. The obtained cell-disrupted solution was centrifuged at 10,000 rpm for 20 minutes at 4°C, thereby collecting the supernatant. The supernatant centrifuged from the cellular extract was introduced into a Ni-NTA resin column (Qiagen, Inc), then washed with 20 ml of a washing buffer (100 mol/l Tris-hydrochloric acid (pH 8.0), 50 mol/l imidazole, 0.5 % Tween 20TM). Then 10 ml of an elution buffer (100 mol/l Tris-hydrochloric acid (pH 8.0), 200 mol/l imidazole) was introduced into the column,

thus fractionating the eluate into 1 ml each.

Protein amounts for each fraction were measured using a kit for quantifying protein amount (Bio-Rad Laboratories), thus obtaining a fraction containing proteins as a purified protein fraction.

② Preparation of a Substrate, 1-deoxy-D-xylulose 5-phosphate

A reaction substrate, 1-deoxy-D-xylulose 5-phosphate was prepared as described below. 1-Deoxy-D-xylulose 5-phosphate was detected by measuring the absorbance at 195 nm using HPLC [Column: Senshu pak NH2-1251-N (4.6 x 250 mm, Senshu), mobile phase: 100 mol/l KH_2PO_4 (pH 3.5)].

The plasmid pQDXS-1 that allows overexpression of *E.coli* *dxs* gene was introduced into *E.coli* M15/pREP4 in the same manner as described above, obtaining a strain M15/pREP4+pQDXS-1.

This strain was cultured in the same way as in Example 8 (2)①, then *dxs* protein was purified using Ni-NTA resin column.

The purified *dxs* protein was added to a 20 ml of reaction solution [100 mol/l Tris- hydrochloric acid (pH 7.5), 10 mol/l sodium pyruvate, 30mol/l DL-glyceraldehyde-3-phosphate, 1.5 mol/l thiamine pyruvate, 10 mol/l MgCl_2 , 1mol/l DL-dithiothreitol] then maintained at 37°C.

After reacting for 12 hours, the reaction solution was diluted with water to 300 ml, introduced into an activated carbon column (2.2 x 8 cm) followed by a Dowex 1-X8 (C1-type, 3.5 x 25 cm), then eluted with 1 % saline solution. After the eluted fraction was concentrated, the fraction was introduced into Sephadex G-10 (1.8 x 100 cm), then eluted with water. Finally fractions containing 1-deoxy-D-xylulose 5-phosphate were freeze-dried, thereby obtaining about 50 mg of white powder.

This powder was confirmed to be 1-deoxy-D-xylulose 5-phosphate by NMR analysis (A-500, JEOL Ltd.).

③ Determination of Enzymatic Activity of *yaeM* Gene Product

0.3 mol/l of 1-deoxy-D-xylulose 5-phosphate (final concentration) synthesized as described above was added to 1 ml of a reaction solution containing 100 mol/l Tris-hydrochloric acid (pH 7.5), 1 mol/l MnCl_2 , 0.3 mol/l NADPH and *yaeM* gene product obtained in Example 8 (2)①, and then incubated at 37°C. The increase and decrease in NADPH during incubation was traced by reading the absorbance at 340 nm using a spectrophotometer (UV-160, SHIMADZU CORP.), suggesting that NADPH decreased with time.

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To confirm the structure of the reaction product, the reaction was carried out similarly, but on a larger scale, thus isolating the product. 200 ml of a reaction solution with a composition the same as that described above except that the concentration of 1-deoxy-D-xylulose 5-phosphate was 0.15mol/L, was incubated for 30 minutes at 37°C. Then the whole amount of the reaction solution was added to an activated carbon column, diluted with water to 1L, then added to a Dowex^{*} 1-X8 (C1-type, 3.5 x 20 cm) column.

The solution was eluted with 400 ml of 1 % saline solution, added to a Sephadex^{*} G-10 (1.8 x 100 cm), then eluted with water. The eluted fraction was freeze-dried, thereby isolating the reaction product.

10 The molecular formula of the reaction product isolated from HR-FABMS analysis was assumed to be $C_5H_{12}O_7P$ [m/z 215.0276 (M-H)⁺, $\Delta - 4.5$ mmu]. NMR analysis for ^1H and ^{13}C resulted in the following chemical shifts.

^1H NMR (D_2O , 500 MHz): δ 4.03 (ddd, $J = 11.5, 6.5, 2.5$ Hz, 1H), 3.84 (ddd, $J = 11.5, 8.0, 6.5$ Hz, 1H), 3.78 (dd, $J = 8.0, 2.5$ Hz, 1H), 3.60 (d, $J = 12.0$ Hz, 1H), 3.50 (d, $J = 12.0$ Hz, 1H), 1.15 (s, 3H); ^{13}C NMR (D_2O , 125 MHz): δ 75.1 (C-2), 74.8 (C-3), 67.4 (C-1), 65.9 (C-4), 19.4 (2-Me)

The chemical shifts resulted from NMR analysis for ^1H and ^{13}C of compounds obtained by treating the reaction products with alkaline phosphatase (TAKARA SHUZO CO., LTD.) were completely identical with that resulted from NMR analysis of 2-C-methyl-D-erythritol synthesized in the method described in Tetrahedron Letter, 38, 6184 (1997).

20 Further the angle of rotation of the former compound was $[\alpha]_D^{21} = + 6.0$ ($c = 0.050$, H_2O), identical with the angle of rotation $[\alpha]_D^{25} = + 7.0$ ($c = 0.13$, H_2O) of 2-C-methyl-D-erythritol, reported in Tetrahedron Letter, 38, 6184 (1997).

These results reveal that the reaction product of yaeM gene product was 2-C-methyl-D-erythritol 4-phosphate. That is, yaeM gene product was found to have activity to yield 2-C-methyl-D-erythritol 4-phosphate from 1-deoxy-D-xylulose 5-phosphate with consumption of NADPH. Based on this catalytic activity, this enzyme was named 1-deoxy-D-xylulose 5-phosphate reductoisomerase.

④ Characteristics of 1-deoxy-D-xylulose 5-phosphate reductoisomerase

The enzymological characteristics of 1-deoxy-D-xylulose 5-phosphate reductoisomerase were examined using 1 ml of the reaction system as described in Example 8 (2) ③. Here, 1 unit is defined

*Trade-mark

as the activity to oxidize 1 mmol of NADPH per a minute.

The activity decreased below 1/100 when NADPH was replaced with NADH.

No reaction occurred when 1-deoxy-D-xylulose was used instead of 1-deoxy-D-xylulose 5-phosphate.

SDS-PAGE analysis showed that this enzyme was consisted of 42 kDa polypeptide.

Table 9 shows effect on the reaction system by the addition of metals.

Table 9

Effect of various metal ions on the activity of
1-deoxy-D-xylulose 5-phosphate reductoisomerase

Additives	Specific Activity (units / mg protein)
none	0.3
EDTA	N.D.
MnCl ₂	11.8
CoCl ₂	6.0
MgCl ₂	4.0
CaCl ₂	0.2
NiSO ₄	0.2
ZnSO ₄	0.3
CuSO ₄	N.D.
FeSO ₄	N.D.

These metal ions and EDTA were added such that the concentration of each was 1mol/l. N.D. indicates that no activity was detected.

K_m for 1-deoxy-D-xylulose 5-phosphate and NADP in the presence of MnCl₂ were 249 μ mol/l and 7.4 μ mol/l, respectively.

Figure 1 shows the effect of reaction temperature and Figure 2 shows the effect of reaction pH.

Example 9 Construction and Characteristics of yaeM - deleted mutant

(1) Construction of yaeM - disrupted mutant

To test whether 1-deoxy-D-xylulose 5-phosphate reductoisomerase is essential for cell growth or not, a 1-deoxy-D-xylulose 5-phosphate reductoisomerase-deleted mutant was constructed as described below.

A kanamycin-resistant gene cassette for insertion into yaeM gene was produced as described below.

The plasmid pMEW41 obtained in Example 1 (2) ② was digested with a restriction enzyme Bal

I, and was subjected to agarose gel electrophoresis, thereby obtaining a Bal I – treated DNA fragment.

Tn5 was digested with restriction enzymes Hind III and Sam I, then the both ends were blunt-ended using a DNA blunting kit (TAKARA SHUZO CO., LTD.).

The resultant blunt-ended DNA fragments were mixed with previously obtained Bal I-treated pMEW41DNA fragments, and then the mixture was treated with ethanol. Next the obtained DNA precipitate was dissolved into 5 μ l of distilled water for ligation reaction to occur, thereby obtaining recombinant DNA.

E. coli JM109 (purchased from TAKARA SHUZO CO., LTD.) was transformed using this recombinant DNA according to standard techniques. Next the transformant was spread on LB agar medium containing 100 μ g/ml of ampicillin and 15 μ g/ml of kanamycin, then cultured overnight at 37°C.

Several ampicillin-resistant transformant colonies grown on the medium were shake-cultured for 16 hours at 37°C in 10 ml of LB liquid medium containing 100 μ g/ml of ampicillin and 15 μ g/ml of kanamycin.

The resulting culture was centrifuged to collect cells.

Plasmids were isolated from the cells according to the standard techniques.

The plasmids isolated as described above were cleaved with various restriction enzymes to test their structure. As a result, the plasmid was confirmed to contain the DNA fragment of interest and was named pMEW41Km.

yaem gene on a chromosomal DNA of *E. coli* was disrupted by homologous recombination using pMEW41Km. Figure 3 shows the schematic diagram for this recombination.

pMEW41Km was digested with restriction enzymes Hind III and Sac I, subjected to agarose gel electrophoresis, thus purifying linear fragments. *E. coli* FS1576 was transformed using the fragments according to standard techniques. The strain FS1576 is available as the strain ME9019 from National Institute of Genetics. The transformants were spread on LB agar medium containing 15 μ g/ml of kanamycin and 1 g/l of 2-C-methyl-D-erythritol, then cultured overnight at 37°C.

Several kanamycin-resistant colonies that grew on the medium were shake-cultured for 16 hours at 37°C in 10 ml of LB liquid medium containing 15 μ g/ml of kanamycin and 1 g/l of 2-C-methyl-D-erythritol.

The resulting culture was centrifuged to collect cells.

Chromosomal DNA was isolated from the cells by the standard techniques.

The chromosomal DNA was digested with a restriction enzyme Sma I or Pst I. Chromosomal DNA of the strain FS1576 was digested with a restriction enzyme in the same way. These DNAs digested with restriction enzymes were subjected to agarose gel electrophoresis by the standard techniques, and then to Southern hybridization analysis using the kanamycin-resistant gene and yaeM gene as probes. Therefore, it was confirmed that the chromosomal DNA of the kanamycin-resistant colonies had a structure as shown in Fig. 3, that is, yaeM gene was disrupted by the kanamycin-resistant gene.

(2) Characteristics of yaeM-disrupted mutant

The yaeM-disrupted strain produced as described above and its parent strain FS1576 were spread on LB agar medium and the same medium containing 1 g/l of 2-C-methyl-D-erythritol, then cultured at 37°C. Table 10 shows the cell growth after 2 days of culture.

Table 10

Effect of deletion of yaeM gene on the *E.coli* growth

Strain	Cell growth on each medium * ¹	
	LB	LB + ME * ²
FS1576	+	+
yaeM-deleted strain	-	+

*1: Cell growth (+ indicates good growth; - indicates no growth)

*2: ME indicates the addition of 1 g/l of 2-C-methyl-D-erythritol.

No yaeM-deleted mutants grew on a medium lacking 2-C-methyl-D-erythritol. Therefore, This gene was shown to be essential for the cell growth in the absence of 2-C-methyl-D-erythritol.

Example 10 Effect of 1-deoxy-D-xylulose 5-phosphate reductoisomerase inhibitor for cell growth.

The following experiments were conducted based on the assumption that fosmidomycin could inhibit 1-deoxy-D-xylulose 5-phosphate reductoisomerase because 2-C-methyl-D-erythritol 4-phosphate, a product from 1-deoxy-D-xylulose 5-phosphate reductoisomerase reaction, or reaction intermediates expected to be produced in this enzyme reaction is structurally analogous to fosmidomycin.

In the presence of fosmidomycin, the activity 1-deoxy-D-xylulose 5-phosphate reductoisomerase was measured by the method as described in Example 8 in order to examine the effect on the enzymatic

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activity.

Fosmidomycin had been synthesized according to the method described in Chem. Pharm. Bull., 30, 111-118 (1982).

Total volume of reaction solution was reduced to 0.2 ml from the volume of reaction solution described in Example 8 (2), but each concentration was kept at the same level as the system of Example 8 (3). Fosmidomycin at various concentration was added to the reaction solution, then the reaction was carried out at 37°C. The increase and decrease in NADPH were measured using Bench^{*} mark micro plate reader (Bio-Rad Laboratories).

As shown in Fig. 4, fosmidomycin was shown to inhibit 1-deoxy-D-xylulose 5-phosphate reductoisomerase.

E. coli W3110 was spread on LB agar medium, the same medium containing 3.13 mg/l of fosmidomycin, and the same medium containing 3.13 mg/l of fosmidomycin and 0.25 g/l of 2-C-methyl-D-erythritol, then cultured at 37°C.

Two days after culturing, the microorganism could grow on the two types of media, that is, the LB agar medium and the same medium containing fosmidomycin and 0.25 g/l of 2-C-methyl-D-erythritol, but no microorganism grew on the LB agar medium supplemented only with fosmidomycin.

These results clearly shows that fosmidomycin inhibited the cell growth by inhibiting 1-deoxy-D-xylulose 5-phosphate reductoisomerase. Accordingly, a substance inhibiting yaeM gene product (1-deoxy-D-xylulose 5-phosphate reductoisomerase) activity can be an effective antibiotic agent or herbicide.

Industrial Applicability

The present invention can provide a process for producing isoprenoid compounds comprising integrating DNA into a vector wherein the DNA contains one or more DNA involved in biosynthesis of isoprenoid compounds useful in pharmaceuticals for cardiac diseases, osteoporosis, homeostasis, prevention of cancer, and immunopotential, health food and anti-fouling paint products against

^{*}Trade-mark

barnacles, introducing the resultant recombinant DNA into a host cell derived from prokaryote, culturing the obtained transformants in a medium, allowing the transformant to produce and accumulate isoprenoid compounds in the culture, and recovering the isoprenoid compounds from the culture; a process for producing a protein having activity to improve efficiency in the biosynthesis of isoprenoid compounds comprising integrating DNA containing one or more DNA encoding the protein into a vector, introducing the resultant recombinant DNA into a host cell, culturing the obtained transformant in a medium, allowing the transformant to produce and accumulate said protein in the culture, and recovering said protein from the culture; the protein; and novel enzymatic protein having activity to catalyze a reaction to produce 2-C-methyl-D-erythritol 4-phosphate from 1-deoxy-D-xylulose 5-phosphate; and a method for screening a compound with antibiotic and/or weeding activity comprising screening a substance inhibiting the enzyme.

Sequence Listing Free Text

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 SEQ ID NO: 13: synthetic DNA
 SEQ ID NO: 14: synthetic DNA
 SEQ ID NO: 15: synthetic DNA
 SEQ ID NO: 16: synthetic DNA
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 SEQ ID NO: 18: synthetic DNA
 SEQ ID NO: 19: synthetic DNA
 SEQ ID NO: 20: synthetic DNA
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SEQ ID NO: 34: synthetic DNA

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<120> A process for producing isoprenoid compounds by microorganisms and
a method for screening compounds with antibiotic or weeding activity

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85 90 95

acc atc cgt cag aaa ggc ggt ctg cac ccg ttc ccg tgg cgc ggc gaa 336

Thr Ile Arg Gln Lys Gly Gly Leu His Pro Phe Pro Trp Arg Gly Glu
100 105 110

agc gaa tat gac gta tta agc gtc ggg cat tca tca acc tcc atc agt 384

Ser Glu Tyr Asp Val Leu Ser Val Gly His Ser Ser Thr Ser Ile Ser
115 120 125

gcc gga att ggt att gcg gtt gct gcc gaa aaa gaa ggc aaa aat cgc 432

Ala Gly Ile Gly Ile Ala Val Ala Ala Glu Lys Glu Gly Lys Asn Arg
130 135 140

cgc acc gtc tgt gtc att ggc gat ggc gcg att acc gca ggc atg gcg 480

Arg Thr Val Cys Val Ile Gly Asp Gly Ala Ile Thr Ala Gly Met Ala

145	150	155	160	
ttt gaa gcg atg aat cac gcg ggc gat atc cgt cct gat atg ctg gtg				528
Phe Glu Ala Met Asn His Ala Gly Asp Ile Arg Pro Asp Met Leu Val				
	165	170	175	
att ctc aac gac aat gaa atg tcg att tcc gaa aat gtc ggc gcg ctc				576
Ile Leu Asn Asp Asn Glu Met Ser Ile Ser Glu Asn Val Gly Ala Leu				
	180	185	190	
aac aac cat ctg gca cag ctg ctt tcc ggt aag ctt tac tct tca ctg				624
Asn Asn His Leu Ala Gln Leu Leu Ser Gly Lys Leu Tyr Ser Ser Leu				
	195	200	205	
cgc gaa ggc ggg aaa aaa gtt ttc tct ggc gtg ccg cca att aaa gag				672
Arg Glu Gly Gly Lys Lys Val Phe Ser Gly Val Pro Pro Ile Lys Glu				
	210	215	220	
ctg ctc aaa cgc acc gaa gaa cat att aaa ggc atg gta gtg cct ggc				720
Leu Leu Lys Arg Thr Glu Glu His Ile Lys Gly Met Val Val Pro Gly				
225	230	235	240	
acg ttg ttt gaa gag ctg ggc ttt aac tac atc ggc ccg gtg gac ggt				768
Thr Leu Phe Glu Glu Leu Gly Phe Asn Tyr Ile Gly Pro Val Asp Gly				
	245	250	255	
cac gat gtg ctg ggc ctt atc acc acg cta aag aac atg cgc gac ctg				816

His Asp Val Leu Gly Leu Ile Thr Thr Leu Lys Asn Met Arg Asp Leu
 260 265 270

aaa ggc ccg cag ttc ctg cat atc atg acc aaa aaa ggt cgt ggt tat 864

Lys Gly Pro Gln Phe Leu His Ile Met Thr Lys Lys Gly Arg Gly Tyr
 275 280 285

gaa ccg gca gaa aaa gac ccg atc act ttc cac gcc gtg cct aaa ttt 912

Glu Pro Ala Glu Lys Asp Pro Ile Thr Phe His Ala Val Pro Lys Phe
 290 295 300

gat ccc tcc agc ggt tgt ttg ccg aaa agt agc ggc ggt ttg ccg agc 960

Asp Pro Ser Ser Gly Cys Leu Pro Lys Ser Ser Gly Gly Leu Pro Ser
 305 310 315 320

tat tca aaa atc ttt ggc gac tgg ttg tgc gaa acg gca gcg aaa gac 1008

Tyr Ser Lys Ile Phe Gly Asp Trp Leu Cys Glu Thr Ala Ala Lys Asp
 325 330 335

aac aag ctg atg gcg att act ccg gcg atg cgt gaa ggt tcc ggc atg 1056

Asn Lys Leu Met Ala Ile Thr Pro Ala Met Arg Glu Gly Ser Gly Met
 340 345 350

gtc gag ttt tca cgt aaa ttc ccg gat cgc tac ttc gac gtg gca att 1104

Val Glu Phe Ser Arg Lys Phe Pro Asp Arg Tyr Phe Asp Val Ala Ile
 355 360 365

gcc gag caa cac gcg gtg acc ttt gct gcg ggt ctg gcg att ggt ggg 1152

Ala Glu Gln His Ala Val Thr Phe Ala Ala Gly Leu Ala Ile Gly Gly
370 375 380

tac aaa ccc att gtc gcg att tac tcc act ttc ctg caa cgc gcc tat 1200

Tyr Lys Pro Ile Val Ala Ile Tyr Ser Thr Phe Leu Gln Arg Ala Tyr
385 390 395 400

gat cag gtg ctg cat gac gtg gcg att caa aag ctt ccg gtc ctg ttc 1248

Asp Gln Val Leu His Asp Val Ala Ile Gln Lys Leu Pro Val Leu Phe
405 410 415

gcc atc gac cgc gcg ggc att gtt ggt gct gac ggt caa acc cat cag 1296

Ala Ile Asp Arg Ala Gly Ile Val Gly Ala Asp Gly Gln Thr His Gln
420 425 430

ggt gct ttt gat ctc tct tac ctg cgc tgc ata ccg gaa atg gtc att 1344

Gly Ala Phe Asp Leu Ser Tyr Leu Arg Cys Ile Pro Glu Met Val Ile
435 440 445

atg acc ccg agc gat gaa aac gaa tgt cgc cag atg ctc tat acc ggc 1392

Met Thr Pro Ser Asp Glu Asn Glu Cys Arg Gln Met Leu Tyr Thr Gly
450 455 460

tat cac tat aac gat ggc ccg tca gcg gtg cgc tac ccg cgt ggc aac 1440

Tyr His Tyr Asn Asp Gly Pro Ser Ala Val Arg Tyr Pro Arg Gly Asn
 465 470 475 480

gcg gtc ggc gtg gaa ctg acg ccg ctg gaa aaa cta cca att ggc aaa 1488

Ala Val Gly Val Glu Leu Thr Pro Leu Glu Lys Leu Pro Ile Gly Lys
 485 490 495

ggc att gtg aag cgt cgt ggc gag aaa ctg gcg atc ctt aac ttt ggt 1536

Gly Ile Val Lys Arg Arg Gly Glu Lys Leu Ala Ile Leu Asn Phe Gly
 500 505 510

acg ctg atg cca gaa gcg gcg aaa gtc gcc gaa tcg ctg aac gcc acg 1584

Thr Leu Met Pro Glu Ala Ala Lys Val Ala Glu Ser Leu Asn Ala Thr
 515 520 525

ctg gtc gat atg cgt ttt gtg aaa ccg ctt gat gaa gcg tta att ctg 1632

Leu Val Asp Met Arg Phe Val Lys Pro Leu Asp Glu Ala Leu Ile Leu
 530 535 540

gaa atg gcc gcc agc cat gaa gcg ctg gtc acc gta gaa gaa aac gcc 1680

Glu Met Ala Ala Ser His Glu Ala Leu Val Thr Val Glu Glu Asn Ala
 545 550 555 560

att atg ggc ggc gca ggc agc ggc gtg aac gaa gtg ctg atg gcc cat 1728

Ile Met Gly Gly Ala Gly Ser Gly Val Asn Glu Val Leu Met Ala His
 565 570 575

cgt aaa cca gta ccc gtg ctg aac att ggc ctg ccg gac ttc ttt att 1776

Arg Lys Pro Val Pro Val Leu Asn Ile Gly Leu Pro Asp Phe Phe Ile
580 585 590

ccg caa gga act cag gaa gaa atg cgc gcc gaa ctc ggc ctc gat gcc 1824

Pro Gln Gly Thr Gln Glu Glu Met Arg Ala Glu Leu Gly Leu Asp Ala
595 600 605

gct ggt atg gaa gcc aaa atc aag gcc tgg ctg gca 1860

Ala Gly Met Glu Ala Lys Ile Lys Ala Trp Leu Ala
610 615 620

<210> 7

<211> 897

<212> DNA

<213> Escherichia coli

<220>

<221> CDS

<222> (1).. (897)

<400> 7

atg gac ttt ccg cag caa ctc gaa gcc tgc gtt aag cag gcc aac cag 48

Met Asp Phe Pro Gln Gln Leu Glu Ala Cys Val Lys Gln Ala Asn Gln
1 5 10 15

gcg ctg agc cgt ttt atc gcc cca ctg ccc ttt cag aac act ccc gtg 96

Ala Leu Ser Arg Phe Ile Ala Pro Leu Pro Phe Gln Asn Thr Pro Val
 20 25 30

gtc gaa acc atg cag tat ggc gca tta tta ggt ggt aag cgc ctg cga 144

Val Glu Thr Met Gln Tyr Gly Ala Leu Leu Gly Gly Lys Arg Leu Arg
 35 40 45

cct ttc ctg gtt tat gcc acc ggt cat atg ttc ggc gtt agc aca aac 192

Pro Phe Leu Val Tyr Ala Thr Gly His Met Phe Gly Val Ser Thr Asn
 50 55 60

acg ctg gac gca ccc gct gcc gcc gtt gag tgt atc cac gct tac tca 240

Thr Leu Asp Ala Pro Ala Ala Ala Val Glu Cys Ile His Ala Tyr Ser
 65 70 75 80

tta att cat gat gat tta ccg gca atg gat gat gac gat ctg cgt cgc 288

Leu Ile His Asp Asp Leu Pro Ala Met Asp Asp Asp Asp Leu Arg Arg
 85 90 95

ggt ttg cca acc tgc cat gtg aag ttt ggc gaa gca aac gcg att ctc 336

Gly Leu Pro Thr Cys His Val Lys Phe Gly Glu Ala Asn Ala Ile Leu
 100 105 110

gct ggc gac gct tta caa acg ctg gcg ttc tcg att tta agc gat gcc 384

Ala Gly Asp Ala Leu Gln Thr Leu Ala Phe Ser Ile Leu Ser Asp Ala
 115 120 125

gat atg ccg gaa gtg tcg gac cgc gac aga att tcg atg att tct gaa 432

Asp Met Pro Glu Val Ser Asp Arg Asp Arg Ile Ser Met Ile Ser Glu
130 135 140

ctg gcg agc gcc agt ggt att gcc gga atg tgc ggt ggt cag gca tta 480

Leu Ala Ser Ala Ser Gly Ile Ala Gly Met Cys Gly Gly Gln Ala Leu
145 150 155 160

gat tta gac gcg gaa ggc aaa cac gta cct ctg gac gcg ctt gag cgt 528

Asp Leu Asp Ala Glu Gly Lys His Val Pro Leu Asp Ala Leu Glu Arg
165 170 175

att cat cgt cat aaa acc ggc gca ttg att cgc gcc gcc gtt cgc ctt 576

Ile His Arg His Lys Thr Gly Ala Leu Ile Arg Ala Ala Val Arg Leu
180 185 190

ggt gca tta agc gcc gga gat aaa gga cgt cgt gct ctg ccg gta ctc 624

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

gac aag tat gca gag agc atc ggc ctt gcc ttc cag gtt cag gat gac 672

Asp Lys Tyr Ala Glu Ser Ile Gly Leu Ala Phe Gln Val Gln Asp Asp
210 215 220

atc ctg gat gtg gtg gga gat act gca acg ttg gga aaa cgc cag ggt 720

Ile Leu Asp Val Val Gly Asp Thr Ala Thr Leu Gly Lys Arg Gln Gly

225	230	235	240	
gcc gac cag caa ctt ggt aaa agt acc tac cct gca ctt ctg ggt ctt				768
Ala Asp Gln Gln Leu Gly Lys Ser Thr Tyr Pro Ala Leu Leu Gly Leu				
	245	250	255	
gag caa gcc cgg aag aaa gcc cgg gat ctg atc gac gat gcc cgt cag				816
Glu Gln Ala Arg Lys Lys Ala Arg Asp Leu Ile Asp Asp Ala Arg Gln				
	260	265	270	
tcg ctg aaa caa ctg gct gaa cag tca ctc gat acc tcg gca ctg gaa				864
Ser Leu Lys Gln Leu Ala Glu Gln Ser Leu Asp Thr Ser Ala Leu Glu				
	275	280	285	
gcg cta gcg gac tac atc atc cag cgt aat aaa				897
Ala Leu Ala Asp Tyr Ile Ile Gln Arg Asn Lys				
	290	295		

<210> 8

<211> 240

<212> DNA

<213> Escherichia coli

<220>

<221> CDS

<222> (1).. (240)

<400> 8

atg ccg aag aaa aat gag gcg ccc gcc agc ttt gaa aag gcg ctg agc 48

Met Pro Lys Lys Asn Glu Ala Pro Ala Ser Phe Glu Lys Ala Leu Ser

1 5 10 15

gag ctg gaa cag att gta acc cgt ctg gaa agt ggc gac ctg ccg ctg 96

Glu Leu Glu Gln Ile Val Thr Arg Leu Glu Ser Gly Asp Leu Pro Leu

20 25 30

gaa gag gcg ctg aac gag ttc gaa cgc ggc gtg cag ctg gca cgt cag 144

Glu Glu Ala Leu Asn Glu Phe Glu Arg Gly Val Gln Leu Ala Arg Gln

35 40 45

ggg cag gcc aaa tta caa caa gcc gaa cag cgc gta caa att ctg ctg 192

Gly Gln Ala Lys Leu Gln Gln Ala Glu Gln Arg Val Gln Ile Leu Leu

50 55 60

tct gac aat gaa gac gcc tct cta acc cct ttt aca ccg gac aat gag 240

Ser Asp Asn Glu Asp Ala Ser Leu Thr Pro Phe Thr Pro Asp Asn Glu

65 70 75 80

<210> 9

<211> 1044

<212> DNA

<213> Escherichia coli

<220>

<221> CDS

<222> (1)... (1044)

<400> 9

gtg act ggg gtg aac gaa tgc agc cgc agc aca tgc aac ttg aag tat 48

Val Thr Gly Val Asn Glu Cys Ser Arg Ser Thr Cys Asn Leu Lys Tyr

1

5

10

15

gac gag tat agc agg agt ggc agc atg caa tac aac ccc tta gga aaa 96

Asp Glu Tyr Ser Arg Ser Gly Ser Met Gln Tyr Asn Pro Leu Gly Lys

20

25

30

acc gac ctt cgc gtt tcc cga ctt tgc ctc ggc tgt atg acc ttt ggc 144

Thr Asp Leu Arg Val Ser Arg Leu Cys Leu Gly Cys Met Thr Phe Gly

35

40

45

gag cca gat cgc ggt aat cac gca tgg aca ctg ccg gaa gaa agc agc 192

Glu Pro Asp Arg Gly Asn His Ala Trp Thr Leu Pro Glu Glu Ser Ser

50

55

60

cgt ccc ata att aaa cgt gca ctg gaa ggc ggc ata aat ttc ttt gat 240

Arg Pro Ile Ile Lys Arg Ala Leu Glu Gly Gly Ile Asn Phe Phe Asp

65

70

75

80

acc gcc aac agt tat tct gac ggc agc agc gaa gag atc gtc ggt cgc 288

Thr Ala Asn Ser Tyr Ser Asp Gly Ser Ser Glu Glu Ile Val Gly Arg

85

90

95

gca ctg cgg gat ttc gcc cgt cgt gaa gac gtg gtc gtt gcg acc aaa 336

Ala Leu Arg Asp Phe Ala Arg Arg Glu Asp Val Val Val Ala Thr Lys
100 105 110

gtg ttc cat cgc gtt ggt gat tta ccg gaa gga tta tcc cgt gcg caa 384

Val Phe His Arg Val Gly Asp Leu Pro Glu Gly Leu Ser Arg Ala Gln
115 120 125

att ttg cgc tct atc gac gac agc ctg cga cgt ctc ggc atg gat tat 432

Ile Leu Arg Ser Ile Asp Asp Ser Leu Arg Arg Leu Gly Met Asp Tyr
130 135 140

gtc gat atc ctg caa att cat cgc tgg gat tac aac acg ccg atc gaa 480

Val Asp Ile Leu Gln Ile His Arg Trp Asp Tyr Asn Thr Pro Ile Glu
145 150 155 160

gag acg ctg gaa gcc ctc aac gac gtg gta aaa gcc ggg aaa gcg cgt 528

Glu Thr Leu Glu Ala Leu Asn Asp Val Val Lys Ala Gly Lys Ala Arg
165 170 175

tat atc ggc gcg tca tca atg cac gct tcg cag ttt gct cag gca ctg 576

Tyr Ile Gly Ala Ser Ser Met His Ala Ser Gln Phe Ala Gln Ala Leu
180 185 190

gaa ctc caa aaa cag cac ggc tgg gcg cag ttt gtc agt atg cag gat 624

Glu Leu Gln Lys Gln His Gly Trp Ala Gln Phe Val Ser Met Gln Asp

195	200	205	
cac tac aat ctg att tat cgt gaa gaa gag cgc gag atg cta cca ctg			672
His Tyr Asn Leu Ile Tyr Arg Glu Glu Glu Arg Glu Met Leu Pro Leu			
210	215	220	
tgt tat cag gag ggc gtg gcg gta att cca tgg agc ccg ctg gca agg			720
Cys Tyr Gln Glu Gly Val Ala Val Ile Pro Trp Ser Pro Leu Ala Arg			
225	230	235	240
ggc cgt ctg acg cgt ccg tgg gga gaa act acc gca cga ctg gtg tct			768
Gly Arg Leu Thr Arg Pro Trp Gly Glu Thr Thr Ala Arg Leu Val Ser			
	245	250	255
gat gag gtg ggg aaa aat ctc tat aaa gaa agc gat gaa aat gac gcg			816
Asp Glu Val Gly Lys Asn Leu Tyr Lys Glu Ser Asp Glu Asn Asp Ala			
260	265	270	
cag atc gca gag cgg tta aca ggc gtc agt gaa gaa ctg ggg gcg aca			864
Gln Ile Ala Glu Arg Leu Thr Gly Val Ser Glu Glu Leu Gly Ala Thr			
275	280	285	
cga gca caa gtt gcg ctg gcc tgg ttg ttg agt aaa ccg ggc att gcc			912
Arg Ala Gln Val Ala Leu Ala Trp Leu Leu Ser Lys Pro Gly Ile Ala			
290	295	300	
gca ccg att atc gga act tcg cgc gaa gaa cag ctt gat gag cta ttg			960

Ala Pro Ile Ile Gly Thr Ser Arg Glu Glu Gln Leu Asp Glu Leu Leu
 305 310 315 320

aac gcg gtg gat atc act ttg aag ccg gaa cag att gcc gaa ctg gaa 1008

Asn Ala Val Asp Ile Thr Leu Lys Pro Glu Gln Ile Ala Glu Leu Glu
 325 330 335

acg ccg tat aaa ccg cat cct gtc gta gga ttt aaa 1044

Thr Pro Tyr Lys Pro His Pro Val Val Gly Phe Lys
 340 345

<210> 10

<211> 1194

<212> DNA

<213> Escherichia coli

<220>

<221> CDS

<222> (1).. (1194)

<400> 10

atg aag caa ctc acc att ctg ggc tcg acc ggc tcg att ggt tgc agc 48

Met Lys Gln Leu Thr Ile Leu Gly Ser Thr Gly Ser Ile Gly Cys Ser
 1 5 10 15

acg ctg gac gtg gtg cgc cat aat ccc gaa cac ttc cgc gta gtt gcg 96

Thr Leu Asp Val Val Arg His Asn Pro Glu His Phe Arg Val Val Ala

20	25	30	
ctg gtg gca ggc aaa aat gtc act cgc atg gta gaa cag tgc ctg gaa			144
Leu Val Ala Gly Lys Asn Val Thr Arg Met Val Glu Gln Cys Leu Glu			
35	40	45	
ttc tct ccc cgc tat gcc gta atg gac gat gaa gcg agt gcg aaa ctt			192
Phe Ser Pro Arg Tyr Ala Val Met Asp Asp Glu Ala Ser Ala Lys Leu			
50	55	60	
ctt aaa acg atg cta cag caa cag ggt agc cgc acc gaa gtc tta agt			240
Leu Lys Thr Met Leu Gln Gln Gln Gly Ser Arg Thr Glu Val Leu Ser			
65	70	75	80
ggg caa caa gcc gct tgc gat atg gca gcg ctt gag gat gtt gat cag			288
Gly Gln Gln Ala Ala Cys Asp Met Ala Ala Leu Glu Asp Val Asp Gln			
85	90	95	
gtg atg gca gcc att gtt ggc gct gct ggg ctg tta cct acg ctt gct			336
Val Met Ala Ala Ile Val Gly Ala Ala Gly Leu Leu Pro Thr Leu Ala			
100	105	110	
gcg atc cgc gcg ggt aaa acc att ttg ctg gcc aat aaa gaa tca ctg			384
Ala Ile Arg Ala Gly Lys Thr Ile Leu Leu Ala Asn Lys Glu Ser Leu			
115	120	125	
gtt acc tgc gga cgt ctg ttt atg gac gcc gta aag cag agc aaa gcg			432

Val Thr Cys Gly Arg Leu Phe Met Asp Ala Val Lys Gln Ser Lys Ala
 130 135 140
 caa ttg tta ccg gtc gat agc gaa cat aac gcc att ttt cag agt tta 480
 Gln Leu Leu Pro Val Asp Ser Glu His Asn Ala Ile Phe Gln Ser Leu
 145 150 155 160
 ccg caa cct atc cag cat aat ctg gga tac gct gac ctt gag caa aat 528
 Pro Gln Pro Ile Gln His Asn Leu Gly Tyr Ala Asp Leu Glu Gln Asn
 165 170 175
 ggc gtg gtg tcc att tta ctt acc ggg tct ggt ggc cct ttc cgt gag 576
 Gly Val Val Ser Ile Leu Leu Thr Gly Ser Gly Gly Pro Phe Arg Glu
 180 185 190
 acg cca ttg cgc gat ttg gca aca atg acg ccg gat caa gcc tgc cgt 624
 Thr Pro Leu Arg Asp Leu Ala Thr Met Thr Pro Asp Gln Ala Cys Arg
 195 200 205
 cat ccg aac tgg tcg atg ggg cgt aaa att tct gtc gat tcg gct acc 672
 His Pro Asn Trp Ser Met Gly Arg Lys Ile Ser Val Asp Ser Ala Thr
 210 215 220
 atg atg aac aaa ggt ctg gaa tac att gaa gcg cgt tgg ctg ttt aac 720
 Met Met Asn Lys Gly Leu Glu Tyr Ile Glu Ala Arg Trp Leu Phe Asn
 225 230 235 240

gcc agc gcc agc cag atg gaa gtg ctg att cac ccg cag tca gtg att 768
 Ala Ser Ala Ser Gln Met Glu Val Leu Ile His Pro Gln Ser Val Ile
 245 250 255

cac tca atg gtg cgc tat cag gac ggc agt gtt ctg gcg cag ctg ggg 816
 His Ser Met Val Arg Tyr Gln Asp Gly Ser Val Leu Ala Gln Leu Gly
 260 265 270

gaa ccg gat atg gta cgc caa ttg ccc aca cca tgg gca tgg ccg aat 864
 Glu Pro Asp Met Val Arg Gln Leu Pro Thr Pro Trp Ala Trp Pro Asn
 275 280 285

cgc gtg aac tct ggc gtg aag ccg ctc gat ttt tgc aaa cta agt gcg 912
 Arg Val Asn Ser Gly Val Lys Pro Leu Asp Phe Cys Lys Leu Ser Ala
 290 295 300

ttg aca ttt gcc gca ccg gat tat gat cgt tat cca tgc ctg aaa ctg 960
 Leu Thr Phe Ala Ala Pro Asp Tyr Asp Arg Tyr Pro Cys Leu Lys Leu
 305 310 315 320

gcg atg gag gcg ttc gaa caa ggc cag gca gcg acg aca gca ttg aat 1008
 Ala Met Glu Ala Phe Glu Gln Gly Gln Ala Ala Thr Thr Ala Leu Asn
 325 330 335

gcc gca aac gaa atc acc gtt gct gct ttt ctt gcg caa caa atc cgc 1056
 Ala Ala Asn Glu Ile Thr Val Ala Ala Phe Leu Ala Gln Gln Ile Arg

340

345

350

ttt acg gat atc gct gcg ttg aat tta tcc gta ctg gaa aaa atg gat 1104

Phe Thr Asp Ile Ala Ala Leu Asn Leu Ser Val Leu Glu Lys Met Asp

355

360

365

atg cgc gaa cca caa tgt gtg gac gat gtg tta tct gtt gat gcg aac 1152

Met Arg Glu Pro Gln Cys Val Asp Asp Val Leu Ser Val Asp Ala Asn

370

375

380

gcg cgt gaa gtc gcc aga aaa gag gtg atg cgt ctc gca agc 1194

Ala Arg Glu Val Ala Arg Lys Glu Val Met Arg Leu Ala Ser

385

390

395

<210> 11

<211> 4390

<212> DNA

<213> Escherichia coli

<220>

<221> CDS

<222> (208).. (447)

<220>

<221> CDS

<222> (450).. (1346)

<220>

<221> CDS

<222> (1374).. (3233)

<220>

<221> CDS

<222> (3344).. (4390)

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ccacacctgc ggctgcatcc aggcgcgga gataccact aacatcgctt tgctgtgcac 120

atcaccttac cattgcgcgt tatttgctat ttgccctgag tccgttacca tgacggggcg 180

aaaaatatg agagtcagac attcatt atg ccg aag aaa aat gag gcg ccc gcc 234

Met Pro Lys Lys Asn Glu Ala Pro Ala

1

5

agc ttt gaa aag gcg ctg agc gag ctg gaa cag att gta acc cgt ctg 282

Ser Phe Glu Lys Ala Leu Ser Glu Leu Glu Gln Ile Val Thr Arg Leu

10

15

20

25

gaa agt ggc gac ctg ccg ctg gaa gag gcg ctg aac gag ttc gaa cgc 330

Glu Ser Gly Asp Leu Pro Leu Glu Glu Ala Leu Asn Glu Phe Glu Arg

30

35

40

ggc gtg cag ctg gca cgt cag ggg cag gcc aaa tta caa caa gcc gaa 378

Gly Val Gln Leu Ala Arg Gln Gly Gln Ala Lys Leu Gln Gln Ala Glu
 45 50 55

cag cgc gta caa att ctg ctg tct gac aat gaa gac gcc tct cta acc 426

Gln Arg Val Gln Ile Leu Leu Ser Asp Asn Glu Asp Ala Ser Leu Thr
 60 65 70

cct ttt aca ccg gac aat gag ta atg gac ttt ccg cag caa ctc gaa 473

Pro Phe Thr Pro Asp Asn Glu Met Asp Phe Pro Gln Gln Leu Glu
 75 80 1 5

gcc tgc gtt aag cag gcc aac cag gcg ctg agc cgt ttt atc gcc cca 521

Ala Cys Val Lys Gln Ala Asn Gln Ala Leu Ser Arg Phe Ile Ala Pro
 10 15 20

ctg ccc ttt cag aac act ccc gtg gtc gaa acc atg cag tat ggc gca 569

Leu Pro Phe Gln Asn Thr Pro Val Val Glu Thr Met Gln Tyr Gly Ala
 25 30 35 40

tta tta ggt ggt aag cgc ctg cga cct ttc ctg gtt tat gcc acc ggt 617

Leu Leu Gly Gly Lys Arg Leu Arg Pro Phe Leu Val Tyr Ala Thr Gly
 45 50 55

cat atg ttc ggc gtt agc aca aac acg ctg gac gca ccc gct gcc gcc 665

His Met Phe Gly Val Ser Thr Asn Thr Leu Asp Ala Pro Ala Ala Ala
 60 65 70

gtt gag tgt atc cac gct tac tca tta att cat gat gat tta ccg gca 713
 Val Glu Cys Ile His Ala Tyr Ser Leu Ile His Asp Asp Leu Pro Ala
 75 80 85
 atg gat gat gac gat ctg cgt cgc ggt ttg cca acc tgc cat gtg aag 761
 Met Asp Asp Asp Asp Leu Arg Arg Gly Leu Pro Thr Cys His Val Lys
 90 95 100
 ttt ggc gaa gca aac gcg att ctc gct ggc gac gct tta caa acg ctg 809
 Phe Gly Glu Ala Asn Ala Ile Leu Ala Gly Asp Ala Leu Gln Thr Leu
 105 110 115 120
 gcg ttc tcg att tta agc gat gcc gat atg ccg gaa gtg tcg gac cgc 857
 Ala Phe Ser Ile Leu Ser Asp Ala Asp Met Pro Glu Val Ser Asp Arg
 125 130 135
 gac aga att tcg atg att tct gaa ctg gcg agc gcc agt ggt att gcc 905
 Asp Arg Ile Ser Met Ile Ser Glu Leu Ala Ser Ala Ser Gly Ile Ala
 140 145 150
 gga atg tgc ggt ggt cag gca tta gat tta gac gcg gaa ggc aaa cac 953
 Gly Met Cys Gly Gly Gln Ala Leu Asp Leu Asp Ala Glu Gly Lys His
 155 160 165
 gta cct ctg gac gcg ctt gag cgt att cat cgt cat aaa acc ggc gca 1001
 Val Pro Leu Asp Ala Leu Glu Arg Ile His Arg His Lys Thr Gly Ala

170	175	180	
ttg att cgc gcc gcc gtt cgc ctt ggt gca tta agc gcc gga gat aaa	1049		
Leu Ile Arg Ala Ala Val Arg Leu Gly Ala Leu Ser Ala Gly Asp Lys			
185	190	195	200
gga cgt cgt gct ctg ccg gta ctc gac aag tat gca gag agc atc ggc	1097		
Gly Arg Arg Ala Leu Pro Val Leu Asp Lys Tyr Ala Glu Ser Ile Gly			
205	210	215	
ctt gcc ttc cag gtt cag gat gac atc ctg gat gtg gtg gga gat act	1145		
Leu Ala Phe Gln Val Gln Asp Asp Ile Leu Asp Val Val Gly Asp Thr			
220	225	230	
gca acg ttg gga aaa cgc cag ggt gcc gac cag caa ctt ggt aaa agt	1193		
Ala Thr Leu Gly Lys Arg Gln Gly Ala Asp Gln Gln Leu Gly Lys Ser			
235	240	245	
acc tac cct gca ctt ctg ggt ctt gag caa gcc cgg aag aaa gcc cgg	1241		
Thr Tyr Pro Ala Leu Leu Gly Leu Glu Gln Ala Arg Lys Lys Ala Arg			
250	255	260	
gat ctg atc gac gat gcc cgt cag tcg ctg aaa caa ctg gct gaa cag	1289		
Asp Leu Ile Asp Asp Ala Arg Gln Ser Leu Lys Gln Leu Ala Glu Gln			
265	270	275	280
tca ctc gat acc tcg gca ctg gaa gcg cta gcg gac tac atc atc cag	1337		

Ser Leu Asp Thr Ser Ala Leu Glu Ala Leu Ala Asp Tyr Ile Ile Gln
 285 290 295

cgt aat aaa taaacaataa gtattaatag gcccctg atg agt ttt gat att gcc 1391

Arg Asn Lys Met Ser Phe Asp Ile Ala
 1 5

aaa tac ccg acc ctg gca ctg gtc gac tcc acc cag gag tta cga ctg 1439

Lys Tyr Pro Thr Leu Ala Leu Val Asp Ser Thr Gln Glu Leu Arg Leu
 10 15 20

ttg ccg aaa gag agt tta ccg aaa ctc tgc gac gaa ctg cgc cgc tat 1487

Leu Pro Lys Glu Ser Leu Pro Lys Leu Cys Asp Glu Leu Arg Arg Tyr
 25 30 35

tta ctc gac agc gtg agc cgt tcc agc ggg cac ttc gcc tcc ggg ctg 1535

Leu Leu Asp Ser Val Ser Arg Ser Ser Gly His Phe Ala Ser Gly Leu
 40 45 50

ggc acg gtc gaa ctg acc gtg gcg ctg cac tat gtc tac aac acc ccg 1583

Gly Thr Val Glu Leu Thr Val Ala Leu His Tyr Val Tyr Asn Thr Pro
 55 60 65 70

ttt gac caa ttg att tgg gat gtg ggg cat cag gct tat ccg cat aaa 1631

Phe Asp Gln Leu Ile Trp Asp Val Gly His Gln Ala Tyr Pro His Lys
 75 80 85

att ttg acc gga cgc cgc gac aaa atc ggc acc atc cgt cag aaa ggc 1679
 Ile Leu Thr Gly Arg Arg Asp Lys Ile Gly Thr Ile Arg Gln Lys Gly
 90 95 100

ggt ctg cac ccg ttc ccg tgg cgc ggc gaa agc gaa tat gac gta tta 1727
 Gly Leu His Pro Phe Pro Trp Arg Gly Glu Ser Glu Tyr Asp Val Leu
 105 110 115

agc gtc ggg cat tca tca acc tcc atc agt gcc gga att ggt att gcg 1775
 Ser Val Gly His Ser Ser Thr Ser Ile Ser Ala Gly Ile Gly Ile Ala
 120 125 130

gtt gct gcc gaa aaa gaa ggc aaa aat cgc cgc acc gtc tgt gtc att 1823
 Val Ala Ala Glu Lys Glu Gly Lys Asn Arg Arg Thr Val Cys Val Ile
 135 140 145 150

ggc gat ggc gcg att acc gca ggc atg gcg ttt gaa gcg atg aat cac 1871
 Gly Asp Gly Ala Ile Thr Ala Gly Met Ala Phe Glu Ala Met Asn His
 155 160 165

gcg ggc gat atc cgt cct gat atg ctg gtg att ctc aac gac aat gaa 1919
 Ala Gly Asp Ile Arg Pro Asp Met Leu Val Ile Leu Asn Asp Asn Glu
 170 175 180

atg tcg att tcc gaa aat gtc ggc gcg ctc aac aac cat ctg gca cag 1967

Met Ser Ile Ser Glu Asn Val Gly Ala Leu Asn Asn His Leu Ala Gln
 185 190 195

ctg ctt tcc ggt aag ctt tac tct tca ctg cgc gaa ggc ggg aaa aaa 2015

Leu Leu Ser Gly Lys Leu Tyr Ser Ser Leu Arg Glu Gly Gly Lys Lys
 200 205 210

gtt ttc tct ggc gtg ccg cca att aaa gag ctg ctc aaa cgc acc gaa 2063

Val Phe Ser Gly Val Pro Pro Ile Lys Glu Leu Leu Lys Arg Thr Glu
 215 220 225 230

gaa cat att aaa ggc atg gta gtg cct ggc acg ttg ttt gaa gag ctg 2111

Glu His Ile Lys Gly Met Val Val Pro Gly Thr Leu Phe Glu Glu Leu
 235 240 245

ggc ttt aac tac atc ggc ccg gtg gac ggt cac gat gtg ctg ggg ctt 2159

Gly Phe Asn Tyr Ile Gly Pro Val Asp Gly His Asp Val Leu Gly Leu
 250 255 260

atc acc acg cta aag aac atg cgc gac ctg aaa ggc ccg cag ttc ctg 2207

Ile Thr Thr Leu Lys Asn Met Arg Asp Leu Lys Gly Pro Gln Phe Leu
 265 270 275

cat atc atg acc aaa aaa ggt cgt ggt tat gaa ccg gca gaa aaa gac 2255

His Ile Met Thr Lys Lys Gly Arg Gly Tyr Glu Pro Ala Glu Lys Asp
 280 285 290

ccg atc act ttc cac gcc gtg cct aaa ttt gat ccc tcc agc ggt tgt 2303
 Pro Ile Thr Phe His Ala Val Pro Lys Phe Asp Pro Ser Ser Gly Cys
 295 300 305 310
 ttg ccg aaa agt agc ggc ggt ttg ccg agc tat tca aaa atc ttt ggc 2351
 Leu Pro Lys Ser Ser Gly Gly Leu Pro Ser Tyr Ser Lys Ile Phe Gly
 315 320 325
 gac tgg ttg tgc gaa acg gca gcg aaa gac aac aag ctg atg gcg att 2399
 Asp Trp Leu Cys Glu Thr Ala Ala Lys Asp Asn Lys Leu Met Ala Ile
 330 335 340
 act ccg gcg atg cgt gaa ggt tcc ggc atg gtc gag ttt tca cgt aaa 2447
 Thr Pro Ala Met Arg Glu Gly Ser Gly Met Val Glu Phe Ser Arg Lys
 345 350 355
 ttc ccg gat cgc tac ttc gac gtg gca att gcc gag caa cac gcg gtg 2495
 Phe Pro Asp Arg Tyr Phe Asp Val Ala Ile Ala Glu Gln His Ala Val
 360 365 370
 acc ttt gct gcg ggt ctg gcg att ggt ggg tac aaa ccc att gtc gcg 2543
 Thr Phe Ala Ala Gly Leu Ala Ile Gly Gly Tyr Lys Pro Ile Val Ala
 375 380 385 390
 att tac tcc act ttc ctg caa cgc gcc tat gat cag gtg ctg cat gac 2591
 Ile Tyr Ser Thr Phe Leu Gln Arg Ala Tyr Asp Gln Val Leu His Asp

395	400	405	
gtg gcg att caa aag ctt ccg gtc ctg ttc gcc atc gac cgc gcg ggc			2639
Val Ala Ile Gln Lys Leu Pro Val Leu Phe Ala Ile Asp Arg Ala Gly			
410	415	420	
att gtt ggt gct gac ggt caa acc cat cag ggt gct ttt gat ctc tct			2687
Ile Val Gly Ala Asp Gly Gln Thr His Gln Gly Ala Phe Asp Leu Ser			
425	430	435	
tac ctg cgc tgc ata ccg gaa atg gtc att atg acc ccg agc gat gaa			2735
Tyr Leu Arg Cys Ile Pro Glu Met Val Ile Met Thr Pro Ser Asp Glu			
440	445	450	
aac gaa tgt cgc cag atg ctc tat acc ggc tat cac tat aac gat ggc			2783
Asn Glu Cys Arg Gln Met Leu Tyr Thr Gly Tyr His Tyr Asn Asp Gly			
455	460	465	470
ccg tca gcg gtg cgc tac ccg cgt ggc aac gcg gtc ggc gtg gaa ctg			2831
Pro Ser Ala Val Arg Tyr Pro Arg Gly Asn Ala Val Gly Val Glu Leu			
475	480	485	
acg ccg ctg gaa aaa cta cca att ggc aaa ggc att gtg aag cgt cgt			2879
Thr Pro Leu Glu Lys Leu Pro Ile Gly Lys Gly Ile Val Lys Arg Arg			
490	495	500	
ggc gag aaa ctg gcg atc ctt aac ttt ggt acg ctg atg cca gaa gcg			2927

Gly Glu Lys Leu Ala Ile Leu Asn Phe Gly Thr Leu Met Pro Glu Ala
 505 510 515

gcg aaa gtc gcc gaa tcg ctg aac gcc acg ctg gtc gat atg cgt ttt 2975

Ala Lys Val Ala Glu Ser Leu Asn Ala Thr Leu Val Asp Met Arg Phe
 520 525 530

gtg aaa ccg ctt gat gaa gcg tta att ctg gaa atg gcc gcc agc cat 3023

Val Lys Pro Leu Asp Glu Ala Leu Ile Leu Glu Met Ala Ala Ser His
 535 540 545 550

gaa gcg ctg gtc acc gta gaa gaa aac gcc att atg ggc ggc gca ggc 3071

Glu Ala Leu Val Thr Val Glu Glu Asn Ala Ile Met Gly Gly Ala Gly
 555 560 565

agc ggc gtg aac gaa gtg ctg atg gcc cat cgt aaa cca gta ccc gtg 3119

Ser Gly Val Asn Glu Val Leu Met Ala His Arg Lys Pro Val Pro Val
 570 575 580

ctg aac att ggc ctg ccg gac ttc ttt att ccg caa gga act cag gaa 3167

Leu Asn Ile Gly Leu Pro Asp Phe Phe Ile Pro Gln Gly Thr Gln Glu
 585 590 595

gaa atg cgc gcc gaa ctc ggc ctc gat gcc gct ggt atg gaa gcc aaa 3215

Glu Met Arg Ala Glu Leu Gly Leu Asp Ala Ala Gly Met Glu Ala Lys
 600 605 610

atc aag gcc tgg ctg gca taatccctac tccactcctg ctatgcttaa 3263

Ile Lys Ala Trp Leu Ala

615 620

gaaattattc atagactcta aataattcga gtigcaggaa ggcggcaaac gagtgaagcc 3323

ccaggagctt acataagtaa gtg act ggg gtg aac gaa tgc agc cgc agc aca 3376

Val Thr Gly Val Asn Glu Cys Ser Arg Ser Thr

1 5 10

tgc aac ttg aag tat gac gag tat agc agg agt ggc agc atg caa tac 3424

Cys Asn Leu Lys Tyr Asp Glu Tyr Ser Arg Ser Gly Ser Met Gln Tyr

15 20 25

aac ccc tta gga aaa acc gac ctt cgc gtt tcc cga ctt tgc ctc ggc 3472

Asn Pro Leu Gly Lys Thr Asp Leu Arg Val Ser Arg Leu Cys Leu Gly

30 35 40

tgt atg acc ttt ggc gag cca gat cgc ggt aat cac gca tgg aca ctg 3520

Cys Met Thr Phe Gly Glu Pro Asp Arg Gly Asn His Ala Trp Thr Leu

45 50 55

ccg gaa gaa agc agc cgt ccc ata att aaa cgt gca ctg gaa ggc ggc 3568

Pro Glu Glu Ser Ser Arg Pro Ile Ile Lys Arg Ala Leu Glu Gly Gly

60 65 70 75

ata aat ttc ttt gat acc gcc aac agt tat tct gac ggc agc agc gaa 3616

Ile Asn Phe Phe Asp Thr Ala Asn Ser Tyr Ser Asp Gly Ser Ser Glu
80 85 90

gag atc gtc ggt cgc gca ctg cgg gat ttc gcc cgt cgt gaa gac gtg 3664

Glu Ile Val Gly Arg Ala Leu Arg Asp Phe Ala Arg Arg Glu Asp Val
95 100 105

gtc gtt gcg acc aaa gtg ttc cat cgc gtt ggt gat tta ccg gaa gga 3712

Val Val Ala Thr Lys Val Phe His Arg Val Gly Asp Leu Pro Glu Gly
110 115 120

tta tcc cgt gcg caa att ttg cgc tct atc gac gac agc ctg cga cgt 3760

Leu Ser Arg Ala Gln Ile Leu Arg Ser Ile Asp Asp Ser Leu Arg Arg
125 130 135

ctc ggc atg gat tat gtc gat atc ctg caa att cat cgc tgg gat tac 3808

Leu Gly Met Asp Tyr Val Asp Ile Leu Gln Ile His Arg Trp Asp Tyr
140 145 150 155

aac acg ccg atc gaa gag acg ctg gaa gcc ctc aac gac gtg gta aaa 3856

Asn Thr Pro Ile Glu Glu Thr Leu Glu Ala Leu Asn Asp Val Val Lys
160 165 170

gcc ggg aaa gcg cgt tat atc ggc gcg tca tca atg cac gct tcg cag 3904

Ala Gly Lys Ala Arg Tyr Ile Gly Ala Ser Ser Met His Ala Ser Gln
175 180 185

ttt gct cag gca ctg gaa ctc caa aaa cag cac ggc tgg gcg cag ttt 3952
 Phe Ala Gln Ala Leu Glu Leu Gln Lys Gln His Gly Trp Ala Gln Phe
 190 195 200
 gtc agt atg cag gat cac tac aat ctg att tat cgt gaa gaa gag cgc 4000
 Val Ser Met Gln Asp His Tyr Asn Leu Ile Tyr Arg Glu Glu Glu Arg
 205 210 215
 gag atg cta cca ctg tgt tat cag gag ggc gtg gcg gta att cca tgg 4048
 Glu Met Leu Pro Leu Cys Tyr Gln Glu Gly Val Ala Val Ile Pro Trp
 220 225 230 235
 agc ccg ctg gca agg ggc cgt ctg acg cgt ccg tgg gga gaa act acc 4096
 Ser Pro Leu Ala Arg Gly Arg Leu Thr Arg Pro Trp Gly Glu Thr Thr
 240 245 250
 gca cga ctg gtg tct gat gag gtg ggg aaa aat ctc tat aaa gaa agc 4144
 Ala Arg Leu Val Ser Asp Glu Val Gly Lys Asn Leu Tyr Lys Glu Ser
 255 260 265
 gat gaa aat gac gcg cag atc gca gag cgg tta aca ggc gtc agt gaa 4192
 Asp Glu Asn Asp Ala Gln Ile Ala Glu Arg Leu Thr Gly Val Ser Glu
 270 275 280
 gaa ctg ggg gcg aca cga gca caa gtt gcg ctg gcc tgg ttg ttg agt 4240

Glu Leu Gly Ala Thr Arg Ala Gln Val Ala Leu Ala Trp Leu Leu Ser
 285 290 295

aaa ccg ggc att gcc gca ccg att atc gga act tcg cgc gaa gaa cag 4288

Lys Pro Gly Ile Ala Ala Pro Ile Ile Gly Thr Ser Arg Glu Glu Gln
 300 305 310 315

ctt gat gag cta ttg aac gcg gtg gat atc act ttg aag ccg gaa cag 4336

Leu Asp Glu Leu Leu Asn Ala Val Asp Ile Thr Leu Lys Pro Glu Gln
 320 325 330

att gcc gaa ctg gaa acg ccg tat aaa ccg cat cct gtc gta gga ttt 4384

Ile Ala Glu Leu Glu Thr Pro Tyr Lys Pro His Pro Val Val Gly Phe
 335 340 345

aaa taa 4390

Lys

<210> 12

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Synthetic DNA

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33

<210> 13

<211> 34

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:Synthetic DNA

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ccgaattctt atttaaattcc tacgacagga tgcg

34

<210> 14

<211> 33

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:Synthetic DNA

<400> 14

ccggatccat gagttttgat attgccaaat acc

33

<210> 15

<211> 33

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:Synthetic DNA

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ccgaattctt atgccagcca ggccttgatt ttg

33

<210> 16

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Synthetic DNA

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ccgaattctt actcattgtc cgggtgtaaaa ggg

33

<210> 17

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Synthetic DNA

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ccggaatccat ggactttccg cagcaactcg aag

33

<210> 18

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Synthetic DNA

<400> 18

ccgaattctt atttattacg ctggatgatg tag

33

<210> 19

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Synthetic DNA

<400> 19

ccggatccta atccctactc cactcctgct atg

33

<210> 20

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Synthetic DNA

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gggggatcca agcaactcac cattctgggc

30

<210> 21

<211> 30

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:Synthetic DNA

<400> 21

gggggatccg cttgcgagac gcatcacctc

30

<210> 22

<211> 32

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:Synthetic DNA

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gggggatcca gttttgatat tgccaaatac cc

32

<210> 23

<211> 32

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:Synthetic DNA

<400> 23

gggggatcct gccagccagg ccttgat ttt gg

32

<210> 24

<211> 30

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:Synthetic DNA

<400> 24

gggggatccg agcaactcac cattctgggc

30

<210> 25

<211> 30

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:Synthetic DNA

<400> 25

gggggatccg ctigcgagac gcatcacctc

30

<210> 26

<211> 637

<212> PRT

<213> Rhodobacter sphaeroides

<400> 26

Met Thr Asp Arg Pro Cys Thr Pro Thr Leu Asp Arg Val Thr Leu Pro

1

5

10

15

Val Asp Met Lys Gly Leu Thr Asp Arg Glu Leu Arg Ser Leu Ala Asp

20

25

30

Glu Leu Arg Ala Glu Thr Ile Ser Ala Val Ser Val Thr Gly Gly His

35

40

45

Leu Gly Ala Gly Leu Gly Val Val Glu Leu Thr Val Ala Leu His Ala

50

55

60

Val Phe Asp Ala Pro Arg Asp Lys Ile Ile Trp Asp Val Gly His Gln

65

70

75

80

Cys Tyr Pro His Lys Ile Leu Thr Gly Arg Arg Asp Arg Ile Arg Thr

85

90

95

Leu Arg Gln Gly Gly Gly Leu Ser Gly Phe Thr Lys Arg Ser Glu Ser

100

105

110

Pro Tyr Asp Cys Phe Gly Ala Gly His Ser Ser Thr Ser Ile Ser Ala
 115 120 125

Ala Val Gly Phe Ala Ala Ala Arg Glu Met Gly Gly Asp Thr Gly Asp
 130 135 140

Ala Val Ala Val Ile Gly Asp Gly Ser Met Ser Ala Gly Met Ala Phe
 145 150 155 160

Glu Ala Leu Asn His Gly Gly His Leu Lys Asn Arg Val Ile Val Ile
 165 170 175

Leu Asn Asp Asn Glu Met Ser Ile Ala Pro Pro Val Gly Ala Leu Ser
 180 185 190

Ser Tyr Leu Ser Arg Leu Tyr Ala Gly Ala Pro Phe Gln Asp Phe Lys
 195 200 205

Ala Ala Ala Lys Gly Ala Leu Gly Leu Leu Pro Glu Pro Phe Gln Glu
 210 215 220

Gly Ala Arg Arg Ala Lys Glu Met Leu Lys Ser Val Thr Val Gly Gly
 225 230 235 240

Thr Leu Phe Glu Glu Leu Gly Phe Ser Tyr Val Gly Pro Ile Asp Gly
 245 250 255

His Asp Leu Asp Gln Leu Leu Pro Val Leu Arg Thr Val Lys Gln Arg
 260 265 270

Ala His Ala Pro Val Leu Ile His Val Ile Thr Lys Lys Gly Arg Gly

275	280	285
Tyr Ala Pro Ala Glu Ala Ala Arg Asp Arg Gly His Ala Thr Asn Lys		
290	295	300
Phe Asn Val Leu Thr Gly Ala Gln Val Lys Pro Val Ser Asn Ala Pro		
305	310	315
Ser Tyr Thr Lys Val Phe Ala Gln Ser Leu Ile Lys Glu Ala Glu Val		
325	330	335
Asp Glu Arg Ile Cys Ala Val Thr Ala Ala Met Pro Asp Gly Thr Gly		
340	345	350
Leu Asn Leu Phe Gly Glu Arg Phe Pro Lys Arg Thr Phe Asp Val Gly		
355	360	365
Ile Ala Glu Gln His Ala Val Thr Phe Ser Ala Ala Leu Ala Ala Gly		
370	375	380
Gly Met Arg Pro Phe Cys Ala Ile Tyr Ser Thr Phe Leu Gln Arg Gly		
385	390	395
Tyr Asp Gln Ile Val His Asp Val Ala Ile Gln Arg Leu Pro Val Arg		
405	410	415
Phe Ala Ile Asp Arg Ala Gly Leu Val Gly Ala Asp Gly Ala Thr His		
420	425	430
Ala Gly Ser Phe Asp Val Ala Phe Leu Ser Asn Leu Pro Gly Ile Val		
435	440	445

Val Met Ala Ala Ala Asp Glu Ala Glu Leu Val His Met Val Ala Thr
 450 455 460

Ala Ala Ala His Asp Glu Gly Pro Ile Ala Phe Arg Tyr Pro Arg Gly
 465 470 475 480

Asp Gly Val Gly Val Glu Met Pro Val Lys Gly Val Pro Leu Gln Ile
 485 490 495

Gly Arg Gly Arg Val Val Arg Glu Gly Thr Arg Ile Ala Leu Leu Ser
 500 505 510

Phe Gly Thr Arg Leu Ala Glu Val Gln Val Ala Ala Glu Ala Leu Arg
 515 520 525

Ala Arg Gly Ile Ser Pro Thr Val Ala Asp Ala Arg Phe Ala Lys Pro
 530 535 540

Leu Asp Arg Asp Leu Ile Leu Gln Leu Ala Ala His His Glu Ala Leu
 545 550 555 560

Ile Thr Ile Glu Glu Gly Ala Ile Gly Gly Phe Gly Ser His Val Ala
 565 570 575

Gln Leu Leu Ala Glu Ala Gly Val Phe Asp Arg Gly Phe Arg Tyr Arg
 580 585 590

Ser Met Val Leu Pro Asp Thr Phe Ile Asp His Asn Ser Ala Glu Val
 595 600 605

Met Tyr Ala Thr Ala Gly Leu Asn Ala Ala Asp Ile Glu Arg Lys Ala
 610 615 620

Leu Glu Thr Leu Gly Val Glu Val Leu Ala Arg Arg Ala
 625 630 635

<210> 27

<211> 1911

<212> DNA

<213> Rhodobacter sphaeroides

<220>

<221> CDS

<222> (1).. (1911)

<400> 27

atg acc gac aga ccc tgc acg ccg acg ctc gac cgg gtg acg ctc ccg 48

Met Thr Asp Arg Pro Cys Thr Pro Thr Leu Asp Arg Val Thr Leu Pro

1

5

10

15

gtg gac atg aag ggc ctc acg gac cgt gag ctg cgc tcg ctg gcc gac 96

Val Asp Met Lys Gly Leu Thr Asp Arg Glu Leu Arg Ser Leu Ala Asp

20

25

30

gag ctg cgg gcc gaa acg atc tcg gcc gtg tcg gtg acg ggc ggg cat 144

Glu Leu Arg Ala Glu Thr Ile Ser Ala Val Ser Val Thr Gly Gly His

35

40

45

ctg ggc gca ggc ctc ggc gtg gtg gag ttg acg gtt gcg ctg cat gcg 192

Leu Gly Ala Gly Leu Gly Val Val Glu Leu Thr Val Ala Leu His Ala

50

55

60

gtc ttc gat gcg ccg cgc gac aag atc atc tgg gac gtg ggc cac cag 240
 Val Phe Asp Ala Pro Arg Asp Lys Ile Ile Trp Asp Val Gly His Gln
 65 70 75 80

tgc tac ccc cac aag atc ctg acc ggg cgg cgc gac cgc atc cgc aca 288
 Cys Tyr Pro His Lys Ile Leu Thr Gly Arg Arg Asp Arg Ile Arg Thr
 85 90 95

ctg cgg cag ggc ggg ggt ctc tcg ggc ttc acc aag cgc tcc gag agc 336
 Leu Arg Gln Gly Gly Gly Leu Ser Gly Phe Thr Lys Arg Ser Glu Ser
 100 105 110

ccc tac gac tgt ttc ggc gcg ggc cat tcc tcg acc tcg atc tcg gcc 384
 Pro Tyr Asp Cys Phe Gly Ala Gly His Ser Ser Thr Ser Ile Ser Ala
 115 120 125

gcg gtg ggc ttt gcc gcg gcg cgc gag atg ggc ggc gac acg ggc gac 432
 Ala Val Gly Phe Ala Ala Ala Arg Glu Met Gly Gly Asp Thr Gly Asp
 130 135 140

gcg gtg gcg gtg atc ggc gat ggc tcg atg tcg gcc ggc atg gcc ttc 480
 Ala Val Ala Val Ile Gly Asp Gly Ser Met Ser Ala Gly Met Ala Phe
 145 150 155 160

gag gcg ctg aac cac ggc ggg cac ctg aag aac cgg gtg atc gtg atc 528
 Glu Ala Leu Asn His Gly Gly His Leu Lys Asn Arg Val Ile Val Ile
 165 170 175

ctg aac gac aat gag atg agc atc gcg ccg ccg gtg ggg gcg ctg tcg 576
 Leu Asn Asp Asn Glu Met Ser Ile Ala Pro Pro Val Gly Ala Leu Ser

180	185	190	
tcc tat ctc tcg cgg ctc tat gcg ggc gcg ccg ttc cag gac ttc aag	624		
Ser Tyr Leu Ser Arg Leu Tyr Ala Gly Ala Pro Phe Gln Asp Phe Lys			
195	200	205	
gcg gcc gcc aag gga gcg ctc ggg ctt ctg ccc gaa ccg ttc cag gag	672		
Ala Ala Ala Lys Gly Ala Leu Gly Leu Leu Pro Glu Pro Phe Gln Glu			
210	215	220	
ggc gcg cgc cgc gcc aag gag atg ctg aag agc gtc acc gtc ggc ggc	720		
Gly Ala Arg Arg Ala Lys Glu Met Leu Lys Ser Val Thr Val Gly Gly			
225	230	235	240
acg ctc ttc gag gag ctg ggt ttc tcc tat gtc ggc ccg atc gac ggg	768		
Thr Leu Phe Glu Glu Leu Gly Phe Ser Tyr Val Gly Pro Ile Asp Gly			
245	250	255	
cac gat ctc gac cag ctt ctg ccg gtg ctg cgg acc gtc aag cag cgg	816		
His Asp Leu Asp Gln Leu Leu Pro Val Leu Arg Thr Val Lys Gln Arg			
260	265	270	
gcg cat gcg ccg gtg ctg atc cat gtc atc acc aag aag ggc agg ggc	864		
Ala His Ala Pro Val Leu Ile His Val Ile Thr Lys Lys Gly Arg Gly			
275	280	285	
tat gct ccg gcc gag gcc gcg cgc gac cgc ggc cat gcc acg aac aag	912		
Tyr Ala Pro Ala Glu Ala Ala Arg Asp Arg Gly His Ala Thr Asn Lys			
290	295	300	
ttc aac gtc ctg acc ggc gcg cag gtg aag ccg gtc tcg aac gcc ccc	960		

Phe	Asn	Val	Leu	Thr	Gly	Ala	Gln	Val	Lys	Pro	Val	Ser	Asn	Ala	Pro
305					310				315					320	
tcc	tac	acc	aag	gtc	ttc	gcc	cag	agc	ctc	atc	aag	gag	gcc	gag	gtc
1008															
Ser	Tyr	Thr	Lys	Val	Phe	Ala	Gln	Ser	Leu	Ile	Lys	Glu	Ala	Glu	Val
			325					330					335		
gac	gag	cgg	atc	tgc	gcg	gtg	acg	gcc	gcc	atg	ccg	gac	ggg	acg	ggg
1056															
Asp	Glu	Arg	Ile	Cys	Ala	Val	Thr	Ala	Ala	Met	Pro	Asp	Gly	Thr	Gly
			340					345					350		
ctc	aac	ctc	ttc	ggc	gag	cgg	ttt	ccg	aag	cgc	acc	ttc	gac	gtg	ggc
1104															
Leu	Asn	Leu	Phe	Gly	Glu	Arg	Phe	Pro	Lys	Arg	Thr	Phe	Asp	Val	Gly
			355					360					365		
atc	gcg	gaa	cag	cat	gcg	gtg	acc	ttc	tcg	gcg	gcg	ctt	gcg	gca	ggc
1152															
Ile	Ala	Glu	Gln	His	Ala	Val	Thr	Phe	Ser	Ala	Ala	Leu	Ala	Ala	Gly
			370					375					380		
ggc	atg	cgg	ccc	ttc	tgc	gcg	atc	tat	tcc	acc	ttc	ctc	cag	cgc	ggc
1200															
Gly	Met	Arg	Pro	Phe	Cys	Ala	Ile	Tyr	Ser	Thr	Phe	Leu	Gln	Arg	Gly
			385					390					395		400
tac	gac	cag	atc	gtg	cat	gac	gtg	gcg	atc	cag	cgc	ctg	ccg	gtg	cgc
1248															
Tyr	Asp	Gln	Ile	Val	His	Asp	Val	Ala	Ile	Gln	Arg	Leu	Pro	Val	Arg
			405					410					415		
ttc	gcc	atc	gat	cgc	gcg	ggc	ctc	gtg	ggg	gcg	gac	ggc	gcc	acc	cat
1296															
Phe	Ala	Ile	Asp	Arg	Ala	Gly	Leu	Val	Gly	Ala	Asp	Gly	Ala	Thr	His
			420					425					430		

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 Val Met Ala Ala Ala Asp Glu Ala Glu Leu Val His Met Val Ala Thr
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gcc gcc gcc cat gac gaa ggg ccc atc gcc ttc cgc tac ccg cgc ggc 1440
 Ala Ala Ala His Asp Glu Gly Pro Ile Ala Phe Arg Tyr Pro Arg Gly
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gac ggc gtg ggg gtc gag atg ccg gtg aag ggc gtg ccg ctc cag atc 1488
 Asp Gly Val Gly Val Glu Met Pro Val Lys Gly Val Pro Leu Gln Ile
 485 490 495

ggc cgc ggc cgt gtg gtg cgc gag ggc acg cga atc gcg ctt ttg tcc 1536
 Gly Arg Gly Arg Val Val Arg Glu Gly Thr Arg Ile Ala Leu Leu Ser
 500 505 510

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 Phe Gly Thr Arg Leu Ala Glu Val Gln Val Ala Ala Glu Ala Leu Arg
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15

Cys Cys Pro Ala Asp Met Lys Ala Leu Ser Asp Ala Glu Leu Glu Arg
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Gly Gly His Leu Gly Ser Ser Leu Gly Val Val Glu Leu Thr Val Ala
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Leu His Ala Val Phe Asn Thr Pro Thr Asp Lys Leu Val Trp Asp Val
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Gly His Gln Cys Tyr Pro His Lys Ile Leu Thr Gly Arg Arg Glu Gln
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		240
Gly Gly Gly Thr Leu Phe Glu Glu Leu Gly Phe Thr Tyr Val Gly Pro		
245	250	255
Ile Asp Gly His Asp Met Glu Ala Leu Leu Gln Thr Leu Arg Ala Ala		
260	265	270
Arg Ala Arg Thr Thr Gly Pro Val Leu Ile His Val Val Thr Lys Lys		
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Gly Lys Gly Tyr Ala Pro Ala Glu Asn Ala Pro Asp Lys Tyr His Gly		
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Val Asn Lys Phe Asp Pro Val Thr Gly Glu Gln Lys Lys Ser Val Ala		
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Asn Ala Pro Asn Tyr Thr Lys Val Phe Gly Ser Thr Leu Thr Glu Glu		
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Ala Ala Arg Asp Pro Arg Ile Val Ala Ile Thr Ala Ala Met Pro Ser		
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Asp Val Gly Ile Ala Glu Gln His Ala Val Thr Phe Ala Ala Gly Leu
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Pro Val Arg Phe Val Ile Asp Arg Ala Gly Leu Val Gly Ala Asp Gly
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Leu Glu Pro Gly Arg Gly Arg Val Val Arg Glu Gly Thr Asp Val Ala
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Ile Leu Ser Phe Gly Ala His Leu His Glu Ala Leu Gln Ala Ala Lys
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Leu Leu Glu Ala Glu Gly Val Ser Val Thr Val Ala Asp Ala Arg Phe
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Ser Arg Pro Leu Asp Thr Gly Leu Ile Asp Gln Leu Val Arg His His
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Ala Ala Leu Val Thr Val Glu Gln Gly Ala Met Gly Gly Phe Gly Ala
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His Val Met His Tyr Leu Ala Asn Ser Gly Gly Phe Asp Gly Gly Leu
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Pro Glu Asp Met Tyr Ala Asp Ala Gly Leu Arg Ala Glu Asp Ile Ala
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Cys Cys Pro Ala Asp Met Lys Ala Leu Ser Asp Ala Glu Leu Glu Arg	
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Leu Ala Asp Glu Val Arg Ser Glu Val Ile Ser Val Val Ala Glu Thr	
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Gly Gly His Leu Gly Ser Ser Leu Gly Val Val Glu Leu Thr Val Ala	
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ctg cat gca gtc ttc aac acg ccc acc gac aag ctc gtc tgg gac gtg	240
Leu His Ala Val Phe Asn Thr Pro Thr Asp Lys Leu Val Trp Asp Val	
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Gly His Gln Cys Tyr Pro His Lys Ile Leu Thr Gly Arg Arg Glu Gln	
85 90 95	
atg cgc acc ctg cgc cag aag ggc ggc ctc tcg ggc ttc acc aag cgc	336
Met Arg Thr Leu Arg Gln Lys Gly Gly Leu Ser Gly Phe Thr Lys Arg	

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Thr Leu Arg Ala Ala Ala Asp Gly Leu Glu Ala Ser Leu Pro Gly Pro			
210	215	220	
ctc cgc gac ggg gcg cgc cgg gcg cgc cag ctc gtg acc ggg atg ccg			720

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Ile	Asp	Gly	His	Asp	Met	Glu	Ala	Leu	Leu	Gln	Thr	Leu	Arg	Ala	Ala		
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Arg	Ala	Arg	Thr	Thr	Gly	Pro	Val	Leu	Ile	His	Val	Val	Thr	Lys	Lys		
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Val	Asn	Lys	Phe	Asp	Pro	Val	Thr	Gly	Glu	Gln	Lys	Lys	Ser	Val	Ala		
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Pro Ala Leu Arg Glu Ala Leu Ala Gly Thr Gly Thr Glu Val Ala Gly
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Leu Lys His Gly Arg Thr Leu Ala Leu Ala Asn Lys Glu Ser Leu Val
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Thr Ala Gly Gln Leu Leu Met Arg Thr Ala Gln Glu Asn Gly Ala Thr
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Gly Pro Phe Arg Asp Trp Ser Leu Glu Arg Ile Arg Ala Cys Thr Val
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Ile Asp Ser Ala Ser Met Phe Asn Lys Ala Leu Glu Leu Ile Glu Thr
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Met Ala His Leu Gly Pro Ala Asp Met Arg His Ala Ile Gly Phe Ala
 260 265 270

Leu Asn Trp Pro Gly Arg Gly Glu Val Pro Val Ala Arg Ile Asp Leu
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Pro Ala Leu Arg Leu Ala Arg Asp Val Met Ala Ala Arg Gly Leu Ser
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Ala Gly Arg Ile Gly Phe Leu Asp Met Ala Ala Val Val Glu Glu Thr
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Leu Ala Gly Val Ser Thr Asp Pro Leu Phe Gly Lys Val Pro Asp Ala
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<212> DNA

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15

acc ttc gac ctc gtc atg cgg aag ggc ggg ccc gag gcg ttc cgc acc 96

Thr Phe Asp Leu Val Met Arg Lys Gly Gly Pro Glu Ala Phe Arg Thr

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25

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gtc gct ctg acc ggc ggg cgc aac atc cgg cga ctg gcc gaa atg gcg 144

Val Ala Leu Thr Gly Gly Arg Asn Ile Arg Arg Leu Ala Glu Met Ala

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cgt gcg ctg aag gcg gag ctt gcc gtc acc gcg cat gag gac tgc ctg 192

Arg Ala Leu Lys Ala Glu Leu Ala Val Thr Ala His Glu Asp Cys Leu

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55

60

ccc gcg ctg cgc gag gcg ctg gcc ggg acg ggc acc gag gtc gcg ggc 240

Pro Ala Leu Arg Glu Ala Leu Ala Gly Thr Gly Thr Glu Val Ala Gly

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atg tcg gcc atc gtg ggc gcc gcg ggc ctc gtg ccc gga atg cgg gcg				336
Met Ser Ala Ile Val Gly Ala Ala Gly Leu Val Pro Gly Met Arg Ala				
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Leu Lys His Gly Arg Thr Leu Ala Leu Ala Asn Lys Glu Ser Leu Val				
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Thr Ala Gly Gln Leu Leu Met Arg Thr Ala Gln Glu Asn Gly Ala Thr				
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Gly Pro Phe Arg Asp Trp Ser Leu Glu Arg Ile Arg Ala Cys Thr Val				
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225	230 235 240
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Pro Gln Ser Ile Val His Ala Met Val Gly Phe Cys Asp Gly Gly Leu	
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Met Ala His Leu Gly Pro Ala Asp Met Arg His Ala Ile Gly Phe Ala	
260	265 270
ctg aac tgg ccg ggt cgc ggc gag gtg ccc gtc gcc cgg atc gac ctc	864
Leu Asn Trp Pro Gly Arg Gly Glu Val Pro Val Ala Arg Ile Asp Leu	
275	280 285
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Ala Gln Ile Ala Ser Leu Thr Phe Gln Lys Pro Asp Glu Glu Arg Phe	
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Pro Ala Leu Arg Leu Ala Arg Asp Val Met Ala Ala Arg Gly Leu Ser	
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23

<210> 34

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic DNA

<400> 34

gcatgctgtt ccgcgatgcc gac

23

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CLAIMS:

1. A process for producing an isoprenoid compound,
which comprises:

integrating DNA into an expression vector, wherein
5 the DNA contains one of the following DNA fragments (e) and
(f):

(e) a DNA fragment encoding a protein which has
activity to catalyze a reaction to produce 2-C-methyl-D-
erythritol 4-phosphate from 1-deoxy-D-xylulose 5-phosphate
10 and has the amino acid sequence of SEQ ID NO: 5 or 30; and

(f) a DNA fragment which hybridizes with the
complement of the nucleotide sequence of SEQ ID NO: 10 or 31
in the presence of 0.7 to 1.0 mol/l NaCl at 65°C, followed
by washing in a 0.1 to 2-fold SSC solution at 65°C, and has
15 activity to catalyze a reaction to produce 2-C-methyl-D-
erythritol 4-phosphate from 1-deoxy-D-xylulose 5-phosphate;

introducing the resultant vector into a prokaryote
host cell;

culturing the obtained prokaryote host cell in a
20 medium;

allowing the prokaryote host cell to produce and
accumulate the isoprenoid compound in a culture; and

recovering the isoprenoid compound from the
culture.

25 2. The process according to claim 1, wherein the DNA
fragment (e) is employed.

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3. The process according to claim 1, wherein the DNA fragment hybridizes with the complement of the nucleotide sequence of SEQ ID NO: 10 or 31.

4. The process according to claim 1, wherein the isoprenoid compound is selected from the group consisting of ubiquinone, vitamin K₂ and carotenoids.

5. The process according to claim 1, wherein the isoprenoid compound is ubiquinone-10.

6. The process according to any one of claims 1 to 5, wherein the prokaryote host cell is a microorganism belonging to the genus Escherichia, Rhodobacter or Erwinia.

7. The process according to any one of claims 1 to 5, wherein the prokaryote host cell is Escherichia coli.

8. The process according to claim 1, wherein the isoprenoid compound is ubiquinone-10; and the prokaryote host cell is Rhodobacter sphaeroides.

9. The process according to claim 3, wherein the isoprenoid compound is ubiquinone-10; and the prokaryote host cell is Rhodobacter sphaeroides.

Fig. 1

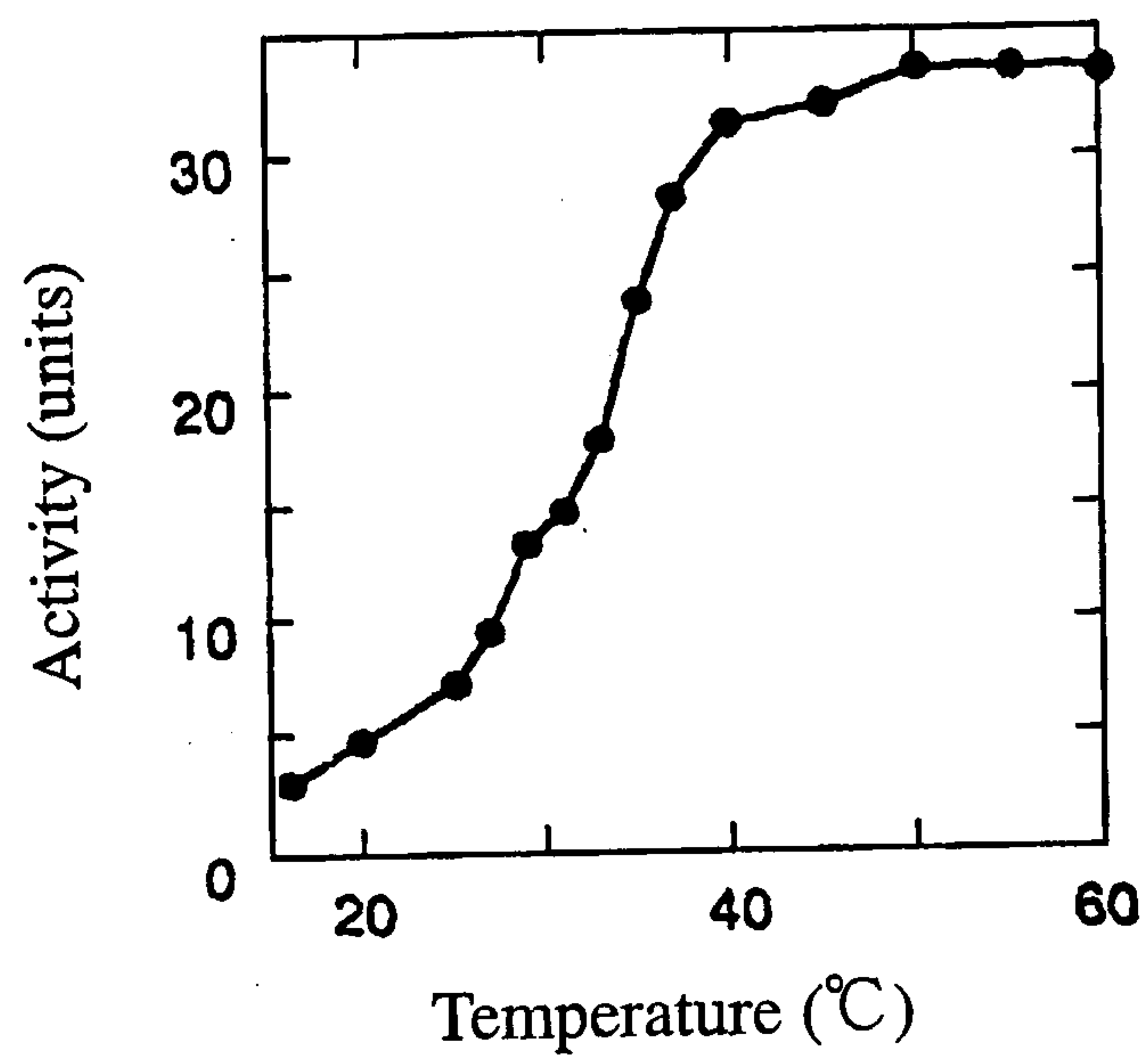


Fig. 2

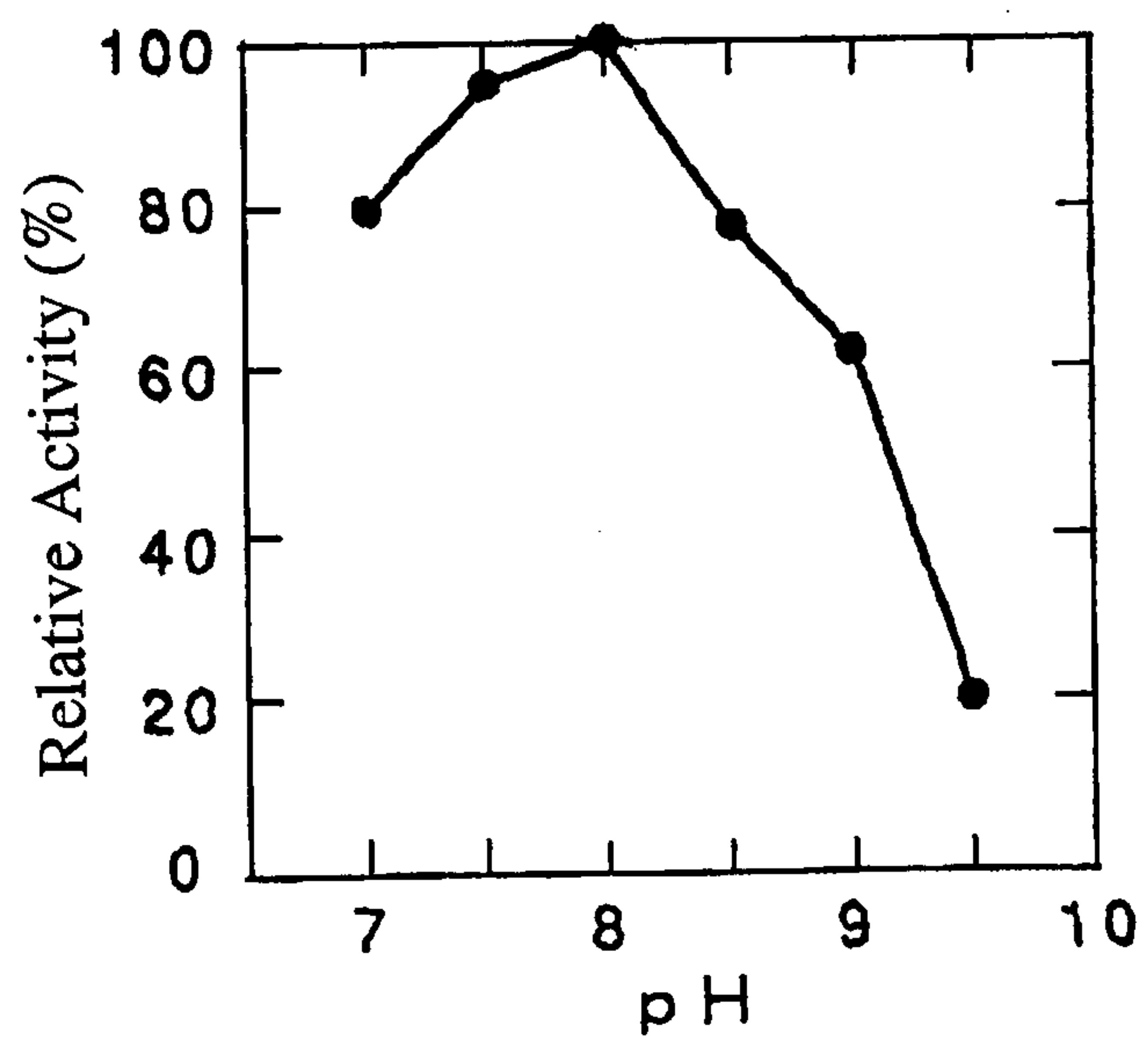


Fig. 3

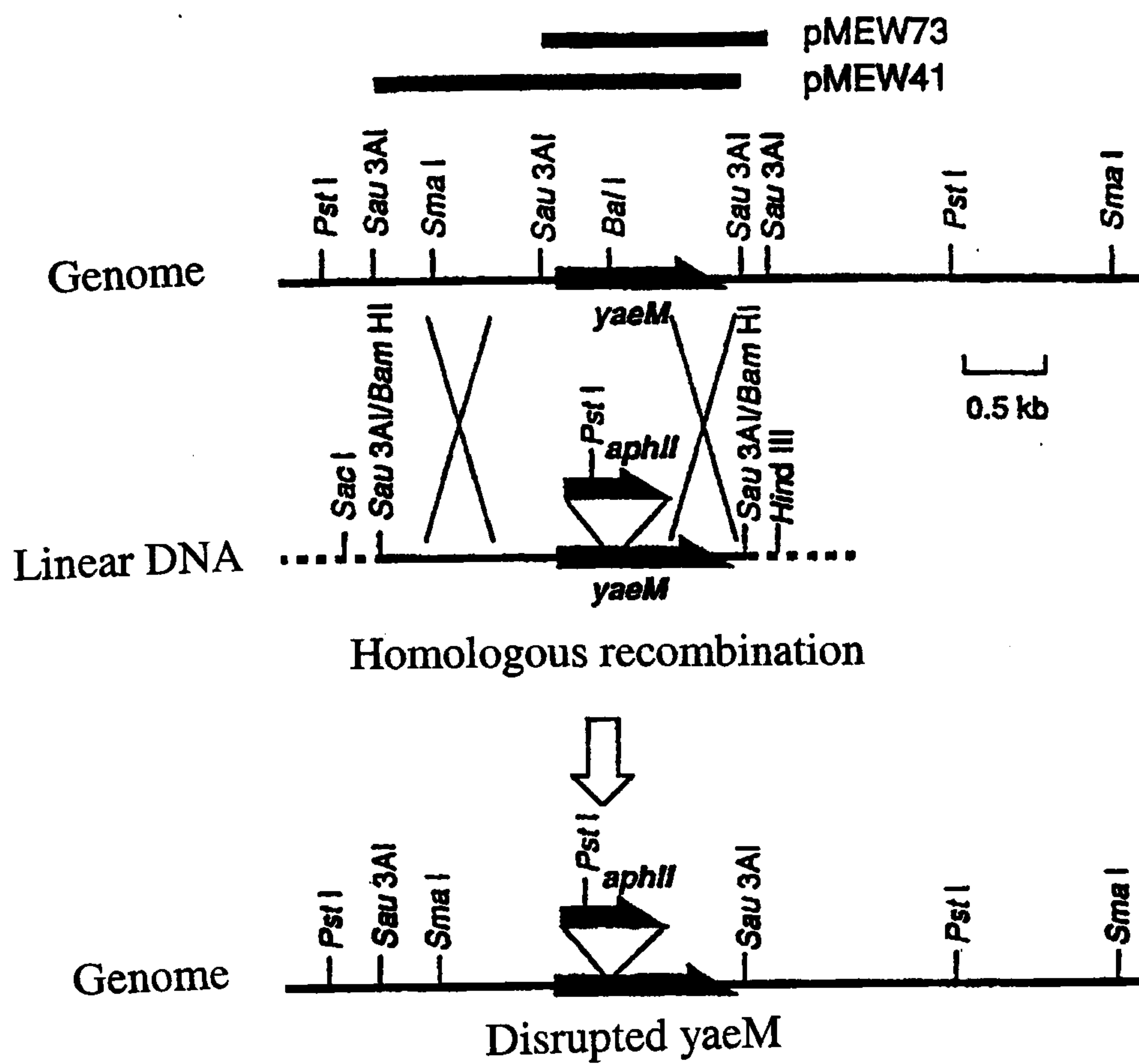


Fig. 4

