United States Patent	12385018
Kind Code	B2
Date of Patent	August 12, 2025
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Transformed microorganism and method of producing polyhydroxyalkanoate

Abstract

Provided is a transformed microorganism that has a polyhydroxyalkanoate synthase gene and in which expression of a minD gene is enhanced. Also provided is a transformed microorganism that has a polyhydroxyalkanoate synthase gene and in which expression of a minC gene and a minD gene is enhanced. In this transformed microorganism, expression of a minE gene may be enhanced or reduced. Also provided is a method of producing a PHA, the method including the step of culturing any of the transformed microorganisms in the presence of a carbon source.

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Appl. No.: **17/412340**

Filed: **August 26, 2021**

Prior Publication Data

Document Identifier	Publication Date
US 20210388324 A1	Dec. 16, 2021

Foreign Application Priority Data

JP	2019-036006	Feb. 28, 2019
JP	2019-036008	Feb. 28, 2019

Related U.S. Application Data

continuation parent-doc WO PCT/JP2020/003156 20200129 PENDING child-doc US 17412340

Publication Classification

Int. Cl.: **C12N9/10** (20060101); **C12P7/625** (20220101)

U.S. Cl.:

CPC **C12N9/1029** (20130101); **C12P7/625** (20130101);

Field of Classification Search

USPC: None

References Cited

U.S. PATENT DOCUMENTS

Patent No.	Issued Date	Patentee Name	U.S. Cl.	CPC
9175317	12/2014	Sato	N/A	C12N 15/74
10072255	12/2017	Arikawa	N/A	C12Y 402/01074
11453896	12/2021	Arikawa	N/A	C12Y 203/01009
2019/0153486	12/2018	Arikawa et al.	N/A	N/A
2020/0087687	12/2019	Aoki et al.	N/A	N/A

FOREIGN PATENT DOCUMENTS

Patent No.	Application Date	Country	CPC
104762311	12/2014	CN	N/A
105779488	12/2015	CN	N/A
4 029 946	12/2021	EP	N/A
WO 2018/021046	12/2017	WO	N/A
WO 2018/216726	12/2017	WO	N/A

OTHER PUBLICATIONS

Chica et al. Curr Opin Biotechnol. Aug. 2005;16(4):378-84. (Year: 2005). cited by examiner
Singh et al. Curr Protein Pept Sci. 2017, 18, 1-11 (Year: 2017). cited by examiner
Accession G0ES24. Oct. 19, 2011 (Year: 2011). cited by examiner
Accession A0A022FNJ8. Jun. 11, 2014. (Year: 2014). cited by examiner
Accession A0A212MH45. Sep. 27, 2017 (Year: 2017). cited by examiner
Accession A0A022FQN4. Jun. 11, 2014 (Year: 2014). cited by examiner
Tan et al., “Engineering *Halomonas* TD01 for the low-cost production of polyhydroxyalkanoates”, Metabolic Engineering, 2014, vol. 26, pp. 34-47. cited by applicant
Uniprot, [online], Accession No. QOKFI4, URL<https://www.uniprot.org/uniprotkb/Q0KFI4/entry >, as cited in the Office Action issued on Jan. 30, 2024 in Japanese counterpart Application No. 2021-501778. 4 pages. cited by applicant
NCBI Database, [online], Accession No. WP_010811452.1, URL<https://www.ncbi.nlm.nih.gov/protein/498510994?sat=47&satkey=62716682 >, as cited in

the Office Action issued on Jan. 30, 2024 in Japanese counterpart Application No. 2021-501778, 1 page. cited by applicant
UniProt, [online], Accession No. Q0KFI3, URL<https://www.uniprot.org/uniprotkb/Q0KFI3/entry >, as cited in the Office Action issued on Jan. 30, 2024 in Japanese counterpart Application No. 2021-501778, 4 pages. cited by applicant
NCBI Database, [online], Accession No. WP_010811453.1, URL<https://www.ncbi.nlm.nih.gov/protein/498510995?sat=47&satkey=68081467 >, as cited in the Office Action issued on Jan. 30, 2024 in Japanese counterpart Application No. 2021-501778, 1 page. cited by applicant
UniProt, [online], Accession No. Q0KFI5, URL<https://www.uniprot.org/uniprotkb/Q0KFI5/entry >, as cited in the Office Action issued on Jan. 30, 2024 in Japanese counterpart Application No. 2021-501778, 4 pages. cited by applicant
NCBI Database, [online], Accession No. WP_010811451.1, URL<https://www.ncbi.nlm.nih.gov/protein/498510993?sat=47&satkey=42867008 >, as cited in the Office Action issued on Jan. 30, 2024 in Japanese counterpart Application No. 2021-501778, 1 page. cited by applicant
International Search Report dated Apr. 14, 2020, in PCT/JP2020/003156, filed Jan. 29, 2020. cited by applicant
Anderson A.J. et al., *Int. J. Biol. Macromol*., 12, 102-105 (1990). cited by applicant
Sato S. et al., *J. Biosci. Bioeng*., 120(3), 246-251 (2015). cited by applicant
Insomphun C. et al., *Metab.Eng*., 27, 38-45 (2015). cited by applicant
Pichoff, s. et al., “Deletion analysis of gene minE which encodes the topological specificity factor of cell division in *Escherichia coil*”, Molecular Microbiology, 1995, vol. 18, No. 2, pp. 321-329. cited by applicant
Sato, Shunsuke et al., “Process development of biodegradable polymer PHBH”, Seibutsu-kogaku kaishi, Feb. 25, 2019, vol. 97, No. 2, pp. 66-74. cited by applicant

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Background/Summary

CROSS REFERENCE TO RELATED APPLICATIONS (1) This application is a continuation of International Application No. PCT/JP2020/003156, filed Jan. 29, 2020, and claims priority to Japanese Application No. 2019-036006, filed Feb. 28, 2019, and Japanese Application No. 2019-036008, filed Feb. 28, 2019, the disclosures of all of which are incorporated herein by reference in their entireties.

TECHNICAL FIELD
(1) The present invention relates to a transformed microorganism capable of elaborating a polyhydroxyalkanoate and a method of producing the polyhydroxyalkanoate using the transformed microorganism.
BACKGROUND ART
(2) There is a growing awareness of environmental issues, food issues, health, and safety, and more and more people are becoming nature-oriented. Against such a background, material production using microorganisms (such as fermentative production and bioconversion) is becoming increasingly significant and important. Microbial material production is applied also to production of protein pharmaceuticals and production of nucleic acids for gene therapy. For example, ethanol

production, acetic acid production, and medical protein production using microorganisms such as yeasts and bacteria are actively employed industrially.

(3) An example of the microbial material production is microbial production of polyhydroxyalkanoates (occasionally referred to as “PHAs” hereinafter) which are considered promising biodegradable plastics for industrial use (see Non Patent Literature 1). PHAs are thermoplastic polyesters produced and accumulated as energy storage materials in cells of many kinds of microorganisms and are biodegradable. Nowadays, the heightened environmental awareness has led to increasing attention to non-petroleum-based plastics. In particular, there is a strong demand for practical use of PHAs produced and accumulated in microorganisms because such PHAs are absorbed into the carbon circulation process in the nature and are therefore expected to have little adverse impact on the ecosystems. A known example of PHA production using microorganisms is to produce a PHA by feeding bacteria of the genus *Cupriavidus* with a carbon source such as a sugar, vegetable oil, or fatty acid and thus allowing the bacteria to accumulate the PHA in their cells (see Non Patent Literatures 2 and 3).

(4) However, microbial material production requires the complicated steps of separating and collecting the microbial cells and the target product and could suffer the problem of high production cost. Improving the efficiency of separation and collection is a major challenge to be addressed for production cost reduction.

CITATION LIST

Non-Patent Literature

(5) NPL 1: Anderson A. J. et al., *Int. J. Biol. Macromol.*, 12, 102-105 (1990) NPL 2: Sato S. et al., *J. Biosci. Bioeng.*, 120 (3), 246-251 (2015) NPL 3: Insomphun C. et al., *Metab. Eng.*, 27, 38-45 (2015)

SUMMARY OF INVENTION

Technical Problem

(6) A PHA is accumulated in microbial cells. To use the PHA accumulated in the microbial cells as a biodegradable plastic, it is necessary first to separate and collect the microbial cells from the culture fluid. The separation and collection of the microbial cells can be conducted by means such as a centrifuge or separation membrane, and the ease and efficiency of the separation and collection depend on the size of the microbial cells. Specifically, a larger size of the microbial cells allows the separation and collection to be more easily and efficiently accomplished by means such as a centrifuge or separation membrane, leading to a lower production cost.

(7) The microbial cells accumulating the PHA are broken to take PHA particles out of the cells, and the PHA particles are separated from other cellular components and collected. Techniques for the separation and collection of the PHA particles are broadly classified into a technique using an organic solvent system and a technique using an aqueous system. Since the use of an organic solvent causes high environmental load and involves high cost, the technique using an aqueous system is preferred from the industrial point of view. With the technique using an aqueous system, for example, the PHA particles contained in the broken cell fluid can be separated from the fluid by means such as a centrifuge or separation membrane. In this case, the efficiency of the separation and collection depends on the size of the PHA particles. Specifically, a larger size of the PHA particles accumulated in the microbial cells allows the separation and collection to be more easily accomplished by means such as a centrifuge or separation membrane, leading to a lower production cost.

(8) In view of the above circumstances, the present invention aims to provide a transformed microorganism that accumulates a PHA and whose size can be large and a method of producing the PHA using the transformed microorganism.

Solution to Problem

(9) As a result of intensive studies, the present inventors have found that when the expression of a particular one of genes expected to be involved in cell division, namely, a minC gene (e.g., a gene

that encodes the amino acid sequence of SEQ ID NO: 1), a minD gene (e.g., a gene that encodes the amino acid sequence of SEQ ID NO: 2), and a minE gene (e.g., a gene that encodes the amino acid sequence of SEQ ID NO: 3), is enhanced or reduced, the size of microbial cells can be increased while ensuring an industrially desired level of PHA accumulation. Based on this finding, the inventors have arrived at the present invention.

(10) That is, the present invention relates to a transformed microorganism having a polyhydroxyalkanoate synthase gene, wherein expression of a minD gene is enhanced. The present invention further relates to a transformed microorganism having a polyhydroxyalkanoate synthase gene, wherein expression of a minC gene and a minD gene is enhanced. In this transformed microorganism, expression of a minE gene may be enhanced or reduced. The above transformed microorganisms preferably belong to the genus *Cupriavidus* and are more preferably transformed *Cupriavidus necator*. The present invention further relates to a method of producing a polyhydroxyalkanoate, the method including the step of culturing any of the transformed microorganisms in the presence of a carbon source. The carbon source preferably contains an oil, a fatty acid, a sugar, or carbon dioxide. The polyhydroxyalkanoate is preferably a copolymer of two or more hydroxyalkanoates, more preferably a copolymer containing 3-hydroxyhexanoate as a monomer unit, and even more preferably a copolymer of 3-hydroxybutyrate and 3-hydroxyhexanoate.

Advantageous Effects of Invention

(11) The present invention can provide a transformed microorganism that accumulates a PHA and whose size can be large and a method of producing the PHA using the transformed microorganism. In the present invention, since the size of microbial cells accumulating the PHA is large, the microbial cells can easily be separated and collected from a culture fluid, and the production cost can be reduced.

(12) A preferred aspect of the present invention can provide a transformed microorganism whose size can be large and that is capable of accumulating large-size PHA particles and a method of producing a PHA using the transformed microorganism. In this aspect, not only are the separation and collection of microbial cells from a culture fluid easy, but also large-size PHA particles are accumulated in the microbial cells. Thus, the PHA can easily be collected separately from other cellular components after cell breakage, and the production cost can be reduced.

Description

BRIEF DESCRIPTION OF DRAWINGS

(1) FIG. **1** is a microscope image of a cultured KNK-005 strain (Comparative Example 1), where the scale bar represents 10 μm (the same applies to FIGS. **2** to **8**).

(2) FIG. **2** is a microscope image of a cultured minE gene-deleted strain (Comparative Example 2).

(3) FIG. **3** is a microscope image of a cultured minC gene expression-enhanced strain (Comparative Example 3).

(4) FIG. **4** is a microscope image of a cultured minD gene expression-enhanced and minE gene-deleted strain (Comparative Example 4).

(5) FIG. **5** is a microscope image of a cultured minD gene expression-enhanced strain (Example 1).

(6) FIG. **6** is a microscope image of a cultured minCD gene expression-enhanced strain (Example 2).

(7) FIG. **7** is a microscope image of a cultured minCDE gene expression-enhanced strain (Example 3).

(8) FIG. **8** is a microscope image of a cultured minCD gene expression-enhanced and minE gene-deleted strain (Example 4).

DESCRIPTION OF EMBODIMENTS

(9) Hereinafter, embodiments of the present invention will be described in detail. A transformed microorganism according to the present invention is a transformed microorganism that has a PHA synthase gene and in which expression of a particular one of minC, minD, and minE genes is enhanced or reduced.

Microorganism

(10) The transformed microorganism according to the present invention may be a microorganism having a PHA synthase gene and transformed to enhance the expression of the minD gene. Alternatively, the transformed microorganism may be a microorganism having a PHA synthase gene and transformed to enhance the expression of the minC and minD genes. Alternatively, the transformed microorganism may be a microorganism having a PHA synthase gene and transformed to enhance the expression of the minC, minD, and minE genes or may be a microorganism having a PHA synthase gene and transformed to enhance the expression of the minC and minD genes and reduce the expression of the minE gene. It should be noted that a microorganism transformed to enhance the expression of the minD gene and reduce the expression of the minE gene and not having enhanced expression of the minC gene is not categorized as the transformed microorganism of the present invention.

(11) The host of the transformed microorganism according to the present invention is not limited to a particular type, and may be any microorganism that has a PHA synthase gene. The host is preferably a bacterium having a minCD or minCDE gene. Examples of the bacterium include bacteria belonging to the genus *Ralstonia*, the genus *Cupriavidus*, the genus *Wautersia*, the genus *Aeromonas*, the genus *Escherichia*, the genus *Alcaligenes*, and the genus *Pseudomonas*. In view of safety and PHA productivity, bacteria belonging to the genus *Ralstonia*, the genus *Cupriavidus*, the genus *Aeromonas*, and the genus *Wautersia* are more preferred. Even more preferred are bacteria belonging to the genus *Cupriavidus* or the genus *Aeromonas*, and still even more preferred are microorganisms belonging to the genus *Cupriavidus*. Particularly preferred is *Cupriavidus necator.*

(12) The host of the transformed microorganism according to the present invention may be a wild strain inherently having a PHA synthase gene, a mutant strain obtained by artificially mutating the wild strain, or a strain having a foreign PHA synthase gene introduced by a genetic engineering technique. The introduction of the foreign PHA synthase gene is not limited to being carried out by a particular method, and the introduction method can be selected from: a method in which the foreign gene is directly inserted onto the chromosome of the host or a gene on the chromosome is replaced by the foreign gene; a method in which the foreign gene is directly inserted onto the megaplasmid of the host or a gene on the megaplasmid is replaced by the foreign gene; and a method in which the foreign gene is placed on a vector such as a plasmid, phage, or phagemid and the vector with the gene is introduced into the host. Two or more of these methods may be used in combination. In view of the stability of the introduced gene, it is preferable to use the method in which the foreign gene is directly inserted onto the chromosome of the host or a gene on the chromosome is replaced by the foreign gene or the method in which the foreign gene is directly inserted onto the megaplasmid of the host or a gene on the megaplasmid is replaced by the foreign gene, and it is more preferable to use the method in which the foreign gene is directly inserted onto the chromosome of the host or a gene on the chromosome is replaced by the foreign gene.

PHA Synthase Gene

(13) The PHA synthase gene is not limited to a particular type, and examples of the PHA synthase gene include PHA synthase genes derived from living organisms belonging to the genus *Ralstonia*, the genus *Cupriavidus*, the genus *Wautersia*, the genus *Alcaligenes*, the genus *Aeromonas*, the genus *Pseudomonas*, the genus *Norcardia*, and the genus *Chromobacterium*, and further include altered genes resulting from alteration of the mentioned PHA synthase genes. Such an altered gene may be a gene having a base sequence that encodes a PHA synthase in which one or more amino acid residues are deleted, added, inserted, or replaced. Examples of the altered gene include a gene having a base sequence that encodes a polypeptide represented by an amino acid sequence of any

one of SEQ ID NOS: 4 to 8 and a gene having a base sequence that encodes a polypeptide having PHA synthase activity and represented by an amino acid sequence that is at least 85% homologous to the amino acid sequence of any one of SEQ ID NOS: 4 to 8. The sequence homology is preferably 90% or more, more preferably 95% or more, even more preferably 97% or more, and particularly preferably 99% or more.

PHA

(14) The PHA produced by the transformed microorganism of the present invention is not limited to a particular type, and may be any PHA that can be produced by microorganisms. The PHA is preferably any one of the following polymers: a homopolymer of one monomer selected from 3-hydroxyalkanoates having 4 to 16 carbon atoms; a copolymer of one monomer selected from 3-hydroxyalkanoates having 4 to 16 carbon atoms and another hydroxyalkanoic acid (such as a 2-hydroxyalkanoic acid, 4-hydroxyalkanoic acid, 5-hydroxyalkanoic acid, or 6-hydroxyalkanoic acid having 4 to 16 carbon atoms); and a copolymer of two or more monomers selected from 3-hydroxyalkanoates having 4 to 16 carbon atoms. Examples of the PHA include, but are not limited to: P(3HB) which is a homopolymer of 3-hydroxybutyrate (abbreviated as 3HB); P(3HB-co-3HV) which is a copolymer of 3HB and 3-hydroxyvaleric acid (abbreviated as 3HV); P(3HB-co-3HH) (abbreviated as PHBH) which is a copolymer of 3HB and 3-hydroxyhexanoate (abbreviated as 3HH); P(3HB-co-4HB) which is a copolymer of 3HB and 4-hydroxybutyrate (abbreviated as 4HB); and PHA containing lactic acid (abbreviated as LA) as a constituent component (an example of this PHA is P(LA-co-3HB) which is a copolymer of 3HB and LA). Among these examples, PHBH is preferred in that this polymer has a wide range of applications. The type of the PHA to be produced can be appropriately selected according to the intended purpose and depending on the type of the PHA synthase gene possessed by or introduced into the microorganism used, the type of the metabolizing gene involved in synthesis of the PHA, and the culture conditions.

minC, minD, and minE Genes

(15) Proteins MinC, MinD, and MinE encoded by the minC, minD, and minE genes are proteins that cooperate in bacteria to control cell division (MinCDE system). For example, it is known that in cells of *Escherichia coli*, the MinD forms a polymer in an ATP-dependent manner, further forms a complex with the MinC, and rapidly oscillates between the cell poles. The MinC serves to inhibit septum formation during cell division. The MinE is known to bind to the MinD competitively against the MinC, and serves to regulate septum formation so that the septum is formed only at the center of the cell.

(16) The minC gene is a gene having a base sequence that encodes a polypeptide (UniProtKB ID Q0KFI3) represented by the amino acid sequence of SEQ ID NO: 1 and a polypeptide represented by an amino acid sequence that is at least 85% homologous to the amino acid sequence of SEQ ID NO: 1. The sequence homology is preferably 90% or more, more preferably 95% or more, even more preferably 97% or more, and particularly preferably 99% or more.

(17) The minD gene is a gene having a base sequence that encodes a polypeptide (UniProtKB ID Q0KFI4) represented by the amino acid sequence of SEQ ID NO: 2 and a polypeptide represented by an amino acid sequence that is at least 85% homologous to the amino acid sequence of SEQ ID NO: 2. The sequence homology is preferably 90% or more, more preferably 95% or more, even more preferably 97% or more, and particularly preferably 99% or more.

(18) The minE gene is a gene having a base sequence that encodes a polypeptide (UniProtKB ID Q0KFI5) represented by the amino acid sequence of SEQ ID NO: 3 and a polypeptide represented by an amino acid sequence that is at least 85% homologous to the amino acid sequence of SEQ ID NO: 3. The sequence homology is preferably 90% or more, more preferably 95% or more, even more preferably 97% or more, and particularly preferably 99% or more.

Gene Expression Enhancement

(19) In the present invention, enhanced gene expression means a state in which the amount of transcription of a target gene or the amount of expression of the polypeptide encoded by the target

gene is increased as compared to that in a strain in which the expression of the target gene is not enhanced. The increase is not limited to a particular level, and it is sufficient that the amount of transcription of the target gene or the amount of expression of the polypeptide be more than that in the strain in which the expression of the target gene is not enhanced. The amount of transcription of the target gene or the amount of expression of the polypeptide is preferably 1.1 or more times, more preferably 1.2 or more times, even more preferably 1.5 or more times, and still even more preferably 2 or more times that in the strain in which the expression of the target gene is not enhanced.

(20) In the present invention, the enhancement of the expression of the min genes is not limited to being carried out by a particular method, and the enhancement method can be selected from a method in which the target gene is introduced into the host and a method in which the amount of expression of the target gene inherently possessed by the host on the genome DNA is increased. Both of the two methods may be used in combination.

(21) The introduction of the target gene into the host is not limited to being carried out by a particular method, and the introduction method can be selected from: a method in which the target gene is directly inserted onto the chromosome of the host or a gene on the chromosome is replaced by the target gene; a method in which the target gene is directly inserted onto the megaplasmid of the host or a gene on the megaplasmid is replaced by the target gene; and a method in which the target gene is placed on a vector such as a plasmid, phage, or phagemid and the vector with the gene is introduced into the host. Two or more of these methods may be used in combination.

(22) In view of the stability of the introduced gene, it is preferable to use the method in which the target gene is directly inserted onto the chromosome of the host or a gene on the chromosome is replaced by the target gene or the method in which the target gene is directly inserted onto the megaplasmid of the host or a gene on the megaplasmid is replaced by the target gene, and it is more preferable to use the method in which the target gene is directly inserted onto the chromosome of the host or a gene on the chromosome is replaced by the target gene. For reliable expression of the introduced gene, it is preferable to introduce the target gene in such a manner that the target gene is downstream of a “gene expression regulatory sequence” inherently possessed by the host or downstream of a foreign “gene expression regulatory sequence”. In the present invention, a “gene expression regulatory sequence” is a DNA sequence including a base sequence that controls the amount of transcription of the gene (an example of this base sequence is a promotor sequence) and/or a base sequence that regulates the amount of translation of a messenger RNA transcribed from the gene (an example of this base sequence is a Shine-Dalgarno sequence). The “gene expression regulatory sequence” used may be any suitable naturally-occurring base sequence or an artificially constructed or altered base sequence.

(23) The increase of the amount of expression of the target gene inherently possessed by the host on the genome DNA is not limited to being achieved by a particular method, and exemplary methods include a method in which a “gene expression regulatory sequence” upstream of the target gene is altered, a method in which a foreign “gene expression regulatory sequence” is introduced upstream of the target gene, and a method in which the target gene and/or a base sequence neighboring the target gene is altered to increase the stability of the transcribed messenger RNA.

(24) Examples of the promotor sequence or Shine-Dalgarno sequence included in the “gene expression regulatory sequence” include, but are not limited to, the base sequences of SEQ ID NOS: 9 to 15 and base sequences including any part of the base sequences of SEQ ID NOS: 9 to 15.

(25) Replacement, deletion, insertion, and/or addition made to at least a part of the genome DNA can be accomplished using a method known to those skilled in the art. Typical methods include a method using a transposon and the mechanism of homologous recombination (Ohman et al., *J. Bacteriol.,* 162:1068-1074 (1985)) and a method based on site-specific integration caused by the mechanism of homologous recombination and on loss due to secondary homologous recombination

(Noti et al., *Methods Enzymol.*, 154:197-217 (1987)). A method may also be used in which a sacB gene derived from *Bacillus subtilis* is allowed to coexist and in which a microorganism strain having lost a gene due to secondary homologous recombination is easily isolated as a sucrose-resistant strain (Schweizer, *Mol. Microbiol.*, 6:1195-1204 (1992) or Lenzet al., *J. Bacteriol.*, 176:4385-4393 (1994)). Another alternative method is to use a CRISPR/Cas9 system-based genome-editing technology for altering the target DNA (Y. Wang et al., *ACS Synth Biol.*, 2016, 5 (7):721-732). In the CRISPR/Cas9 system, the guide RNA (gRNA) has a sequence capable of binding to a part of the base sequence of the genome DNA to be altered, and serves to transport the Cas9 to the target.

(26) The introduction of a vector into a cell is not limited to being carried out by a particular method, and exemplary methods include calcium chloride transformation, electroporation, polyethylene glycol transformation, and spheroplast transformation.

Gene Expression Reduction

(27) In the present invention, “reduced gene expression” means a state in which the amount of transcription of a target gene or the amount of expression of the polypeptide encoded by the target gene is decreased as compared to that in a strain in which the expression of the target gene is not reduced. The decrease is not limited to a particular level, and it is sufficient that the amount of transcription of the target gene or the amount of expression of the polypeptide be less than that in the strain in which the expression of the target gene is not reduced. The amount of transcription of the target gene or the amount of expression of the polypeptide is preferably 0.8 or less times, more preferably 0.5 or less times, even more preferably 0.3 or less times, and still even more preferably 0.2 or less times that in the strain in which the expression of the target gene is not reduced. The amount of transcription of the target gene or the amount of expression of the polypeptide encoded by the target gene may be zero. The gene expression can be considered to have been reduced also when the polypeptide encoded by the target gene cannot exhibit the original function for a reason such as alteration of the base sequence of the gene. In the case where the transformed microorganism of the present invention is a transformed microorganism with enhanced expression of the minC and minD genes, the expression of the target gene can be reduced by using a drug or protein that inhibits the function of the corresponding polypeptide.

(28) In the present invention, the reduction of the gene expression is not limited to being achieved by a particular method, and exemplary methods include: a method in which a part or the entire length of the target gene is deleted; a method in which the “gene expression regulatory sequence” involved in the expression of the target gene is altered; and a method in which the target gene and/or a base sequence neighboring the target gene is altered to decrease the stability of the transcribed messenger RNA. The base sequence alteration is not limited to being carried out by a particular method, and can be accomplished through replacement, deletion, insertion, and/or addition made to at least a part of the target gene and/or the neighboring base sequence. The replacement, deletion, insertion, and/or addition can be made by a method known to those skilled in the art. In the case where the transformed microorganism of the present invention is a transformed microorganism with enhanced expression of the minC and minD genes, an antisense RNA, RNA interference (RNAi), or CRISPR interference (CRISPRi) may be used to reduce the expression of the target gene without altering the target gene and/or the neighboring base sequence.

(29) Culturing the transformed microorganism of the present invention allows the microorganism to accumulate a PHA therein. The culture of the transformed microorganism of the present invention can be conducted according to a common microbial culture method, and it is sufficient that the transformed microorganism be cultured in a culture medium containing a suitable carbon source. There are no particular limitations on the composition of the culture medium, the method of adding the carbon source, the scale of the culture, the conditions of aeration and stirring, the culture temperature, and the culture time. It is preferable to add the carbon source continuously or intermittently to the culture medium.

(30) The carbon source used for the culture may be any carbon source that can be assimilated by the transformed microorganism of the present invention. Examples of the carbon source include, but are not limited to: sugars such as glucose, fructose, and sucrose; palm and palm kernel oils (including palm olein, palm double olein, and palm kernel olein which are low-melting fractions obtained through fractionation of palm oil and palm kernel oil); oils such as corn oil, coconut oil, olive oil, soybean oil, rapeseed oil, and Jatropha oil; fractions of these oils; by-products formed during refining of these oils; fatty acids such as lauric acid, oleic acid, stearic acid, palmitic acid, and myristic acid; derivatives of these fatty acids; and glycerol. In the case where the transformed microorganism of the present invention can assimilate gases such as carbon dioxide, carbon monoxide, and methane or alcohols such as methanol and ethanol, any of these gases or alcohols can be used as the carbon source.

(31) In the PHA production of the present invention, it is preferable to culture the microorganism using a culture medium containing the carbon source and other nutrient sources including a nitrogen source, an inorganic salt, and another organic nutrient source. Examples of the nitrogen source include, but are not limited to: ammonia; ammonium salts such as ammonium chloride, ammonium sulfate, and ammonium phosphate; peptone; meat extracts; and yeast extracts. Examples of the inorganic salt include potassium dihydrogen phosphate, sodium dihydrogen phosphate, magnesium phosphate, magnesium sulfate, and sodium chloride. Examples of the other organic nutrient source include: amino acids such as glycine, alanine, serine, threonine, and proline; and vitamins such as vitamin B1, vitamin B12, and vitamin C.

(32) After the microorganism is cultured for an adequate time to allow the microorganism to accumulate a PHA therein, the PHA is collected from the microorganism using a known method. The PHA collection is not limited to being carried out by a particular method. For example, the PHA can be collected by a method consisting of: after the culture, separating the microorganism from the culture fluid by means such as a centrifuge or separation membrane; drying the separated microorganism; extracting the PHA from the dried microorganism using an organic solvent such as chloroform; removing cellular components from the PHA-containing organic solvent solution by a process such as filtration; adding a poor solvent such as methanol or hexane to the filtrate to precipitate the PHA; removing the supernatant by a process such as filtration or centrifugation; and drying the precipitated PHA. Alternatively, the PHA may be collected by dissolving cellular components other than the PHA in water with the aid of a surfactant, an alkali, or an enzyme, then separating the PHA particles from the aqueous phase by a process such as filtration or centrifugation, and drying the separated PHA particles.

(33) In the present invention, large-size microbial cells accumulating PHA can be obtained, and the microbial cells can be separated from the culture fluid easily and efficiently thanks to their large size. Large-size PHA particles producible according to a preferred aspect of the present invention are preferred because such PHA particles are easy to separate and collect using an aqueous system as described above.

EXAMPLES

(34) Hereinafter, the present invention will be described more specifically using examples. The present invention is not limited to the examples. The overall genetic manipulation can be carried out, for example, in a manner as taught in Molecular Cloning (Cold Spring Harbor Laboratory Press (1989)). The enzymes and cloning hosts used in the gene manipulation can be purchased from market suppliers and used according to the instructions given by the suppliers. The enzymes are not limited to particular types and may be any enzymes that can be used for gene manipulation.

(Production Example 1) Preparation of minE Gene-Deleted Strain

(35) First, a gene deletion plasmid was prepared. The preparation was done as follows. PCR using a synthetic oligo DNA was carried out to obtain a DNA fragment (SEQ ID NO: 16) having base sequences upstream and downstream of the minE structural gene. The DNA fragment was digested by a restriction enzyme SwaI, and the resulting DNA fragment was joined by a DNA ligase

(Ligation High, manufactured by Toyobo Co., Ltd.) to a vector pNS2X-sacB which is described in Japanese Laid-Open Patent Application Publication No. 2007-259708 and which was also digested by SwaI. Thus, a gene deletion plasmid vector pNS2X-sacB+minEUD having base sequences upstream and downstream of the minE structural gene was prepared.

(36) Subsequently, a minE gene-deleted strain was prepared using the gene deletion plasmid vector pNS2X-sacB+minEUD as follows. An *Escherichia coli* S17-1 strain (ATCC 47055) was transformed with the gene deletion plasmid vector pNS2X-sacB+minEUD, and the resulting transformed microorganism was cocultured with a KNK-005 strain on Nutrient Agar (manufactured by Difco Laboratories) to effect conjugal transfer. The KNK-005 strain is a transformed strain produced by introducing an *Aeromonas caviae*-derived PHA synthase gene (a gene that encodes a PHA synthase that has the amino acid sequence of SEQ ID NO: 6) onto the chromosome of a *Cupriavidus necator* H16 strain, and can be prepared according to the method described in U.S. Pat. No. 7,384,766.

(37) The culture fluid obtained as above was inoculated into a Simmons agar medium (2 g/L sodium citrate, 5 g/L sodium chloride, 0.2 g/L magnesium sulfate heptahydrate, 1 g/L ammonium dihydrogen phosphate, 1 g/L potassium dihydrogen phosphate, 15 g/L agar, pH=6.8) containing 250 mg/L of kanamycin, and a strain grown on the agar medium was selectively collected. Thus, a strain having the plasmid integrated into the chromosome of the KNK-005 strain was obtained. The obtained strain was cultured on Nutrient Broth (manufactured by Difco Laboratories) for two generations, after which the culture broth was diluted and applied onto Nutrient Agar containing 15% sucrose. A strain grown on Nutrient Agar was obtained as a strain having lost the plasmid. PCR and analysis using a DNA sequencer were further carried out to isolate one strain from which the start to stop codons of the minE structural gene on the chromosome were deleted. In this manner, a minE gene-deleted strain was obtained.

(Production Example 2) Preparation of minC Gene Expression-Enhanced Strain

(38) First, a minC gene expression plasmid pCUP2-PA-minC was prepared. The preparation was done as follows.

(39) PCR using a synthetic oligo DNA was carried out to obtain a DNA fragment (SEQ ID NO: 17) having a promotor sequence and a minC gene sequence. The DNA fragment was digested by restriction enzymes MunI and SpeI, and the resulting DNA fragment was joined to a plasmid vector pCUP2 which is described in WO 2007/049716 and which was cleaved by MunI and SpeI. Thus, the minC gene expression plasmid pCUP2-PA-minC was obtained.

(40) Subsequently, the minC gene expression plasmid pCUP2-PA-minC was introduced into the KNK-005 stain to obtain a minC gene expression-enhanced strain. The introduction of the plasmid vector into the cells was accomplished by electroporation. The gene introduction device used was Gene Pulser manufactured by Bio-Rad Laboratories, Inc., and the cuvette used was a 0.2-cm-gap cuvette also manufactured by Bio-Rad Laboratories, Inc. The cuvette was charged with 400 μl of competent cells and 20 μl of an expression vector and set on the pulse device, by which electric pulse was applied to the contents of the cuvette at a capacitance of 25 μF, a voltage of 1.5 kV, and a resistance value of 800Ω. After the pulse application, the fluid in the cuvette was subjected to shake culture on Nutrient Broth (manufactured by Difco Laboratories) at 30° C. for 3 hours and then to culture on a selection plate (Nutrient Agar manufactured by Difco Laboratories, containing 100 mg/L kanamycin) at 30° C. for 2 days. The minC gene expression-enhanced strain thus grown was collected.

(Production Example 3) Preparation of minD Gene Expression-Enhanced Strain

(41) First, a minD gene expression plasmid pCUP2-PA-minD was prepared. The preparation was done as follows.

(42) PCR using a synthetic oligo DNA was carried out to obtain a DNA fragment (SEQ ID NO: 18) having a promotor sequence and a minD gene sequence. The DNA fragment was digested by restriction enzymes MunI and SpeI, and the resulting DNA fragment was joined to a plasmid vector

pCUP2 which is described in WO 2007/049716 and which was cleaved by MunI and SpeI. Thus, the minD gene expression plasmid pCUP2-PA-minD was obtained.
(43) Subsequently, the minD gene expression plasmid pCUP2-PA-minD was introduced into the KNK-005 strain in the same manner as the plasmid pCUP2-PA-minC was introduced in Production Example 2. Thus, a minD gene expression-enhanced strain was obtained.
(Production Example 4) Preparation of minCD Gene Expression-Enhanced Strain
(44) First, a minCD gene expression plasmid pCUP2-PA-minCD was prepared. The preparation was done as follows.
(45) PCR using a synthetic oligo DNA was carried out to obtain a DNA fragment (SEQ ID NO: 19) having a promotor sequence and a minCD gene sequence. The DNA fragment was digested by restriction enzymes MunI and SpeI, and the resulting DNA fragment was joined to a plasmid vector pCUP2 which is described in WO 2007/049716 and which was cleaved by MunI and SpeI. Thus, the minCD gene expression plasmid pCUP2-PA-minCD was obtained.
(46) Subsequently, the minCD gene expression plasmid pCUP2-PA-minCD was introduced into the KNK-005 strain in the same manner as the plasmid pCUP2-PA-minC was introduced in Production Example 2. Thus, a minCD gene expression-enhanced strain was obtained.
(Production Example 5) Preparation of minD Gene Expression-Enhanced and minE Gene-Deleted Strain
(47) The minD gene expression plasmid pCUP2-PA-minD prepared in Production Example 3 was introduced into the minE gene-deleted strain prepared in Production Example 1 in the same manner as the plasmid pCUP2-PA-minC was introduced into the KNK-005 strain in Production Example 2. Thus, a minD gene expression-enhanced and minE gene-deleted strain was obtained.
(Production Example 6) Preparation of minCDE Gene Expression-Enhanced Expression
(48) First, a minCDE gene expression plasmid pCUP2-PA-minCDE was prepared. The preparation was done as follows.
(49) PCR using a synthetic oligo DNA was carried out to obtain a DNA fragment (SEQ ID NO: 20) having a promotor sequence and a minCDE gene sequence. The DNA fragment was digested by restriction enzymes MunI and SpeI, and the resulting DNA fragment was joined to a plasmid vector pCUP2 which is described in WO 2007/049716 and which was cleaved by MunI and SpeI. Thus, the minCDE gene expression plasmid pCUP2-PA-minCDE was obtained.
(50) Subsequently, the minCDE gene expression plasmid pCUP2-PA-minCDE was introduced into the KNK-005 strain in the same manner as the plasmid pCUP2-PA-minC was introduced in Production Example 2. Thus, a minCDE gene expression-enhanced strain was obtained.
(Production Example 7) Preparation of minCD Gene Expression-Enhanced and minE Gene-Deleted Strain
(51) The minCD gene expression plasmid pCUP2-PA-minCD prepared in Production Example 4 was introduced into the minE gene-deleted strain prepared in Production Example 1 in the same manner as the plasmid pCUP2-PA-minC was introduced into the KNK-005 strain in Production Example 2. Thus, a minCD gene expression-enhanced and minE gene-deleted strain was obtained.
(Comparative Example 1) PHA Production by KNK-005 Strain
(52) Culture examination using the KNK-005 strain was conducted under the conditions described below.
Culture Media
(53) The seed culture medium was composed of 1 w/v % Meat-extract, 1 w/v % Bacto-Tryptone, 0.2 w/v % Yeast-extract, 0.9 w/v % Na.sub.2HPO.sub.4.Math.12H.sub.2O, and 0.15 w/v % KH.sub.2PO.sub.4 (pH=6.8). The preculture medium was composed of 1.1 w/v % Na.sub.2HPO.sub.4.Math.12H.sub.2O, 0.19 w/v % KH.sub.2PO.sub.4, 1.29 w/v % (NH.sub.4).sub.2SO.sub.4, 0.1 w/v % MgSO.sub.4.Math.7H.sub.2O, 2.5 w/v % palm olein oil, and 0.5 v/v % trace metal salt solution (solution of 1.6 w/v % FeCl.sub.3.Math.6H.sub.2O, 1 w/v % CaCl.sub.2.Math.2H.sub.2O, 0.02 w/v % CoCl.sub.2.Math.6H.sub.2O, 0.016 w/v %

CuSO.sub.4.Math.5H.sub.2O, and 0.012 w/v % NiCl.sub.2.Math.6H.sub.2O in 0.1N hydrochloric acid). Palm olein oil was added as a carbon source in a concentration of 10 g/L at one time. The PHA production culture medium was composed of 0.385 w/v % Na.sub.2HPO.sub.4.Math.12H.sub.2O, 0.067 w/v % KH.sub.2PO.sub.4, 0.291 w/v % (NH.sub.4).sub.2SO.sub.4, 0.1 w/v % MgSO4.Math.7H.sub.2O, and 0.5 v/v % trace metal salt solution (solution of 1.6 w/v % FeCl.sub.3.Math.6H.sub.2O, 1 w/v % CaCl.sub.2.Math.2H.sub.2O, 0.02 w/v % CoCl.sub.2.Math.6H.sub.2O, 0.016 w/v % CuSO.sub.4.Math.5H.sub.2O, and 0.012 w/v % NiCl.sub.2.Math.6H.sub.2O in 0.1N hydrochloric acid).

Method of Measuring Accumulated PHA Percentage

(54) The accumulated PHA percentage was measured as follows. The microorganism was collected from the culture fluid by centrifugation. The collected microorganism was washed with ethanol and freeze-dried to give a dried microorganism, the weight of which was measured. To 1 g of the dried microorganism was added 100 ml of chloroform, and the microorganism in chloroform was stirred at room temperature for a day to extract a PHA from the microorganism. The residual microorganism was removed by filtration, and the filtrate was concentrated using an evaporator to a total volume of 30 ml. To the concentrate was slowly added 90 ml of hexane, and the mixture was left for 1 hour under gentle stirring. The PHA precipitated was collected by filtration and vacuum-dried at 50° C. for 3 hours. The weight of the dried PHA was measured, and the percentage of the accumulated PHA to the dried microorganism was calculated.

Method of Measuring Cell Size

(55) The cell size was measured as follows. After the culture, the culture fluid was treated at 65° C. for 60 minutes to inactivate the microbial cells. The treated fluid was analyzed with a laser diffraction-scattering particle size distribution analyzer (Microtrac MT3300EXII) to measure the mean volume diameter (MV) of the cells. The measurement was conducted using standard settings (Permeability: Transparent, Particle refractive index: 1.81, Particle shape: Non-spherical, Solvent refractive index: 1.333).

Method of Measuring PHA Particle Size

(56) The PHA particle size was measured as follows. After the culture, the culture fluid was treated at 65° C. for 60 minutes to inactivate the microbial cells. The culture fluid was diluted to 150 times the original volume with a 3.3 w/v % aqueous solution of sodium dodecyl sulfate, and subjected to ultrasonic disintegration to obtain a liquid containing the extracted PHA. The ultrasonic disintegration was conducted using Ultrasonic Homogenizer UH-600 manufactured by SMT Co., Ltd. and consisted of four repetitions of ultrasonic stirring performed at a maximum output for 40 seconds. The resulting liquid containing the extracted PHA was analyzed with a laser diffraction-scattering particle size distribution analyzer (Microtrac MT3300EXII) to measure the mean volume diameter (MV) of the PHA particles. The measurement was conducted using standard settings (Permeability: Transparent, Particle refractive index: 1.81, Particle shape: Non-spherical, Solvent refractive index: 1.333).

Microscopic Observation of Cells

(57) Microscopic observation of the cells was conducted as follows. After the culture, the culture fluid was diluted as appropriate. The dilution was placed and dried on a glass slide, and then the cells were stained with fuchsin. The stained cells were observed with an optical microscope.

PHA Production Culture

(58) PHA production culture was performed as follows. First, a glycerol stock (50 μl) of the KNK-005 strain was inoculated into the seed culture medium (10 ml) and cultured for 24 hours to accomplish seed culture. Subsequently, the seed culture fluid was inoculated at a concentration of 1.0 v/v % into a 3 L jar fermenter (MDL-300, manufactured by B. E. Marubishi Co., Ltd.) containing 1.8 L of the preculture medium. The fermenter was operated at a culture temperature of 33° C., a stirring speed of 500 rpm, and an aeration of 1.8 L/min, and the preculture was conducted for 28 hours during which the pH was controlled between 6.7 and 6.8. For the pH control, a 14%

aqueous solution of ammonium hydroxide was used.

(59) Next, the preculture fluid was inoculated at a concentration of 5.0 v/v % into a 5 L jar fermenter (MDS-U50, manufactured by B. E. Marubishi Co., Ltd.) containing 2.5 L of the PHA production culture medium. The fermenter was operated at a culture temperature of 33° C., a stirring speed of 420 rpm, and an aeration of 2.1 L/min, and the pH was controlled between 6.7 and 6.8. For the pH control, a 25% aqueous solution of ammonium hydroxide was used. The carbon source was added intermittently. Palm olein oil was used as the carbon source. The culture was continued until the accumulated PHA percentage reached around 90%. The accumulated PHA percentage, the cell size, and the PHA particle size were measured as previously described. The results are listed in Table 1. An image taken by the microscopic cell observation conducted as previously described is shown in FIG. **1**.

(Comparative Example 2) PHA Production by minE Gene-Deleted Strain

(60) Culture examination using the minE gene-deleted strain was conducted under the same conditions as the culture examination in Comparative Example 1. The measurement results of the accumulated PHA percentage, the cell size, and the PHA particle size are listed in Table 1. An image taken by the microscopic cell observation conducted as previously described is shown in FIG. **2**.

(61) The results of the culture examination revealed that the cell size of the minE gene-deleted strain was little different from that of the KNK-005 strain which was a parent strain.

(Comparative Example 3) PHA Production by minC Gene Expression-Enhanced Strain

(62) Culture examination using the minC gene expression-enhanced strain was conducted under the same conditions as the culture examination in Comparative Example 1. The measurement results of the accumulated PHA percentage, the cell size, and the PHA particle size are listed in Table 1. An image taken by the microscopic cell observation conducted as previously described is shown in FIG. **3**.

(63) The results of the culture examination revealed that the cell size of the minC gene expression-enhanced strain was smaller than that of the KNK-005 strain which was a parent strain. Additionally, the PHA productivity of the minC gene expression-enhanced strain was considerably low, and the accumulated PHA percentage was only 83% despite the culture time being longer than in Comparative Example 1.

(Comparative Example 4) PHA Production by minD Gene Expression-Enhanced and minE Gene-Deleted Strain

(64) Culture examination using the minD gene expression-enhanced and minE gene-deleted strain was conducted under the same conditions as the culture examination in Comparative Example 1. The measurement results of the accumulated PHA percentage, the cell size, and the PHA particle size are listed in Table 1. An image taken by the microscopic cell observation conducted as previously described is shown in FIG. **4**.

(65) The results of the culture examination revealed that the cell size of the minD gene expression-enhanced and minE gene-deleted strain was little different from that of the KNK-005 strain which was a parent strain.

(Example 1) PHA Production by minD Gene Expression-Enhanced Strain

(66) Culture examination using the minD gene expression-enhanced strain was conducted under the same conditions as the culture examination in Comparative Example 1. The measurement results of the accumulated PHA percentage, the cell size, and the PHA particle size are listed in Table 1. An image taken by the microscopic cell observation conducted as previously described is shown in FIG. **5**.

(67) The results of the culture examination revealed that the cell size of the minD gene expression-enhanced strain was more than 10% above that of the KNK-005 strain which was a parent strain. Additionally, the PHA productivity was comparable to that of the KNK-005 strain.

(Example 2) PHA Production by minCD Gene Expression-Enhanced Strain

(68) Culture examination using the minCD gene expression-enhanced strain was conducted under the same conditions as the culture examination in Comparative Example 1. The measurement results of the accumulated PHA percentage, the cell size, and the PHA particle size are listed in Table 1. An image taken by the microscopic cell observation conducted as previously described is shown in FIG. **6**.
(69) The results of the culture examination revealed that the cell size of the minCD gene expression-enhanced strain was more than 15% above that of the KNK-005 strain which was a parent strain. Additionally, the PHA productivity was comparable to that of the KNK-005 strain. The particle size of the PHA produced by the minCD gene expression-enhanced strain was greater than the particle size of the PHA produced by the KNK-005 strain.
(Example 3) PHA Production by minCDE Gene Expression-Enhanced Strain
(70) Culture examination using the minCDE gene expression-enhanced strain was conducted under the same conditions as the culture examination in Comparative Example 1. The measurement results of the accumulated PHA percentage, the cell size, and the PHA particle size are listed in Table 1. An image taken by the microscopic cell observation conducted as previously described is shown in FIG. **7**.
(71) The results of the culture examination revealed that the cell size of the minCDE gene expression-enhanced strain was more than 20% above that of the KNK-005 strain which was a parent strain. Additionally, the particle size of the PHA produced by the minCDE gene expression-enhanced strain was greater than the particle size of the PHA produced by the KNK-005 strain.
(Example 4) PHA Production by minCD Gene Expression-Enhanced and minE Gene-Deleted Strain
(72) Culture examination using the minCD gene expression-enhanced and minE gene-deleted strain was conducted under the same conditions as the culture examination in Comparative Example 1. The measurement results of the accumulated PHA percentage, the cell size, and the PHA particle size are listed in Table 1. An image taken by the microscopic cell observation conducted as previously described is shown in FIG. **8**.
(73) The results of the culture examination revealed that the cell size of the minCD gene expression-enhanced and minE gene-deleted strain was more than 55% or more above that of the KNK-005 strain which was a parent strain. Additionally, the PHA productivity was almost comparable to that of the KNK-005 strain.
(74) The PHA produced in the culture examinations in Comparative Examples and Examples was found to be PHBH by HPLC analysis.
(75) TABLE-US-00001 TABLE 1 Percentage of accumulated PHA PHA to dried Cell particle microorganism size size Strain (%) (μm) (μm) Comp. Example 1 KNK-005 strain 90 1.89 1.74 Comp. Example 2 minE gene- 90 1.93 1.72 deleted strain Comp. Example 3 minC gene 83 1.58 1.34 expression- enhanced strain Comp. Example 4 minD gene 88 1.94 1.71 expression- enhanced and minE gene- deleted strain Example 1 minD gene 90 2.09 1.78 expression- enhanced strain Example 2 minCD gene 90 2.24 1.94 expression- enhanced strain Example 3 minCDE gene 90 2.34 1.87 expression- enhanced strain Example 4 minCD gene 87 3.00 1.73 expression- enhanced and minE gene- deleted strain

Claims

1. A transformed microorganism, belonging to the genus *Cupriavidus*, and comprising a polyhydroxyalkanoate synthase gene, wherein expression of a minC gene and a minD gene is enhanced as compared to a wild strain of the microorganism, wherein the minC gene is a gene having a nucleotide sequence that encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 1, or a polypeptide comprising an amino acid sequence that is at least 90% homologous to the amino acid sequence of SEQ ID NO: 1, the minD gene is a gene having a

nucleotide sequence that encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 2, or a polypeptide comprising an amino acid sequence that is at least 90% homologous to the amino acid sequence of SEQ ID NO: 2, and the polyhydroxyalkanoate synthase gene is a gene having a nucleotide sequence that encodes a polypeptide comprising the amino acid sequence of SEQ ID NOS: 4, 5, 6, 7, or 8, or a polypeptide having polyhydroxyalkanoate synthase activity and comprising an amino acid sequence that is at least 90% homologous to the amino acid sequence of SEQ ID NOS: 4, 5, 6, 7, or 8, and a size of microbial cells of the transformed microorganism that accumulates polyhydroxyalkanoate is larger compared to a size of microbial cells of the wild strain that accumulates the polyhydroxyalkanoate, and the transformed microorganism accumulates larger-size particles of the polyhydroxyalkanoate compared to the wild strain.

2. The transformed microorganism according to claim 1, wherein expression of a minE gene is enhanced as compared to a wild strain of the microorganism, wherein the minE gene is a gene having a nucleotide sequence that encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 3, or a polypeptide comprising an amino acid sequence that is at least 90% homologous to the amino acid sequence of SEQ ID NO: 3.

3. The transformed microorganism according to claim 2, which is transformed *Cupriavidus necator.*

4. A method of producing a polyhydroxyalkanoate, comprising culturing the transformed microorganism of claim 2 in the presence of a carbon source.

5. The method according to claim 4, wherein the carbon source comprises an oil or a fatty acid.

6. The method according to claim 4, wherein the carbon source comprises a sugar.

7. The method according to claim 4, wherein the carbon source contains comprises carbon dioxide.

8. The method according to claim 4, wherein the polyhydroxyalkanoate is a copolymer of at least two hydroxy alkanoates.

9. The method according to claim 8, wherein the polyhydroxyalkanoate is a copolymer comprising 3-hydroxyhexanoate as a monomer unit.

10. The method according to claim 9, wherein the polyhydroxyalkanoate is a copolymer of 3-hydroxybutyrate and 3-hydroxyhexanoate.

11. The transformed microorganism according to claim 1, which is transformed *Cupriavidus necator.*

12. A method of producing a polyhydroxyalkanoate, comprising culturing the transformed microorganism of claim 1 in the presence of a carbon source.

13. The method according to claim 12, wherein the carbon source comprises an oil or a fatty acid.

14. The method according to claim 12, wherein the carbon source comprises a sugar.

15. The method according to claim 12, wherein the carbon source comprises carbon dioxide.

16. The method according to claim 12, wherein the polyhydroxyalkanoate is a copolymer of at last two hydroxyalkanoates.

17. The method according to claim 16, wherein the polyhydroxyalkanoate is a copolymer comprising 3-hydroxyhexanoate as a monomer unit.

18. The method according to claim 17, wherein the polyhydroxyalkanoate is a copolymer of 3-hydroxybutyrate and 3-hydroxyhexanoate.

19. The transformed microorganism according to claim 1, wherein the size of microbial cells of the transformed microorganism that accumulates the polyhydroxyalkanoate is at least 2.24 μm, and the size of polyhydroxyalkanoate particles that are accumulated by the transformed microorganism is at least 1.94 μm.

20. The transformed microorganism according to claim 2, wherein the size of microbial cells of the transformed microorganism that accumulates the polyhydroxyalkanoate is at least 2.34 μm, and the size of polyhydroxyalkanoate particles that are accumulated by the transformed microorganism is at least 1.87 μm.