US011359217B2

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

(10) **Patent No.: US 11,359,217 B2**
(45) **Date of Patent: Jun. 14, 2022**

(54) **TRANSFORMANT OF CORYNEFORM BACTERIUM AND PRODUCTION METHOD FOR USEFUL COMPOUND USING SAME**

(71) Applicant: **RESEARCH INSTITUTE OF INNOVATIVE TECHNOLOGY FOR THE EARTH**, Kyoto (JP)

(72) Inventors: **Masayuki Inui**, Kyoto (JP); **Kazumi Hiraga**, Kyoto (JP); **Masako Suda**, Kyoto (JP); **Takeshi Kubota**, Kyoto (JP)

(73) Assignee: **RESEARCH INSTITUTE OF INNOVATIVE TECHNOLOGY FOR THE EARTH**, Kyoto (JP)

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

(21) Appl. No.: **17/051,514**

(22) PCT Filed: **Feb. 18, 2019**

(86) PCT No.: **PCT/JP2019/005902**
§ 371 (c)(1),
(2) Date: **Oct. 29, 2020**

(87) PCT Pub. No.: **WO2019/211937**
PCT Pub. Date: **Nov. 7, 2019**

(65) **Prior Publication Data**

US 2021/0222211 A1 Jul. 22, 2021

(30) **Foreign Application Priority Data**

May 1, 2018 (JP) JP2018-088424

(51) **Int. Cl.**

C12N 9/88	(2006.01)
C12P 7/22	(2006.01)
C12N 9/02	(2006.01)
C12N 9/10	(2006.01)
C12R 1/15	(2006.01)

(52) **U.S. Cl.**
CPC ***C12P 7/22*** (2013.01); ***C12N 9/0069*** (2013.01); ***C12N 9/1085*** (2013.01); ***C12N 9/88*** (2013.01); ***C12Y 113/11001*** (2013.01); ***C12Y 205/01054*** (2013.01); ***C12Y 401/01063*** (2013.01); ***C12Y 402/03004*** (2013.01); *C12R 2001/15* (2021.05)

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

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,272,073	A *	12/1993	Frost	C12N 9/1022 435/155
5,487,987	A	1/1996	Frost et al.	
5,616,496	A	4/1997	Frost et al.	
5,629,181	A	5/1997	Frost et al.	
8,809,583	B2	8/2014	Bui et al.	
9,453,248	B2 *	9/2016	Yukawa	C12N 9/88
2012/0196339	A1	8/2012	Koppisch et al.	
2013/0030215	A1	1/2013	Bui et al.	
2013/0252294	A1	9/2013	Koppisch et al.	
2015/0203880	A1	7/2015	Stephanopoulos et al.	
2019/0119664	A1	4/2019	Inui et al.	
2019/0194629	A1	6/2019	Inui et al.	

FOREIGN PATENT DOCUMENTS

JP	9-505463	6/1997
JP	9-506242	6/1997
JP	2013-516196	5/2013
WO	95/07979	3/1995
WO	95/07996	3/1995
WO	2011/085311	7/2011
WO	2012/106257	8/2012
WO	2015/069847	5/2015
WO	2016/207403	12/2016
WO	2017/146241	8/2017
WO	2017/169399	10/2017

OTHER PUBLICATIONS

International Search Report (ISR) dated May 21, 2019 in International (PCT) Application No. PCT/JP2019/005902.

K.M. Draths et al., "Environmentally Compatible Synthesis of Catechol from D-Glucose", J. Am. Chem. Soc., vol. 117, No. 9, pp. 2395-2400, 1995, cited in the specification.

Victor E. Balderas-Hernandez et al., "Catechol biosynthesis from glucose in *Escherichia coli* anthranilate-overproducer strains by heterologous expression of anthranilate 1,2-dioxygenase from *Pseudomonas aeruginosa* PAO1", Microbial Cell Factories, 13:136, 2014, cited in the specification.

Wensheng Li et al., "Benzene-Free Synthesis of Catechol: Interfacing Microbial and Chemical Catalysis", J. Am. Chem. Soc., vol. 127, No. 9, pp. 2874-2882, 2005, cited in the specification.

Xi-Hui Shen et al., "Genomic Analysis and Identification of Catabolic Pathways for Aromatic Compounds in *Corynebacterium glutamicum*", Microbes and Environments, vol. 20, No. 3, pp. 160-167, 2005, cited in CA.

Xihui Shen et al., "Key enzymes of the protocatechuate branch of the β-ketoadipate pathway for aromatic degradation in *Corynebacterium glutamicum*", Science in China Series C: Life Sciences, vol. 48, No. 3, pp. 241-249, 2005, cited in CA.

Junya Maeda et al., "characterization of phenol 2-monooxygenase from *Corynebacterium glutamicum*", Annual Meeting of the Japan Society for Bioscience, Biotechnology, and Agrochemisty, 2F201, 2016, with machine translation, cited in CA.

(Continued)

Primary Examiner — Hope A Robinson

(74) *Attorney, Agent, or Firm* — Wenderoth, Lind & Ponack, L.L.P.

(57) **ABSTRACT**

Provided is a transformant of a microorganism that has improved catechol productivity.

6 Claims, 1 Drawing Sheet

Specification includes a Sequence Listing.

(56) **References Cited**

OTHER PUBLICATIONS

Masao Tsuji et al., "Characterization of a Mutant Strain for Catechol Accumulation in *Corynebacterium glutamicum*", J. Ferment. Technol., vol. 54, No. 11, pp. 789-794, 1976, cited in CA.

Extended European Search Report dated Jan. 18, 2022, in Application No. 19796525.4.

Shin et al., "Characterization of a non-phosphotransferase system for cis, cis-muconic acid production in Corynebacterium glutamicum". Biochemical and Biophysical Research Communications, vol. 499, No. 2 (2018), pp. 279-284, cited in CA.

Johnson et al., "Enhancing muconic acid production from glucose and lignin-derived aromatic compounds viz increased protocatechuate decarboxylase activity", Metabolic Engineering Communications, vol. 3, (2016), pp. 111-119, cited in CA.

* cited by examiner

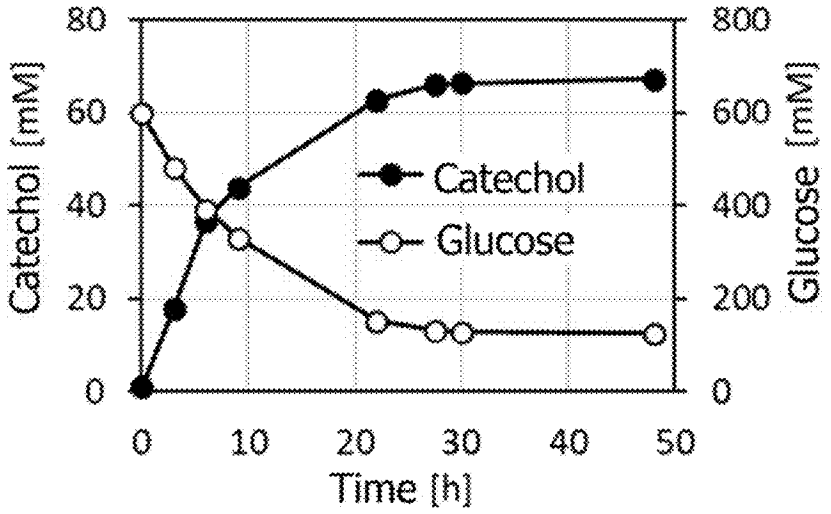
Catechol [mM]
Glucose [mM]
Time [h]
Catechol
Glucose
0
20
40
60
80
0
200
400
600
800
0
10
20
30
40
50

FIG. 1

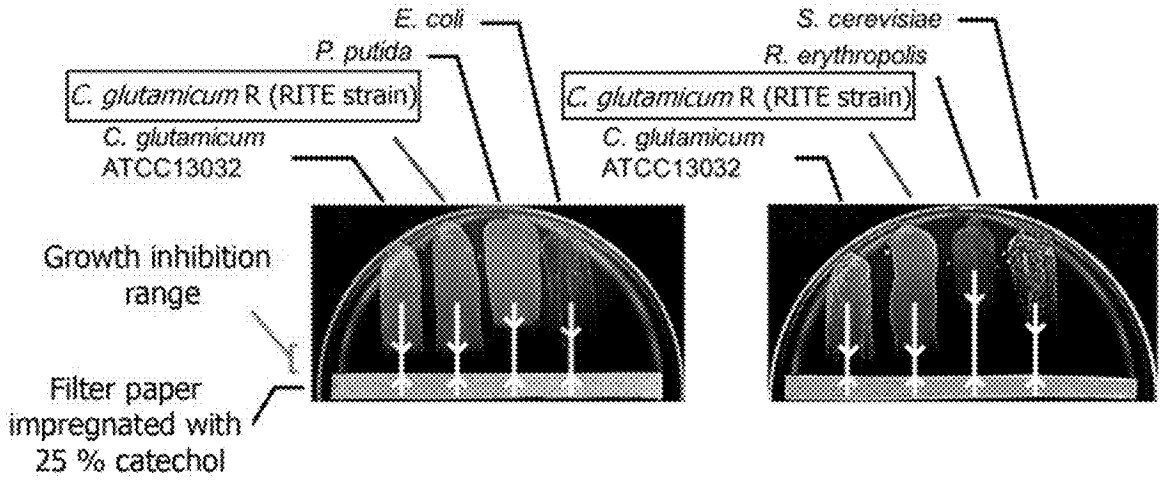
E. coli
P. putida
C. glutamicum R (RITE strain)
C. glutamicum ATCC13032
S. cerevisiae
R. erythropolis
C. glutamicum R (RITE strain)
C. glutamicum ATCC13032
Growth inhibition range
Filter paper impregnated with 25 % catechol

FIG. 2

TRANSFORMANT OF CORYNEFORM BACTERIUM AND PRODUCTION METHOD FOR USEFUL COMPOUND USING SAME

TECHNICAL FIELD

The present disclosure relates to a transformant of a coryneform bacterium. The present disclosure also relates to a method for producing a useful compound (for example, catechol) using the transformant.

BACKGROUND ART

Against the backdrop of global warming and exhaustion of fossil resources, production of chemical products using renewable resources has been recognized to be an important measure with view to realizing a low-carbon society, as new industrial biorefinery, along with biofuel, and has attracted attention.

Catechol is used as a raw material for synthesis of flavoring agents, polymerization inhibitors, antioxidants, pharmaceutical products, and a raw material for synthesis of agricultural chemicals. Catechol is also used as a raw material for removers for a resist (a photosensitive resin applied when a printed substrate is manufactured), deoxygenating agents (activated carbon adsorbents), and plating treatment agents.

Catechol is produced by an oxidation reaction using phenol as a main raw material. However, the production of catechol from renewable resources is earnestly desired, towards the realization of the above-described low-carbon society.

Catechol exists on the metabolic pathway of microorganisms. Catechol is produced through two-stage oxidation of benzene or a decarboxylation reaction with respect to dihydroxybenzoic acid. Thereafter, the decomposition of catechol is promoted through ortho-cleavage or meta-cleavage, and is incorporated in the tricarboxylic acid (TCA) cycle.

Patent Documents 1 and 2 discloses a technique for producing catechol from glucose using a transformed bacterium obtained by using a microorganism of the genus *Escherichia* or the genus *Klebsiella* as a host into which transketolase, DAHP synthase, and 3-dehydroquinate synthase are introduced, and further, dehydroshikimate dehydratase and protocatechuic acid decarboxylase derived from *Klebsiella pneumoniae* are introduced.

The invention disclosed by Patent Document 3 intends to produce adipic acid and cis,cis-muconate using microorganisms. The document, in the discussion, discloses an exemplary production of catechol using the strain having the same configuration as that disclosed in Patent Document 2.

Patent Documents 4 and 5 disclose methods for producing compounds using dehydroshikimic acid as a precursor, and propose a catechol producing method wherein protocatechuic acid decarboxylase derived from *Klebsiella pneumoniae, Enterobacter cloacae, Lactobacillus plantarum*, or *Clostridium butyricum* is caused to express. In the examples disclosed therein, a transformed bacterium obtained by causing 3,4-DHB decarboxylase derived from *Enterobacter cloacae* to express in *Escherichia coli* used.

The invention disclosed by Patent Document 6 intends to produce three types of isomers of muconic acid. The document, in the discussion, discloses an exemplary production of catechol using the strain having the same configuration as that disclosed in Patent Document 2.

Non-Patent Document 1 discloses a technique for producing catechol from glucose using a transformed bacterium obtained by introducing a protocatechuic acid decarboxylase gene of *Klebsiella pneumoniae* into *Escherichia coli*.

Non-Patent Document 2 discloses a technique for producing catechol from glucose using a transformed bacterium obtained by introducing an anthranilate 1,2-dioxygenase gene of *Pseudomonas aeruginosa* into *Escherichia coli*.

Non-Patent Document 3 discloses a technique for producing catechol from glucose using a transformed bacterium obtained by introducing a protocatechuic acid decarboxylase gene of *Klebsiella pneumoniae* into *Escherichia coli*.

PRIOR ART DOCUMENT

Patent Document

Patent Document 1: U.S. Pat. No. 5,272,073
Patent Document 2: JP-T-hei-9(1997)-506242
Patent Document 3: JP-T-hei-9(1997)-505463
Patent Document 4: US Patent No. 2012-0196339
Patent Document 5: US Patent No. 2013-0252294
Patent Document 6: JP-T-2013-516196

Non-Patent Document

Non-Patent Document 1: J. Am. Chem. Soc. (1995) 117: 2395-2400
Non-Patent Document 2: Microb. Cell Fact. (2014) 13:136
Non-Patent Document 3: J. Am. Chem. Soc. (2005) 127: 2874-2882

SUMMARY OF THE INVENTION

Problem to be Solved by the Invention

Regarding the catechol producing method based on a biological method, further improvement in the productivity is expected, toward practical use of the same.

The present disclosure, in one aspect, provides a microorganism that is able to efficiently produce catechol from a saccharide as a raw material, and a method of efficiently producing catechol by using the microorganism.

Means to Solve the Problem

The present disclosure, in one aspect, relates to a transformant of a coryneform bacterium,

wherein the transformant is obtained by introducing, into the coryneform bacterium as a host, at least one gene selected from the group consisting of:

(1) a decarboxylase gene ubiD of *Lactobacillus rhamnosus;*

(2) an ortholog of the gene (1) in at least one of the genus *Lactobacillus*, the genus *Bacillus*, the genus *Enterobacter*, the genus *Escherichia*, the genus *Paenibacillus*, the genus *Citrobacter*, and the genus *Pantoea*; and

(3) a gene in which an enzyme that has an amino acid sequence identity of 70% or more with an amino acid sequence of an enzyme encoded by the gene (1) or (2), and that has a decarboxylation activity, is encoded

wherein a mutation is introduced into a catechol 1,2-dioxygenase gene catA and a protocatechuic acid dehydrogenase gene pcaHG of the coryneform bacterium as a host, and functions of enzymes encoded by the two genes are degraded or lost.

The present disclosure, in another aspect, relates to a catechol producing method that includes:

the step of causing the transformant of a coryneform bacterium according to the present disclosure react in a reaction solution from which at least one of factors necessary for growth, or in a reaction solution under reducing conditions; and

the step of collecting catechol in a reaction medium.

Effect of the Invention

According to the present disclosure, in one aspect, the production of catechol in a coryneform bacterium can be made efficient. For example, the production rate and/or yield in the catechol production can be improved.

BRIEF DESCRIPTION OF DRAWINGS

FIG. **1** is a graph showing an exemplary catechol production using a strain CAT21.

FIG. **2** shows an exemplary experiment that indicates high resistance of a coryneform bacterium against catechol.

MODE FOR CARRYING OUT THE INVENTION

As a result of earnest studies, the present inventors found that the catechol productivity can be improved by causing a predetermined decarboxylase to be expressed in a coryneform bacterium into which a mutation that suppresses decomposition of protocatechuic acid and catechol is introduced.

It is estimated that the decarboxylation reaction of protocatechuic acid is accelerated by causing the predetermined decarboxylase to be expressed, whereby the catechol productivity is improved. The present disclosure, however, is not limited to this mechanism.

According to the present disclosure, in one aspect, the production concentration and/or yield of catechol can be improved.

[Host]

In the present disclosure, the host into which a predetermined decarboxylase is introduced is a coryneform bacterium.

In the present disclosure, the coryneform bacteria are a group of microorganisms defined in Bergey's Manual of Determinative Bacteriology Vol. 8, 599 (1974), and are not particularly limited as long as they grow under normal aerobic conditions. The specific examples include bacteria of the genus *Corynebacterium*, bacteria of the genus *Brevibacterium*, bacteria of the genus *Arthrobacter*, bacteria of the genus *Mycobacterium* and bacteria of the genus *Micrococcus* Among the coryneform bacteria, bacteria of the genus *Corynebacterium* are preferred.

Examples of the genus *Corynebacterium* include *Corynebacterium glutamicum, Corynebacterium efficiens, Corynebacterium ammomagenes, Corynebacterium halotolerance*, and *Corynebacterium alkanolyticum*. Among them, *Corynebacterium glutamicum* is preferred for safety and high xylooligosaccharide utilization.

Examples of preferred strains include *Corynebacterium glutamicum* R (FERM P-18976), ATCC13032, ATCC13869, ATCC13058, ATCC13059, ATCC13060, ATCC13232, ATCC13286, ATCC13287, ATCC13655, ATCC13745, ATCC13746, ATCC13761, ATCC14020, ATCC31831, MJ-233 (FERM BP-1497), MJ-233AB-41 (FERM BP-1498). Among them, strains R (FERM P-18976), ATCC13032, and ATCC13869 are preferred.

These strains are available from NBRC (NITE Biological Resource Center), ATCC (American Type Culture Collection), etc., which are microorganism culture collections.

Further, these microorganisms are not only wild strains that exist in the natural world, but may be mutant strains or gene recombinant strains of the same.

With a view to improving the catechol productivity, the transformant according to the present disclosure is configured so that mutations are introduced into the gene catA that encodes an enzyme having a catechol 1,2-dioxygenase activity, and into the gene pcaHG that encodes an enzyme having a protocatechuic acid dehydrogenase activity, in the genome of the coryneform bacterium as a host; and functions of these two enzymes are degraded or lost. Examples of the mutations include substitution, deletion, and insertion of a base sequence.

These mutations may be introduced in advance into a coryneform bacterium to be used as a host, or may be introduced in a process of producing the transformant according to the present disclosure.

Further, with a view to improving the catechol productivity, a gene-modified strain that would improve the production of the protocatechuic acid may be used as a coryneform bacterium as a host (for example, WO2017/169399).

[Introduction of Decarboxylase]

In the present disclosure, a decarboxylase that is introduced into a coryneform bacterium as a host is preferably an enzyme that has a decarboxylation activity with respect to protocatechuic acid.

Examples of the introduction of an enzyme having a decarboxylation activity with respect to protocatechuic acid, into a coryneform bacterium as a host, include the introduction of any one of the following genes (1) to (3) below:

(1) a decarboxylase gene ubiD of *Lactobacillus rhamnosus;*

(2) an ortholog of the gene (1) in the genus *Lactobacillus*, the genus *Bacillus*, the genus *Enterobacter*, the genus *Escherichia*, the genus *Paenibacillus*, the genus *Citrobacter*, and the genus *Pantoea*; and

(3) a gene in which an enzyme that has an amino acid sequence identity of 70% or more with an amino acid sequence of an enzyme encoded by the gene (1) or (2), and that has a decarboxylation activity, is encoded.

In the present disclosure, the introduction of the genes (1) to (3) into a host coryneform bacterium can be performed by using a common gene recombination technique (for example, the method proposed by Michael R Green & Joseph Sambrook, "Molecular cloning", Cold spring Harbor Laboratory Press); it can be implemented in the form of the introduction of a gene by using a plasmid vector, or the incorporation of a gene into a host coryneform bacterium chromosome.

In the present disclosure, "incorporating/introducing a gene" refers to incorporating or introducing a gene into a host in such a manner that the gene can express in the host, in one or a plurality of embodiments.

For example, to introduce the ubiDX gene into a host coryneform bacterium, it is preferable to introduce an appropriate promoter in an upstream region on the 5' side of the gene, and it is more preferable to additionally introduce a terminator in a downstream region on the 3' side.

[Decarboxylase Gene ubiD of *Lactobacillus rhamnosus*]

In the present disclosure, a decarboxylase gene ubiD of *Lactobacillus rhamnosus* is registered as LGG_02656 or LGG_RS12695 in a database such as NCBI, in one or a plurality of embodiments.

A decarboxylase gene to be introduced into a host may be an ortholog of the above-described ubiD of *Lactobacillus rhamnosus*. Examples of orthologs of ubiD of *Lactobacillus rhamnosus* include orthologs of the genus *Lactobacillus*, the genus *Bacillus*, the genus *Enterobacter*, the genus *Escherichia*, the genus *Paenibacillus*, the genus *Citrobacter*, and the genus *Pantoea*; with a view to improving the catechol productivity, orthologs of the genus *Lactobacillus*, the genus *Bacillus*, and the genus *Enterobacter* are preferred; among these, orthologs of the genus *Lactobacillus* and the genus *Bacillus* are more preferred; among these, orthologs of the genus *Lactobacillus* are further preferred; and the genes used in Examples are still further preferred.

Examples of the ortholog of the genus *Lactobacillus* for the ubiD gene of *Lactobacillus rhamnosus* include, though not limited to, the ubiD gene of *Lactobacillus pentosus*, the ubiD gene of *Lactobacillus plantarum*, the ubiD gene of *Lactobacillus pobuzihii*, and the ubiD gene of *Lactobacillus composti*.

Examples of the ortholog of the genus *Bacillus* for the ubiD gene of *Lactobacillus rhamnosus* include, though not limited to, the ubiD gene of *Bacillus megaterium*, the ubiD gene of *Bacillus licheniformis*; the ubiD gene of *Bacillus atrophaeus*, the ubiD gene of *Bacillus subtilis* subsp. *subtilis*; and the ubiD gene of *Bacillus subtilis* subsp. *spizizenii*.

Examples of the ortholog of the genus *Enterobacter* for the ubiD gene of *Lactobacillus rhamnosus* include, though not limited to, the ubiD gene of *Enterobacter aerogenes* the ubiD gene of *Enterobacter cloacae*, the ubiD gene of *Enterobacter sakazakii*; and the ubiD gene of *Enterobacter hormaechei*.

Examples of the ortholog of the genus *Escherichia* for the ubiD gene of *Lactobacillus rhamnosus* include, though not limited to, the ubiD gene of *Escherichia coli*, and the ubiD gene of *Escherichia fergusonii*.

Examples of the ortholog of the genus *Paenibacillus* for the ubiD gene of *Lactobacillus rhamnosus* include, though not limited to, the ubiD gene of *Paenibacillus polymyxa*.

Examples of the ortholog of the genus *Citrobacter* for the ubiD gene of *Lactobacillus rhamnosus* include, though not limited to, the ubiD gene of *Citrobacter koseri*.

Examples of the ortholog of the genus *Pantoea* for the ubiD gene of *Lactobacillus rhamnosus* include, though not limited to, the ubiD gene of *Pantoea ananatis*.

It should be noted that the "ortholog gene" in the present disclosure means an analog gene that encodes a protein having a homologous function, existing in a different organism (for example, a different species, a different genus).

A decarboxylase gene to be introduced into a host may be a gene in which an enzyme that has an amino acid sequence identity of 70% or more with an amino acid sequence of an enzyme encoded by the ubiD gene of *Lactobacillus rhamnosus* described above or an ortholog of the same, and that has a decarboxylation activity, is encoded.

The identity of the amino acid sequence is 70% or more, preferably 75% or more, more preferably 80% or more, and further preferably 85% or more, with a view to improving the catechol productivity.

In the present disclosure, it is preferable that, together with the ubiD gene, the ubiX gene, located in the same genome as that of the ubiD gene, is introduced into a host coryneform bacterium together with the ubiD gene, with a view to improving the catechol productivity. Besides, in a case where the ubiH gene is present in the same genome as that of the ubiD gene, it is preferable that the ubiH gene is also introduced into a host coryneform bacterium together with the ubiD gene and the ubiX gene, with a view to improving the catechol productivity.

The ubiD gene and the ubiX gene of *Lactobacillus rhamnosus*, arrayed in this order, constitute an operon, and in such a case, they are described as an ubiDX gene in the present disclosure. In one or a plurality of embodiments, an exemplary base sequence of the ubiDX gene of *Lactobacillus rhamnosus* is the base sequence of SEQ ID NO: 1 in the sequence listing.

In the case where a ubiD gene of *Lactobacillus rhamnosus* is to be introduced into a host coryneform bacterium, the same is preferably introduced as the ubiDX gene of *Lactobacillus rhamnosus*, with a view to improving the catechol productivity. Besides, in the case where an ortholog of a ubiD gene of *Lactobacillus rhamnosus* to be introduced into a host coryneform bacterium, similarly, the ubiX gene and the ubiD gene are preferably introduced into a host coryneform bacterium, with a view to improving the catechol productivity; if there is the ubiH gene in the genome, it is preferable that the ubiH gene, the ubiD gene and the ubiX gene are also introduced into a host coryneform bacterium.

The ubiX gene of *Lactobacillus pentosus*, together with the ubiH gene, constitutes an operon (an ubiHX gene), independently from the ubiD gene. In the case where a ubiD gene of *Lactobacilluspentosus* to be introduced into a host coryneform bacterium, the ubiHX gene and the ubiD gene are preferably introduced, with a view to improving the catechol productivity. In one or a plurality of embodiments, exemplary base sequences of the ubiXH gene and the ubiD gene of *Lactobacillus pentosus* are the base sequences of SEQ ID NOs: 2 and 3 in the sequence listing, respectively.

In the case where a ubiD gene of *Lactobacillus plantarum* is to be introduced into a host coryneform bacterium, similarly, the ubiHX gene and the ubiD gene are preferably introduced therein, with a view to improving the catechol productivity, as is the case with *Lactobacillus pentosus* In one or a plurality of embodiments, exemplary base sequences of the ubiXH gene and the ubiD gene of *Lactobacillus plantarum* are the base sequences of SEQ ID NOs: 4 and 5 in the sequence listing, respectively.

In the case where the ubiD gene of *Lactobacillus pobuzihii* or that of *Lactobacillus composti*, is to be introduced into a host coryneform bacterium, similarly, it is preferably introduced as the ubiDX gene of *Lactobacillus pobuzihii* or *Lactobacillus composti*, with a view to improving the catechol productivity. In one or a plurality of embodiments, exemplary base sequences of the ubiDX gene of *Lactobacillus pobuzihii* and *Lactobacillus composti* are the base sequences of SEQ ID NOs: 6 and 7 in the sequence listing.

Regarding the ubiD gene of *Bacillus megaterium*, the ubiX gene, the ubiD gene, and the ubiH gene, arrayed in this order, constitute an operon, and in such a case, they are described as an ubiXDH gene in the present disclosure. In the case where the ubiD gene of *Bacillus megaterium* is to be introduced into a host coryneform bacterium, the same is preferably introduced as the ubiXDH gene, with a view to improving the catechol productivity. In one or a plurality of embodiments, an exemplary base sequence of the ubiXDH gene of *Bacillus megaterium* is the base sequence of SEQ ID NO: 10 in the sequence listing.

In the case where the ubiD gene of *Bacillus licheniformis* is to be introduced into a host coryneform bacterium, the same is preferably introduced as the ubiXDH gene, with a view to improving the catechol productivity. In one or a plurality of embodiments, an exemplary base sequence of the ubiXDH gene of *Bacillus licheniformis* is the base sequence of SEQ ID NO: 11 in the sequence listing.

In the case where the ubiD gene of *Bacillus atrophaeus* is to be introduced into a host coryneform bacterium, the same is preferably introduced as the ubiXDH gene, with a view to improving the catechol productivity. In one or a plurality of embodiments, an exemplary base sequence of the ubiXDH gene of *Bacillus atrophaeus* is the base sequence of SEQ ID NO: 12 in the sequence listing.

In the case where the ubiD gene of *Bacillus subtilis* subsp. *subtilis* is to be introduced into a host coryneform bacterium, the same is preferably introduced as the ubiXDH gene, with a view to improving the catechol productivity. In one or a plurality of embodiments, an exemplary base sequence of the ubiXDH gene of *Bacillus subtilis* subsp. *subtilis* is the base sequence of SEQ ID NO: 13 in the sequence listing.

In the case where the ubiD gene of *Bacillus subtilis* subsp. *spizizenii* is to be introduced into a host coryneform bacterium, the same is preferably introduced as the ubiXDH gene, with a view to improving the catechol productivity. In one or a plurality of embodiments, an exemplary base sequence of the ubiXDH gene of *Bacillus subtilis* subsp. *spizizenii* is the base sequence of SEQ ID NO: 14 in the sequence listing.

In the case where the ubiD gene of *Enterobacter aerogenes* is to be introduced into a host coryneform bacterium, the same is preferably introduced as the ubiXDH gene, with a view to improving the catechol productivity. In one or a plurality of embodiments, an exemplary base sequence of the ubiXDH gene of *Enterobacter aerogenes* is the base sequence of SEQ ID NO: 15 in the sequence listing.

In the case where the ubiD gene of *Enterobacter cloacae* is to be introduced into a host coryneform bacterium, the same is preferably introduced as the ubiXDH gene, with a view to improving the catechol productivity. In one or a plurality of embodiments, an exemplary base sequence of the ubiXDH gene of *Enterobacter cloacae* is the base sequence of SEQ ID NO: 16 in the sequence listing.

In the case where the ubiD gene of *Enterobacter sakazakii* is to be introduced into a host coryneform bacterium, the same is preferably introduced as the ubiXDH gene, with a view to improving the catechol productivity. In one or a plurality of embodiments, an exemplary base sequence of the ubiXDH gene of *Enterobacter sakazakii* is the base sequence of SEQ ID NO: 17 in the sequence listing.

In the case where the ubiD gene of *Enterobacter hormaechei* is to be introduced into a host coryneform bacterium, the same is preferably introduced in the form of the ubiXDH gene, with a view to improving the catechol productivity. In one or a plurality of embodiments, an exemplary base sequence of the ubiXDH gene of *Enterobacter hormaechei* is the base sequence of SEQ ID NO: 18 in the sequence listing.

In the case where the ubiD gene of *Escherichia coli* W is to be introduced into a host coryneform bacterium, the same is preferably introduced as the ubiXDH gene, with a view to improving the catechol productivity. In one or a plurality of embodiments, an exemplary base sequence of the ubiXDH gene of *Escherichia coli* W is the base sequence of SEQ ID NO: 19 in the sequence listing.

In the case where the ubiD gene of *Escherichia fergusonii* is to be introduced into a host coryneform bacterium, the same is preferably introduced as the ubiXDH gene, with a view to improving the catechol productivity. In one or a plurality of embodiments, an exemplary base sequence of the ubiXDH gene of *Escherichia fergusonii* is the base sequence of SEQ ID NO: 20 in the sequence listing.

In the case where the ubiD gene of *Paenibacillus polymyxa* is to be introduced into a host coryneform bacterium, the same is preferably introduced as the ubiXDH gene, with a view to improving the catechol productivity. In one or a plurality of embodiments, an exemplary base sequence of the ubiXDH gene of *Paenibacillus polymyxa* is the base sequence of SEQ ID NO: 21 in the sequence listing.

In the case where the ubiD gene of *Citrobacter koseri* is to be introduced into a host coryneform bacterium, the same is preferably introduced as the ubiXDH gene, with a view to improving the catechol productivity. In one or a plurality of embodiments, an exemplary base sequence of the ubiXDH gene of *Citrobacter koseri* is the base sequence of SEQ ID NO: 22 in the sequence listing.

In the case where the ubiD gene of *Pantoea ananatis* is to be introduced into a host coryneform bacterium, the same is preferably introduced as the ubiXDH gene, with a view to improving the catechol productivity. In one or a plurality of embodiments, an exemplary base sequence of the ubiXDH gene of *Pantoea ananatis* is the base sequence of SEQ ID NO: 23 in the sequence listing.

[Transformant]

The present disclosure, in one aspect, relates to a transformant obtained by introducing any one of the above-described genes (1) to (3) into a host coryneform bacterium, wherein functions of two enzymes in the host genome, which are catechol 1,2-dioxygenase (catA) and protocatechuic acid dehydrogenase (pcaHG), are degraded or lost.

The transformant according to the present disclosure, in one or a plurality of embodiments, is capable of efficiently producing catechol.

In the transformant according to the present disclosure, in one or a plurality of embodiments, the ubiX gene and/or the ubiH gene are preferably introduced, with a view to improving the catechol productivity.

The transformant according to the present disclosure may be further characterized in that another gene (or genes) is introduced therein, or that a gene (or genes) is deleted and/or mutated, to produce catechol or to make the production more efficient.

In one or a plurality of embodiments for making the production of catechol more efficient, the introduction or disruption of a gene for improving the production of protocatechuic acid is performed, for example. Exemplary introduction of a gene for improving the production of protocatechuic acid is the introduction of a gene that encodes an enzyme having 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase activity (for example, aroG), and/or a gene that encodes an enzyme having 3-dehydroquinate synthase activity (for example, qsuB).

The transformant according to the present disclosure, in one or a plurality of embodiments, can be used in the production of catechol. The transformant according to the present disclosure, in one or a plurality of embodiments, can be used in the production of an organic compound from catechol as an intermediate.

[Method for Producing Catechol]

The transformant according to the present disclosure is capable of producing catechol at a high efficiency in a reaction solution without bacterial cell growth, using saccharides as raw materials.

The present disclosure, therefore, in another aspect, relates to a catechol producing method that includes the steps of causing the transformant of the coryneform bacterium according to the present disclosure to react in a reaction solution in which at least one of factors necessary for growth is removed, or in a reaction solution under reducing conditions; and collecting catechol in a reaction medium.

In the catechol producing method according to the present invention, first of all, the above-described transformant according to the present disclosure is cultured to grow under aerobic conditions.

The transformant according to the present disclosure can be cultured by using a normal nutrient medium that contains a carbon source, a nitrogen source, inorganic salts, and the like. In the culture, as a carbon source, for example, glucose, waste molasses, or the like can be used alone or in mixture, and as a nitrogen source, for example, ammonium, ammonium sulfate, ammonium chloride, ammonium nitrate, urea, or the like can be used alone or in mixture. Further, as an inorganic salt, for example, dibasic potassium phosphate, potassium dihydrogen phosphate, magnesium sulfate, or the like can be used. In addition to these, nutrients such as peptone, meat extract, yeast extract, corn steep liquor, various types of vitamins such as casamino acid, biotin, or thiamine can be appropriately added to the medium as required.

Generally, the culturing can be carried out under aerobic conditions such as aeration stirring or shaking, at a temperature of about 20° C. to about 60° C., preferably about 25° C. to about 35° C. The pH during the culturing is in a range of, for example, around 5 to 10, preferably around 7 to 8, and the pH adjustment during the culturing can be carried out by adding acid or alkali. The carbon source concentration at the start of the culturing is about 1% (W/V) to about 20% (W/V), preferably about 2% (W/V) to about 5% (W/V). Further, the culturing period is usually about 1 to 7 days.

Next, cultured bacterial cells of the transformant according to the present disclosure are collected. A method for collecting and separating cultured bacterial cells from the cultured substance thus obtained as described above is not limited particularly, and a known method such as centrifugation or membrane separation can be used, for example.

The cultured bacterial cells thus collected may be processed, and the processed bacterial cells thus obtained may be used in the next step. Examples of the processed bacterial cells include cultured bacterial cells subjected to a certain processing operation, for example, immobilized bacterial cells that are obtained by immobilizing bacterial cells with acrylamide, carrageenan, or the like.

In the catechol production reaction by the cultured bacterial cells of the transformant according to the present disclosure, collected and separated from the cultured substance thus obtained as described above, or by the processed bacterial cells obtained from the same, any production process under aerobic conditions or reducing conditions may be used, as long as it is in a solution of a reaction without bacterial cell growth. The catechol production process may be of a batch type, or of a continuous type.

In the present disclosure, "does not grow" encompasses "substantially does not grow", and "hardly grows". For example, in a reaction under aerobic conditions, growth of the transformant can be avoided or inhibited by the use of a reaction solution in which one or more of compounds essential for the growth of the microorganism, for example, vitamins, such as biotin and thiamine, nitrogen sources, etc. is depleted or limited.

Besides, under reducing conditions, coryneform bacteria substantially do not grow, and therefore, the composition of the reaction solution is not limited. The oxidation-reduction potential of the reaction solution under reducing conditions is preferably about-200 mV to about −500 mV, and more preferably about −150 mV to −500 mV. The reduced state of the reaction solution can be simply estimated using a resazurin indicator (in a reduced state, decolorization from blue to colorless is observed). However, for precise measurement, a redox-potential meter (for example, ORP Electrodes made by BROADLEY JAMES) may be used.

In the present disclosure, it is preferable that reducing conditions are maintained immediately after bacterial cells or processed bacterial cells are added to a reaction solution until catechol is collected; however, a reaction solution may be in a reduced state at least at the point in time when catechol is collected. It is desirable that a reaction solution is kept under reducing conditions during about 50% or more of a reaction period, preferably during about 70% or more of the same, and more preferably during about 90% or more of the same. Particularly, it is more desirable that a reaction solution has an oxidation-reduction potential kept at about −200 mV to about −500 mV during about 50% or more of a reaction period, preferably during about 70% or more of the same, and more preferably during about 90% or more of the same.

The reaction solution contains an organic carbon source (for example, saccharides) that are raw materials used in the production of catechol. Examples of the organic carbon source include materials that the transformant according to the present disclosure can utilize in a biochemical reaction.

Specifically, examples of saccharides include monosaccharides, such as glucose, xylose, arabinose, galactose, fructose, and mannose; disaccharides, such as cellobiose, sucrose, lactose, and maltose; and polysaccharides, such as dextrin and soluble starch; etc. Among these, glucose is preferable.

The present disclosure, therefore, in one aspect, relates to a catechol producing method that includes the steps of causing the transformant of the coryneform bacterium according to the present disclosure to react in a reaction solution in which at least one of factors necessary for growth is removed, or in a reaction solution under reducing conditions; and collecting catechol in a reaction medium.

Finally, the catechol produced in the reaction medium as described above is collected. For doing so, a known method that is used in bioprocessing can be used. Examples of such a known method include the salting-out method, the recrystallization method, the organic solvent extraction method, the distillation method (reactive distillation by esterification etc.), the chromatography separation method, and the electrodialysis method, which can be used with respect to a solution of catechol. The method for separating and purifying catechol may be decided appropriately.

The present disclosure relates to the following, in one or a plurality of embodiments:

[1] A transformant of a coryneform bacterium that is obtained by introducing, into the coryneform bacterium as a host, at least one gene selected from the group consisting of:

(1) a decarboxylase gene ubiD of *Lactobacillus rhamnosus;*

(2) an ortholog of the gene (1) in at least one of the genus *Lactobacillus*, the genus *Bacillus*, the genus *Enterobacter*, the genus *Escherichia*, the genus *Paenibacillus*, the genus *Citrobacter*, or the genus *Pantoea*; and

(3) a gene in which an enzyme that has an amino acid sequence identity of 70% or more with an amino acid sequence of an enzyme encoded by the gene (1) or (2), and that has a decarboxylation activity, is encoded.

wherein mutations are introduced into a catechol 1,2-dioxygenase gene catA, and a protocatechuic acid dehydrogenase gene pcaHG in the coryneform bacterium as a host; and functions of enzymes encoded by the gene catA and functions of enzymes encoded by the gene pcaHG are degraded or lost.

[2] The transformant according to Item [1],

wherein the transformant has a catechol producing ability.

[3] The transformant according to Item [1] or [2],

wherein at least one of a gene that encodes an enzyme having 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase activity, and a gene that encodes an enzyme having 3-dehydroquinate synthase activity, is additionally introduced.

[4] The transformant according to any one of Items [1] to [3],

wherein the coryneform bacterium as a host is *Corynebacterium glutamicum.*

[5] The transformant according to any one of Items [1] to [4],

wherein the coryneform bacterium as a host is *Corynebacterium glutamicum* R (FERM P-18976), ATCC13032, or ATCC13869.

[6] A transformant of *Corynebacterium glutamicum* CAT21 (Accession Number: NITE BP-02689).

[7] A catechol producing method including the steps of

causing the transformant of a coryneform bacterium according to any one of Items [1] to [6] to react in a reaction solution in which at least one of factors necessary for growth is removed, or in a reaction solution under reducing conditions; and

collecting catechol in a reaction medium.

[8] The catechol producing method according to Item [7],

wherein, in the reaction solution, at least one saccharide selected from the group consisting of glucose, fructose, cellobiose, xylobiose, sucrose, lactose, maltose, dextrin, xylose, arabinose, galactose, mannose, and soluble starch is converted into catechol with use of the transformant according to any one of Items [1] to [6], and catechol is collected from the reaction solution.

EXAMPLE

The following description describes the present invention in detail, while referring to examples, but the present invention is not limited to these examples.

Example 1

Construction of Catechol Producing Strain

(1) Preparation/Obtainment of Chromosomal DNA

Corynebacterium glutamicum R(FERM P-18976), *Lactobacillus rhamnosus* NBRC 3425, *Lactobacillus pentosus* JCM 1558, *Lactobacillus plantarum* NBRC 3070, *Lactobacillus pobuzihii* JCM 18084, *Lactobacillus composti* JCM 14202, *Lactobacillus hokkaidonensis* JCM 18461, *Lactobacillus sakei* subsp. *sakei* JCM 1157, *Bacillus megaterium* JCM 2506, *Bacillus licheniformis* JCM 2505, *Bacillus atrophaeus* JCM 9070, *Bacillus subtilis* subsp. *subtilis* NBRC 14144, *Bacillus subtilis* subsp. *spizizenii* NBRC 101239, *Enterobacter aerogenes* NBRC 13534, *Enterobacter cloacae* NBRC 13535, *Enterobacter hormaechei* ATCC 49162, *Escherichia coli* W NBRC 13500, *Escherichia fergusonii* NBRC 102419, *Paenibacillus polymyxa* NBRC 15309, and *Pantoea ananatis* LMG 20103 were cultured according to information obtained from organizations from which the strains are available, and thereafter, chromosomal DNAs thereof were prepared by using DNA genome extraction kit (trade name: "GenomicPrep Cells and Tissue DNA Isolation Kit", manufactured by Amersham PLC). Chromosomal DNAs of *Enterobacter sakazakii* ATCC BAA-894D-5 and *Citrobacter koseri* ATCC BAA-895D-5 were obtained from ATCC.

(2) Construction of Plasmid for Expression of Catechol-Production-Related Gene

Primer sequences used for isolating target enzyme genes are shown in Table 1. In PCR, Veriti Thermal Cycler (manufactured by Applied Biosystems Inc.) was used, and PrimeSTAR HS DNA Polymerase (manufactured by Takara Bio Inc.) was used as a reaction reagent.

DNA fragments obtained were introduced into cloning vectors containing PgapApromoters (pCRB209 [WO2012/033112], pCRB210 [WO2012/033112]). It should be noted that in *Lactobacillus pentosus* and those of *Lactobacillus plantarum*, the ubiD gene and the ubiXH gene are located at different positions on the chromosome, and therefore they were separately cloned, and then transferred onto the same plasmid.

The names of the cloning vectors introduced and the plasmids obtained are shown in Table 2.

TABLE 1

Primer for Isolation of Catechol-Production-Related Gene

Gene Source	Enzyme Gene	Forward	Reverse	Gene Sequence
Lactobacillus rhamnosus	ubiDX	SEQ ID NO. 24	SEQ ID NO. 25	SEQ ID NO. 1
Lactobacillus pentosus	ubiXH	SEQ ID NO. 26	SEQ ID NO. 27	SEQ ID NO. 2
Lactobacillus pentosus	ubiD	SEQ ID NO. 28	SEQ ID NO. 29	SEQ ID NO. 3
Lactobacillus plantarum	ubiXH	SEQ ID NO. 30	SEQ ID NO. 31	SEQ ID NO. 4
Lactobacillus plantarum	ubiD	SEQ ID NO. 32	SEQ ID NO. 33	SEQ ID NO. 5
Lactobacillus pobuzihii	ubiDX	SEQ ID NO. 34	SEQ ID NO. 35	SEQ ID NO. 6
Lactobacillus composti	ubiDX	SEQ ID NO. 36	SEQ ID NO. 37	SEQ ID NO. 7
Lactobacillus hokkaidonensis	ubiDXH	SEQ ID NO. 38	SEQ ID NO. 39	SEQ ID NO. 8
Lactobacillus sakei subsp. *sakei*	ubiDXH	SEQ ID NO. 40	SEQ ID NO. 41	SEQ ID NO. 9
Bacillus megaterium	ubiXDH	SEQ ID NO. 42	SEQ ID NO. 43	SEQ ID NO. 10
Bacillus licheniformis	ubiXDH	SEQ ID NO. 44	SEQ ID NO. 45	SEQ ID NO. 11
Bacillus atrophaeus	ubiXDH	SEQ ID NO. 46	SEQ ID NO. 47	SEQ ID NO. 12
Bacillus subtilis subsp. *subtilis*	ubiXDH	SEQ ID NO. 48	SEQ ID NO. 49	SEQ ID NO. 13
Bacillus subtilis subsp. *spizizenii*	ubiXDH	SEQ ID NO. 50	SEQ ID NO. 51	SEQ ID NO. 14
Enterobacter aerogenes	ubiXDH	SEQ ID NO. 52	SEQ ID NO. 53	SEQ ID NO. 15
Enterobacter cloacae	ubiXDH	SEQ ID NO. 54	SEQ ID NO. 55	SEQ ID NO. 16
Enterobacter sakazakii	ubiXDH	SEQ ID NO. 56	SEQ ID NO. 57	SEQ ID NO. 17
Enterobacter hormaechei	ubiXDH	SEQ ID NO. 58	SEQ ID NO. 59	SEQ ID NO. 18

TABLE 1-continued

Primer for Isolation of Catechol-Production-Related Gene				
Gene Source	Enzyme Gene	Forward	Reverse	Gene Sequence
Escherichia coli W	ubiXDH	SEQ ID NO. 60	SEQ ID NO. 61	SEQ ID NO. 19
Escherichia fergusonii	ubiXDH	SEQ ID NO. 62	SEQ ID NO. 63	SEQ ID NO. 20
Paenibacillus polymyxa	ubiXDH	SEQ ID NO. 64	SEQ ID NO. 65	SEQ ID NO. 21
Citrobacter koseri	ubiXDH	SEQ ID NO. 66	SEQ ID NO. 67	SEQ ID NO. 22
Pantoea ananatis	ubiXDH	SEQ ID NO. 68	SEQ ID NO. 69	SEQ ID NO. 23

TABLE 2

Plasmid for Expression of Catechol-Production-Related Gene			
Gene Source	Enzyme Gene	Introduced Vector	Plasmid
Lactobacillus rhamnosus	ubiDX	pCRB209	Pani37
Lactobacillus pentosus	ubiXH	pCRB209	Pani277
Lactobacillus pentosus	ubiD	pCRB209	Pani278
Lactobacillus pentosus	ubiXH + ubiD	Pani277	Pani279
Lactobacillus plantarum	ubiXH	pCRB209	Pani33
Lactobacillus plantarum	ubiD	pCRB209	Pani34
Lactobacillus plantarum	ubiXH + ubiD	Pani33	Pani40
Lactobacillus pobuzihii	ubiDX	pCRB209	Pani284
Lactobacillus composti	ubiDX	pCRB209	Pani283
Lactobacillus hokkaidonensis	ubiDXH	pCRB210	Pani282
Lactobacillus sakei subsp. *sakei*	ubiDXH	pCRB210	Pani281
Bacillus megaterium	ubiXDH	pCRB209	PGadi21
Bacillus licheniformis	ubiXDH	pCRB209	PGadi20
Bacillus atrophaeus	ubiXDH	pCRB209	Pani63
Bacillus subtilis subsp. *subtilis*	ubiXDH	pCRB209	pCRG34
Bacillus subtilis subsp. *spizizenii*	ubiXDH	pCRB209	Pani60
Enterobacter aerogenes	ubiXDH	PCRB209	Pani86
Enterobacter cloacae	ubiXDH	pCRB209	Pani26
Enterobacter sakazakii	ubiXDH	PCRB209	Pani81
Enterobacter hormaechei	ubiXDH	pCRB209	Pani88
Escherichia coli W	ubiXDH	pCRB209	Pani80
Escherichia fergusonii	ubiXDH	PCRB209	Pani85
Paenibacillus polymyxa	ubiXDH	PCRB209	Pani84
Citrobacter koseri	ubiXDH	pCRB209	Pani83
Pantoea ananatis	ubiXDH	pCRB209	Pani82

(3) Construction of Plasmid for Chromosomal Gene Disruption of *Corynebacterium Glutamicum* Strain R

A DNA region necessary for markerless chromosomal gene disruption of a *Corynebacterium glutamicum* strain R was amplified by the PCR method. Each PCR fragment is linkable in overlap regions. The DNA fragment thus obtained was introduced into the plasmid pCRA725 [J. Mol. Microbiol. Biotechnol. 8: 243-254 (2004), (JP-A-2006-124440)] for markerless gene disruption. Obtained plasmids for chromosomal gene disruption are shown in Table 3.

TABLE 3

Plasmid for Chromosomal Disruption of *Corynebacterium Glutamicum* Strain R			
Plasmid for Chromosomal Disruption	Disruptied Gene	Forward	Reverse
pCRG33	catA	SEQ ID NO. 70 SEQ ID NO. 72*	SEQ ID NO. 71* SEQ ID NO. 73

*Primer including overlap region

(4) Construction of Catechol Producing Strains by Chromosomal Gene Recombination

The vector pCRA725 for markerless chromosomal gene introduction is a plasmid that cannot be replicated in *Corynebacterium glutamicum* R. In a case of a single crossover strain that has a crossover with the homologous region on the chromosome introduced into the plasmid pCRA725, the strain exhibits the kanamycin resistance due to the expression of the kanamycin-resistant gene on pCRA725, and the lethality in a sucrose-containing medium due to the expression of the sacR-sacB gene of the *Bacillus subtilis*. In contrast, in a case of a double crossover strain, the strain exhibits the kanamycin sensitivity due to the loss of the kanamycin-resistant gene on pCRA725, and the viability in a sucrose-containing medium due to the loss of the sacR-sacB gene. A markerless chromosomal gene introduced strain, therefore, exhibits the kanamycin sensitivity and the viability in the sucrose-containing medium.

By the above-described methods, PCA-production-related gene chromosome integrated strains were constructed by using the above-described plasmids for catechol-production-related gene chromosomal integration and the plasmids for chromosomal genes disruption. *A Corynebacterium glutamicum* strain PCA3 [WO2017/169399], which is a coryneform bacterium that produces protocatechuic acid, was used as a host strain. Further, the plasmid pCRG3 [WO2017/169399] for gene pcaHG disruption, the plasmid pCRB295 [WO2017/169399] for the qsuB gene chromosome integration, and the plasmid pCRB285 [WO2017/169399] for the aroG gene (S180F) chromosome integration were also used. This chromosomal gene recombination is outlined in Tables 4 and 5.

TABLE 4

Construction of Catechol Producing Strains by Chromosomal Gene Recombination		
Constructed Strain	Host Strain	Recombinant Plasmid
LHglc1367	*Corynebacterium glutamicum* PCA3	pCRG33
ESglc1590	*Corynebacterium glutamicum* R	pCRG33, pCRG3
ESglc1609	ESglc1590	PCRB295, pCRB285

TABLE 5

Outline of Strain Constructed by Chromosomal Gene Recombination		
Constructed Strain	Chromosome integrated gene	Disrupted chromosomal gene
LHglc1367	xylABx4, bglF(V317A)A, araBAD, araE, tkt-tal, aroG(S180F)x2, aroCKBx3, aroAx2, aroDx2, aroEx2, qsuB, pobAx2, ubiC	qsuD, poxF, pcaHG, catA, ldhA
ESglc1590		pcaHG, catA
ESglc1609	qsuB, aroG(S180F)	pcaHG, catA

x2, x3: indicating the number of genes introduced into chromosome

(5) Construction of Strain in which Plasmid for Expression of Catechol-Producing Gene is Introduced

Catechol-producing strains were constructed by introducing a protocatechuic acid decarboxylase into the above-described chromosomal gene recombinant strains. Besides, pCRB22 (Appl Microbiol Biotechnol. 2015 June; 99(11): 4679-89) was used for carrying out control experiments. The strains thus constructed are outlined in Table 6.

TABLE 6

Outline of Catechol Producing Strain

Constructed Strain	Host Strain	Introduced Plasmid	Source of Protocatechuic Acid Decarboxylase Gene
CAT21	LHglc1367	Pani37	*Lactobacillus rhamnosus*
CAT41	LHglc1367	Pani279	*Lactobacillus pentosus*
CAT24	LHglc1367	Pani40	*Lactobacillus plantarum*
CAT42	LHglc1367	Pani284	*Lactobacillus pobuzihii*
CAT45	LHglc1367	Pani283	*Lactobacillus composti*
CAT6	LHglc1367	PGadi21	*Bacillus megaterium*
CAT5	LHglc1367	PGadi20	*Bacillus licheniformis*
CAT39	LHglc1367	Pani63	*Bacillus atrophaeus*
CAT2	LHglc1367	pCRG34	*Bacillus subtilis* subsp. *subtilis*
CAT38	LHglc1367	Pani60	*Bacillus subtilis* subsp. *spizizenii*
CAT37	LHglc1367	Pani86	*Enterobacter aerogenes*
CAT1	LHglc1367	Pani26	*Enterobacter cloacae*
CAT32	LHglc1367	Pani81	*Enterobacter sakazakii*
CAT40	LHglc1367	Pani88	*Enterobactor hormaechei*
CAT31	LHglc1367	Pani80	*Escherichia coli* W
CAT36	LHglc1367	Pani85	*Escherichia fergusonii*
CAT35	LHglc1367	Pani84	*Paenibacillus polymyxa*
CAT34	LHglc1367	Pani83	*Citrobacter koseri*
CAT33	LHglc1367	Pani82	*Pantoea ananatis*
CAT158	LHglc1367	pCRB22	—
CAT91	ESglc1590	Pani37	*Lactobacillus rhamnosus*
CAT92	ESglc1609	Pani37	*Lactobacillus rhamnosus*

Corynebacterium glutamicum CAT21 was deposited in Incorporated Administrative Agency National institute of Technology and Evaluation, NITE Patent Microorganisms Depositary (2-5-8-122 Kazusakamatari, Kisarazu-shi, Chiba 292-0818 Japan) as an international depositary authority (International deposit date: Apr. 17, 2018, Accession Number: NITE BP-02689 under the Budapest Treaty).

Example 21

Catechol Production Test (in Test Tube, 10 mL Scale) (Combination of Protocatechuic Acid Decomposition Pathway Disruption, Catechol Decomposition Pathway Disruption)

By using a strain CAT91, which is a catechol producing strain, which was constructed on the basis of a *Corynebacterium glutamicum* strain R (see Tables 5 and 6), experiments of producing catechol in an aerobic batch reaction using a test tube were carried out by the method described below.

Each strain CAT91 was applied to A-agar plate [obtained by dissolving the following in 1 liter of distilled water: $(NH_2)_2CO$ 2 g; $(NH_4)_2SO_4$ 7 g; KH_2PO_4 0.5 g; K_2HPO4 0.5 g; $MgSO_4.7H_2O$ 0.5 g; 0.06% (w/v) $FeSO_4.7H_2O$, 0.042% (w/v) $MnSO_4.2H_2O$ 1 ml; 0.02% (w/v) biotin solution 1 ml; 0.01% (w/v) thiamin solution 2 ml; yeast extract 2 g; vitamin assay casamino acid 7 g; and agar 15 g] containing kanamycin of final concentration 50 μg/mL and 4% glucose, and it was incubated at 33° C. for 15 hours in a dark place.

One platinum loop of the strain CAT91 grown on the above-described plate was inoculated in a test tube containing 10 ml of A-liquid medium [obtained by dissolving the following in 1 liter of distilled water: $(NH_2)_2CO$ 2 g; $(NH_4)_2SO_4$ 7 g; KH_2PO_4 0.5 g; K_2HPO_4 0.5 g; $MgSO_4.7H_2O$ 0.5 g; 0.06% (w/v) $FeSO_4.7H_2O$, 0.042% (w/v) $MnSO_4.2H_2O$ 1 ml; 0.02% (w/v) biotin solution 1 ml; 0.01% (w/v) thiamin solution 2 ml; yeast extract 2 g; and vitamin assay casamino acid 7 g] containing kanamycin of final concentration 50 μg/mL and 2% glucose, and aerobic shaking culture was carried out at 33° C. for 7 to 15 hours.

Each strain grown under the above-described conditions was suspended in 10 ml of A-liquid medium containing kanamycin of final concentration 50 μg/mL and 4% glucose so that the initial bacterial cell concentration OD_{610}=0.5. 200 mg of $CaCO_3$ was added thereto and aerobic shaking culture was carried out at 33° C. for 48 hours. The culture solution obtained after 48 hours was centrifuged (4° C., 15,000×g, 5 minutes), whereby supernatant of culture was obtained. The concentration of metabolite in the supernatant of culture was analyzed by using a high-performance liquid chromatography system (Prominence HPLC (manufactured by Shimadzu Corporation), COSMOSIL Packed column 5C18-AR-II, separation using 10% methanol and 0.1% phosphoric acid for the mobile phase). Consequently, this strain produced 0.1 mM of catechol after 48 hours.

Example 31

Catechol Production Test (in Test Tube, 10 mL Scale) (Combination of Protocatechuic Acid Decomposition Pathway Disruption, Catechol Decomposition Pathway Disruption, DAHP Synthesis Enzyme Reinforcement, and Protocatechuic Acid Synthesis Enzyme Reinforcement)

By using the strain CAT92, which is a catechol producing strain constructed on the basis of the strain CAT91 (see Tables 5 and 6)), experiments of producing catechol in an aerobic batch reaction using a test tube were carried out by the method described below.

Each strain CAT92 was applied to A-agar plate [obtained by dissolving the following in 1 liter of distilled water: $(NH_2)_2CO$ 2 g; $(NH_4)_2SO_4$ 7 g; KH_2PO_4 0.5 g; K_2HPO_4 0.5 g; $MgSO_4.7H_2O$ 0.5 g; 0.06% (w/v) $FeSO_4.7H_2O$+0.042% (w/v) $MnSO_4.2H_2O$ 1 ml; 0.02% (w/v) biotin solution 1 ml; 0.01% (w/v) thiamin solution 2 ml; yeast extract 2 g; vitamin assay casamino acid 7 g; and agar 15 g] containing kanamycin of final concentration 50 μg/mL and 4% glucose, and it was incubated at 33° C. for 15 hours in a dark place.

One platinum loop of the strain CAT92 grown on the above-described plate was inoculated in a test tube containing 10 ml of A-liquid medium [obtained by dissolving the following in 1 liter of distilled water: $(NH_2)_2CO$ 2 g; $(NH_4)_2SO_4$ 7 g; KH_2PO_4 0.5 g; K_2HPO_4 0.5 g; $MgSO_4.7H_2O$ 0.5 g; 0.06% (w/v) $FeSO_4.7H_2O$+0.042% (w/v) $MnSO_4.2H_2O$ 1 ml; 0.02% (w/v) biotin solution 1 ml; 0.01% (w/v) thiamin solution 2 ml; yeast extract 2 g; and vitamin assay casamino acid 7 g] containing kanamycin of final concentration 50 μg/mL and 2% glucose, and aerobic shaking culture was carried out at 33° C. for 7 to 15 hours.

Each strain grown under the above-described conditions was suspended in 10 ml of A-liquid medium containing kanamycin of final concentration 50 μg/mL and 4% glucose so that the initial bacterial cell concentration OD_{610}=0.5. 200 mg of $CaCO_3$ was added thereto and aerobic shaking culture was carried out at 33° C. for 48 hours. The culture solution obtained after 48 hours was centrifuged (4° C., 15,000×g, 5 minutes), whereby supernatant of culture was obtained. The concentration of metabolite in the supernatant of culture was analyzed by using a high-performance liquid chromatography system (Prominence HPLC (manufactured by Shimadzu Corporation), COSMOSIL Packed column

5C18-AR-II, separation using 10% methanol and 0.1% phosphoric acid for the mobile phase). Consequently, this strain produced 18.4 mM of catechol after 24 hours.

Example 41

Catechol Production Test (in Test Tube, 10 mL Scale) (Influence on Catechol Production by Genes From Various Organisms that Encode Enzymes Having Decarboxylation Activity with Respect to Protocatechuic Acid Derived)

In order to examine effects of the introduction of a gene that encodes an enzyme having a decarboxylation activity with respect to protocatechuic acid in the production of catechol by a *Corynebacterium glutamicum* transformant, a strain LHglc1367 in which a gene encoding a catechol degrading enzyme was disrupted was constructed on the basis of *Corynebacterium glutamicum* strain PCA3 [WO2017/169399], which produces protocatechuic acid (Table 5). Plasmids in which respective genes were incorporated were introduced in these strains, respectively, whereby decarboxylase-introduced strains CAT01 to CAT47 were obtained (Table 6). Respective catechol productivities were compared. Each strain was applied to the above-described A-agar plate containing kanamycin of final concentration 50 μg/mL and 4% glucose, and it was incubated at 33° C. for 15 hours in a dark place.

One platinum loop of each strain grown on the above-described plate was inoculated in a test tube containing 10 ml of the A-liquid medium containing kanamycin of final concentration 50 μg/mL and 2% glucose, and aerobic shaking culture was carried out at 33° C. for 7 to 15 hours.

Each strain grown under the above-described conditions was inoculated in 10 ml of A-liquid medium containing kanamycin of final concentration 50 μg/mL and 4% glucose so that the initial bacterial cell concentration OD_{610}=0.5. 200 mg of $CaCO_3$ was added thereto and aerobic shaking culture was carried out at 33° C. for 24 hours. The culture solution obtained after 24 hours was centrifuged (4° C., 15000×g, 5 minutes), and the supernatant of culture obtained was subjected to quantitative analysis of catechol, using the above-mentioned high-performance liquid chromatography system. The results are shown in Table 7.

Incidentally, the "amino acid sequence identity" shown in Table 7 indicates results of comparison between amino acid sequences encoded by the ubiD gene of *Lactobacillus rhamnosus*, and amino acid sequences encoded by other ubiD genes.

TABLE 7

	Introduced Decarboxylase		Catechol Production Concentration	Amino Add Sequence Identity (%) vs. *L.*	vs. *B.*
Strain	Species	Gene	(mM)	*rhamnosus*	*megaterium*
CAT 21	*Lactobacillus rhamnosus*	ubiDX	44.3	100	
CAT 41	*Lactobacillus pentosus*	ubiXH + ubiD	33.6	85	—
CAT 24	*Lactobacillus plantarum*	ubiXH + ubiD	33.2	85	—
CAT 42	*Lactobacillus pobuzihii*	ubiDX	29.4	82	—
CAT 45	*Lactobacillus composti*	ubiDX	26.3	80	—
CAT 06	*Bacillus megaterium*	ubiXDII	29.1	—	100
CAT 05	*Bacillus licheniformis*	ubiXDH	27.1	—	81
CAT 39	*Bacillus atrophaeus*	ubiXDII	23.5	—	81
CAT 02	*Bacillus subtilis* subsp. *subtilis*	ubiXDII	21.8	—	82
CAT 38	*Bacillus subtilis* subsp. *spizizenii*	ubiXDH	22.4	—	82
CAT 37	*Enterobactor aerogenes*	ubiXDH	25.4	—	—
CAT 01	*Enterobactor cloacae*	ubiXDII	25.4	—	—
CAT 32	*Enterobactor sakazakii*	ubiXDH	24.9	—	—
CAT 40	*Enterobactor hormaechei*	ubiXDH	21.4	—	—
CAT 31	*Escherichia coli* W	ubiXDH	21.9	—	—
CAT 36	*Escherichia fergusonii*	ubiXDH	21.9	—	—
CAT 35	*Paenibacillus polymyxa*	ubiXDH	26.4	—	—
CAT 34	*Citrobacter koseri*	ubiXDH	24.5	—	—
CAT 33	*Pantoea ananatis*	ubiXDH	21.2	—	—
CAT 158	Control	—	0	—	—

The results shown in Table 7 indicate that the introduction of the ubiDX gene of *Lactobacillus rhamnosus* or an ortholog of the same causes the amount of produced catechol to increase. It is indicated that the amount of produced catechol was particularly increased in the case where the strain in which the gene ubiDX of *Lactobacillus rhamnosus* or the gene having high homology with the gene ubiDX is introduced is used (for example, the strain CAT21, the strain CAT41, the strain CAT24).

Example 51

Catechol Production Test (in Jar Fermenter, 400 mL Scale) (Study on Optimal pH for Production)

By using strain CAT21 (see Tables 5 to 7), experiments of producing catechol in an aerobic batch reaction using a jar fermenter were carried out by the method described below.

The strain CAT21 was inoculated in 10 ml of the A-liquid medium containing kanamycin of final concentration 50

μg/mL and 2% glucose, and thereafter, aerobic shaking culture was carried out at 33° C. for 18 hours.

The strain CAT21 was inoculated in 100 ml of the A-liquid medium containing kanamycin of final concentration 50 μg/mL and 2% glucose, and thereafter, aerobic shaking culture was carried out at 33° C. for 12 hours.

Bacterial cells grown under the above-described conditions were collected by centrifugation (4° C., 3000×g, 10 minutes), and the bacterial cells thus obtained were suspended in 400 ml of a culture solution [obtained by dissolving the following in 1 liter of distilled water: $(NH_4)_2SO_4$ 7 g; KH_2PO_4 0.5 g; K_2HPO_4 0.5 g; $MgSO_4.7H_2O$ 0.5 g; 0.06% (w/v) $FeSO_4.7H_2O$+0.042% (w/v) $MnSO_4.2H_2O$ 1 ml; 0.02% (w/v) biotin solution 25 μl; 0.01% (w/v) thiamine solution 2 ml; yeast extract 2 g; and vitamin assay casamino acid 7 g] containing kanamycin of final concentration 50 μg/mL, 8% glucose, and 3 g/L of an antifoam agent (ADEKANOL L126, manufactured by Adeka Corporation) in a 1000-ml jar fermenter culture vessel so that OD_{610}=0.2. Each of these was subjected to 24-hour aerated agitated culture in a 1000-ml jar fermenter under the conditions of 33° C., pH control by addition of 5.0 N aqueous ammonia, aeration amount of 0.4 L/min (air, 1 vvm), and dissolved oxygen concentration (DO) of 10% (assuming that the saturated dissolved oxygen concentration under atmospheric pressure is 100%). The concentration of metabolite in the supernatant of culture was analyzed by using the high-performance liquid chromatography system described above. The results are shown in Table 8.

TABLE 8

Comparison of Amounts of Catechol Produced in Jar Fermenter with Varied pH

pH	Catechol Production Concentration (mM)
6.0	39
6.5	51
7.0	60
7.5	50
8.0	0

The strain CAT21, in a case of being cultured with pH 7.0 being maintained, had produced 60 mM of catechol when 24 hours passed after the start of culturing, and exhibited the highest concentration, among the examined cases of various values of pH. In addition, in a case where it was cultured with pH 8.0 being maintained, the concentration of produced catechol was 0 mM at the point in time when 24 hours passed. These results indicate that in a case where catechol is produced with use of this strain, pH set in the vicinity of 7.0 leads to the highest productivity.

Example 6

Catechol Production Test (in Jar Fermenter, 400 mL Scale) (Growth-Independent Production Test)

By using the strain CAT21 (see Tables 5 to 7), experiments of producing catechol in an aerobic batch reaction using a jar fermenter were carried out by the method described below.

The strain CAT21 was inoculated in 10 ml of the A-liquid medium containing kanamycin of final concentration 50 μg/mL and 2% glucose, and thereafter, aerobic shaking culture was carried out at 33° C. for 18 hours.

The strain CAT21 was inoculated in 100 ml of the A-liquid medium containing kanamycin of final concentration 50 μg/mL and 2% glucose, and thereafter, aerobic shaking culture was carried out at 33° C. for 12 hours.

Bacterial cells grown under the above-described conditions were collected by centrifugation (4° C., 3000×g, 10 minutes), and the bacterial cells thus obtained were suspended in 400 ml of a culture solution [obtained by dissolving the following in 1 liter of distilled water: $(NH_4)_2SO_4$ 7 g; KH_2PO_4 0.5 g; K_2HPO_4 0.5 g; $MgSO_4.7H_2O$ 0.5 g; 0.06% (w/v) $FeSO_4.7H_2O$+0.042% (w/v) $MnSO_4.2H_2O$ 1 ml; 0.02% (w/v) biotin solution 25 μl; 0.01% (w/v) thiamine solution 2 ml; yeast extract 2 g; and vitamin assay casamino acid 7 g] containing kanamycin of final concentration 50 μg/mL, 8% glucose, and 3 g/L of an antifoam agent (ADEKANOL L126) in a 1000-ml jar fermenter culture vessel so that OD_{610}=0.2. Each of these was subjected to 18-hour aerated agitated culture in the 1000-ml jar fermenter under the conditions of 33° C., pH 7.0 (controlled by addition of 5.0 N aqueous ammonia), aeration amount of 0.4 L/min (air, 1 vvm), and dissolved oxygen concentration (DO) of 5% (assuming that the saturated dissolved oxygen concentration under atmospheric pressure is 100%).

Bacterial cells of the strain grown under the above-described conditions were collected by centrifugation (4° C., 5000×g, 10 minutes), and the bacterial cells thus obtained were washed with 0.9% sodium chloride aqueous solution once. Thereafter, the bacterial cells were suspended in 250 ml of a reaction solution [obtained by dissolving the following in 1 liter of distilled water: $(NH_4)_2SO_4$ 7 g; KH_2PO_4 0.5 g; K_2HPO_4 0.5 g; $MgSO_4.7H_2O$ 0.5 g; 0.06% (w/v) $Fe_2SO_4.7H_2O$+0.042% (w/v) $MnSO_4.2H_2O$ 1 ml; and 0.01% (w/v) thiamine solution 2 ml] containing 10% glucose so that 100 g of wet bacterial cells were contained per liter (5% of the medium volume in terms of weight of wet bacterial cells), and a catechol producing reaction was caused under the conditions of 33° C., pH 7.0 (controlled by adding 5.0 N aqueous ammonia), aeration amount of 0.25 L/min (air, 1 vvm), DO 5%, by using a 1000-ml jar fermenter. The concentration of metabolite in the supernatant of culture was analyzed by using the high-performance liquid chromatography system described above. The results are shown in FIG. **1**.

As illustrated in FIG. **1**, the strain CAT21 had produced 66 mM (7.25 g/l) of catechol when 27.5 hours had passed after the start of the catechol producing reaction. The results indicate that this strain has a very high catechol productivity in a reaction process without bacterial cell growth using an inorganic salt minimal medium. The catechol productivity of this strain significantly exceeded the productivity of *Escherichia coli* recombinant strain, 38 mM (4.2 g/L) in 36 hours (Non-Patent Document 3) and 41 mM (4.5 g/L) in 84 hours (Non-patent Document 2), which is the highest productivity among the productivities by the processes of fermentation from saccharides that have been reported so far.

Example 7

Catechol Production Test (in Jar Fermenter) (Utilization of Resin Adsorption)

By using the strain CAT21 (see Tables 5 to 7), experiments of producing catechol performed in an aerobic batch reaction using a jar fermenter, with use of a resin adsorption in combination, were carried out by the method described below.

The strain CAT21 was inoculated in 10 ml of the A-liquid medium containing kanamycin of final concentration 50

μg/mL and 2% glucose, and thereafter, aerobic shaking culture was carried out at 33° C. for 18 hours.

The strain CAT21 was inoculated in 100 ml of the A-liquid medium containing kanamycin of final concentration 50 μg/mL and 2% glucose, and thereafter, aerobic shaking culture was carried out at 33° C. for 12 hours.

Bacterial cells grown under the above-described conditions were collected by centrifugation (4° C., 3000×g 10 minutes), and the bacterial cells thus obtained were suspended in 400 ml of a culture solution [obtained by dissolving the following in 1 liter of distilled water: $(NH_4)_2SO4$ 7 g; KH_2PO_4 0.5 g; K_2HPO_4 0.5 g; $MgSO_4.7H_2O$ 0.5 g; 0.06% (w/v) $FeSO_4.7H_2O$+0.042% (w/v) $MnSO_4.2H_2O$ 1 ml; 0.02% (w/v) biotin solution 25 μl; 0.01% (w/v) thiamine solution 2 ml; yeast extract 2 g; and vitamin assay casamino acid 7 g] containing kanamycin of final concentration 50 μg/mL, 8% glucose, and 3 g/L of an antifoam agent (ADEKANOL L126) in a 1000-ml jar fermenter culture vessel so that OD_{610}=0.2. Each of these was subjected to 18-hour aerated agitated culture in the 1000-ml jar fermenter under the conditions of 33° C., pH 7.0 (controlled by addition of 5.0 N aqueous ammonia), aeration amount of 0.4 L/min (air, 1 vvm), and dissolved oxygen concentration (DO) of 5% (assuming that the saturated dissolved oxygen concentration under atmospheric pressure is 100%).

Bacterial cells of the strain grown under the above-described conditions were collected by centrifugation (4° C., 5000×g, 10 minutes), and the bacterial cells thus obtained were washed with 0.9% sodium chloride aqueous solution once. Thereafter, the bacterial cells were suspended in 300 ml of a reaction solution [obtained by dissolving the following in 1 liter of distilled water: $(NH_4)_2SO_4$ 7 g; KH_2PO_4 0.5 g; K_2HPO_4 0.5 g; $MgSO_4.7H_2O$ 0.5 g; 0.06% (w/v) $Fe_2SO_4.7H_2O$+0.042% (w/v) $MnSO_4.2H_2O$ 1 ml; and 0.01% (w/v) thiamine solution 2 ml] containing 10% glucose so that 100 g of wet bacterial cells were contained per liter (5% of the medium volume in terms of weight of wet bacterial cells), and a catechol producing reaction was caused under the conditions of 33° C., pH 7.0 (controlled by adding 5.0 N aqueous ammonia), aeration amount of 0.3 L/min (air, 1 vvm), DO 5%, by using a 1000-ml jar fermenter. At this time, a flow passage filled with the reaction solution from the jar fermenter in advance, and a peristaltic pump, were connected, so that circulation of the culture solution was started simultaneously. A cross flow filtration apparatus (Microza Pencil module) and another peristaltic pump were arranged in the middle of the flow passage, so that filtrate that does not contain bacterial cells was discharged. This filtrate was passed through a column packed with 60 g of an adsorption resin (SP850), and flow-through liquid was returned to the jar fermenter. After 48 hours, the experiment was ended; all the reaction solution contained in the flow passage was returned to the jar fermenter, and the volume thereof was measured. The concentration of metabolite in the supernatant of culture was analyzed by using the high-performance liquid chromatography system described above. The metabolite adsorbed to the resin was extracted by causing water, then, 100% ethanol to pass therethrough, and the aqueous extract, as it was, and the ethanol extract, dried and solidified by an evaporator and dissolved in water of the same volume, were analyzed with the above-described high-performance liquid chromatography system. The results are shown in Table 9.

TABLE 9

Amount of Catechol Contained in Culture Solution and Resin After End of Reaction

Analyzed Sample	Volume (mb)	Concentration (mM)	Amount of Catechol (mmole)
In Culture Solution	475	40.5	19.2
Resin 1	100	42.4	4.2
Resin 2	40	915.6	36.6
Resin 3	50	81.1	4.1
Resin 4	50	0.9	0.0
Total			64.2

The total mass of catechol products divided by the volume of the reaction solution was assumed to be the catechol production concentration. Consequently, this strain CAT21 produced 135 mM (14.9 g/L) of catechol in 48 hours. The yield with respect to consumed glucose in that case was 18% (molar ratio).

As an exemplary case of the catechol production by the process of fermentation from saccharides wherein an adsorption resin was used in combination, a case where 77 mM (8.5 g/L) of catechol was produced with use of a *Escherichia coli* recombinant strain in 36 hours, resulting in the yield of 7%, was reported (Non-Patent Document 3); however, the catechol productivity of the strain CAT21 significantly exceeded the above-described results in terms of concentration and yield.

Reference Example 1

Verification that Coryneform Bacterium Exhibits Higher Catechol Resistance, as Compared with Other Microorganisms

Coryneform bacteria (*Corynebacterium glutamicum*), colon bacteria (*Escherichia coli*), yeast (*Saccharomyces cerevisiae*), *Pseudomonas* (*Pseudomonas putida*), and *Rhodococcus* (*Rhodococcus erythropolis*) were subjected to cross-streak assay on agar plates, so that their resistances against catechol were compared.

The *Corynebacterium glutamicum* strain R, and the strain ATCC 13032, were applied to the above-described A-agar plates containing 4% glucose, and were incubated at 33° C. for 15 hours in a dark place. One platinum loop of *Corynebacterium glutamicum* grown on the plate described above was inoculated in a test tube having therein 10 ml of the A-liquid medium containing 2% glucose, and aerobic shaking culture was carried out at 33° C. for 13 hours.

The *Escherichia coli* strain K-12 MG1655 was applied to a LB-agar plate [containing 1% polypeptone, 0.5% yeast extract, 0.5% sodium chloride, and 1.5% agar], and was incubated at 37° C. for 15 hours in a dark place. *Escherichia coli* grown on the plate described above was inoculated in an LB-liquid medium [containing 1% polypeptone, 0.5% yeast extract, and 0.5% sodium chloride], and aerobic shaking culture was carried out at 37° C. for 13 hours.

The *Pseudomonas putida* strain ATCC 700801 was applied to the above-described LB-agar plate, and was incubated at 30° C. for 15 hours in a dark place. *Pseudomonas putida* grown on the plate described above was inoculated in the LB-liquid medium, and aerobic shaking culture was carried out at 30° C. for 13 hours.

Further, the *Saccharomyces cerevisiae* strain NBRC2376 was applied to a YEPD agar plate [2% polypeptone, 1% yeast extract, 2% glucose, and 1.5% agar], and was incu-

bated at 30° C. for 20 hours in a dark place. *Saccharomyces cerevisiae* grown on the plate described above was inoculated in a YEPD liquid medium [2% polypeptone, 1% yeast extract, and 2% glucose], and aerobic shaking culture was carried out at 30° C. for 13 hours.

The *Rhodococcus erythropolis* strain ATCC 27854 was applied to the LB-agar plate, and was incubated at 30° C. for 15 hours in a dark place. *Rhodococcus erythropolis* grown on the plate described above was inoculated in the LB-liquid medium, and aerobic shaking culture was carried out at 30° C. for 13 hours.

Each strain preliminary cultured as described above was uniformly applied in a line form onto the above-described A-agar plates containing 4% glucose, and filter paper impregnated with 25% catechol was placed on each plate at the center thereof so as to intersect with the lines. After being incubated at 30° C. or 26° C. for 24 hours in a dark place, growth inhibition ranges of the strains from the filter paper were compared so that resistances thereof were compared. The results are shown in FIG. **2**.

As illustrated in FIG. **2**, the results indicate that the coryneform bacteria have narrower growth inhibition ranges than any of the other bacteria, and have relatively high resistances. In addition, no clear difference was seen between the results of the coryneform bacterium strain R and those of the strain ATCC 13032.

INDUSTRIAL APPLICABILITY

The present disclosure is useful for, for example, producing catechol.

[Sequence Listing]

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 73

<210> SEQ ID NO 1
<211> LENGTH: 2058
<212> TYPE: DNA
<213> ORGANISM: Lactobacillus rhamnosus

<400> SEQUENCE: 1

atgacagcat caccttggga cttaagaaaa gtattggatg aactaaaaca ggatccgcag 60

caatatcatg aaacagaggt gcaagtcgat cccgatgcag agcttgctgg cgtttatcgt 120

tacatcggtg ccggtgggac ggtcgaacgt ccgacacagg aaggtccggc aatgatgttt 180

aacaacgttg tcggcttccc aacgacaagg gttttgatcg gtttaatggc cagtcgcaag 240

cgggttggca agatgtttca ccaagactat cacacacttg gtcgattctt gaacaaagcg 300

gttttaaatc ctattcaacc cgttacagtc gaagaatcag cagcgcctgc gcatgaagtc 360

gttgccaagg ctagtgaccc ggactttgac attagaaaac tcgttgcagc accaaccaat 420

acgccacaag atgccggccc atacatcaca tgcggcgtag ttttgggttc caatatggcc 480

aaaacaatga ctgatgtgac gattcatcgc atggttttgg aagataagga tacgcttggt 540

atttatatca tgcccggtgg tcgccacatt ggtcattttg ctgaagaata tgaaaaagcc 600

aataagccga tgccggtgac catcaacatt ggcttggatc cggccattac cattggtgcc 660

acttttgaac cgcctaccac gccgcttggc tacgatgaac taggagttgc cggagccatt 720

cgccaagaac ccgtgcaact ggttcaggct gtgaccgtca atgaaaaagc cattgcgcgt 780

tcagaattta cactggaagg ctatatcatg cctaacacgc gtatccaaga agatatcaat 840

acccataccg gcaaagccat gccagagttt cccggctatg acggtgatgc caatccggct 900

ttgcaagtga ttaaagtgac ggctgtaacc catcggcgcg atcatcccat tatgcaaagt 960

gtcatcggac ctagtgaaga acatgtctcc atggccggca ttccaaccga agccagcatt 1020

ttacaacttg ttgatcgtgc catccccggc aaggtcaaga atgtgtacaa tcccccagct 1080

ggtggcggca aactcatgac catcatgcaa attcacaaag ataatccagc tgatgaaggg 1140

attcaacgtc aagctgcatt actcgctttt tcggcattca aagaactaaa aactgtttgg 1200

ctggtcgatg atgatgtcga tatttttgac atgaatgatg tcgtctggac aatgaacacg 1260

cgttttcaag gtgatcagga catcatggta ttacctggca tgcgcaacca tccgcttgat 1320

ccgtcagaac gaccgcaata tgatcccaag tctattcggg tacgcggaat gagttcgaag 1380

acggtcattg atggtaccgt accatttgat atgcgcgatc aattcaaacg agcagccttt 1440

-continued

aaaaaagttt ccgactggca aaaatatttg aaataggtga ttgaattgaa acgtattatc 1500

gtagggatta ccggggcatc cggaactatt tacgctgtta acctgctcca gcatttacat 1560

cgcctgcctg atgtcgaagt tcatttggtg atgagtgctt gggcaaagca aaacctgtca 1620

cttgagaccg acatgaaaca aagcgaactc gaagctttgg cggattatgt ttatcctgtt 1680

caaaaccaag gggcaaccat tgcaagcggc agtttttaa ccgatgcaat ggtcattgtt 1740

ccggcaagca tgaaaaccat tgcgggcatt gcgatgggct ttgatgataa tctcattgga 1800

cgagcagccg atgtcacgat taaagaacag cggcaattga ttattgtgcc gcgggaaaca 1860

ccgcttagtc caattcatct ggataacctc gctaaactag cccacattgg cgttcaaatc 1920

attccgccta ttccagcttt ctatcagcat ccccaaacca tccaggattt aattgagcat 1980

cacaccatga aactattaga cgccttgcat attaaaaccg aaaccgctag tcgctggaat 2040

ggagcgtcgt taagatga 2058

<210> SEQ ID NO 2
<211> LENGTH: 977
<212> TYPE: DNA
<213> ORGANISM: Lactobacillus pentosus

<400> SEQUENCE: 2

atgaagcgaa ttgttgtcgg aattacaggg gcgtcaggaa cgatttatgc cgttgattta 60

ttagagaagt tacaccaact ccctgatgtt gaagtgcatc tagttatgag tgcttgggcg 120

aagaagaact tggagctcga gacggattac tcacttgcac aattgacggc attggcggat 180

gcgacttacc gtgctaacga ccaggggggcg gcaatcgcga gcggttcatt tttaaatgac 240

ggtatggtga tcgttccagc cagcatgaag accgttgccg gaattgctta cggctttggc 300

gataatctga tttcacgtgc agccgatgtg acgatcaagg agcaacgtaa actcgtcatt 360

gttccacgtg aaacaccctt gagtgtgatt catttggaga atctgacgaa attagccaag 420

ttgggtgcac aaatcattcc accgattccg gccttttata accatccgac cacgattcaa 480

gacttggtta accatcagac gatgaaaatc ttagatgcct tccatattca taatgaaacg 540

gatcgccgtt gggaaggaga ttagccatgc ccacttttac gactgaacaa gcaggttatc 600

agatgcaagc caccgttcag gttatcggat atgacttatt gattgtcgtt acgggcggca 660

ccaatccgca tattggtgat gtgaccacca ttacagcaac gatgccggcc caaaccgtca 720

aatttcctag tcacgatggc cgttttcaca aggataactt catttcggat cgaatggcga 780

agcgcctgca gtcgtcgttg ccgggaagtt gcacgattac tgctggaatt catgtcaacc 840

agattactaa ggcacagatt gcggccgctg caccaatgac ggatgattta agccagcaaa 900

ttattacttg gctacaagca caccccattc aggctgctcg gccggaatac tacggggatg 960

atgagcagcc gaagtag 977

<210> SEQ ID NO 3
<211> LENGTH: 1473
<212> TYPE: DNA
<213> ORGANISM: Lactobacillus pentosus

<400> SEQUENCE: 3

atggcagaac aaccatggga tttacgccgg gtacttgatg agatcaaaga tgatcccaag 60

aactatcatg aaaccgacgt tgaagtagat ccaaatgcgg aactttctgg tgtttatcgg 120

tatattggtg ctggtgggac cgttgaacgg ccaacacaag aaggtccagc aatgatgttc 180

aacaacgtga agggttttcc tgacacgcgt gtcttgactg gtttaatggc tagccgtcgt 240

-continued

cgggttggta agatgttcca tcatgattac caaacgttag gtcaatatct taacgatgca 300

gtttcaaatc cagttgcacc ggaaacggtc gctgaaaagg atgcaccggc tcacgaagtc 360

atttacaagt caactgatga aggctttgat attcggaagt tagttgcagc gccaactaat 420

acgccacaag atgctggtcc atatatcacg gtcggtgttg tcttcggttc aagcatggac 480

aagtccaaga gtgacgttac gattcaccgg atggttcttg aagacaagga caagctcgga 540

atctacatca tgcctggtgg ccgtcatatc ggtgcctttg ccgaagaata tgaaaaggcc 600

aataaaccaa tgccaatcac gatcaacatt ggtttggatc ctgccattac cattggtgcc 660

acctttgaac cacctaccac cccatttggt tacaacgaat taggggttgc tggtgccatt 720

cggaatcaag ccgttcaatt agtcgatggg gttaccgttg atgaaaaggc gattgcacgt 780

tctgaataca cgttggaagg ctacatcatg cctaacgaac ggattcaaga agacatcaac 840

acccatacgg gtaaagcgat gcctgaattc ccaggttatg atggtgatgc taacccagcc 900

ttacaagtca tcaaggtgac ggcggtaacg catcgggaaa atgccatcat gcaaagtgtt 960

atcggaccat ccgaagaaca tgtcagcatg gctggtattc caaccgaagc aagtatctta 1020

cagttagtta accgcgccat tcctggcaaa gtgactaacg tttacaatcc gccggctggt 1080

ggtggtaagt tgatgaccat catgcagatt cataaggata atgaagcgga tgaagggatt 1140

caacggcaag cagccttatt ggcgttctca gcgttcaagg aattgaagac ggtcatttta 1200

gttgatgaag acgttgatat ttttgatatg aacgatgtga tttggaccat gaatacccgt 1260

ttccaagctg accaagactt gatggtctta tctggcatgc ggaaccatcc attggatcca 1320

tcagaacggc cacaatacga tccaaaatcg attcgtttcc gtgggatgag ttcgaagttg 1380

gttatcgatg gcactgtacc attcgatatg aaagaccaat ttgaacgggc tcaattcatg 1440

aaggtcgatg actgggaaaa gtatttgaaa taa 1473

<210> SEQ ID NO 4
<211> LENGTH: 977
<212> TYPE: DNA
<213> ORGANISM: Lactobacillus plantarum

<400> SEQUENCE: 4

atgaaacgaa ttgttgtggg aatcacggga gcgtccggta cgatttacgc ggtcgactta 60

ttagaaaagt tacatcagcg gccagatgtt gaagttcacc tggtaatgag tgcgtgggct 120

aaaaaaaact tggagttaga gactgattac tcgctcgcgc agttgacggc gctcgcggat 180

gctacttatc gggctaatga ccaaggcgca gcgattgcca gtggttcgtt tttgaatgac 240

ggaatggtca ttgtcccagc tagtatgaag acggtagcgg ggattgcgta cggcttcggt 300

gataatttaa tatcgcgggc tgctgatgtc acgattaaag aacaacgtaa acttgtgatt 360

gttccacgtg aaacaccgtt aagcgtgatt catttagaaa atctaacaaa gttggcaaaa 420

ctcggtgccc aaattattcc accgattccc gctttttata atcatccaca gtccattcag 480

gatctggtca atcatcaaac aatgaaaatt ttggatgcgt ttcatattca taatgaaact 540

gatcgccgtt gggaggggga ttaagtatgg caacttttac gactgagcag gccgggtatc 600

aaatgcaagc aacactccaa gtgattggat atgacttgtt gatcgtcgtt accggtggga 660

ccaatcccca tattggtgac gtgaccacac taactgccag cacggttccc gaaacggtta 720

agtttcccag ccatgatggt cgcttccata aagataactt tatttcggaa cgaatggcca 780

agcggattca gcgttatcta gctggaagct gtacaattac tgcgggaatt catgtcaacc 840

-continued

aaattactaa agcacaaata gcagctgcgg caccaatgac ggatgacctc agccgccaga 900

ttattagctg gttacaggcc catcccgtcc aggctgaaaa gccggaatat tatggacaag 960

atgagcaacc gcggtag 977

<210> SEQ ID NO 5
<211> LENGTH: 1473
<212> TYPE: DNA
<213> ORGANISM: Lactobacillus plantarum

<400> SEQUENCE: 5

atggcagaac aaccatggga tttgcgtcgc gtgcttgatg agatcaagga tgatccaaag 60

aactatcatg aaactgacgt cgaagttgat ccaaatgcgg aactttctgg tgtttatcgg 120

tatatcggtg ctggtgggac cgttcaacgg ccaacgcaag agggtccagc aatgatgttt 180

aacaacgtta aggggtttcc tgatacgcgg gtcttgactg gattgatggc gagtcgccgg 240

cgcgttggta agatgttcca ccacgattat cagacgttag ggcaatactt gaacgaagca 300

gtctctaatc cagtggcgcc agaaacggtt gctgaagcgg atgcgccagc tcacgatgtt 360

gtttataaag cgacggatga aggctttgat attcggaagt tagtggcagc accaacgaat 420

acgccccaag atgctggacc atatattacg gtcggtgtgg tgtttggctc aagcatggac 480

aagtctaaga gtgatgtgac gattcaccga atggtccttg aagataagga taagttaggg 540

atttatatca tgcctggcgg tcggcacatt ggtgcgtttg cggaagagta tgagaaagct 600

aacaagccaa tgccaattac aattaatatt ggtttggatc cagccattac gattggtgca 660

actttcgaac caccgaccac gccattcggt tataacgaat taggtgttgc tggtgcgatt 720

cggaaccaag ctgttcaatt agttgacggg gtgaccgtcg atgaaaaggc gattgcgcgt 780

tctgaatata cgcttgaggg gtacattatg cctaacgaac gtattcagga agatatcaat 840

acgcatacgg gcaaggcgat gcctgaattc ccgggttatg atggtgacgc caacccagct 900

ttacaagtga ttaaggtgac ggcggtgact catcggaaga atgccatcat gcaaagcgtg 960

attggaccat ccgaagaaca tgtcagcatg gcgggaattc caactgaagc tagtatctta 1020

caattggtta accgtgccat tcctggtaaa gtgacgaatg tttataatcc gccggctggt 1080

ggtggtaagt tgatgaccat catgcagatt cacaaggata atgaagcgga tgaaggaatt 1140

caacggcaag ctgccttgct tgcgttctca gcctttaagg aattgaagac tgttatcctg 1200

gttgatgaag atgttgatat ttttgatatg aatgatgtga tttggacgat gaatacccgt 1260

ttccaagccg atcaggactt gatggtctta tcaggcatgc ggaatcatcc gttggaccca 1320

tcggaacgcc cacaatatga tccaaagtcg attcgtttcc gtgggatgag ttctaaacta 1380

gtgattgatg gcaccgtacc attcgatatg aaggaccaat ttgaacgggc ccaattcatg 1440

aaagtggctg actgggagaa gtatttgaag taa 1473

<210> SEQ ID NO 6
<211> LENGTH: 2043
<212> TYPE: DNA
<213> ORGANISM: Lactobacillus pobuzihii

<400> SEQUENCE: 6

atgggtgaag acaaatggga tttgcgtaaa gttttgtctg agatcaaaga tgatcccaaa 60

aactatcatg aaacagatgt cgaagttgat ccagaagctg aattagccgg tgtttatcga 120

tacattggtg ctggtgggac agttgaacgt ccaacacaag aaggacctgc gatgatgttt 180

aataatgtca aaggctttcc tagtacacgt gttttgattg gcttaatggc cagtcggaga 240

-continued

cgtgtaggaa aaatgctcca tcatgattat cagacattag gtcaattctt taatgaagca 300

gtttcgaaac cggttcctcc agttttggtt gacgaaaagg atgcacctac gcatgaagtt 360

gtgcaccatg caacagataa gaattttgat attcgtaagt tagtcgctgc tcctacaaac 420

acaccccaag atgctggtcc ttatattaca gttggtgtag ttttagggtc taacatggat 480

aagacgatgt cagatgtgac tatccatcgt atgtgcattg aaggaaaaga taagttggga 540

atttatatta tgcctggcgg aagacatatt ggggcttttg ctgaagaata cgaaaaggct 600

aataagccga tgcctgttac gatcaatatt ggacttgacc cagcagtaac gattggtaca 660

acattcgagc cgccaacaac tcctcttggt tacaatgagt taggggttgc aggttcgatt 720

cgtaaccagc ctgttgaatt ggtcaatggt gtttcagtag atgaaaaagc aattgcacgg 780

gctgaatata ctttagaagg ctatattatg cctaacgaaa gaatgcaaga agatatcaat 840

actcgtacag gtaaagcaat gcccgaattt ccaggttatg atggtgatgc taatcctgca 900

gttcaagtta taaaagttac ggccgttacc catcgtaaag atccaattat gcaaagtgtg 960

atcgggccaa gtgaagaaca cgtcagcatg gcaggaattc cgactgaagc aagcatttta 1020

caattagtca ataaggctat tcctggtaag gttactaagg tttataatcc atcagcgggc 1080

ggtggcaagt tgatgactat tatgcagatt cataaagaaa acgaagcaga tgaaggtatt 1140

caacgtcaag cagctttact tgcattctct gcttttaaag aactaaagac tgtatttta 1200

gttgacgaag atgttgatat tttcgatatg aatgatgttg tttggacgat taataccagg 1260

ttccaggctg ataaagatat catggtactt tctgggatgc gtaatcatcc attggatccc 1320

tctgaacgtc ccgaatatga tccgaaatca attcgttttc gtggaatgag ttccaagtta 1380

gttgttgatg ggactgttcc attcgacatg aaagaccagt ttgaaagagc gcagtttatg 1440

aaggttccag actggaaaaa atatttggag taatcaacaa tgaaaaaaat tattattgga 1500

gtttccggag catcgggaac aatctatgca gttgatcttt tgaaaaaaac tcagcagtta 1560

tccaatgttg aaactcacct agtaatgagt aagtgggcta agcaaaatct tgcactggag 1620

acaaattatc agttaaatga aatcaactct ttggctgatt atgtatacga cgagcgagat 1680

caagctgcca aaattgctag cggttctttt ttagttgatg gtatggtcgt tgttcccgca 1740

agtatgaaaa ctatcgcagg tatagccaat ggatttgctg ataatttggt tggtcgggca 1800

gccgatgtca tacttaagga acaacgaaaa cttgtgatag ttcctcgtga atcgccttta 1860

agcgtgatcc atcttgaaaa tttaactaaa ttagctaaaa ttggggccca gatcattccc 1920

ccgattccag ccttttataa ccatccttct tctattcaag accttgtgga ccatcagacg 1980

atgaaaacct tagatgcatt gggaatcaac aatgatatat cttcacgttg ggatggcaga 2040

tga 2043

<210> SEQ ID NO 7
<211> LENGTH: 2055
<212> TYPE: DNA
<213> ORGANISM: Lactobacillus composti

<400> SEQUENCE: 7

atgtcagatt attatgattt gagacgggtc ttaaaggaat tagcagctga ggcccctaag 60

caatatcacg ccaccgatga attggtggac cctaatgcgg aattagctgg agtttaccgc 120

tacattggcg ctgggggcac ggtcaagcgg cccactcaag caggaccggc attgatgttc 180

aacaacgtca agggctttgc cggcacccgg gtcttgattg gcttattggc cagtcgcaag 240

-continued

cgggtgggtt tgctatttca tcacgattac catacgctag gccaatttct aaatgatgca 300

gtggaccatc ccctaaaccc ggtgacagtt tctgaagctg acgccccggc ccatgaagtc 360

atccacaaag tcgacgaccc tgattttgat atccgcaaac tcatcgccgc ccccaccaat 420

accgaatacg acgcagggcc ttacatcacc atgggcttag tttatgggtc taatcgggcc 480

aaaaccaaaa gtgatgtgac cattcatcgc atggttttag aggataaaga taccattggc 540

atctacatca tgcctggggg ccggcatatc ggcgcttttg ctgaagaata tgagcaagcc 600

aacgaaccca tgcccattac tgtgaacatc ggcttagatc cggccatcac gttgggggcc 660

acctttgaac cgcctacaac gcccttaggt tataacgaat tgggagttgc cggggctatt 720

cgccaagaac ccgtccagct ggtagacgct atcaccgttg ccgaaaaggc catcgcccgt 780

tctgaattca ccatcgaagg ctacatcatg ccccaccaac gcatgcaaga agacatcaac 840

accaacacgg gtaaagccat gccagaattt cctggctatg acggtgatgc taatcccgcc 900

gtgcaggtca tcaaagtaac ggcggtcacc catcgtcaag accagcccat tatgcagaca 960

gtgattggtc ccagcgaaga acatgtgaac ttagccggaa ttcccacgga agctagcatt 1020

ctagaactca ccaataaagc catccccggt aaagtcttaa atgtctacaa tccccctgct 1080

ggcggcggta aattgatgac gattctccag atccacaaaa ctaatcccgc tgatgaaggc 1140

atccagcgtc aggccgccct cttagccttt gcggctttta aagaattgaa gacagtgatt 1200

ttagtggacg aggatgtgga cattttcgac atgaacgatg tgatatggac tgtcaatacc 1260

cgttttcagg ccgaccagga cattgtggtg ctgccgggga tgcgcaatca tcccctggac 1320

ccttcagagc ggcccgctta tgacccgaag tctattcgag ttcaggggat gtccagcaag 1380

ttgattttag atggcaccgt cccctgggac atgaaggctc agttcatccg agcccggttt 1440

aaagaagttc aagactggcg caagtatttg caataggagg tctgttaaac tatgaagaaa 1500

attgtcgttg ctatgaccgg cgcgtcaggc gtgatttacg gccttgattt attgcgccat 1560

ttgcaggcca ttccgtcggt tgaaacccac ctggtgctca gtccctgggc taaaaagaat 1620

attcaactag aaaccgatct gaccttgcag caggtccaag atttgtccga tgtggtctac 1680

cacgacaatg accaaggggc ggcgattgcc agtggttctt tcctgcacga tggcatggtg 1740

atcgtcccgg ccagcatgaa gaccgtggcc gaaatcgcct acggcttcgg ggaaaatctg 1800

attggccggg cggctgatgt gaccattaaa gaatgccgac cgctgatcat cgtacctaga 1860

gaaacgccct tgagcgtgat tcatttggaa aatctcacca agctggccaa attaggagct 1920

caaatcattc cccctattcc cagtttttat acccagccta agaccatcgc cgatttggtg 1980

accaaacaaa ccatgcactt attagacgct ctaaagatcc ccaacgactt ggctcaacgc 2040

tggacgggag ggtaa 2055

<210> SEQ ID NO 8
<211> LENGTH: 2517
<212> TYPE: DNA
<213> ORGANISM: Lactobacillus hokkaidonensis

<400> SEQUENCE: 8

atgacagaac aaccatatga tttaagaaaa gtacttgcag aaattaaaga tgaccccatg 60

caataccacg aaacaaatcg agaaatcgat ccaaatgcag atttagctgg tgtctatcga 120

tatattggtg cgggtggaac cgttaagcgt cccaccactg aagggccaac aatgatgttt 180

aataatgtta aaggatttcc aggtagtcgg gtgctgattg gattacaagc ctctcgtaaa 240

cgggttggta agatcttgca tcatgattac aaaacgttgg gtcaaatgct aaacgaggct 300

-continued

gtttcaaatc ctgtcaaacc agtagaagtt aaaagagaag atgcacctgc tcaagaagta 360

gtacacctag cgtcagattc taactttgat attcgtaaat tactagctgc gccgacgaat 420

actcctgagg acgcaggtcc atatatcaca atgggggttg tgtatggtca tagcgtggat 480

ggtaagcaaa gtgatgttac aattcaccga atggttttag aagataaaga tacaatcggt 540

atgtatatca tgccaggtgg ccgccacatt ggtgcatttt taaaagacta tgagcaacaa 600

aacaagccaa tgccgatcac aattaatatt ggtttagatc ccgcagttac gattggggca 660

acctttgagc caccgacgac accactgggc tacaatgagc ttcaggtagc tggtgcattg 720

agaaatgagc cagtacaagt ggttcccgga gttgcagtaa acgcgttagg tattgcgcgc 780

tcagaatgga ttattgaggc tgaaattcaa cctaatcaaa caatgcaaga agacatcaat 840

acaaataccg gttacgcaat gcctgaattt cctggctaca atggaaccgc gaatccagcc 900

gttaatgtcg ttaaaattaa ggctgtgacg caccggaaag ataatccaat cgtccaaaca 960

actattgggc catctgaaga gcatgtttcc atggctggaa ttccaactga agcttcaatt 1020

ttaagtttag ttgatcgtgc cattccgggt aaagttttga atgtttataa cgctcccgct 1080

ggtggtggta aattaatgac catcatgcaa atccataagg ataatgaagc ggatgaagga 1140

attcaacgac aagcagcatt attagcattt tcagcgttta aagaattaaa aacggtcatt 1200

ttagttgacg aagatgttga tatttttgac tggaacgatg taatgtggac gatcaatacg 1260

agattccaag ctgatcgtga cattatggta ctcgagggga tgcggaatca tccgcttgac 1320

ccatcagaac tgccggaata tgatcctgct cgaatccgag tgaagggaat gagttctaaa 1380

ttagtccttg atggtacctt cccttatgat atgcgtgaca aatttaaacg ggcagaattt 1440

aaagaaattc cggattggaa gaaatatttg gattaattaa ggaggggcaa atgaaaaagg 1500

ttgtcgttgg aattactggt gctagtggaa caatctacgg gattcgttta ttagaagttt 1560

tgcacgcaat gccaaatgtg gagacacacc tagtgatgag tcagtgggcc aaggaaaatt 1620

tagcagtaga agatactggt tatacggaaa ctcaagtcaa agcactggct gatttgactt 1680

attctgagca aaatatgggt gccaaaattg ctagtggcag ctttattcat gatggaatgg 1740

ttattattcc agctagtatg aagactgttg catcgattgc tactggtgta ggagaaaact 1800

taattgcacg agcagccgac gtgactctaa aagaacagcg acaattgatc attgtgcctc 1860

gtgagtcgcc gtttaaccaa attcatttgg agaatatgct caaactttcc aaaatgggtg 1920

tgggtattat tccaccaatt ccggcatttt ataataatcc aaaaacggta gatgacatta 1980

tcaatcattc cgtgatgaag atcttggatc atttacagat tgagaattct gttagtacac 2040

gatgggaggg attggctcat gcccgcaaag atgcccaaaa caaataacga gattccgaac 2100

acttttaaag tagatgtaac aaaaagtggt tatacgatgg ttgccatttt ggaacgtcaa 2160

aatcaagatg tgctgatcca attaattgga ggcgatgtgc cgcactacgg ggttgtgatg 2220

tcgattgata atacaggcaa tactgagaca attcggttgc ctagccgccc aggacatgta 2280

catcaggaaa agattctgat tgaacaagtt gctggcgcca tcaaaccagt tttgcaaaat 2340

aatgcgatca ttgtttctgg aatgcatgtc aatgatatct ccacagaaca gatgcatgcc 2400

gctattccaa tggcgcaaaa gttgggcgca cgactagcag tttggttaaa acaaaatccg 2460

gttgatccat taccgatgag tttcgctaag aaaaacagtg tgaacaaccg cgtttag 2517

<210> SEQ ID NO 9
<211> LENGTH: 2491
<212> TYPE: DNA

-continued

<213> ORGANISM: Lactobacillus sakei subsp. sakei

<400> SEQUENCE: 9

atgagtgaac aaccttatga tttacgtaaa gtattggctg aaatcaaaga tttaccaggc 60

caatatcatg aaacggatgt tgaaattgat cccaatgctg atttagcggg tgtctatcgt 120

tatattggtg ctggcgggac agtcatgcgg ccaacaaccg aaggcccaac aatgatgttt 180

aataatgtta aaggcttccc tggaagtcgt gttttaattg gcttgcaagc ttcacgccaa 240

cgggtcgcaa cgattttaca tcatgactac aaaacgctgg gtcaaatgtt aaacgaagcg 300

gtaaccaaac cagttgcccc cgtcgaagtg acacgcgaac aagcaccagc acaagaagtc 360

gttcatttag cgagcgatgc tgattttgat atccgcaaat tattagcagc accaaccaat 420

acggaagacg atgctggtcc atacatcaca atgggtgtcg tgtatggtca tagcgtggat 480

caccaacaaa gcgacgttac gattcaccga atggttcttg aagataaaga tacaattggg 540

atgtatatca tgcccggtgg tcgccatatc ggtgcgttct taaaagaata tgaagcaatc 600

aacgaaccaa tgccaattac cattaacatt ggtttggacc cagcgattac cattggggct 660

acttttgaac cacctacaac gccacttggt tataatgaac tccaaattgc cggtgcttta 720

agaaatgaag ccgttcaagt agtgcctggt gtcgctgttg atgccttagg gattgcccgt 780

gctgaatgga ttatcgaagc cgaaatttta ccaaaccaat caatgcaaga agatattaat 840

acgaattctg ggtatgcgat gccagaattc cctggttata acggctcagc aaatcctgcc 900

gtgaatgtga ttaaagttaa agcggtcact catcgtcaag ataatccaat tgttcaaaca 960

acaattggtc cttcagaaga acacgtttcg atggccggta ttccaactga agcatcaatt 1020

ttgagcttag tggagcgcgc tattccaggt aaagtattga acgtctataa cgcccctgct 1080

ggtggtggga aattaatgac tatcatgcaa attcataagg ataacgaagc cgatgaaggg 1140

attcaaagac aagcagcatt gctcgccttt tctgccttca aagaattaaa gacggtcatc 1200

ttagtggatg aagatgttga tatcttcgat tggaacgacg tgatgtggac catcaataca 1260

cggttccaag ccgatcggga catcatggtc ttagaaggtt tacggaatca tccattagat 1320

ccatctgaat taccagaata cgatccagct cggattcgtg tgcgtgggat gagttctaag 1380

ttagtattag acgggacttt cccatatgac atgcaaaaga gtttcaagcg tgcgcaattt 1440

aaagaaatcg cagattggca aaaatattta aattaattag aagaaaaggg gaataatatg 1500

cgtaaaatag tcgtcggtat ttcgggtgcc agtgggacaa tttatgggat tcgtttgcta 1560

gaggcattac accaagtgcc ggatgttgaa acacatctgg tcatgagtcg ctgggccaaa 1620

gaaaatttag ccatcgaaaa aactgggtac actgaaaagc aagtcgtggc gctagctgat 1680

tttgtccatc cagagcaaaa tatgggcgca acaattgcca gcggtagttt caaacacgat 1740

gggatggtga ttgtacccac tagcatgaaa actttagctt cgattgcaac cggtctgggc 1800

gaaaacttga ttgccagagc tgccgatgtt actttgaaag aacgacgacc attaattatt 1860

gtgccccgtg aatctccttt taatcagatt caccttgaaa atatgttgaa gttggcccaa 1920

atgggtgtgg cgattgtacc gccgattcca gctttttata atcaaccaca aacgatagat 1980

gatatcgtca atcatacggt aatgaaatta ttggatcaac tgcatatcga gaccaacctt 2040

ggttcacgtt gggaggggtt agctaatgca cgtcaaaacg ctcgctagta ccgaacaaac 2100

cgtacaattt gcggtcactg aaacggatta tacaatgcac ttaaaattag agcgccaaac 2160

gtctgattta ttaattcaga ttatcggtgg cgacgtgccc cattacggcg tcatcacgac 2220

ggtcgataaa acgggtaagg cattgaccac ggcacttcct agccgcccag gacatgtcca 2280

-continued

tcaagaaaaa gtattaattg atcaggtttt aaaaactgtt aagccactga ttacgaataa 2340

tgcggtctta gtgtctggga tgcacgtcaa tgagattaca ccggcgcaaa tgcgcgcggc 2400

aatgctaatg gcacatgaac taggagtcgc tttagccaaa tggttaaaag cacatccaga 2460

tcaaacccaa gtggttacct atgctaaata a 2491

<210> SEQ ID NO 10
<211> LENGTH: 2247
<212> TYPE: DNA
<213> ORGANISM: Bacillus megaterium

<400> SEQUENCE: 10

atgaaaatag ctgtaggaat cacaggagcc acgggagcta ttttaggtat tcgaatttta 60

gagctgttaa agaaggcttc tgttgaaacg catttagtca tgtcgccttg ggctcatgcc 120

acaattaagc tggaaacatc ctatagcgtt cagcaagtag aagcgctagc agattattgt 180

tattcatatc aagaccaagc agcaaaaatt tcgagcggct cttttcgtat agatggaatg 240

attgttagtc cttgcagtat gaaaacatta gcatctattc gaatgggact agcagataac 300

ttaatagcga gaacggcaga tgtgatgtta aaagagagaa agccacttgt gctacttcct 360

cgcgaaacgc ctttaaatac gattcattta gaaaacatgc tggatctttc aaaaatggga 420

gctatcctgg tgccgcctat gccggctttt tataacaaac ctaagacgat cgacgatatt 480

gtcacacata ttgctgttcg aacgttagat cagttgggaa ttgagcttcc tgaagcaaaa 540

agatggcaag gaattaaaca tttatcacaa ggaggaaaat aaaaatggct tataaagact 600

ttagagattt ccttgatacg ctacataaag aagggcagct gctgacgatt actgatgaag 660

tgcagcctga tcctgatttg ggttcagcag gtcaagccat cagtaattta ggagatcaaa 720

cgccgggatt attgtttact aatatttatg gatatcacaa tgcaaaggta gctctaaacg 780

taatgggttc ctggtcaaat cacgcgttaa tgatggggct gcctaaatcg actcctgtaa 840

aagaacagtt ctttgagttt gctcggagat atgaaaaatt tcctgtcaaa gtgaaaagag 900

aagagacagc gccgtttcat gagtgtgaaa taacagacga tattaactta ttcgatcttt 960

tgccgttgtt tcgcttaaat caaggagacg gaggctatta tctagataaa gcgtgtgtta 1020

tttctcgtga tcagcatgat aaggagaact tcggtaaaca aaatgtaggg atataccgta 1080

tgcaggtcaa agggaaagat cgtctaggca ttcagcccgt gccacagcat gatattgcca 1140

ttcatttaaa acaagccgaa gaaaaaggtg aaaacctccc tgtatcaatt gctttaggat 1200

gtgaacctgc gattgttaca gcagccgcta cgccgcttca ttacgatcaa tctgagtatg 1260

aaatggcagg agctattcaa ggtgagccgt acagaattgt gaagtctcag ctttctgatt 1320

tagatgtacc ttggggagca gaagtgattt tagagggaga aattttagct ggtgaacgtg 1380

aatatgaagg tccttttggc gaatttacag gtcattactc aggcggcaga agcatgcctg 1440

ttatcaaaat caatcatgta taccatcgca aagatcctat ttttgaaagt ttatatatcg 1500

gtatgccttg gacagaaaca gattatttaa ttggaattaa tacaagcgtt cctttatatc 1560

agcagctaaa agaagcatat cctgaagaga ttgaagctgt gaatgcgatg tatactcatg 1620

gtctcgttgc tatcatttca acgaaaagcc gatacggagg atttgctaag gcggttggta 1680

tgagggcatt gactacgccg catggattag gatattgcaa gctagttatt ttagtagacg 1740

aagatgtgga tccgtttaat ttaccgcaag tcatgtgggc gctatcaaca aaaatgcacc 1800

caaaacacga tgttataaca gtgcctaatc tttcagttct gccacttgat ccaggatctg 1860

-continued

agccggctgg tattacagat aaaatgattt tggatgctac aacaccagtt gcaccggaaa 1920

caagaggcca ctattctcag cctttagata caccacttga aactgaaaaa tgggaaaaaa 1980

tcttaacgaa tatgatgcaa aaataaacaa ggaggaatcg acatgcatac ttgtccaaga 2040

tgtgaagcca aacaagcgaa cttagtatct aaatcaccag ttgaaggagc ctgggagatt 2100

tacttatgca acgtgtgttt gtttacctgg cgttcttctg aacctgagac catcaccaat 2160

cctgaaaaat accctcgacc atttaaagtc aatccaaaag acgtaccgct ggcaacgcac 2220

gtgcctcctg tgccaccccg atcttaa 2247

<210> SEQ ID NO 11
<211> LENGTH: 2252
<212> TYPE: DNA
<213> ORGANISM: Bacillus licheniformis

<400> SEQUENCE: 11

atgaacatca tcgtcggaat cacgggcgcg accggcgcgg tatttggcgt gcggatgctg 60

gagtggctga agaaaaccga cgcagagaca caccttgtca tctctccgtg ggcagcggca 120

acgatcctgc acgaaacggg atatacgatg aaagacgtgg aaaagctcgc atcttttacg 180

tattcccaca aagaccaggc ggcccgcatt tcaagcggtt cctttcaaac ggacggaatg 240

attgtcgcac cgtgcagtat gaagacgttg gcgggcatcc gcaccggtat ggcggataac 300

ctcttgaccc gttcggcgga cgtcatgctg aaggaacgga aaaagctcgt tctgttaaca 360

agggagacgc cgctgaacca gattcatctt gaaaacatgc ttgagctgac aaaaatgggg 420

gcggtgatcc tgccgccgat gccggctttt tataatcatc cccaaaatct gaccgaaatg 480

gtcgatcata tcgtatttcg gacgctggac caatttggca tccatctgtc tgaagcgaag 540

cgctgggaag gtatgaaaca ggagaaataa ggaggataac agaatggctt atcaagattt 600

tagagatttt ttaaatacgc tgaaaaaaga aggacagctt cttgaagtcc aggaagaggt 660

gaagccggaa cccgatttgg gagcagctgc acgcgccgcc aacaacctcg gagacaaatc 720

acccgctctt ttattcaaca acatttacgg ctataacaat gcccaaatcg cgctgaatgt 780

aatcggctcc tggccgaacc acgcattaat gctcggcctt ccaaaagaca cgccggttaa 840

agaacaattc tttgagttcg cgcgccgtta taatcagttt ccagtaaaag tgcaaagaga 900

ggagacagcg ccgtttcacg aaaacgaaat cacagaagac atcaacctgt ttgacattct 960

gccgctcttc cgcatcaatc agggcgacgg cggcttttat ttagacaagg catgcgtcat 1020

ttcgagagat gtcgaggatc cggaccactt cggcaagcaa aacgtcggca tgtacagatt 1080

gcaggtaaaa ggcaaagacc gcctcggcat tcagcccgtc ccgcagcatg acattgcgat 1140

ccacctgcgt caggctgaag agcgcggcga aaacctgcct gtcacgattg cgctcggctg 1200

cgaaccggtt attgcaacgg cggcatccac accgctctta tacgatcaat cagaatacga 1260

gatggcaggg gccctgcaag gcgaaccata taaaatcgtc aaatcaaaac tgtctaactt 1320

agatatccca tggggcgcag aagtggttct cgaaggtgag atccttgcgg gcgaacgcga 1380

gtatgaaggt ccgttcggcg agtttaccgg ccactattca ggcggacgaa gcatgccgat 1440

catcaaaatc aagcgcgtct gccaccgcaa caatccgatt tttgaacacc tgtatttagg 1500

catgccttgg actgaggttg actatatggt cggcattaat acatgtgtgc cgctttacca 1560

gcagcttaaa gaggcgtatc cgaatgaaat tgtcgcggta aacgcgatgt atacgcacgg 1620

cttgatcgcc attgtatcaa cgaaaagccg ctacggcgga ttcgccaaag ccgtcggcat 1680

gcgcgcgctg acaactccgc acggcctcgg ctactgcaaa atggtgatcc tcgttgacga 1740

-continued

agacgtcgat ccgtttaacc tcccgcaagt gatgtgggca atctcaacga aaatgcatcc 1800

gaaacacgat gcagtcatca tcccggattt atccgttttg gcgctagatc caggttctga 1860

accggcggga atcacccaca aaatgatatt ggacgcgaca acgccggctg caccggaaac 1920

aaggggacac tattcacagc cgctcgattc ccctatagga acgaaagagt gggaagcaaa 1980

attaatgaat ctgctaaatc aataaaagag gagagtgttt catcatgcat acatgtccgc 2040

gctgcgactt aaaaaaagcg gaaaccgtca gcaaatcacc cgttgaagga gcctgggaag 2100

tctatcaatg ccagcactgc tttttcactt ggaggtcatc agagccggag acgatcacaa 2160

atcctgaaaa atacaatccg gcctttaaaa tcgatcccgc tgaagttgaa acagctgtac 2220

aagtgccggc gattccagac cggaaaatct aa 2252

<210> SEQ ID NO 12
<211> LENGTH: 2249
<212> TYPE: DNA
<213> ORGANISM: Bacillus atrophaeus

<400> SEQUENCE: 12

atgaaactcg ttgtcgggat gaccggagct acaggggcta ttttcggagt caggctttta 60

gaatggctga aggccgcagg agcggaaact caccttgtcg tttctccttg ggctcatgtc 120

acaatcaaac atgaaacagg ttatagctta aaagaagttg aagagcttgc ctcatatacg 180

tactctcata aggatcaggc ggctgccatt tcaagcgggt cttttcaaac ggacggcatg 240

atcgtcgccc cgtgcagtat gaagtcgctc gcaagcattc gcacggggat ggcggacaat 300

ctgttgaccc gggctgcaga tgtcatgctg aaagagagaa aaaagcttgt cctgctgacg 360

agagaaacgc cgcttaacca gattcattta gagaatatgc tcgcattaac aaagatggga 420

accattattc ttccgccaat gccggctttt tataatcagc cggcaagtct ggatgaaatg 480

gtggaccata ttgtattcag aacgctggat caattcggca ttcgccttcc tgaggcaaaa 540

cgctggaatg gaattgaaaa agaaaaagga ggagcttgat catggcttat caagatttca 600

gagaatttct cgctgccctg gaaaaagagg gacagctatt aaaagtggat gaagaggtga 660

agccggagcc ggatttagga gccgcagccc gcgcagccaa caacctcggc gataaaagcc 720

cggctctttt atttaacaat atttacggct acaacaatgc acaaatcgcg atgaatgtca 780

tcggttcttg gccgaaccac gcgatgatgc ttggcttgcc gaaagataca ccggtgaaag 840

agcagttttt tgaatttgcg aagcgatatg aacagtttcc gatgccggtc aaacgcgaag 900

aaactgcacc atttcatgaa aatgaaatca cagaggacat caacctgttc gatctattgc 960

ctcttttcag aattaaccag ggtgacggcg gctattattt agataaagcg tgtgtcattt 1020

cccgtgatct ggatgaccct gacaatttcg gcaagcagaa cgtcggaatt taccggatgc 1080

aggtaaaagg gaaagaccgc ctcggcattc agccagttcc gcagcatgac atcgcgattc 1140

atcttcgcca agcagaagaa cgcggcgtca atcttccggt cactatcgcg cttggctgtg 1200

agcctgtcat tacgaccgcg gcgtcaactc cgctcctata tgaccaatca gaatacgaaa 1260

tggcgggagc gatccaaggc gaaccgtata gaatcgtcaa atcaaacctg tctgaccttg 1320

atattccttg gggcgcagaa gtcgtgcttg aaggagaaat cattgccgga gaacgggaat 1380

atgaaggacc gttcggcgaa tttaccggcc attattcagg cggacgcagc atgccgatta 1440

tcaaaatcaa acgcgtatct catagaaatc atccggtatt tgaacattta tatctcggca 1500

tgccttggac agagtgcgat tacatgatcg gcattaatac atgcgtgccg ctttatcagc 1560

-continued

agctgaaaga agcatatccg agtgaaattg tcgctgtgaa cgcaatgtac acacatggct 1620

taatcgccat tgtatctgca aaaacccgtt acggaggatt tgcaaaagct gtcggaatga 1680

gagccctgac tacaccgcac ggactcggct actgtaagat ggtgatcgtc gtggatgaag 1740

atgttgatcc gttcaacctc ccgcaagtca tgtgggcgct ttcaacaaag atgcatccga 1800

agcatgatgc cgtaaccatt cctgatttat ccgtgctgcc gcttgatccg ggatcagacc 1860

catccggcat tactcataaa atgattctcg atgccacaac gcctgttgcg ccggaaacaa 1920

gaggccatta ttcacagccg cttgactctc ctttaacaac aaaagaatgg gaacaaaaac 1980

taatggactt gatgaataaa taagagaaag gatgatctga catgcataca tgtcctcgat 2040

gtgattcaaa aaagggagaa atcatgagca aatcgcctgt agaaggcgct tgggaagtct 2100

accaatgtca aacgtgtttc ttcacatgga gatcatgtga accggaaagc attacaaacc 2160

cgaaaaaata caatccatca tttaagatcg atccgaagga aacagaaaca gctgttgaag 2220

tgccggctgt tccggaaaga aaggcctga 2249

<210> SEQ ID NO 13
<211> LENGTH: 2283
<212> TYPE: DNA
<213> ORGANISM: Bacillus subtilis subsp. subtilis

<400> SEQUENCE: 13

atgaaagcag aattcaagcg taaaggaggg ggcaaagtga aactcgttgt cggaatgaca 60

ggggcaacag gggccatttt cggggtcagg ctgctgcagt ggctgaaggc cgccggagtg 120

gaaacccatc tcgttgtgtc tccttgggca aacgtcacga tcaaacacga aacaggctat 180

acgttacaag aagtagaaca actggccaca tacacttact cacataagga tcaggcggca 240

gccatttcaa gcgggtcgtt tgataccgat ggaatgattg ttgcgccgtg cagcatgaaa 300

tctctcgcaa gcattcgcac aggaatggcg gataatctgc tgacacgtgc ggcggatgtc 360

atgctcaagg agagaaaaaa actcgtcctc ttaacgagag agacgccttt gaaccaaatt 420

catctcgaaa atatgctagc gcttacgaaa atgggcacca tcattcttcc tccgatgccg 480

gcattttata atcggccgag aagcttagag gaaatggttg accatattgt ttttagaacg 540

ttggaccaat tcggcattcg gcttcctgaa gcgaagcgct ggaatgggat tgaaaaacaa 600

aaaggaggag cttgatcatg gcttatcaag atttcagaga atttctcgct gcccttgaaa 660

aagaaggaca gctgcttaca gtgaatgaag aggtaaagcc ggaaccggat ttaggggcct 720

ccgcacgggc agccagcaat cttggcgata aaagccctgc gctcttattt aacaacattt 780

acggctatca taacgcgcga attgcgatga atgtcatcgg ctcttggcca aaccatgcca 840

tgatgctggg catgccgaaa gacacaccgg taaaagaaca gttttttgaa ttcgcaaagc 900

gttatgacca gtttccgatg ccggtcaaac gtgaggaaac agcgccattt catgaaaatg 960

aaatcacaga agatatcaat ttgttcgata tactgcctct tttcagaatt aaccagggtg 1020

atggaggcta ctatttagac aaagcatgtg tcatttcccg tgatcttgag gaccctgaca 1080

acttcggcaa acaaaatgtc ggcatttaca gaatgcaagt caaaggaaaa gaccgccttg 1140

gcattcagcc tgtcccgcag cacgatattg caatccatct gcgccaagct gaagaacgcg 1200

gcatcaacct tccggtcact attgcgctcg gctgtgagcc ggtcattaca acggcggcat 1260

cgactccgct tctctatgat caatcagaat acgaaatggc aggtgcgatt caaggcgaac 1320

catatcgcat cgtcaaatca aagctgtctg atcttgatgt tccgtggggc gctgaagtgg 1380

tgcttgaagg tgagattatt gccggagagc gcgaatatga agggccgttc ggtgaattca 1440

-continued

caggccatta ttccggcgga cgcagcatgc cgattatcaa aattaaacgc gtctatcaca 1500

gaaacaatcc gatctttgaa catttatact taggcatgcc ttggacagaa tgcgattaca 1560

tgatcggcat taacacatgc gtgccgcttt atcagcagtt aaaagaagcg tatccgaacg 1620

aaattgtggc agtgaacgcc atgtacacac acggtttaat cgcgattgtt tccacaaaaa 1680

cccgctatgg cggatttgcg aaagcggtcg gcatgcgcgc actcacaacg ccgcacggac 1740

tcggctactg caaaatggtc atagtcgttg atgaggatgt cgatccattc aaccttccgc 1800

aggtcatgtg ggcgctttcg accaaaatgc atccgaaaca tgatgcggtc atcattccgg 1860

acttatctgt cctgccgctt gatccgggat ccaatccatc aggaatcact cacaaaatga 1920

ttctcgacgc cactacaccg gttgcgccgg aaacaagagg ccattattca cagccgcttg 1980

attctccgct aacaacgaaa gaatgggaac aaaaactaat ggacttaatg aataaataag 2040

gaaaggatgt tcgaaatgca tacatgtcct cgatgcgact caaaaaaggg agaagtcatg 2100

agcaaatcgc ctgtagaagg cgcatgggaa gtttatcagt gccaaacatg ctttttttaca 2160

tggagatcct gtgaaccgga aagcattaca aatcccgaaa aatacaatcc agcgtttaaa 2220

attgatccaa aggaaacaga aacagcaatt gaagttccgg cggtgccgga acgaaaggct 2280

tga 2283

<210> SEQ ID NO 14
<211> LENGTH: 2283
<212> TYPE: DNA
<213> ORGANISM: Bacillus subtilis subsp. spizizenii

<400> SEQUENCE: 14

atgaaagcag aattcaagcg taaaggaggg ggcaaagtga aactcgttgt cggaatgaca 60

ggggcaacag gggctatttt cggggtcagg ctgctggagt ggctgaaggc ggccgaagta 120

gaaacccatc tcgtcgtgtc tccttgggct aacgtcacga tcaaacacga aacaggctat 180

accttaaaag aagtagaaca acttgccaca tacacgtatt cgcataagga ccaggcggca 240

gccatttcaa gcgggtcgtt tgataccgat ggcatgattg ttgcgccatg cagcatgaaa 300

tctctcgcaa gcattcgcac cgggatggcg gataatctgc tgacgcgtgc ggcggatgtc 360

atgctcaagg agagaaaaaa actcgtcctc ttaacgagag agacgccttt gaaccagatt 420

catctcgaaa atatgctagc gcttacgaaa atgggtacca tcattcttcc tccgatgccg 480

gcattttata atcagccgag cagcttagag gaaatggttg accatattgt attcagaacg 540

ttggaccaat tcggcattcg ccttcctgaa gcgaaacgct ggaatgggat tgaaaaacaa 600

aaaggaggag cttgatcatg gcttatcaag atttcagaga atttctcgct gcccttgaaa 660

aagaaggaca gctgctaaca gtgaatgaag aggtaaagcc ggagccggat ataggggctg 720

cagcacgcgc agccagcaat cttggcgata aaagccccgc gctcttattt aataacattt 780

atggctatca caacgcgcaa attgcgatga atgtgatcgg ctcctggccg aaccatgcaa 840

tgatgctggg catgccgaaa gacacgccgg tgaaagaaca gttttttgaa tttgcgaaac 900

gttatgacca gtttccgatg ccagtcaaac gtgaggaatc agcgccgttt catgaaaatg 960

aaatcacaga agatatcaat ttgttcgata tactgcctct tttcagaatt aaccaaggag 1020

acggcggtta ctatctagac aaagcatgtg tcatttcccg cgatcttgaa gatcctgaga 1080

atttcggcaa acaaaacgtc gggatttaca gaatgcaggt caaaggaaaa gaccgccttg 1140

gcattcagcc tgtgccgcag cacgatattg cgatccatct gcgtcaagct gaagaacgcg 1200

-continued

gcatcaatct tccggtcacc attgcgctcg gctgtgagcc ggtcataaca acggcggcat 1260

cgactccgct tctttatgat caatcagaat acgaaatggc aggcgcaatt caaggtgaac 1320

catatcgcat cgtgaaatct aagctgtctg atcttgatgt tccatggggc gctgaagtag 1380

tgcttgaagg tgaaatcatt gccggagagc gtgaatatga aggcccgttc ggtgagttca 1440

caggccatta ttccggcgga cgcagcatgc cgattattaa aattaaacga gtgtatcata 1500

gaaacaatcc gatttttgaa catttatact taggcatgcc ttggacagaa tgcgattaca 1560

tgattggcat taacacttgt gtgccgcttt atcagcagtt aaaagaagcg tatccgaatg 1620

aaattgtggc tgtgaacgcc atgtacacac acggtttgat cgcgattgtt tccacaaaaa 1680

cacgctatgg cggatttgcg aaagcagtcg gcatgcgcgc gctcacaaca ccgcacggac 1740

tcggctactg caaaatggtc attgtcgttg acgaggatgt cgatccattc aatctgccgc 1800

aggtcatgtg ggcgctttcg accaaaatgc atccgaagca cgatgcggtc atcattccag 1860

acttatctgt cctgccgctt gacccgggat ctaatccatc aggaatcact cacaaaatga 1920

ttcttgacgc cactacaccg gttgcgccgg aaacaagagg ccattattca cagccgcttg 1980

attcaccatt aacaacgaaa gaatgggaac aaaaactaat ggacttaatg aataaataag 2040

aaaaggatga tcgaaatgca tatatgtcct cgttgcgatt cgaaaaaggg agaagtcatg 2100

agcaaatcgc ctgtagaagg cgcatgggaa gtttatcagt gtcaaacatg ttttttcaca 2160

tggagatcct gtgagccgga aagtattaca aatccggcga aatacaatcc agcgtttaaa 2220

attgatccga aggaaacaga aacagcaatt gaagttccgg ctgtgccgga acgaaaggct 2280

tga 2283

<210> SEQ ID NO 15
<211> LENGTH: 2268
<212> TYPE: DNA
<213> ORGANISM: Enterobacter aerogenes

<400> SEQUENCE: 15

atgaaactga ttattgggat gaccggggcg accggcgcgc cgttaggcgt cgcgctgtta 60

caggcgctga atgaaatgcc ggatgtggaa acgcatctgg tcatgtcgaa atgggcaaaa 120

accaccattg agctggaaac gccctatagc gctcgtgatg tcgccgcgct ggcggacttc 180

tgccatagcc ctgcggatca ggccgcgacc atctcatcag gatcgtttcg taccgacggc 240

atgattgtta tcccctgcag catgaaaacg ctggcgggta ttcgcgctgg ctatgcggaa 300

gggttagtcg gccgcgcggc ggacgtggtg ctgaaagagg ggcgcaagct ggttctggtg 360

ccgcgtgaaa tgccgctgag caccattcat ctggagaaca tgctggcgct gtcgcgcatg 420

ggcgtggcga tggtgccgcc catgcctgcc tattacaacc acccggaaac ggtagaggat 480

atcaccaacc atatcgtgac ccgggtgctg gatcagtttg gtctcgaata tcacaaagcg 540

cgccgctgga acggcctgcg cgcggtcgag aatttatcac aggagaatta atcatggctt 600

ttgatgattt acgcagcttt ttgcaggcgc ttgatgagca ggggcaactg ctaaaaatta 660

gcgaagaggt gaatgccgag ccggatctcg ccgctgccgc taacgccaca gggcgcatcg 720

gtgacggcgc gccagcgttg tggtttgata acattcgcgg ctttaccgac gcccgtgtcg 780

ccatgaacac catcggttcc tggcaaaacc acgcgatttc gctggggctg ccgccaaaca 840

cgccggtgaa aaagcagatt gatgaattta ttcgccgctg ggataaattc ccggtaacgc 900

cggagcgtcg cgctaatcca gcgtgggcgg aaaacaccgt tgatggcgac gatatcaacc 960

tgttcgatat tctgccgctg ttccgcctga acgatggcga cggtggtttc tatctcgaca 1020

-continued

aagcctgtgt ggtttcgcgc gatccgcttg acccggacca ctttggcaaa cagaacgtcg 1080

gtatttaccg gatggaagtg aaaggcaagc gcaagctggg cctgcagccg gtaccgatgc 1140

acgatatcgc gctgcatctg cataaagcgg aagagcgcgg tgaggatctg cccattgcca 1200

tcaccctggg taacgacccg attattaccc tgatgggcgc gacgccgctg aaatatgacc 1260

agtcagaata tgagatggcg ggcgcgctgc gcgaaagccc gtatcccatc gccaccgcgc 1320

cgctgaccgg ctttgacgtt ccctggggct cagaggtgat ccttgaaggg gtgattgaag 1380

ggcgcaagcg tgaaatcgaa gggccgttcg gcgagttcac cggccactac tcaggcggcc 1440

gcaatatgac ggtggtgcgt atcgataaag tctcttatcg cacaaaaccg atttttgaat 1500

cgttgtatct cggaatgccg tggaccgaaa tcgactatct gatgggcccg gcgacctgcg 1560

tgccgctgta ccagcagctg aaggcggagt tcccggaggt gcaggcggtc aatgccatgt 1620

acacccatgg tctgctggcg attatctcca ccaaaaaacg ctacggcggt tttgcccgcg 1680

cggtgggatt acgggcaatg actaccccgc acggcctcgg ttacgtgaaa atggtgatca 1740

tggtcgatga agatgtcgat ccgttcaacc tgccgcaggt gatgtgggcg ctctcctcga 1800

aggtcaaccc ggcgggcgac ctggtacagt tgccgaacat gtcggtgctg gagcttgacc 1860

ctggttccag tccggcgggg atcaccgaca aactgattat cgacgccacc accccggttg 1920

cgcctgacct tcgcggtcac tacagccagc cggttcagga tttaccggaa accaaagcct 1980

gggctgaaaa actgaccgcc atgttggcca accgtaaata aggagaagaa gatgatttgt 2040

ccacgttgcg ctgatgagca gattgaagtg atggcgacgt cgccggtaaa aggggtgtgg 2100

atcgtttacc agtgccagca ctgcctctat acctggcgta ataccgaacc gctgcgtcgt 2160

accagccgcg aacattatcc ggaagcgttc cgcatgacgc agaaagatat tgatgaggcg 2220

ccgcaggtgc cgcatattcc accgctgttg gcggcagata agcgttaa 2268

<210> SEQ ID NO 16
<211> LENGTH: 2252
<212> TYPE: DNA
<213> ORGANISM: Enterobacter cloacae

<400> SEQUENCE: 16

atgagattga tcgtgggaat gacgggagca acaggtgctc cgctgggtgt ggctttactg 60

caggcgttac gtgacatgcc agaggttgaa acccatctgg tgatgtcgaa atgggcgaaa 120

accaccattg agctggaaac gccttatacc gcgcaggatg tcgccgccct ggcagatgtc 180

gttcacagtc ctgccgatca ggctgccacc atctcctccg gctcgtttcg taccgacggc 240

atgatcgtca ttccctgcag catgaaaacg ctggcgggta tccgcgcggg ctatgccgaa 300

gggctggtgg gccgtgcggc agacgtggtg ctgaaagagg ggcgcaagct ggtgctggtc 360

ccgcgtgaaa cgccgctcag caccattcat ctggagaaca tgctcgcgct ttcccgcatg 420

ggggtggcga tggtgccgcc catgcctgcg tattacaacc acccgcaaac cgccgatgat 480

atcacccagc atatcgtgac ccgcgtactc gaccagtttg gtctggagca caaaaaggcg 540

cgtcgctgga acggcctgca ggcggcgaaa cattttcac aggagaataa cgatggcatt 600

tgatgatttg agaagcttcc tgcaggcgct agatgagcaa gggcaactgc tgaaaattga 660

agaagaggtc aatgcggagc cggatctggc ggcggccgct aacgcgacgg gacgtatcgg 720

tgatggtgcg cctgcgctgt ggttcgataa cattcgcggg tttaccgatg ccagggtggt 780

gatgaacacc atcggctcct ggcagaacca cgccatttcg atggggctgc cggcgaatac 840

-continued

cccggtcaaa aagcagatcg atgagtttat tcgccgctgg gataaattcc cggtcgcacc 900

ggagcgccgg gccaaccccg catgggcgca gaatacggtg gacggtgagg agattaacct 960

gttcgacatc ctgccgctgt ttcgcctgaa cgacggggac ggcggttttt atctcgacaa 1020

agcgtgcgtt gtctcgcgcg atccgctcga cccggaccat ttcggcaagc agaacgtcgg 1080

tatttaccgc atggaagtga agggcaaacg taagctcggc ctgcagccgg tgccgatgca 1140

tgatatcgcc ctgcatctgc ataaagccga agagcgtggt gaagacctgc cgattgcgat 1200

tacgttgggc aacgatccga tcatcaccct gatgggcgca acgccgctga aatacgatca 1260

gtccgagtat gaaatggccg gggcgctgcg tgaaagcccg tacccgattg cgaccgcgcc 1320

gttgaccggc ttcgatgtgc cgtgggggtc tgaagtgatc ctggaagggg tgattgaagg 1380

ccgtaaacgt gaaattgaag ggccgttcgg tgagtttacc gggcactatt cgggcggacg 1440

caatatgacg gtggtccgta ttgataaagt ctcgtaccgc accaaaccga ttttcgaatc 1500

cctctatctc gggatgccct ggaccgagat cgactacctg atggggccag ccacctgtgt 1560

gccgctttac cagcaactga aagcggagtt ccctgaagtg caggcggtga acgcgatgta 1620

tacccacggt ctgctggcga tcatctccac caaaaaacgc tacggtggtt ttgcccgcgc 1680

ggtcggttta cgcgccatga ccacgccgca tggcctgggc tatgtgaaga tggtgattat 1740

ggtggatgaa gatgtcgatc cgttcaacct gccgcaggtg atgtgggcgc tgtcatcaaa 1800

agtgaacccg gcaggggatc tggtgcagct gccgaacatg tcggttcttg agcttgatcc 1860

tgggtccagc ccggcaggca tcaccgacaa gctgattatt gatgccacca cgcctgttgc 1920

gccggataac cgcggtcact acagccagcc ggtgcaggat ttacctgaaa ccaaagcctg 1980

ggctgaaaag ctgactgcga tgctggcagc acgccaataa ggaggaaaag atgatttgtc 2040

cacgttgtgc cgatgagcaa attgaggtga tggccacatc accggtgaaa gggatctgga 2100

cggtttatca gtgccagcat tgcctgtata cctggcgcga tactgagccg ctgcgtcgta 2160

ccagccgcga acattaccct gaagcgttcc gcatgacgca gaaggatatt gatgaggcgc 2220

cgcaggtacc gaccattccg ccattgctgt aa 2252

<210> SEQ ID NO 17
<211> LENGTH: 2284
<212> TYPE: DNA
<213> ORGANISM: Enterobacter sakazakii

<400> SEQUENCE: 17

atgaggctaa ttgtcggaat gacgggcgca accggcgcgc cgcttggggt cgcgctgttg 60

caggcgctga aagcgatgcc tgaggtggaa acccatctgg tgatgtcaaa gtgggcgaaa 120

accacgatcg aactggaaac gccgttctcc tggcaggatg tcgcggggct ggcagatgtg 180

gtgcacagcc cggcggatca ggccgcgacg atctcctcag gatcgtttcg caccgacggc 240

atggtgatca ttccgtgcag catgaaaacc ctggcgggca tccgcgcggg ctacgccgac 300

gggctggtgg gccgcgccgc tgatgtggtg ctgaaagaga accgtaaact ggtgctggtg 360

ccgcgcgaaa caccgcttag caccattcat ctggaaaacc tgctggcgct ctcgaagatg 420

ggcgtggcca tcgtgccgcc catgcccgcc tggtacaacc atcccgcgac gatcgacgac 480

atcatcaacc atatcgtcgc gcgcgtgctc gatcagttcg ggctcgatgc ccgcaacgcc 540

cgccgctggc aggggctaaa tcctgcgaaa acagccgaca cccattcatc acgaggagga 600

aacacgcatg gcgtttgacg atctgcgcag ctttttgcag gcgcttgaag agcaggggca 660

actgctgagg atcagcgaag aggtgcaggc ggagccggat atcgcggcgg ccgccaacgc 720

-continued

gaccggacgc atcggcgaag gcgcgcccgc gctctggttt gacaatatcc gcggctttac 780

tgacgcgcgg gtggcgatga acaccattgg ttcatggccg aaccacgcga tctcgctcgg 840

tctgccgcct gccacaccgg taaagcagca gatagaagaa tttattcgcc gctgggatac 900

cttcccggtc gcgccggaac gccgcgataa tccgccatgg gcggaaaaca gcgtcgacgg 960

cgacgacatt aacctgttcg acattctgcc gctgtttcgc ttaaacgacg gcgacggcgg 1020

gttctacctt gataaagcgt gtgtggtctc gcgcgatccg ctcgatcccg aacacttcgg 1080

caagcagaat gtcggcatct accggatgga agtgaaaggc aagcgcaagc tcgggctgca 1140

accggtgccg atgcatgaca tcgcgctgca tctgcataag gccgaagagc gtggcgagga 1200

tttgccggtt gcgattacgc ttggcaacga tccgatcatc acgctgatgg gcgccacgcc 1260

gctgaaatac gatcagtcgg aatatgaaat ggcgggcgcg ctgcgcgaaa gcccgtaccc 1320

gatagccacc gcgccgctga ccggtttcga cgtgccgtgg gggtcggaag tgatccttga 1380

aggggtgatt gaaggacgca agcgcgagat agaagggccg ttcggcgagt ttaccgggca 1440

ctactccggc gggcgtaaca tgaccgtggt gcgtatcgat aaagtctctt atcgcaccaa 1500

accgattttc gaatcgctct atctcggcat gccgtggacc gaaatcgact acctgattgg 1560

cccggcgacc tgcgtgccgc tttaccagca gcttaaagcg gagttcccgg aagtgcaggc 1620

ggtgaacgcg atgtataccc acgggctgct cgcgattatc tccaccaaga aacgctacgg 1680

cggtttcgcc cgcgcggtgg gcctgcgtgc gatgaccacg ccgcacgggc ttggctacgt 1740

gaagatggtg attatggtgg atgaggatgt cgatccgttc gatctgccgc aggtgatgtg 1800

ggcgctgtcg tcaaaagtga acccggcggg cgatctggtg cagttgccga atatgtcggt 1860

gctggagctt gatcctggct caagcccggc ggggattacc gacaagctga ttatcgacgc 1920

cactacgccg gttgcgccgg ataaccgcgg gcattacagc cagccggtga aagacctgcc 1980

ggaaaccccg cagtgggtag agaagctgac cgccatgctg gctaaccgta aaaaataagg 2040

agacgagatg atttgtccac gttgtgccga tgaaaccatc gaaatcatgg cgacgtcgcc 2100

ggtgaaaggc gtctggacgg tgtatcagtg ccagcattgt ttgtacacct ggcgcgacac 2160

cgagccgctg cgccgtacca gccgcgagca ttaccccgag gcgttccgga tgacgcaggc 2220

cgatatcgat aacgcgccgg aagtgccaac ggtgccgccg ctgctggcgg atggtaagcg 2280

ttaa 2284

<210> SEQ ID NO 18
<211> LENGTH: 2252
<212> TYPE: DNA
<213> ORGANISM: Enterobacter hormaechei

<400> SEQUENCE: 18

atgagattga ttgtgggaat gacgggcgcg acgggtgcgc cattaggcgt ggcgttgttg 60

caggcgctgc gggaaatgcc ggaggtggaa acgcacctgg tgatgacgaa gtgggcaaaa 120

accacgattg agctggaaac gcccttcact gcgcatgacg ttgctgcact ggcggatgtc 180

gtccacagtc cggccgatca ggctgccacc atctcctccg gctcgtttcg caccgacggc 240

atgatcgtca tcccgtgcag catgaaaacg ctggcgggga tccgcgcggg ctacgccgaa 300

gggctggtag ggcgtgcggc agacgtggtg ctgaaagagg gacgcaagct ggtgctggtt 360

ccccgcgaga cgccgctcag caccattcat cttgagaaca tgcttgccct ttcccgcatg 420

ggcgtggcga tggtgccgcc tatgcctgcg tactacaacc acccgcaaac cgccgatgac 480

-continued

attacccagc atatcgtgac ccgcgttctc gaccagtttg gtctggagca taaaaaagcc 540

cgacgctggg aaggtttgca ggcagcgaaa cattttcac aggagaataa agatggcatt 600

tgatgatttg agaagcttct tgcaggcgct cgatgagcaa gggcagctgc tgaaaattga 660

ggaagaggta aacgcggagc cggatttagc ggcggccgcc aacgctaccg ggcgcattgg 720

cgatggcgcg cctgcgctgt ggttcgataa tattcgcggc ttcaccgatg cccgagtggt 780

gatgaacacc atcggctcgt ggcaaaacca cgccatttcg atggggctgc cagcgaatac 840

ttcggtgaaa aaacagatcg acgagtttat tcgtcgctgg gacaaattcc ccgtcacgcc 900

agagcgtcgt gccaatcctg cctgggcgca gaacacggtg gacggagaag atatcaacct 960

gttcgacatt ttgccgctgt tccgcctgaa cgacggtgac gggggctttt atctcgataa 1020

agcgtgcgtt gtctcccgcg atccgctcga ccccgaccac ttcggcaagc agaacgtcgg 1080

catttaccgt atggaagtga agggcaagcg taagctcggc ctgcaaccgg tgccgatgca 1140

tgatattgcg ctgcatctgc ataaggcaga agagcgtggc gaagacctgc ccattgccat 1200

tacgctgggt aacgatccga tcatcaccct gatgggcgcc acgccgctga aatacgatca 1260

atccgagtat gagatggctg gcgcgctacg cgaaagcccg tatccgattg cgacggctcc 1320

gctgaccggt tttgatgtgc cgtgggggtc ggaagtgatc ctggaagggg tgattgaagg 1380

ccggaaacgt gaaattgaag gaccattcgg tgagtttacc ggacactact ctggcgggcg 1440

caacatgacc gttgtgcgca ttgataaagt ctcttaccgc accaaaccca ttttcgaatc 1500

tctctacctg gggatgcctt ggaccgagat tgattatctg atgggacccg ccacctgcgt 1560

gccgctctat cagcaactga aggcggaatt cccggaagtg caggcggtaa acgccatgta 1620

cacccacggt ctgctggcaa ttatctccac taaaaagcgt tacggcggtt ttgcccgtgc 1680

ggtcgggcta cgcgccatga ccacaccgca cggtctgggt tacgtgaaga tggtgattat 1740

ggtggatgaa gatgtcgatc cgtttaacct gccgcaggtc atgtgggcgc tttcatcgaa 1800

ggttaatccg gcgggcgatc tggtgcagct tccgaatatg tctgtgctgg aacttgaccc 1860

tggctccagc ccggcgggga tcaccgacaa gctgatcatt gatgccacca cccctgttgc 1920

cccggacaac cgtggtcact acagccagcc ggtacaggac ctccctgaaa ccaaagcctg 1980

ggccgaaaaa ctgaccgcga tgctggcagc acgtcaataa ggaggaaaaa atgatttgtc 2040

cacgttgtgc cgatgaacat attgaagtaa tggcaacatc accggtgaaa ggtgtctgga 2100

cggtatatca gtgccagcac tgtctgtata cctggcgcga taccgaaccg ctacgccgta 2160

ccagccgcga gcattacccg gaagccttcc gcatgacgca gaaggatatt gatgaggcgc 2220

cgcaggtgcc aacaatcccg ccgctgctgt aa 2252

<210> SEQ ID NO 19
<211> LENGTH: 2268
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 19

atgaaactga tcgtcgggat gacaggggct accggtgcgc ctcttggtgt ggcattactg 60

caagcgctgc gggagatgcc gaatgtcgag actcatctgg tgatgtcgaa gtgggcgaaa 120

accaccattg aactggaaac gccttacagc gctcgcgatg ttgctgccct cgcagacttc 180

agccataacc cggcggatca ggcggcgatc atctcatccg gttcttttcg taccgacggc 240

atgatcgtta ttccgtgcag tatgaaaacg ctcgccggta tccgcgctgg ttacgccgat 300

ggcctggtag ggcgcgcggc ggacgtcgtg ctcaaagaag gccgcaaact ggtgctggtg 360

-continued

ccgcgtgaaa tgccgcttag caccatccat ctcgaaaata tgctcgcact ttcacgcatg 420

ggcgtggcga tggtgccgcc gatgcctgcc ttttataacc atcccgaaac ggtagatgac 480

attgtccacc atgtggtagc ccgcgtgctg gatcaatttg gcctcgaaca tccccacgcc 540

aggcgctggc aaggattgcc gcaggcccgg aatttttctc aggagaatga ataatggcat 600

ttgatgattt acgcagcttt ttacaggcgc ttgatgacca cggccagtta ctgaaaatca 660

gcgaagaagt gaacgccgag ccggatctgg cagcagcagc taacgccacc gggcgtatcg 720

gcgacggcgc gcccgcgctg tggtttgata atattcgcgg ctttaccgat gcccgcgtgg 780

cgatgaacac catcggttcc tggcagaacc acgcgatttc cctcggcctg ccgccaaatg 840

ccccggttaa aaagcagatt gatgagttta tccgccgctg ggataacttc ccgattgccc 900

cggagcgccg cgccaatcca gcctgggcgc agaacaccgt tgatggcgac gagatcaacc 960

tgttcgatat cctgccgctg tttcgtttaa acgatggcga tggcggtttc tatctcgaca 1020

aagcgtgcgt ggtttcccgc gatccgctcg acccggataa cttcggcaag cagaacgtcg 1080

gcatctaccg catggaagtg aagggcaagc gtaagctcgg cctgcaaccg gtgccgatgc 1140

acgatatcgc cctgcatctg cataaagcag aagagcgcgg tgaagatctg ccgattgcga 1200

tcacgctcgg taacgatccg atcatcacgc tgatgggggc cacgccgctg aaatatgatc 1260

agtccgagta cgaaatggca ggcgcgctgc gtgaaagccc gtacccgatc gccaccgccc 1320

cgttgaccgg ttttgatgtg ccgtggggtt cagaagtgat cctcgaaggg gtcatcgaaa 1380

gccgtaaacg cgaaatcgaa gggccgttcg gtgagtttac cgggcactac tccggcgggc 1440

gtaacatgac cgtggtgcgc atcgataaag tctcttaccg caccaggccg attttcgaat 1500

cgctgtacct cggtatgccg tggaccgaaa tcgactacct gatggggcca gccacctgcg 1560

tgccgctgta tcagcagctg aaagccgagt tccctgaagt gcaggcggta aacgccatgt 1620

acacccatgg cctgctggcg attatctcca ccaaaaaacg ctacggcggc tttgcccgcg 1680

cggtgggcct gcgcgcaatg accacgccgc atggtctggg ctacgtgaag atggtgatta 1740

tggtcgatga agacgttgac ccgttcaacc tgccgcaggt gatgtgggcg ctctcctcga 1800

aagtgaaccc ggcaggggat ttggtgcagt tgccgaatat gtccgtgctg gaactcgatc 1860

caggctcaag ccctgcgggg atcaccgaca agctgattat cgacgccact acgcctgtcg 1920

ccccggacaa ccgtggtcac tacagccaac cggtggtgga tttaccggaa accaaagcct 1980

gggctgaaaa actgaccgct atgctggctg cacgtaaata aggagaagaa gatgatttgt 2040

ccacgttgtg ccgatgaaca gattgaagtg atggcgaaat cgccggtgaa agatgtctgg 2100

acggtatatc agtgccagca ttgcctttat acctggcgcg ataccgaacc gctgcgccgt 2160

accagccgcg aacattatcc cgaagcgttc cgcatgacgc agaaagatat tgatgacgcg 2220

ccaatggtgc cgagcatccc gccgctgctg gtggaaggta agcgctaa 2268

<210> SEQ ID NO 20
<211> LENGTH: 2268
<212> TYPE: DNA
<213> ORGANISM: Escherichia fergusonii

<400> SEQUENCE: 20

atgagactga tcgtcgggat gacaggggcc accggagcgc ctcttggtgt ggcattactg 60

caagcgctgc gggagatgcc gaatgtcgag actcatctgg tgatgtcgaa gtgggcgaaa 120

accaccattg aactggaaac gccttacaac gcccgcgatg ttgctgccct cgcagacttc 180

-continued

tgccataacc cggcggatca ggccgcaacc atctcctcag gttcctttcg taccgacggt 240

atgatcgtta ttccgtgcag tatgaaaacg ctcgccggta tccgcgctgg ttacgccgat 300

ggcctggtag ggcgcgcggc ggacgtcgtg ctcaaagaag gccgcaaact ggtgctggtg 360

ccgcgtgaaa tgccgcttag caccatccat ctcgaaaata tgctcgcact ttcgcgcatg 420

ggcgtggcga tggtgccgcc gatgcctgcc ttttataacc atcccgaaac ggtagatgac 480

attgtccacc acgtggtagc ccgcgtgctg gatcaatttg gcctcgaaca tcctcacgcc 540

aggcgctggc aaggattgcc gcaggcccgg aatttttccc aggagaatga ataatggcat 600

ttgatgattt acgcagcttt ttacaggcgc ttgatgacta cggtcagtta ctgaaaatca 660

gtgaagaagt gaacgccgag ccggatctgg cagccgctgc caacgccacc gggcgtatcg 720

gcgacggtgc accggcgctg tggtttgaca atattcgcgg ctttaccgat gcccgcgtgg 780

caatgaacac catcggctcc tggcagaacc acgcgatttc cctcggcctg ccgccaaaca 840

ccccggttaa aaaacagatt gatgagttta tccgccgctg ggataacttt cccattgccc 900

cggagcgccg tgcgaatccg gtctgggcgc agaacaccgt cgatggcgac gagattaatt 960

tgttcgatat tctgccgctg tttcgtttaa acgatggcga tggcggtttc tatctcgaca 1020

aagcgtgcgt ggtttcccgc gatccgctcg acccggataa tttcggcaag cagaatgtcg 1080

gcatctaccg catggaagtg aagggcaagc gtaagctcgg cctgcaaccg gtgccgatgc 1140

acgatatcgc cctgcatctg cataaagcag aagagcgcgg tgaagatctg ccgattgcga 1200

tcacgctcgg taacgatccg atcatcaccc tgatgggggc caccccgctg aaatacgatc 1260

aatcagagta cgaaatggct ggcgcactac gcgaaagccc gtacccgatc gccaccgccc 1320

cgctgaccgg ttttgatgtg ccgtggggct cagaagtgat cctcgaaggc gttatcgaaa 1380

gccgtaaacg cgagattgaa gggccgttcg gtgaatttac cggccactac tccggcgggc 1440

gcaacatgac cgtagtgcgc atcgataaag tctcttaccg caccaaaccg atttttgaat 1500

cgctctatct cggtatgccg tggaccgaaa tcgactacct gatggggcca gccacctgtg 1560

tgccgctgta tcagcaactg aaagccgagt tcccggaagt gcaggcggtg aacgccatgt 1620

acacccacgg cctgctggcg attatctcca ccaaaaaacg ctacggcggc tttgcccgcg 1680

cggtgggcct gcgtgcgatg accacgccgc acggtctggg ctacgtgaag atggtgatta 1740

tggtcgatga agacgttgat ccgttcaacc tgccgcaggt gatgtgggcg ctttcgtcga 1800

aagtgaaccc ggcaggggat ctggtgcagt tgccgaatat gtcagtactg gaactcgacc 1860

ctggctcaag cccggcgggg atcaccgata agctgattat cgacgccact acgcctgtcg 1920

ccccggacaa ccgtggtcac tacagccagc cggtggtgga cttaccggaa accaaagcct 1980

gggctgaaaa actgaccgct atgctggccg cacgtaaata aggagaacaa gatgatttgt 2040

ccacgttgtg ccgatgaaca gattgaagtg atggcgaaat cgccggtgaa agatgtctgg 2100

acggtctacc agtgccagca ttgcctttat acctggcgcg atactgaacc gctacgccgc 2160

accagccgcg aacattaccc gcaagcgttc cgtatgactc aaaaagatat tgatgacgcg 2220

ccaatggtgc cgagcattcc gccgctgctg gcggcagata agcgctaa 2268

<210> SEQ ID NO 21
<211> LENGTH: 2304
<212> TYPE: DNA
<213> ORGANISM: Paenibacillus polymyxa

<400> SEQUENCE: 21

atgaagaaaa tcattgtagg aatatcggga gcgacagggt caatctttgg tatccgtata 60

-continued

ttgcaaaaat tacgggaggc tggagtccaa agccatctgg tgctatcccc gtgggctatt 120

gccaacattc cctatgagac aggctacacg gtgaaggatg tgaaggcaat ggcggatgca 180

gtctactcgt ataaggatca ggccgcacgt atttctagcg gctccttccg ggtagatggt 240

atggtcgtcg ctccttgcag tatgaagact cttgcctcta ttcgtatcgg tatggcggac 300

aacctgctta cccgatcagc ggatgtgata ctgaaggagc gaaagaagct gctgctcatg 360

accagagaaa caccattaag cagtatccat ctggaaaata tgctggagct gtcacgtatg 420

ggcgtgatga tcctgccgcc gatgcctgcc ttttataatc atcctgcaag tatcgaggaa 480

ttagtggatc atattgtttt tcgcgcattg gatcagttcg gtattgtcac aaccgcagcc 540

aaacgctggg atgggatgaa gcagaatgac tccaggctgc accagaattg agaaatcgaa 600

agacgaagga gaatgaatga tggcttataa agactttcgc gattttctac acaccttgga 660

aaaggaggga caattactca cgatcagcga tgaggtaaag ccggagccgg acctcgcagc 720

agctaacaga gcattaaaca atcttggaga taagacgcct gctctctttt tcaacaacat 780

ctatggatat acggatgctc gtattgcaat gaatgtgatg ggctcctggc ccaatcatgc 840

cctcatgatg ggaatgccca aaaatacgcc gctcaaggag cagttttttg aatttgccag 900

acgctatgaa caatttccgg tgcccgtgaa gcgggaagaa gccgctcctt ttcatgaagt 960

cgaaattacg gagaatatta atttgtttga tattttgccg ttgtttcgtt tgaatcaggg 1020

ggacggaggg ttttatttgg ataaagcaat tctaatttca cgcgatctgg atgacccgga 1080

cacctacggt aagcaaaatg tcggcttata ccggatgcag gtgaaaggca agaaccgttt 1140

gggcatccag cctgtaccac agcatgatat tgcgatccat atccgtcagg ctgaggagcg 1200

tggcgaaaat ctgaaggtgg ctattgccct cggatgtgag cctgtgatta caacggctgc 1260

ttctacgcca ctgctgtacg atcaatccga atatgagatg gcgggcgcca ttcagggcga 1320

gccttatcgt gtggtcaaag cgaaggatgc agatctggat ctgccttggg gagccgaggt 1380

cattttggaa ggcgaagtgt tagcaggtga acgtgagtat gaaggtccat tcggtgaatt 1440

cacaggtcac tattccggcg gtcgcgcgat gccagtcatt cagattaatc gtgtatatca 1500

ccgcaaacag cctatctttg agcatctgta catcgggatg ccttggacgg aaacggatta 1560

tatgatcggt gtgaatacaa gtgtaccgtt gtttcagcag cttaaggatg cttttcctaa 1620

tgaaatcgta gctgttaatg ccatgtatac gcatgggctg gtcgctatta tttccacgaa 1680

aacccggtat ggcggctttg cgaaggctgt gggaatgcgt gcgttaacga ctccgcatgg 1740

attggggtat tgcaagctgg tgattgtggt ggacgaggag gtcgatccgt tcaatctgcc 1800

gcaagtcatg tgggctttat ccaccaagct tcatccaaag catgatgctg tcattgttcc 1860

tggcttgtct attttaccgc ttgaccccgg ctctgatccg gcaggtatga cgcacaaaat 1920

gatactggat gcgacgacac ctgtagcacc ggatattaga ggccattact cgcagccgct 1980

cgattccccg ctgggtgtag cggaatggga gaaaaagttg agccaaatgc ttcgctaaat 2040

atttttaaaa acaaagaaaa tttaaaggag tgctgacaga tgcatatttg tccccgttgt 2100

gagtccaatc gttcagaagt cgtttcccat tcgccggtta aaggtgcctg ggaggttttg 2160

ttgtgccctg tatgcctgtt cacatggcga acctcagaac cggatagcat tactgatcca 2220

gcaaagtata aatcggcgtt caaggtaaac ccccaagata ttccggatgc tgctcatgtt 2280

cctcctattc cagagcggat atag 2304

<210> SEQ ID NO 22

-continued

<211> LENGTH: 2268
<212> TYPE: DNA
<213> ORGANISM: Citrobacter koseri

<400> SEQUENCE: 22

atgagactga ttgtggggat gaccggcgca acgggggcgc cgctaggcat tgcgctgcta 60

caggcgctgc ggcaaatgcc gacagtagaa acacacctgg taatgtctaa gtgggccaaa 120

acgaccattg agctggaaac gccttacagt gcgcgagatg ttgccggact ggctgattac 180

tgccataacc cggcggatca ggcggcgacg atctcttccg gctcatttcg caccgacggc 240

atgatcatta tgccttgcag tatgaaaacg ctggcgggga ttcgcgcagg atatgccgag 300

gggttagttg gccgtgccgc cgatgtggtg ctgaaagaag ggcgcaaact ggtgctggtg 360

ccgcgtgaaa tgccgctcag cacgatccat ctggaaaaca tgctcgccct ttcccgcatg 420

ggggtcgcga tggtgccgcc catgcctgct ttctacaacc atccgcaaac tattgatgat 480

attacgcagc atattgtggc gcgtgtgctg gatcagtttg gtctggagca tccgcgtgcc 540

cggcgctggc aggggttgca gcaggcgcag aatttttcac aggagaatga ataatggcat 600

ttgatgactt acgcagcttt ttgcaggcgc tcgacgagca ggggcaactg ctgaaaatca 660

gtgaagaagt gaatgcagag ccggatctgg ctgctgcggc taacgcaacc gggcgcattg 720

gcgacggcgc gcctgcgctg tggttcgata atatccgtgg cttcacggat gcgcgcgtgg 780

cgatgaacac cattggttcc tggcagaacc atgccatctc tttaggcttg ccgcctaatg 840

cgccagtaaa aaagcaaatt gatgaattta tccgccgctg ggacacgttc cccgtcgccc 900

ccgagcgccg agccaacccg gcgtgggcgg aaaacaccgt tgatggcgag gcgatcaacc 960

tgtttgatat tctgccgctg tttcgcctca acgatggcga tggcggcttc tatctggata 1020

aagcctgtgt cgtctcccgc gatccgctcg acccggatca cttcggcaag cagaatgtgg 1080

gtatctaccg gatggaagtg aaaggcaagc gcaagctggg cctgcaaccg gtgccaatgc 1140

acgatatcgc gctgcatctg cataaggcgg aagagcgtgg cgaagatctg ccgattgcta 1200

ttacgctcgg taacgatccg atcatcactc tgatgggcgc cacgccgctg aaatacgatc 1260

agtctgagta tgaaatggcg ggcgcgctgc gcgaaagccc atacccgatc gccaccgcgc 1320

cgctgaccgg ctttgatgtg ccgtggggtt cagaagtgat ccttgaaggg gtgatcgaaa 1380

gccgtaagcg tgaaattgaa gggccgtttg gcgagtttac cggccactat tctggtgggc 1440

gcaatatgac ggtggtgcgc atcgacaaag tgtcttatcg cactaaaccg atttttgaat 1500

cactctatct ggggatgccg tggactgaaa tcgactacct gatggggcca gcgacctgtg 1560

tgccgctgta tcagcagttg aaagcggaat tcccggaagt gcaggcggtt aacgccatgt 1620

atacccacgg tctgctggcg attatctcga ccaaaaaacg ctacggcgga tttgcccgcg 1680

cgatcggcct gcgggcaatg accacgccgc acggtctggg ctatgtgaag atggtgatta 1740

tggttgatga ggatgtcgat ccgttcaacc tgccgcaggt gatgtgggcg ctgtcgtcga 1800

aggtcaaccc ggcaggcgat ctggtgcagc tgccgaacat gtcggtgctg gaactggacc 1860

caggctcaag cccggcgggg atcactgaca aactgatcat cgacgccaca acgccggttg 1920

cgccggataa tcgcggccac tacagccagc cggtatgtga tttaccggaa accaaagcct 1980

gggctgaaaa gctgactgcc atgctggcca accgtaaata aggagtagca gatgatttgt 2040

ccacgttgtg ctgatgaaca tattgaattg atggcgacct ctccggtcaa agggatctgg 2100

acggtgtatc agtgccagca ttgtctgtac acctggcgtg ataccgagcc gctacgccgt 2160

accagccgtg aacattatcc gcaagcgttt cgcatgacgc agaaagatat tgatcaagcg 2220

-continued

ccgatggtgc cgggcattcc accgctgctg gcggaagata agcgttaa 2268

<210> SEQ ID NO 23
<211> LENGTH: 2318
<212> TYPE: DNA
<213> ORGANISM: Pantoea ananatis

<400> SEQUENCE: 23

atgagtagat tactgttaat ttcattcgta cacgaacgtt atttgcaagg aagtcagatg 60

agaattgtaa tcggtatgac gggagcaaca ggtgcccctt taggggtggc tctgctcagc 120

attttgcagg aaatcaaaga ggttgaaact catctgattt tgagcaagtg ggctaaaacc 180

acaattgaac tcgaaacgcc tttttcatcg cgtgaggtga tgagcatggc tgatgttgtg 240

tatggcccgt ccgaccaggc cgctactctc tcgtcaggtt cttttcacac cgatgggatg 300

gtcattattc cttgcagtat gaaaacctta gcgggaattc gcatgggata cgcggaaggc 360

cttattggac gggctgctga tgtcgtcatt aaagaaggca gaaaacttgt gctggtcccc 420

agagagacgc ctctcagcac cattcacctg gaaaatatgc tagccctttc ccgtcttggc 480

gtatccatgg ttccgcccat gcccgctttt tataaccacc ccgcagtaat tgatgatgtg 540

atcgatcatg tcgtttctcg tgttctcgac cagtttggga ttgcctcgcc aaaggcaaat 600

cgctggaaag gcctgaacaa ttctaagaaa tccctgagta tggagagtaa ataatggctt 660

ttgatgacct acgtagcttc cttaaggctc tggacgagca ggggcagctt cttgagattg 720

atgaagaggt tttacccgaa cctgatattg ccgcggccgc taatgctaca ggccgaattg 780

gtgaaggtgc accggcaatc tcattcaaaa aaataaaggg gttcaatcat gctcatgttg 840

tgatgaacac tattggttcc tggcaaaacc atgcaatttc actgggcctc ccaatgaata 900

ccccagtgaa acagcagata gatgaattca ttcgtcgctg ggacactttt cctgtggcac 960

cagagcggcg cgacaatgcg ccctggtcag aaaataccgt tgattgtgaa gagatcaatc 1020

tcttcgacat ccttcccctg ttccgcctga acgacggcga cggcggtttc tatcttgata 1080

aggcctgcgt agtatcacgt gacccgcttg atccagaaca tttcggtaag caaaacgtcg 1140

gcatttaccg gatggaggtg aaaggtaaac gtaaactcgg gctccagccc gtgccgatgc 1200

atgacattgc acttcatctc cataaggccg aagaacgcgg cgacgatctg ccagtggcta 1260

ttacgctggg caatgacccc attattacat tgatgggcgc cacgccgctg aaatacgacc 1320

agtcagaata tgagatggca ggtgcgctgc gtgaaagccc gtaccccatc gcctccgcgc 1380

ctctgaccgg ctttgatgtg ccgtggggat cggaagtcat tcttgaaggc gtgatagaag 1440

ggcgcaaacg tgagattgaa ggaccgtttg gcgaattcac cggccattat tccggcggtc 1500

gcaatatgac cgttgtgcgg attgataagg tctcctaccg cactaagcca atattcgagt 1560

cattgtatct gggaatgccc tggaccgaaa ttgattatct gatgggcccg gcaacctgtg 1620

tccctttgta tcaacagctg aaagcggatt tccctgaggt gcaggctgta aatgcaatgt 1680

atacacacgg attactggcc attatttcta caaagaaacg ttatggtgga tttgcccgtg 1740

ctgtaggcgt acgggcgatg acaaccccgc atggtctggg ctacgtcaag atggtgatca 1800

tggtcgatga ggatgtcgat ccctttaacc tgcctcaggt gatgtgggcg ctgtcttcaa 1860

aggtcaatcc gcaaggcgat ctcgttcaac tgccaaacat gtccgtactg gaactggacc 1920

cgggttccag ccctgcggga atcacggata aacttgtgat cgatgcgacg actcccgtgg 1980

caccggatac ccgcggccac tacagtcagc cggtaaaaga cctgccagaa acttcaatct 2040

-continued

gggttgagaa gttaacgtcc ctgttatcaa atcgcggtta aggagaaagt atgatttgtc 2100

cacgttgtgc tgatgaacac attgaaatca tggcaacatc cccagttgag gggatatgga 2160

cggtgcatca gtgtcagcat tgcctgtaca catggcgcaa tacagagcca gcccgaagaa 2220

cggagcggga acattatcct gaagccttcc ggatgactca acgtgatatt gataatgcgc 2280

cggaagtccc gtctgtccct cctctgttag ctaagtaa 2318

<210> SEQ ID NO 24
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 24

ctctcatatg acagcatcac cttggg 26

<210> SEQ ID NO 25
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 25

ctctcatatg tcatcttaac gacgctccat tc 32

<210> SEQ ID NO 26
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 26

ggaattccat atgaagcgaa ttgttgtcgg aattac 36

<210> SEQ ID NO 27
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 27

ggaattccat atgctacttc ggctgctcat catc 34

<210> SEQ ID NO 28
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 28

ggaattccat atggcagaac aaccatggga tttac 35

<210> SEQ ID NO 29
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 29

ggaattccat atggacggca taactaatcg catc 34

<210> SEQ ID NO 30
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 30

ctctcatatg aaacgaattg ttgtgggaat c 31

<210> SEQ ID NO 31
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 31

ctctcatatg ctaccgcggt tgctcg 26

<210> SEQ ID NO 32
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 32

ctctcatatg gcagaacaac catggg 26

<210> SEQ ID NO 33
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 33

ctctcatatg ttacttcaaa tacttctccc agtc 34

<210> SEQ ID NO 34
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 34

agttgagaca tatgggtgaa gacaaatggg atttgc 36

<210> SEQ ID NO 35
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 35

ttattttaca tatgtcatct gccatcccaa cgtg 34

<210> SEQ ID NO 36
<211> LENGTH: 40
<212> TYPE: DNA

-continued

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 36

aggtcatcat atgtcagatt attatgattt gagacgggtc 40

<210> SEQ ID NO 37
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 37

acgtggcgca tatggtcatc attaccctcc cgtc 34

<210> SEQ ID NO 38
<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 38

gaggcccggg atgacagaac aaccatatga tttaagaaaa gtac 44

<210> SEQ ID NO 39
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 39

actccccggg ctaaacgcgg ttgttcacac 30

<210> SEQ ID NO 40
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 40

aggcagtact atgagtgaac aaccttatga tttacgtaaa g 41

<210> SEQ ID NO 41
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 41

atgaagtact ttatttagca taggtaacca cttggg 36

<210> SEQ ID NO 42
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 42

ctctcatatg aaaatagctg taggaatcac ag 32

<210> SEQ ID NO 43
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 43

ctctcatatg ttaagatcgg ggtggcaca 29

<210> SEQ ID NO 44
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 44

ctctcatatg aacatcatcg tcggaatc 28

<210> SEQ ID NO 45
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 45

ctctcatatg ttagattttc cggtctggaa tcg 33

<210> SEQ ID NO 46
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 46

ctctcatatg aaactcgttg tcgggatg 28

<210> SEQ ID NO 47
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 47

ctctcatatg tcaggccttt ctttcc 26

<210> SEQ ID NO 48
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 48

agtatgattc atatgaaagc agaattcaag cgtaaag 37

<210> SEQ ID NO 49
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 49

acatacagtt catatggatc aagcctttcg 30

<210> SEQ ID NO 50
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 50

ctctcatatg aaagcagaat tcaagcgtaa ag 32

<210> SEQ ID NO 51
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 51

ctctcatatg tcaagccttt cgttccgg 28

<210> SEQ ID NO 52
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 52

ctctcatatg aaactgatta ttgggatgac cg 32

<210> SEQ ID NO 53
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 53

ctctcatatg ttaacgctta tctgccgcc 29

<210> SEQ ID NO 54
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 54

ctctcatatg agattgatcg tgggaatgac 30

<210> SEQ ID NO 55
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 55

ctctcatatg ttacagcaat ggcggaatgg 30

-continued

<210> SEQ ID NO 56
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 56

ctctcatatg aggctaattg tcggaatgac 30

<210> SEQ ID NO 57
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 57

ctctcatatg ttaacgctta ccatccgcc 29

<210> SEQ ID NO 58
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 58

ctctcatatg agattgattg tgggaatgac 30

<210> SEQ ID NO 59
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 59

ctctcatatg gagtctggtt tagttctctg c 31

<210> SEQ ID NO 60
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 60

ctctcatatg aaactgatcg tcgggatg 28

<210> SEQ ID NO 61
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 61

ctctcatatg ttagcgctta ccttccgc 28

<210> SEQ ID NO 62
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

-continued

<400> SEQUENCE: 62

ctctcatatg agactgatcg tcgggat 27

<210> SEQ ID NO 63
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 63

ctctcatatg ttagcgctta tctgccgc 28

<210> SEQ ID NO 64
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 64

ctctcatatg aagaaaatca ttgtaggaat atcgg 35

<210> SEQ ID NO 65
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 65

ctctcatatg ctatatccgc tctggaatag g 31

<210> SEQ ID NO 66
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 66

ctctcatatg agactgattg tggggatg 28

<210> SEQ ID NO 67
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 67

ctctcatatg ttaacgctta tcttccgcca g 31

<210> SEQ ID NO 68
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 68

ctctcatatg agtagattac tgttaatttc attcgtac 38

<210> SEQ ID NO 69
<211> LENGTH: 32

-continued

<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 69

ctctcatatg ttacttagct aacagaggag gg 32

<210> SEQ ID NO 70
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 70

ctctctgcag catcgttgct gaatgtccag 30

<210> SEQ ID NO 71
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 71

gatcaacgat ctgttcagct g 21

<210> SEQ ID NO 72
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 72

agctgaacag atcgttgatc agaactgatc ctgcaccctg 40

<210> SEQ ID NO 73
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Amplification Primer

<400> SEQUENCE: 73

ctctgagctc gttgatgtca atgcgcagag 30

The invention claimed is:

1. A transformant of a *Corynebacterium* obtained by introducing,

into the *Corynebacterium*, at least one gene selected from the group consisting of:

(1) a decarboxylase gene ubiD of *Lactobacillus rhamnosus*; and

(2) at least one ortholog of the decarboxylase gene ubiD of *Lactobacillus rhamnosus*,

wherein one or more substitutions, deletions and/or insertions are introduced into a catechol 1,2-dioxygenase gene catA, and a protocatechuic acid dehydrogenase gene pcaHG in the *Corynebacterium*, thereby degrading or losing functions of enzymes encoded by the catechol 1,2-dioxygenase gene catA and the protocatechuic acid dehydrogenase gene pcaHG,

wherein the *Corynebacterium* is selected from the group consisting of: *Corynebacterium glutamicum, Corynebacterium efficiens, Corynebacterium ammoniagenes*, and *Corynebacterium halotolerance,*

wherein the ortholog of the decarboxylase gene ubiD of *Lactobacillus rhamnosus* is selected from the group consisting of: an ubiD gene of *Lactobacillus pentosus*, an ubiD gene of *Lactobacillus plantarum*, an ubiD gene of *Lactobacillus pobuzihii*, an ubiD gene of *Lactobacillus composti*, an ubiD gene of *Bacillus megaterium*, an ubiD gene of *Bacillus licheniformis*, an ubiD gene of *Bacillus atrophaeus*, an ubiD gene of *Bacillus subtilis* subsp. *subtilis*, an ubiD gene of *Bacillus subtilis* subsp. *Spizizenii*, an ubiD gene of *Enterobacter aerogenes*, an ubiD gene of *Enterobacter cloacae*, an ubiD gene of *Enterobacter sakazakii*, an ubiD gene of *Enterobacter hormaechei*, an ubiD gene of *Escherichia coli* W, the ubiD gene of *Escherichia fergusonii*, an ubiD gene of *Paenibacillus polymyxa*, the ubiD gene of *Citrobacter koseri*, and an ubiD gene of *Pantoea ananatis*, and

wherein the transformant has a catechol producing ability.

2. The transformant of claim **1**,

wherein the *Corynebacterium* is *Corynebacterium glutamicum* R (FERM P-18976), ATCC13032, or ATCC13869.

3. A transformant of *Corynebacterium glutamicum* CAT21 deposited under Accession Number: NITE BP-02689.

4. A method for producing catechol comprising:

reacting the transformant of claim **1** in a reaction solution under reducing conditions; and

collecting catechol in a reaction solution.

5. The method of claim **4**,

wherein the reaction solution comprises at least one saccharide selected from the group consisting of glucose, fructose, cellobiose, xylobiose, sucrose, lactose, maltose, dextrin, xylose, arabinose, galactose, mannose, and soluble starch.

6. The transformant of claim **1**, wherein the *Corynebacterium* is *Corynebacterium glutamicum*.

* * * * *