

[DESCRIPTION]

[Title of Invention] LACTIC ACID BACTERIUM DETECTION PRIMER SET, AND DETECTION METHOD USING SAID PRIMER SET

[Technical Field]

5 [0001]

The present invention relates to a method for detecting a specific lactic acid bacterium. Specifically, the present invention relates to a primer set for detecting an *Enterococcus faecalis* EF-2001 strain and a detection method using the primer set.

[Background Art]

[0002]

A lactic acid bacterium is known to have various actions. In recent years, a lactic acid bacterium is utilized as a useful microorganism resource deeply involved in humans' health because a lactic acid bacterium has various health retaining effects such as the effect of regulating the intestinal function by improving a bacterial flora in the gastrointestinal tract as probiotics, the immunity improving effect, or the anti-tumor effect.

[0003]

Meanwhile, *Enterococcus faecalis*, which is one type of lactic acid bacterium, also includes a bacterial cell that is known to cause an opportunistic infection. Such a bacterial cell may cause, as an opportunistic pathogen, various diseases including a urinary tract infectious disease and a life-threatening infectious disease such as bacteremia, endocarditis, or meningitis. Thus, it is extremely important to distinguish a bacterial cell which can be utilized as probiotics from a pathogenic bacterial cell.

[Summary of Invention]

[Solution to Problem]

[0004]

35 It is reported that an EF-2001 strain, which is one

Enterococcus faecalis strain, has various biological modulating activities including anti-inflammation and immunomodulation. Said strain is widely utilized as probiotics of supplements or functional food. The present invention provides a primer set which can rapidly and simply perform specific identification of a lactic acid bacterium Enterococcus faecalis EF-2001 strain, and a detection method using the primer set.

[0005]

Thus, the present disclosure provides the following items.

(Item 1)

A primer set for specifically detecting a nucleic acid derived from a lactic acid bacterium Enterococcus faecalis EF-2001 strain, the primer set comprising:

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 1 and an oligonucleotide comprising the base sequence of SEQ ID NO: 2 or 3;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 4 and an oligonucleotide comprising the base sequence of SEQ ID NO: 5 or 6;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 7 and an oligonucleotide comprising the base sequence of SEQ ID NO: 8 or 9;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 10 and an oligonucleotide comprising the base sequence of SEQ ID NO: 11 or 12;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 13 and an oligonucleotide comprising the base sequence of SEQ ID NO: 14 or 15;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 16 and an oligonucleotide comprising the base sequence of SEQ ID NO: 17 or 18;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 19 and an oligonucleotide comprising the base

sequence of SEQ ID NO: 20 or 21;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 22 and an oligonucleotide comprising the base sequence of SEQ ID NO: 23 or 24;

5 a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 25 and an oligonucleotide comprising the base sequence of SEQ ID NO: 26 or 27; or

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 28 and an oligonucleotide comprising the base
10 sequence of SEQ ID NO: 29 or 30.

(Item 2)

A primer set for specifically detecting a nucleic acid derived from a lactic acid bacterium *Enterococcus faecalis* EF-2001 strain, the primer set selected from the group
15 consisting of

(a) a primer set (1) for amplifying a part of the base sequence of SEQ ID NO: 51:

a forward primer CGAAAAGGATGTAGTCAGCGG (SEQ ID NO: 1); and

20 a reverse primer CCGAAGGCGAAACAGAGGAT (SEQ ID NO: 2),

(b) a primer set (2) for amplifying a part of the base sequence of SEQ ID NO: 51:

a forward primer CGAAAAGGATGTAGTCAGCGG (SEQ ID NO: 1); and

25 a reverse primer CCCAGACATAATCGCATGGC (SEQ ID NO: 3),

(c) a primer set (3) for amplifying a part of the base sequence of SEQ ID NO: 52:

a forward primer GCGGCTGCACAATTTATTGC (SEQ ID NO: 4); and

30 a reverse primer AGAATACTTGGGCGGTCGTG (SEQ ID NO: 5),

(d) a primer set (4) for amplifying a part of the base sequence of SEQ ID NO: 52:

a forward primer GCGGCTGCACAATTTATTGC (SEQ ID NO: 4); and

35 a reverse primer AATTCAGCTTCGCTAGATAAGGC (SEQ ID NO:

6),

(e) a primer set (5) for amplifying a part of the base sequence of SEQ ID NO: 53:

5 a forward primer CGCGTATGACTTGCAATCGA (SEQ ID NO: 7); and

a reverse primer AGGATTGTTTGACGGTGCAA (SEQ ID NO: 8),

(f) a primer set (6) for amplifying a part of the base sequence of SEQ ID NO: 53:

10 a forward primer CGCGTATGACTTGCAATCGA (SEQ ID NO: 7); and

a reverse primer ACATGAGATAGTTGGGGTAGACA (SEQ ID NO: 9),

(g) a primer set (7) for amplifying a part of the base sequence of SEQ ID NO: 54:

15 a forward primer CTTAGAGAGCTGGGCGAAG (SEQ ID NO: 10); and

a reverse primer TACTTTTCTAGCTGCCCCGCC (SEQ ID NO: 11),

20 (h) a primer set (8) for amplifying a part of the base sequence of SEQ ID NO: 54:

a forward primer CTTAGAGAGCTGGGCGAAG (SEQ ID NO: 10); and

a reverse primer GGGTTGTAGCCCTACCCGAT (SEQ ID NO: 12),

25 (i) a primer set (9) for amplifying a part of the base sequence of SEQ ID NO: 55:

a forward primer CGTAACGTGACATTGCGGAC (SEQ ID NO: 13); and

30 a reverse primer ATGCCAGTACGTCGCGTTAA (SEQ ID NO: 14),

(j) a primer set (10) for amplifying a part of the base sequence of SEQ ID NO: 55:

a forward primer CGTAACGTGACATTGCGGAC (SEQ ID NO: 13); and

35 a reverse primer CGATTGTCAACTAATTGTGCCGA (SEQ ID NO:

15),

(k) a primer set (11) for amplifying a part of the base sequence of SEQ ID NO: 56:

5 a forward primer CATGGCTTGCCGTTTCACAA (SEQ ID NO: 16); and

a reverse primer ACCGCAACAACCTACATACTACCA (SEQ ID NO: 17),

(l) a primer set (12) for amplifying a part of the base sequence of SEQ ID NO: 56:

10 a forward primer CATGGCTTGCCGTTTCACAA (SEQ ID NO: 16); and

a reverse primer ACCAAAAGGAACGCTACCAGT (SEQ ID NO: 18),

(m) a primer set (13) for amplifying a part of the base sequence of SEQ ID NO: 57:

a forward primer TCAGCATAATCCCCAGACGT (SEQ ID NO: 19); and

a reverse primer AATGAACGCCCTTCAGCAGA (SEQ ID NO: 20),

20 (n) a primer set (14) for amplifying a part of the base sequence of SEQ ID NO: 57:

a forward primer TCAGCATAATCCCCAGACGT (SEQ ID NO: 19); and

25 a reverse primer GGCTCCTCTACCTGAACAAACT (SEQ ID NO: 21),

(o) a primer set (15) for amplifying a part of the base sequence of SEQ ID NO: 58:

a forward primer GCGTTCAAACCTGTTCTGGTGT (SEQ ID NO: 22); and

30 a reverse primer TACAAGGCTTGCGAGGTAGC (SEQ ID NO: 23),

(p) a primer set (16) for amplifying a part of the base sequence of SEQ ID NO: 58:

35 a forward primer GCGTTCAAACCTGTTCTGGTGT (SEQ ID NO: 22); and

a reverse primer GCTGCAATGGAAAGCAAATCG (SEQ ID NO: 24),

(q) a primer set (17) for amplifying a part of the base sequence of SEQ ID NO: 59:

5 a forward primer AGGCATATGGGTCATCTGCT (SEQ ID NO: 25); and

a reverse primer GAGCATCACAGAGCCTCGAA (SEQ ID NO: 26),

10 (r) a primer set (18) for amplifying a part of the base sequence of SEQ ID NO: 59:

a forward primer AGGCATATGGGTCATCTGCT (SEQ ID NO: 25); and

a reverse primer AGAGATTTTTCAGTATTGCTGGGT (SEQ ID NO: 27),

15 (s) a primer set (19) for amplifying a part of the base sequence of SEQ ID NO: 60:

a forward primer CGTTGGGTGTGCAGAAATGG (SEQ ID NO: 28); and

20 a reverse primer TGTACCGTCAACCTCGTTCG (SEQ ID NO: 29), or

(t) a primer set (20) for amplifying a part of the base sequence of SEQ ID NO: 60:

a forward primer CGTTGGGTGTGCAGAAATGG (SEQ ID NO: 28); and

25 a reverse primer AACGGGTTGCGACTCTTTTT (SEQ ID NO: 30).

(Item 3)

A kit for detecting a lactic acid bacterium *Enterococcus faecalis* EF-2001 strain, characterized by comprising the primer set of item 1 or 2.

(Item 4)

A method for specifically detecting a nucleic acid derived from a lactic acid bacterium *Enterococcus faecalis* EF-2001 strain, the method comprising:

35 the step of amplifying a nucleic acid fragment using the

primer set of item 1 or 2; and

the step of detecting a nucleic acid fragment obtained in the amplification step.

(Item 5)

5 A lactic acid bacterium composition comprising a nucleic acid fragment,

wherein the nucleic acid fragment is not amplified by one or more primer sets selected from the group consisting of:

10 a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 1 and an oligonucleotide comprising the base sequence of SEQ ID NO: 2;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 4 and an oligonucleotide comprising the base
15 sequence of SEQ ID NO: 5;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 7 and an oligonucleotide comprising the base sequence of SEQ ID NO: 8;

a pair of an oligonucleotide comprising the base sequence
20 of SEQ ID NO: 10 and an oligonucleotide comprising the base sequence of SEQ ID NO: 11;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 13 and an oligonucleotide comprising the base sequence of SEQ ID NO: 14;

25 a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 16 and an oligonucleotide comprising the base sequence of SEQ ID NO: 17;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 19 and an oligonucleotide comprising the base
30 sequence of SEQ ID NO: 20;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 22 and an oligonucleotide comprising the base sequence of SEQ ID NO: 23;

a pair of an oligonucleotide comprising the base sequence
35 of SEQ ID NO: 25 and an oligonucleotide comprising the base

sequence of SEQ ID NO: 26; and/or

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 28 and an oligonucleotide comprising the base sequence of SEQ ID NO: 29, and

5 wherein the nucleic acid fragment is amplified by one or more primer sets selected from the group consisting of:

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 1 and an oligonucleotide comprising the base sequence of SEQ ID NO: 3;

10 a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 4 and an oligonucleotide comprising the base sequence of SEQ ID NO: 6;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 7 and an oligonucleotide comprising the base
15 sequence of SEQ ID NO: 9;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 10 and an oligonucleotide comprising the base sequence of SEQ ID NO: 12;

20 a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 13 and an oligonucleotide comprising the base sequence of SEQ ID NO: 15;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 16 and an oligonucleotide comprising the base sequence of SEQ ID NO: 18;

25 a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 19 and an oligonucleotide comprising the base sequence of SEQ ID NO: 21;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 22 and an oligonucleotide comprising the base
30 sequence of SEQ ID NO: 24;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 25 and an oligonucleotide comprising the base sequence of SEQ ID NO: 27; and/or

35 a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 28 and an oligonucleotide comprising the base

sequence of SEQ ID NO: 30.

(Item 6)

The composition of item 5, comprising a nucleic acid fragment which is not amplified by a primer set of an oligonucleotide comprising the base sequence of SEQ ID NO: 16 and an oligonucleotide comprising the base sequence of SEQ ID NO: 17 and is amplified by a primer set of an oligonucleotide comprising the base sequence of SEQ ID NO: 16 and an oligonucleotide comprising the base sequence of SEQ ID NO: 18.

(Item 7)

The composition of item 5 or 6, comprising a heat-treated lactic acid bacterium product resulting from subjecting a lactic acid bacterium *Enterococcus faecalis* EF-2001 strain to heat treatment at at least about 70°C.

(Item 8)

The composition of any one of items 5 to 7, comprising a heat-treated lactic acid bacterium product resulting from subjecting a lactic acid bacterium *Enterococcus faecalis* EF-2001 strain to heat treatment at at least about 90°C.

(Item 9)

A method for manufacturing a lactic acid bacterium composition, the method comprising:

the step of heating a lactic acid bacterium *Enterococcus faecalis* EF-2001 strain to obtain a plurality of lots of a heat-treated lactic acid bacterium product;

the step of extracting a nucleic acid fragment in one of the lots of the heat-treated lactic acid bacterium product;

the step of amplifying the nucleic acid fragment using at least one of the primer sets of item 1 or 2;

the step of detecting a nucleic acid fragment obtained in the amplification step; and

the step of selecting a lot of the heat-treated lactic acid bacterium product in which the nucleic acid fragment has been detected.

(Item 10)

The method of item 9, wherein the heating is heating at at least about 70°C.

(Item 11)

5 The method of item 9 or 10, wherein the heating is heating at at least about 90°C.

(Item 12)

10 The method of any one of items 9 to 11, wherein the nucleic acid fragment is amplified by one or more primer sets selected from the group consisting of:

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 1 and an oligonucleotide comprising the base sequence of SEQ ID NO: 3;

15 a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 4 and an oligonucleotide comprising the base sequence of SEQ ID NO: 6;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 7 and an oligonucleotide comprising the base sequence of SEQ ID NO: 9;

20 a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 10 and an oligonucleotide comprising the base sequence of SEQ ID NO: 12;

25 a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 13 and an oligonucleotide comprising the base sequence of SEQ ID NO: 15;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 16 and an oligonucleotide comprising the base sequence of SEQ ID NO: 18;

30 a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 19 and an oligonucleotide comprising the base sequence of SEQ ID NO: 21;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 22 and an oligonucleotide comprising the base sequence of SEQ ID NO: 24;

35 a pair of an oligonucleotide comprising the base sequence

of SEQ ID NO: 25 and an oligonucleotide comprising the base sequence of SEQ ID NO: 27; and/or

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 28 and an oligonucleotide comprising the base sequence of SEQ ID NO: 30, and

wherein the nucleic acid fragment is not amplified by one or more primer sets selected from the group consisting of:

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 1 and an oligonucleotide comprising the base sequence of SEQ ID NO: 2;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 4 and an oligonucleotide comprising the base sequence of SEQ ID NO: 5;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 7 and an oligonucleotide comprising the base sequence of SEQ ID NO: 8;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 10 and an oligonucleotide comprising the base sequence of SEQ ID NO: 11;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 13 and an oligonucleotide comprising the base sequence of SEQ ID NO: 14;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 16 and an oligonucleotide comprising the base sequence of SEQ ID NO: 17;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 19 and an oligonucleotide comprising the base sequence of SEQ ID NO: 20;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 22 and an oligonucleotide comprising the base sequence of SEQ ID NO: 23;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 25 and an oligonucleotide comprising the base sequence of SEQ ID NO: 26; and/or

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 28 and an oligonucleotide comprising the base sequence of SEQ ID NO: 29.

(Item 13)

5 A polynucleotide characterized by being amplified by a primer set for detecting a lactic acid bacterium *Enterococcus faecalis* EF-2001 strain, wherein the polynucleotide is:

the polynucleotide set forth in SEQ ID NO: 31 which is amplified by a primer set consisting of a pair of an
10 oligonucleotide comprising the base sequence of SEQ ID NO: 1 and an oligonucleotide comprising the base sequence of SEQ ID NO: 2;

the polynucleotide set forth in SEQ ID NO: 32 which is amplified by a primer set consisting of a pair of an
15 oligonucleotide comprising the base sequence of SEQ ID NO: 1 and an oligonucleotide comprising the base sequence of SEQ ID NO: 3;

the polynucleotide set forth in SEQ ID NO: 33 which is amplified by a primer set consisting of a pair of an
20 oligonucleotide comprising the base sequence of SEQ ID NO: 4 and an oligonucleotide comprising the base sequence of SEQ ID NO: 5;

the polynucleotide set forth in SEQ ID NO: 34 which is amplified by a primer set consisting of a pair of an
25 oligonucleotide comprising the base sequence of SEQ ID NO: 4 and an oligonucleotide comprising the base sequence of SEQ ID NO: 6;

the polynucleotide set forth in SEQ ID NO: 35 which is amplified by a primer set consisting of a pair of an
30 oligonucleotide comprising the base sequence of SEQ ID NO: 7 and an oligonucleotide comprising the base sequence of SEQ ID NO: 8;

the polynucleotide set forth in SEQ ID NO: 36 which is amplified by a primer set consisting of a pair of an
35 oligonucleotide comprising the base sequence of SEQ ID NO:

7 and an oligonucleotide comprising the base sequence of SEQ ID NO: 9;

the polynucleotide set forth in SEQ ID NO: 37 which is amplified by a primer set consisting of a pair of an
5 oligonucleotide comprising the base sequence of SEQ ID NO: 10 and an oligonucleotide comprising the base sequence of SEQ ID NO: 11;

the polynucleotide set forth in SEQ ID NO: 38 which is amplified by a primer set consisting of a pair of an
10 oligonucleotide comprising the base sequence of SEQ ID NO: 10 and an oligonucleotide comprising the base sequence of SEQ ID NO: 12;

the polynucleotide set forth in SEQ ID NO: 39 which is amplified by a primer set consisting of a pair of an
15 oligonucleotide comprising the base sequence of SEQ ID NO: 13 and an oligonucleotide comprising the base sequence of SEQ ID NO: 14;

the polynucleotide set forth in SEQ ID NO: 40 which is amplified by a primer set consisting of a pair of an
20 oligonucleotide comprising the base sequence of SEQ ID NO: 13 and an oligonucleotide comprising the base sequence of SEQ ID NO: 15;

the polynucleotide set forth in SEQ ID NO: 41 which is amplified by a primer set consisting of a pair of an
25 oligonucleotide comprising the base sequence of SEQ ID NO: 16 and an oligonucleotide comprising the base sequence of SEQ ID NO: 17;

the polynucleotide set forth in SEQ ID NO: 42 which is amplified by a primer set consisting of a pair of an
30 oligonucleotide comprising the base sequence of SEQ ID NO: 16 and an oligonucleotide comprising the base sequence of SEQ ID NO: 18;

the polynucleotide set forth in SEQ ID NO: 43 which is amplified by a primer set consisting of a pair of an
35 oligonucleotide comprising the base sequence of SEQ ID NO:

19 and an oligonucleotide comprising the base sequence of
SEQ ID NO: 20;

the polynucleotide set forth in SEQ ID NO: 44 which is
amplified by a primer set consisting of a pair of an
5 oligonucleotide comprising the base sequence of SEQ ID NO:
19 and an oligonucleotide comprising the base sequence of
SEQ ID NO: 21;

the polynucleotide set forth in SEQ ID NO: 45 which is
amplified by a primer set consisting of a pair of an
10 oligonucleotide comprising the base sequence of SEQ ID NO:
22 and an oligonucleotide comprising the base sequence of
SEQ ID NO: 23;

the polynucleotide set forth in SEQ ID NO: 46 which is
amplified by a primer set consisting of a pair of an
15 oligonucleotide comprising the base sequence of SEQ ID NO:
22 and an oligonucleotide comprising the base sequence of
SEQ ID NO: 24;

the polynucleotide set forth in SEQ ID NO: 47 which is
amplified by a primer set consisting of a pair of an
20 oligonucleotide comprising the base sequence of SEQ ID NO:
25 and an oligonucleotide comprising the base sequence of
SEQ ID NO: 26;

the polynucleotide set forth in SEQ ID NO: 48 which is
amplified by a primer set consisting of a pair of an
25 oligonucleotide comprising the base sequence of SEQ ID NO:
25 and an oligonucleotide comprising the base sequence of
SEQ ID NO: 27;

the polynucleotide set forth in SEQ ID NO: 49 which is
amplified by a primer set consisting of a pair of an
30 oligonucleotide comprising the base sequence of SEQ ID NO:
28 and an oligonucleotide comprising the base sequence of
SEQ ID NO: 29; or

the polynucleotide set forth in SEQ ID NO: 50 which is
amplified by a primer set consisting of a pair of an
35 oligonucleotide comprising the base sequence of SEQ ID NO:

28 and an oligonucleotide comprising the base sequence of
SEQ ID NO: 30.

[0006]

5 The present disclosure is intended so that one or more
of the aforementioned features can be provided not only as
the explicitly shown combinations, but also as other
combinations thereof. Additional embodiments and advantages
of the present disclosure are recognized by those skilled in
the art by reading and understanding the following detailed
10 description, as needed.

[0007]

Features other than those described above and
significant action/effect of the present disclosure become
clear to those skilled in the art by referring to the
15 following section of the embodiments and the drawings.

[Advantageous Effects of Invention]

[0008]

The present invention can simply and rapidly detect a
lactic acid bacterium *Enterococcus faecalis* EF-2001 strain
20 without recognizing a bacterial strain closely related to a
lactic acid bacterium *Enterococcus faecalis* EF-2001 strain.
This is very useful.

[Brief Description of Drawings]

[0009]

25 [Figure 1] Figure **1** shows the results of electrophoresis
showing the results of detecting a nucleic acid using the
primer sets of one embodiment of the present invention and
using the DNA of a lactic acid bacterium EF-2001 strain and
various *Enterococcus faecalis* subspecies as a template. (A)
30 A lactic acid bacterium EF-2001 strain, and (B) *E. faecalis*
NBRC3971, (C) *E. faecalis* NBRC3989, (D) *E. faecalis* NBRC12970,
(E) *E. faecalis* NBRC100480, (F) *E. faecalis* NBRC100482, (G)
E. faecalis NBRC100483 and (H) *E. faecalis* NBRC100484, which
are *Enterococcus faecalis* subspecies.

35 [Figure 2] Figure **2** shows the results of electrophoresis

showing the results of detecting a nucleic acid using the primer sets of one embodiment of the present invention and using the DNA of a lactic acid bacterium EF-2001 strain and various other lactic acid bacteria as a template. (B) E. faecium Aus0004, (C) E. gallinarum LMG13129, (D) Lactobacillus rhamnosus NBRC3425, (E) L. plantarum NBRC15891, (F) L. paracasei NBRC15906, and (G) L. lactis NBRC102622.

[Figure 3] Figure 3 shows the results of electrophoresis showing the results of detecting a live bacterium and a dead bacterium using the primer sets (long and short) of one embodiment of the present invention and using the DNA of manufacturing step liquid of a lactic acid bacterium EF-2001 strain as a template. (A) Culture liquid (live bacterium), (B) liquid after heat treatment (dead bacterium), and (C) product bulk (dead bacterium).

[Figure 4] Figure 4 shows the results of electrophoresis confirming the disappearance of a band of the primer set of the present invention due to heat treatment by using a lactic acid bacterium EF-2001 strain.

[Figure 5] Figure 5 shows a graph confirming the change in the TNF- α production amount of a lactic acid bacterium EF-2001 strain due to heat treatment.

[Description of Embodiments]

[0010]

The present disclosure is described hereinafter while showing the best mode thereof. Throughout the entire specification, a singular expression should be understood as encompassing the concept thereof in the plural form, unless specifically noted otherwise. Thus, singular articles (e.g., "a", "an", "the", and the like in the case of English) should also be understood as encompassing the concept thereof in the plural form, unless specifically noted otherwise. Further, the terms used herein should be understood as being used in the meaning that is commonly used in the art, unless specifically noted otherwise. Therefore, unless defined

otherwise, all terminologies and scientific technical terms that are used herein have the same meaning as the general understanding of those skilled in the art to which the present disclosure pertains. In case of a contradiction, the
5 present specification (including the definitions) takes precedence.

[0011]

The definitions of the terms and/or basic technical concepts that are particularly used herein are described
10 hereinafter when appropriate.

[0012]

As used herein, "about" means $\pm 10\%$ of a numerical value that follows.

[0013]

15 (Preferred embodiments)

Preferred embodiments of the present disclosure are described below. Embodiments provided below are provided to facilitate the understanding of the present disclosure. The scope of the present disclosure should not be limited to the
20 following descriptions. Thus, it is apparent that those skilled in the art can make appropriate modifications within the scope of the present disclosure by referring to the descriptions herein. The following embodiments of the present disclosure can be used alone or in combination.

25 [0014]

The primer set of the present invention can amplify a chromosomal DNA region having a base sequence specific to a lactic acid bacterium *Enterococcus faecalis* EF-2001 strain by PCR (polymerase chain reaction), and can specifically
30 recognize and detect a lactic acid bacterium *Enterococcus faecalis* EF-2001 strain without recognizing a bacterial strain closely related to a lactic acid bacterium *Enterococcus faecalis* EF-2001 strain.

[0015]

35 The primer set of the present invention was designed by

the following method.

First, the sequence of a full length genome of a lactic acid bacterium EF-2001 strain was determined by using a hybrid assembly approach using Short Read from ThermoFisher IonPGM and Long Read from OxfordNanopore MinION. When the Draft Genome sequence obtained from this Short Read was determined, gene fragments of 1 kbp or greater which did not match when compared with 42 strains of Table 1 below were narrowed as a gene sequence specific to an EF-2001 strain.

[Table 1]

Enterococcus faecalis Complete Genome sequence information
of 42 strains used for comparison
GenBank (dated September 1, 2020)

<i>E. faecalis</i> strain	Host/Source	Accession	Collection date
EF-2001	Human		1995
R712	Human	GCA_009662495.1	missing
C25	Pig	GCA_005979285.1	2017
OG1RF	missing	GCA_000172575.2	missing
62	missing	GCA_000211255.2	missing
DENG1	Human	GCA_000550745.1	missing
ATCC29212	missing	GCA_000742975.1	missing
111540027-2	Environment (ISS)	GCA_013344645.1	2011
111540047-1	Environment (ISS)	GCA_013344625.1	2011
NCTC8745	missing	GCA_900636775.1	missing
FDAARGOS338	Human	GCA_002208945.2	2015
NCTC8732	missing	GCA_901553725.1	missing
FDAARGOS324	Human	GCA_003030425.1	2015
W11	Food	GCA_002355755.1	missing
ARO1/DG	missing	GCA_002814115.1	2001
SGAir0397	Environment (air)	GCA_005484525.1	2015
FDAARGOS611	Food	GCA_006364815.1	missing
D32	Pig	GCA_000281195.1	2001
133170041-3	Environment (air at ISS)	GCA_013344605.1	2013
C54	Pig	GCA_005979305.1	2017
Symbioflor1	missing	GCA_000317915.1	missing
LD33	Food	GCA_001598635.1	2010
KUB3006	Human	GCA_003966385.1	2017
KUB3007	Human	GCA_003966405.1	2017
FC	Milk	GCA_005158025.1	2017
H25	missing	GCA_002289045.2	missing
KB1	Mouse	GCA_001689055.2	2015
L8	Pig	GCA_009498175.1	2013
TH4125	Human	GCA_012594215.1	missing
HA-1	Human	GCA_006349345.1	2019
JY32	Human	GCA_010103655.1	2016
L15	Pig	GCA_009498155.1	2013
L12	Pig	GCA_001886675.1	2013
ES1	Pig	GCA_011193375.1	2017
V563	missing	GCA_000007785.1	missing
BFFF11	Fish	GCA_009685155.1	2018
serialis	Human	GCA_002163735.1	2014
092160007-3	Environment (air at ISS)	GCA_013344665.1	2009
DM01	Food	GCA_006766145.1	2019
FDAARGOS528	Human	GCA_003812665.1	2016
CLB21560	Human	GCA_001989555.1	2017
TY1	Fish	GCA_003345275.1	2011

From each of these sequences, PCR primers which can acquire a PCR product having a length of about 100 to about 250 bp, and PCR primers which can acquire a PCR product having a length of about 500 to about 600 bp, with the same forward primer, were created. PCR using said primers was performed using the genomic DNA prepared from an EF-2001 strain and a comparative bacterial strain as a template, thereby selecting a primer set which can acquire a PCR

product of interest in only the EF-2001 strain and cannot acquire the PCR product even when the DNA of another bacterial strain is used as a template.

[0016]

5 <Regarding the primer set of the present invention>

In one aspect of the present invention, the primer set of the present invention can comprise the following:

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 1 and an oligonucleotide comprising the base
10 sequence of SEQ ID NO: 2 or 3;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 4 and an oligonucleotide comprising the base sequence of SEQ ID NO: 5 or 6;

a pair of an oligonucleotide comprising the base sequence
15 of SEQ ID NO: 7 and an oligonucleotide comprising the base sequence of SEQ ID NO: 8 or 9;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 10 and an oligonucleotide comprising the base sequence of SEQ ID NO: 11 or 12;

a pair of an oligonucleotide comprising the base sequence
20 of SEQ ID NO: 13 and an oligonucleotide comprising the base sequence of SEQ ID NO: 14 or 15;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 16 and an oligonucleotide comprising the base
25 sequence of SEQ ID NO: 17 or 18;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 19 and an oligonucleotide comprising the base sequence of SEQ ID NO: 20 or 21;

a pair of an oligonucleotide comprising the base sequence
30 of SEQ ID NO: 22 and an oligonucleotide comprising the base sequence of SEQ ID NO: 23 or 24;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 25 and an oligonucleotide comprising the base sequence of SEQ ID NO: 26 or 27;

35 a pair of an oligonucleotide comprising the base sequence

of SEQ ID NO: 28 and an oligonucleotide comprising the base sequence of SEQ ID NO: 29 or 30.

[0017]

In another aspect of the present invention, the primer set of the present invention can comprise the following:

(a) a primer set (1) for amplifying a part of the base sequence (contig 1) of SEQ ID NO: 51 (contig 1 long):

a forward primer CGAAAAGGATGTAGTCAGCGG (SEQ ID NO: 1); and

a reverse primer CCGAAGGCGAAACAGAGGAT (SEQ ID NO: 2),

(b) a primer set (2) for amplifying a part of the base sequence (contig 1) of SEQ ID NO: 51 (contig 1 short):

a forward primer CGAAAAGGATGTAGTCAGCGG (SEQ ID NO: 1); and

a reverse primer CCCAGACATAATCGCATGGC (SEQ ID NO: 3),

(c) a primer set (3) for amplifying a part of the base sequence (contig 4) of SEQ ID NO: 52 (contig 4 long):

a forward primer GCGGCTGCACAATTTATTGC (SEQ ID NO: 4); and

a reverse primer AGAATACTTGGGCGGTCGTG (SEQ ID NO: 5),

(d) a primer set (4) for amplifying a part of the base sequence (contig 4) of SEQ ID NO: 52 (contig 4 short):

a forward primer GCGGCTGCACAATTTATTGC (SEQ ID NO: 4); and

a reverse primer AATTCAGCTTCGCTAGATAAGGC (SEQ ID NO: 6),

(e) a primer set (5) for amplifying a part of the base sequence (contig 7) of SEQ ID NO: 53 (contig 7 long):

a forward primer CGCGTATGACTTGCAATCGA (SEQ ID NO: 7); and

a reverse primer AGGATTGTTTGACGGTGCAA (SEQ ID NO: 8),

(f) a primer set (6) for amplifying a part of the base sequence (contig 7) of SEQ ID NO: 53 (contig 7 short):

a forward primer CGCGTATGACTTGCAATCGA (SEQ ID NO: 7); and

a reverse primer ACATGAGATAGTTGGGGTAGACA (SEQ ID NO: 9),

(g) a primer set (7) for amplifying a part of the base sequence (contig 11) of SEQ ID NO: 54 (contig 11 long):

5 a forward primer CTTCAGAGAGCTGGGCGAAG (SEQ ID NO: 10); and

a reverse primer TACTTTTCTAGCTGCCCCGCC (SEQ ID NO: 11),

10 (h) a primer set (8) for amplifying a part of the base sequence (contig 11) of SEQ ID NO: 54 (contig 11 short):

a forward primer CTTCAGAGAGCTGGGCGAAG (SEQ ID NO: 10); and

a reverse primer GGGTTGTAGCCCTACCCGAT (SEQ ID NO: 12),

15 (i) a primer set (9) for amplifying a part of the base sequence (contig 13) of SEQ ID NO: 55 (contig 13 long):

a forward primer CGTAACGTGACATTGCGGAC (SEQ ID NO: 13); and

20 a reverse primer ATGCCAGTACGTCGCGTTAA (SEQ ID NO: 14),

(j) a primer set (10) for amplifying a part of the base sequence (contig 13) of SEQ ID NO: 55 (contig 13 short):

a forward primer CGTAACGTGACATTGCGGAC (SEQ ID NO: 13); and

25 a reverse primer CGATTGTCAACTAATTGTGCCGA (SEQ ID NO: 15),

(k) a primer set (11) for amplifying a part of the base sequence (contig 16) of SEQ ID NO: 56 (contig 16 long):

30 a forward primer CATGGCTTGCCGTTTCACAA (SEQ ID NO: 16); and

a reverse primer ACCGCAACAACCTACATACTACCA (SEQ ID NO: 17),

(l) a primer set (12) for amplifying a part of the base sequence (contig 16) of SEQ ID NO: 56 (contig 16 short):

35 a forward primer CATGGCTTGCCGTTTCACAA (SEQ ID NO:

16); and

a reverse primer ACCAAAAGGAACGCTACCAGT (SEQ ID NO: 18),

(m) a primer set (13) for amplifying a part of the base sequence (contig 22) of SEQ ID NO: 57 (contig 22 long):

a forward primer TCAGCATAATCCCCAGACGT (SEQ ID NO: 19); and

a reverse primer AATGAACGCCCTTCAGCAGA (SEQ ID NO: 20),

(n) a primer set (14) for amplifying a part of the base sequence (contig 22) of SEQ ID NO: 57 (contig 22 short):

a forward primer TCAGCATAATCCCCAGACGT (SEQ ID NO: 19); and

a reverse primer GGCTCCTCTACCTGAACAACT (SEQ ID NO: 21),

(o) a primer set (15) for amplifying a part of the base sequence (contig 25) of SEQ ID NO: 58 (contig 25 long):

a forward primer GCGTTCAAACCTGTTCTGGTGT (SEQ ID NO: 22); and

a reverse primer TACAAGGCTTGCGAGGTAGC (SEQ ID NO: 23),

(p) a primer set (16) for amplifying a part of the base sequence (contig 25) of SEQ ID NO: 58 (contig 25 short):

a forward primer GCGTTCAAACCTGTTCTGGTGT (SEQ ID NO: 22); and

a reverse primer GCTGCAATGGAAAGCAAATCG (SEQ ID NO: 24),

(q) a primer set (17) for amplifying a part of the base sequence (contig 31) of SEQ ID NO: 59 (contig 31 long):

a forward primer AGGCATATGGGTCATCTGCT (SEQ ID NO: 25); and

a reverse primer GAGCATCACAGAGCCTCGAA (SEQ ID NO: 26),

(r) a primer set (18) for amplifying a part of the base sequence (contig 31) of SEQ ID NO: 59 (contig 31 short):

a forward primer AGGCATATGGGTCATCTGCT (SEQ ID NO: 25); and

a reverse primer AGAGATTTTTCAGTATTGCTGGGT (SEQ ID NO: 27),

5 (s) a primer set (19) for amplifying a part of the base sequence (contig 43) of SEQ ID NO: 60 (contig 43 long):

a forward primer CGTTGGGTGTGCAGAAATGG (SEQ ID NO: 28); and

10 a reverse primer TGTACCGTCAACCTCGTTTCG (SEQ ID NO: 29), or

(t) a primer set (20) for amplifying a part of the base sequence (contig 43) of SEQ ID NO: 60 (contig 43 short):

a forward primer CGTTGGGTGTGCAGAAATGG (SEQ ID NO: 28); and

15 a reverse primer AACGGGTTGCGACTCTTTTT (SEQ ID NO: 30).

[0018]

Further, a primer set which can be used in the detection method of the present invention also includes a primer set
20 consisting of an oligonucleotide having a base sequence that is substantially homologous to the base sequences of SEQ ID NOs: 1 to 30 depending on the base length, the PCR condition and the like. In this regard, a sequence which is substantially homologous means that the sequence has a
25 fragment length and homology to the extent that it can function as a primer for PCR or the like, and the sequence preferably has about 90% or greater sequence identity.

[0019]

For example, the primer set of the present invention
30 does not necessarily need to have 100% homology to the base sequences of SEQ ID NOs: 1 to 30 depending on the purpose of use and the condition. A few bases may be different near the 5' terminal of a primer for a region of interest. Even when such a primer is used, it is possible to appropriately
35 amplify a DNA fragment of interest by considering the

annealing temperature or the like.

[0020]

For example, when high specificity is required in detecting a lactic acid bacterium EF-2001 strain, a fully homologous sequence site (the base sequences of SEQ ID NOs: 1 to 30) should be used, and a PCR condition under which annealing occurs only with such a sequence should be selected. On the other hand, when a condition with relatively low specificity is allowed, a sequence which is different from the base sequences of SEQ ID NOs: 1 to 30 in a few bases (e.g., a sequence having about 90% or greater sequence identity) can be used, and a PCR condition under which annealing occurs even with such a sequence can be selected.

[0021]

The primer set of the present invention can be synthesized by a common DNA synthesizing method that is well known to those skilled in the art, e.g., a DNA synthesizer. The primer of the present invention can also be obtained by entrusting synthesis to a DNA synthesis operator.

[0022]

A lactic acid bacterium EF-2001 strain in a sample can be detected by using the primer set of the present invention to perform PCR using the chromosomal DNA of a test bacterium contained in the sample as a template and determining the presence or absence of an amplification product. Specifically, when a reaction of PCR specific to the sequence of the primers occurs, a region (target region) sandwiched by the sequences corresponding to the sequence of each of the primers that are a pair in the chromosomal DNA in the lactic acid bacterium EF-2001 strain is amplified.

[0023]

Thus, when an amplification product is obtained using the primer set of the present invention, the test bacterium is identified to be a lactic acid bacterium EF-2001 strain, or the test bacterium is determined to at least comprise a

lactic acid bacterium EF-2001 strain.

[0024]

Further, when an amplification product is obtained, determination of the presence or absence of the amplification product may comprise determination of the length of the amplification product. For example, when a primer set for detecting a lactic acid bacterium EF-2001 strain which comprises oligonucleotides consisting of each of the combinations of SEQ ID NO: 1 and SEQ ID NO: 2, SEQ ID NOS: 4 and 5, SEQ ID NOS: 7 and 8, SEQ ID NOS: 10 and 11, SEQ ID NOS: 13 and 14, SEQ ID NOS: 16 and 17, SEQ ID NOS: 19 and 20, SEQ ID NOS: 22 and 23, SEQ ID NOS: 25 and 26, or SEQ ID NOS: 28 and 29 is used, an amplification product of about 500 to about 600 bp is generally obtained for each of the sequences if the test bacterium comprises a lactic acid bacterium EF-2001 strain. Further, when a primer set for detecting a lactic acid bacterium EF-2001 strain which comprises oligonucleotides consisting of each of the combinations of SEQ ID NO: 1 and SEQ ID NO: 3, SEQ ID NOS: 4 and 6, SEQ ID NOS: 7 and 9, SEQ ID NOS: 10 and 12, SEQ ID NOS: 13 and 15, SEQ ID NOS: 16 and 18, SEQ ID NOS: 19 and 21, SEQ ID NOS: 22 and 24, SEQ ID NOS: 25 and 27, or SEQ ID NOS: 28 and 30 is used, an amplification product of about 100 to about 250 bp is generally obtained for each of the sequences if the test bacterium comprises a lactic acid bacterium EF-2001 strain.

[0025]

The following amplification products are obtained when the primer set for detecting a lactic acid bacterium EF-2001 strain comprising oligonucleotides of the present invention is used.

SEQ ID NO: 31: an amplification product amplified by a primer set consisting of a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 1 and an oligonucleotide comprising the base sequence of SEQ ID NO: 2

SEQ ID NO: 32: an amplification product amplified by a primer set consisting of a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 1 and an oligonucleotide comprising the base sequence of SEQ ID NO: 3

5 SEQ ID NO: 33: an amplification product amplified by a primer set consisting of a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 4 and an oligonucleotide comprising the base sequence of SEQ ID NO: 5

10 SEQ ID NO: 34: an amplification product amplified by a primer set consisting of a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 4 and an oligonucleotide comprising the base sequence of SEQ ID NO: 6

15 SEQ ID NO: 35: an amplification product amplified by a primer set consisting of a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 7 and an oligonucleotide comprising the base sequence of SEQ ID NO: 8

20 SEQ ID NO: 36: an amplification product amplified by a primer set consisting of a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 7 and an oligonucleotide comprising the base sequence of SEQ ID NO: 9

SEQ ID NO: 37: an amplification product amplified by a primer set consisting of a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 10 and an oligonucleotide comprising the base sequence of SEQ ID NO: 11

25 SEQ ID NO: 38: an amplification product amplified by a primer set consisting of a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 10 and an oligonucleotide comprising the base sequence of SEQ ID NO: 12

30 SEQ ID NO: 39: an amplification product amplified by a primer set consisting of a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 13 and an oligonucleotide comprising the base sequence of SEQ ID NO: 14

35 SEQ ID NO: 40: an amplification product amplified by a primer set consisting of a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 13 and an oligonucleotide

comprising the base sequence of SEQ ID NO: 15

SEQ ID NO: 41: an amplification product amplified by a primer set consisting of a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 16 and an oligonucleotide

5 comprising the base sequence of SEQ ID NO: 17

SEQ ID NO: 42: an amplification product amplified by a primer set consisting of a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 16 and an oligonucleotide comprising the base sequence of SEQ ID NO: 18

10 SEQ ID NO: 43: an amplification product amplified by a primer set consisting of a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 19 and an oligonucleotide comprising the base sequence of SEQ ID NO: 20

15 SEQ ID NO: 44: an amplification product amplified by a primer set consisting of a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 19 and an oligonucleotide comprising the base sequence of SEQ ID NO: 21

20 SEQ ID NO: 45: an amplification product amplified by a primer set consisting of a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 22 and an oligonucleotide comprising the base sequence of SEQ ID NO: 23

25 SEQ ID NO: 46: an amplification product amplified by a primer set consisting of a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 22 and an oligonucleotide comprising the base sequence of SEQ ID NO: 24

SEQ ID NO: 47: an amplification product amplified by a primer set consisting of a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 25 and an oligonucleotide comprising the base sequence of SEQ ID NO: 26

30 SEQ ID NO: 48: an amplification product amplified by a primer set consisting of a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 25 and an oligonucleotide comprising the base sequence of SEQ ID NO: 27

35 SEQ ID NO: 49: an amplification product amplified by a primer set consisting of a pair of an oligonucleotide comprising

the base sequence of SEQ ID NO: 28 and an oligonucleotide comprising the base sequence of SEQ ID NO: 29

SEQ ID NO: 50: an amplification product amplified by a primer set consisting of a pair of an oligonucleotide comprising
5 the base sequence of SEQ ID NO: 28 and an oligonucleotide comprising the base sequence of SEQ ID NO: 30

[0026]

Further, each of the base sequences of SEQ ID NOs: 31 to 50 is a part of the base sequence of any of SEQ ID NOs: 51
10 to 60.

[0027]

Each primer sequence of SEQ ID NOs: 1 to 30, and the base sequences of SEQ ID NOs: 31 to 50 and the base sequences of SEQ ID NOs: 51 to 60 are sequences specific to a lactic
15 acid bacterium EF-2001 strain. Thus, it is also possible to perform FISH method, Southern hybridization, dot hybridization or the like using a part of these sequences as a DNA probe.

[0028]

In the present invention, detection of a lactic acid bacterium EF-2001 strain comprises detecting whether or not a lactic acid bacterium EF-2001 strain is present in a sample, and when a test bacterium is a single species, detection of a lactic acid bacterium EF-2001 strain comprises identifying
20 whether or not the test bacterium is a lactic acid bacterium EF-2001 strain.

[0029]

In one embodiment of the present invention, any sample can be used for detection of a lactic acid bacterium EF-2001 strain as long as a lactic acid bacterium EF-2001 strain is present or may be present in the sample. Examples of the
30 sample can include a mixed culture of lactic acid bacteria, fermented food comprising a microorganism such as miso, soy sauce, alcohol such as wine, pickles, yoghurt, or cheese, feces of experimental animals such as mice or rats or humans,
35

bacteria that are present in an environment such as soil, and the like. A test bacterium may be an isolated single species, or may be a mixture comprising a plurality of bacterial species.

5 [0030]

In one embodiment of the present invention, when a dead bacterium is used as a test bacterium, DNA may be directly prepared from a sample and used for PCR. When a live bacterium is used, a test bacterial cell may be isolated
10 from a sample by colony isolation or the like, and DNA may be prepared from the test bacterial cell and subjected to PCR. Methods for extracting a nucleic acid from these samples are not particularly limited. For example, it is possible to use a highly versatile kit, such as Kaneka Easy DNA
15 Extraction Kit version 2, which is capable of extracting a nucleic acid from a cultured cell including an animal cell, a plant cell, a microorganism and the like, or use a commercially available extraction kit (such as DNeasy Blood & Tissue kit (QIAGEN) or Isogen) as commonly used for
20 preparation of a genomic DNA of a lactic acid bacterium after degrading a cell wall by an enzyme such as Lysozyme or physically destroying a cell wall by a bead or the like.

[0031]

<Regarding the detection method of the present invention>

25 In one aspect of the present invention, the method for detecting a lactic acid bacterium EF-2001 strain of the present invention can comprise (1) the step of amplifying a nucleic acid fragment using the primer set of the present invention and (2) the step of detecting a nucleic acid
30 fragment obtained in the step (1).

[0032]

Specific examples of the detection method of the present invention can include PCR method, FISH method, Southern hybridization, dot hybridization and the like. PCR method is
35 particularly preferable. Among these methods, a method for

detecting a lactic acid bacterium EF-2001 strain using PCR method is not particularly limited. Apart from the use of the primer set of the present invention, commonly used PCR reagents and apparatus such as DNA polymerase can also be used.

[0033]

In one embodiment, a DNA polymerase which is used for PCR is not particularly limited, but is preferably, for example, KOD FX Neo (TOYOBO), ExTaq (Takara Bio), KOD DNA polymerase (TOYOBO), KOD-plus-polymerase (TOYOBO) or the like.

[0034]

In one embodiment, a PCR reaction condition can be appropriately set depending on the optimal temperature for a DNA polymerase that is used, the length or the type of DNA to be synthesized, or the like. A condition under which 20 to 40 cycles of a cycle of "5 to 30 seconds at 90 to 98°C (thermal denaturation/dissociation) → 5 to 30 seconds at 55 to 60°C (annealing) → 30 to 60 seconds at 65 to 80°C (synthesis/extension)" are performed in total can be set as a cycle condition. For example, 25 cycles of "10 seconds at 98°C → 30 seconds at 60°C → 30 seconds at 68°C" can also be performed. In this case, a temperature which was used for design of a primer can be used as the annealing temperature. For example, when KOD FX Neo is used as a polymerase, the reaction can be configured as two-step reaction which omits the annealing temperature depending on the primer size (e.g., 20 mer or greater). Regarding the quantity ratio of a template DNA to a primer, for example, in the case of a live bacterium, 0.3 μM of each primer can be used for 200 ng of the template DNA. In the case of a dead bacterium, a band in electrophoresis can be made clearer by increasing the template DNA depending on the situation of thermal treatment.

[0035]

The presence or absence or the size of an amplification

product obtained by PCR can be determined by a common nucleic acid detection method. For example, an amplification product can be detected by electrophoresis by agarose gel electrophoresis, followed by staining with ethidium bromide or SYBR Green I. Further, the amount of an amplification product can be determined by fluorescence intensity, and the molecular weight of an amplification product can be determined by comparison with a molecular weight marker. The presence or absence of an amplification product can also be confirmed by subjecting a PCR product to cycle sequencing, followed by measuring the base sequence and the length using a DNA sequencer. Further, real-time PCR method enables detection of an amplification reaction over time.

[0036]

In one aspect of the present invention, the primer set of the present invention can be configured to be a kit for detecting a lactic acid bacterium EF-2001 strain in combination with other elements. Examples of other elements include extraction of a nucleic acid, PCR, and any one or more types of reagents necessary for detection of an amplification product. This kit for detecting a lactic acid bacterium EF-2001 strain may comprise a DNA fragment which has a part of the sequence of the chromosomal DNA of the lactic acid bacterium EF-2001 strain and can be amplified by the primer set of the present invention as a positive control, and/or a DNA fragment having a base sequence which corresponds to the primer set of the present invention but has a mismatch in one base or a few bases as a negative control.

[0037]

The primer set of the present invention can be used for detection of a lactic acid bacterium EF-2001 strain as described above. Further, when industrially manufacturing a lactic acid bacterium EF-2001 strain bacterial cell or food and drink or the like comprising the bacterial cell, use of

the primer set of the present invention facilitates measurement of the number of bacteria and control of the fermentation state.

[0038]

5 <Regarding the lactic acid bacterium composition of the present invention>

In one aspect of the present invention, the lactic acid bacterium composition of the present invention can comprise a heat-treated lactic acid bacterium product which can be
10 obtained by heating a live bacterium of a lactic acid bacterium *Enterococcus faecalis* EF-2001 strain. In one embodiment, the lactic acid bacterium composition of the present invention preferably comprises a nucleic acid fragment which is not amplified when any one or more of
15 primer sets for detecting a lactic acid bacterium EF-2001 strain comprising oligonucleotides consisting of each of the combinations of SEQ ID NO: 1 and SEQ ID NO: 2, SEQ ID NOs: 4 and 5, SEQ ID NOs: 7 and 8, SEQ ID NOs: 10 and 11, SEQ ID NOs: 13 and 14, SEQ ID NOs: 16 and 17, SEQ ID NOs: 19 and
20 20, SEQ ID NOs: 22 and 23, SEQ ID NOs: 25 and 26, and/or SEQ ID NOs: 28 and 29 are used, and which is amplified when any one or more of primer sets for detecting a lactic acid bacterium EF-2001 strain comprising oligonucleotides consisting of each of the combinations of SEQ ID NO: 1 and
25 SEQ ID NO: 3, SEQ ID NOs: 4 and 6, SEQ ID NOs: 7 and 9, SEQ ID NOs: 10 and 12, SEQ ID NOs: 13 and 15, SEQ ID NOs: 16 and 18, SEQ ID NOs: 19 and 21, SEQ ID NOs: 22 and 24, SEQ ID NOs: 25 and 27, or SEQ ID NOs: 28 and 30 are used.

[0039]

30 As used herein, "a nucleic acid fragment not being amplified" or "a band disappearing" refers to an amplification efficiency or an amplification amount being less than about 30% in comparison to those of a nucleic acid fragment derived from an untreated (e.g., without heat
35 treatment) lactic acid bacterium EF-2001 strain when a

nucleic acid fragment of a lactic acid bacterium EF-2001 strain is amplified by using the primer set for detecting a lactic acid bacterium EF-2001 strain of the present invention. In one embodiment, "a nucleic acid fragment not being amplified" or "a band disappearing" refers to an amplification efficiency or an amplification amount being less than about 20%, preferably less than about 10%, or further preferably less than about 5% in comparison to those of a nucleic acid fragment derived from an untreated (e.g., without heat treatment) lactic acid bacterium EF-2001 strain when a nucleic acid fragment of a lactic acid bacterium EF-2001 strain is amplified by using the primer set for detecting a lactic acid bacterium EF-2001 strain of the present invention.

[0040]

In one embodiment, the lactic acid bacterium composition of the present invention can be heated to be a dead bacterial cell and then dried by lyophilization, spray drying, or a drum dryer to be a powdery dry bacterial cell. Safety and preservability can be thereby improved, and said composition can be utilized for various food. Heating of a lactic acid bacterium EF-2001 strain can be performed for any duration of time and at any temperature which can result in a dead bacterial cell. Heating can be performed, for example, for about 5 minutes or longer, about 10 minutes or longer, about 15 minutes or longer, about 20 minutes or longer, about 25 minutes or longer, about 30 minutes or longer, about 45 minutes or longer, or about 60 minutes or longer, for example, at a temperature of about 50°C or greater, about 60°C or greater, about 70°C or greater, about 80°C or greater, about 90°C or greater, about 100°C or greater, about 110°C or greater, or about 120°C or greater or the like. Heating may be performed with any combination of temperature and duration of time as long as a sterilized or dead bacterial cell can be obtained by heating.

[0041]

In one embodiment of the present invention, when obtaining the lactic acid bacterium composition of the present invention, a lactic acid bacterium *Enterococcus faecalis* EF-2001 strain is heated at the above-described temperature and for the above-described duration of time to obtain a plurality of lots of a heat-treated lactic acid bacterium product, and a nucleic acid fragment is extracted from these heat-treated lactic acid bacterium products. The nucleic acid fragment is then amplified using at least one of the primer sets of the present invention described in other portions herein. A lot of the heat-treated lactic acid bacterium product in which the amplified nucleic acid fragment has been detected is then selected, and the heat-treated lactic acid bacterium product contained in the lot can be made as the lactic acid bacterium composition of the present invention. An apparatus or an approach commonly used in the art can be employed for heating and nucleic acid amplification. For example, an apparatus or an approach which can be used in the detection method described in other portions herein can be employed. When heating a bacterial cell, a class-I pressure vessel equipped with a stirring apparatus can be commonly used, and a class-II pressure vessel can also be used depending on the temperature. A small pressure vessel or the like can also be used if the liquid amount is small. In other embodiments, a continuous sterilizing apparatus utilizing a plate or a tube can also be used as a heat conductor.

[0042]

In one embodiment of the present invention, the TNF- α production amount of the lactic acid bacterium composition of the present invention can be increased by heating and stabilizing the composition. The lactic acid bacterium composition of the present invention preferably comprises a nucleic acid fragment which is not amplified when any one or

more of primer sets for detecting a lactic acid bacterium EF-2001 strain comprising oligonucleotides consisting of each of the combinations of SEQ ID NO: 1 and SEQ ID NO: 2, SEQ ID NOS: 4 and 5, SEQ ID NOS: 7 and 8, SEQ ID NOS: 10 and 11, SEQ ID NOS: 13 and 14, SEQ ID NOS: 16 and 17, SEQ ID NOS: 19 and 20, SEQ ID NOS: 22 and 23, SEQ ID NOS: 25 and 26, and/or SEQ ID NOS: 28 and 29 are used, and which is amplified when any one or more of primer sets for detecting a lactic acid bacterium EF-2001 strain comprising oligonucleotides consisting of each of the combinations of SEQ ID NO: 1 and SEQ ID NO: 3, SEQ ID NOS: 4 and 6, SEQ ID NOS: 7 and 9, SEQ ID NOS: 10 and 12, SEQ ID NOS: 13 and 15, SEQ ID NOS: 16 and 18, SEQ ID NOS: 19 and 21, SEQ ID NOS: 22 and 24, SEQ ID NOS: 25 and 27, and/or SEQ ID NOS: 28 and 30 are used.

[0043]

In one embodiment, it is possible to confirm whether or not the TNF- α production amount of the lactic acid bacterium composition of the present invention is increased by using a band of a primer set for detecting a long sequence disappearing and a band of a primer set for detecting a short sequence remaining as an indicator. For example, by using a nucleic acid fragment not being amplified by a primer set (long) of SEQ ID NOS: 16 and 17 amplifying a part of the base sequence of contig 16 and the nucleic acid fragment being amplified by a primer set (short) of SEQ ID NOS: 16 and 18 as an indicator, it is possible to determine that the lactic acid bacterium composition of the present invention subjected to heat treatment showing such an amplification result has an increased TNF- α production amount.

[0044]

In one embodiment of the present invention, any diluent can be mixed into the lactic acid bacterium composition of the present invention to prevent individual bacterial cells which are placed in an isolated state from being thereby

aggregated when each bacterial cell is added to food or the like and absorbs water. Preferably, a diluent is not only fine powdery but also fluidic as highly as possible in order to facilitate homogeneous mixing with bacterial cells. Given
5 the safety upon addition to food, the effect on a flavor or a property of food and the like, a diluent is preferably a polysaccharide or a protein. Examples of the polysaccharide can include naturally-occurring polysaccharides such as lactose, polydextrose, cyclodextrin, corn starch,
10 microcrystalline cellulose, starch, or galactomannan, enzyme-treated hydrolyzate thereof and the like, and examples of the protein can include soy bean protein, casein, wheat gluten, egg white albumin and the like. It is also possible to use any two or more types thereof in combination.

15 [0045]

As used herein, "or" is used when "at least one or more" of the matters listed in the sentence can be employed. When explicitly described herein as "within the range" of "two values", the range also includes the two values themselves.

20 Reference literatures such as scientific literatures, patents, and patent applications cited herein are incorporated herein by reference to the same extent that the entirety of each document is specifically described.

[0046]

25 As described above, the present disclosure has been described while showing preferred embodiments to facilitate understanding. The present disclosure is described below based on Examples. The aforementioned description and the following Examples are not provided to limit the present
30 disclosure, but for the sole purpose of exemplification. Thus, the scope of the present disclosure is not limited to the embodiments and Examples specifically described herein and is limited only by the scope of claims.

[EXAMPLES]

35 [0047]

(Example 1: Detection 1 of a lactic acid bacterium EF-2001 strain (comparison with *Enterococcus faecalis* subspecies))

(1) Test bacterial strain

(A) A lactic acid bacterium EF-2001 strain, and (B) *E. faecalis* NBRC3971, (C) *E. faecalis* NBRC3989, (D) *E. faecalis* NBRC12970, (E) *E. faecalis* NBRC100480, (F) *E. faecalis* NBRC100482, (G) *E. faecalis* NBRC100483 and (H) *E. faecalis* NBRC100484, which are *Enterococcus faecalis* subspecies, were used as test bacterial strains.

[0048]

(2) Preparation of a lactic acid bacterium DNA

Buffer comprising an enzyme such as lysozyme was added to the above bacterial cells collected from culture liquid of each bacterial cell, and the cell membrane was degraded. After treatment with proteinase and RNase, sodium acetate was added and a precipitate precipitated with ethanol was rinsed with ethanol, followed by drying the product thereof. Buffer such as TE was added thereto and dissolved to prepare solution, which was used as DNA solution for PCR. DNA solution can also be prepared using a commercially available DNA extracting kit.

[0049]

(3) PCR reaction

The DNA solution of the test bacterial strains obtained in the above (2) was used as a template, and a primer set consisting of a pair of oligonucleotides comprising the base sequences of SEQ ID NO: 1 and SEQ ID NO: 2 (contig 1), SEQ ID NOs: 4 and 5 (contig 4), SEQ ID NOs: 7 and 8 (contig 7), SEQ ID NOs: 10 and 11 (contig 11), SEQ ID NOs: 13 and 14 (contig 13), SEQ ID NOs: 16 and 17 (contig 16), SEQ ID NOs: 19 and 20 (contig 22), SEQ ID NOs: 22 and 23 (contig 25), SEQ ID NOs: 25 and 26 (contig 31), and SEQ ID NOs: 28 and 29 (contig 43) and a primer set consisting of a pair of oligonucleotides comprising the base sequences of SEQ ID NO: 1 and SEQ ID NO: 3 (contig 1), SEQ ID NOs: 4 and 6 (contig

4), SEQ ID NOs: 7 and 9 (contig 7), SEQ ID NOs: 10 and 12 (contig 11), SEQ ID NOs: 13 and 15 (contig 13), SEQ ID NOs: 16 and 18 (contig 16), SEQ ID NOs: 19 and 21 (contig 22), SEQ ID NOs: 22 and 24 (contig 25), SEQ ID NOs: 25 and 27 (contig 31), and SEQ ID NOs: 28 and 30 (contig 43) were used to perform a PCR reaction according to the method of KOD FX Neo (TOYOBO), which is a PCR reaction reagent kit. The total amount of PCR reaction liquid was 50 μ L, and 25 cycles of a cycle of preheating reaction liquid comprising 25.0 μ L of 2 $\times$ PCR buffer accompanying the kit, 10.0 μ L of 2 mM dNTPs, 1.0 U of DNA polymerase, 0.2 μ L of primer mixture, and 10 to 200 ng of the template DNA (in the case of a live bacterium) or 10 to 400 ng of the template DNA (in the case of a dead bacterium) for 10 seconds at 94°C followed by denaturation for 10 seconds at 98°C, annealing for 30 seconds at 60°C, and extension for 30 seconds at 68°C were performed using Thermal cycler GenAtlas (ASTECH).

[0050]

As a positive control, primers (F: SEQ ID NO: 61 and R: SEQ ID NO: 62) which were designed so that the amplification product would be 200 to 300 bp were used, with the 16S rDNA of a lactic acid bacterium EF-2001 strain as a template.

[0051]

(4) Agarose electrophoresis

The PCR product obtained by the PCR reaction was subjected to electrophoresis for 30 minutes at 100 V with 1.4% agarose gel comprising GelRed Nucleic Acid Gel Stain (FUJIFILM Co., Ltd.). Next, the band showing amplification of the PCR product was observed with WSE-5400-UP Printgraph Classic (ATTO Co., Ltd.). Figure 1 shows the results.

[0052]

As shown Figure 1, a band of the positive control of about 284 bp was observed in all subspecies of *Enterococcus faecalis* which were used in this test. A band of about 100 to about 250 bp and a band of about 500 to about 600 bp were

observed only when the DNA of the EF-2001 strain was used (Figure **1(A)**), and it was confirmed that the primer set of the present invention can specifically detect only a lactic acid bacterium EF-2001 strain from *Enterococcus faecalis* subspecies.

[0053]

<Example 2: Detection 2 of a lactic acid bacterium EF-2001 strain (comparison with other lactic acid bacteria)>

All steps were performed in the same manner as Example 1 except that (A) a lactic acid bacterium EF-2001 strain, and (B) *E. faecium* Aus0004, (C) *E. gallinarum* LMG13129, (D) *Lactobacillus rhamnosus* NBRC3425, (E) *L. plantarum* NBRC15891, (F) *L. paracasei* NBRC15906, and (G) *L. lactis* NBRC102622, which are other lactic acid bacteria, were used as test bacterial strains. Figure **2** shows the results.

[0054]

As shown in Figure **2**, a band of the positive control of about 284 bp, a band of about 100 to about 250 bp, and a band of about 500 to about 600 bp were observed only when the DNA of the lactic acid bacterium EF-2001 strain was used (Figure **2(A)**). No band was observed in the lactic acid bacteria other than the lactic acid bacterium EF-2001 strain. It was confirmed from these results that the primer set of the present invention can specifically detect only a lactic acid bacterium EF-2001 strain from other lactic acid bacteria.

[0055]

<Example 3: Detection 3 of a lactic acid bacterium EF-2001 strain (live bacterium and heated bacterium)>

(1) Test bacterial strain

A lactic acid bacterium EF-2001 strain was used as a test bacterial strain, and a nucleic acid was detected using the DNA extracted from three types of bacterial cells, a live bacterium (Figure **3(A)** culture liquid), a dead bacterium (Figure **3(B)** liquid after heat treatment), and a dead bacterium (Figure **3(C)** product bulk) as a template.

(2) The test was performed using the same DNA preparation, PCR condition, and PCR primers as those of Examples 1 and 2. Figure 3 shows the results of performing agarose electrophoresis under the same condition as that of Examples 1 and 2.

[0056]

As shown in Figure 3, a band of about 100 to about 250 bp was observed for both the live bacterium and the dead bacterium of the lactic acid bacterium EF-2001 strain. It was revealed that the primer set of the present invention exhibits a specific detection ability on not only a live bacterium of a lactic acid bacterium EF-2001 strain but also on a heat-treated bacterium (dead bacterium). These results mean that a lactic acid bacterium EF-2001 strain can be detected in a product or the like utilizing a heat-treated lactic acid bacterium EF-2001 strain, let alone a product or the like utilizing a live bacterium of a lactic acid bacterium EF-2001 strain, which is very useful.

[0057]

<Example 4: Disappearance of a band due to heating>

Disappearance of a band of the primer set of the present invention due to heat treatment was confirmed using a lactic acid bacterium EF-2001 strain as a test bacterial strain. In addition to an untreated bacterium (live bacterium), lactic acid bacterium EF-2001 strains that were heat-treated at 70°C, 90°C, and 110°C were used.

[0058]

As one example, primers of SEQ ID NOs: 16 and 17 amplifying a part of the base sequence of contig 16, and SEQ ID NOs: 16 and 18 were used to study the amplification ability of a nucleic acid fragment under the same PCR condition and the like as that of Example 1. Figure 4 shows the results.

[0059]

As shown in Figure 4, it was found that a band of the

primer set of SEQ ID NOs: 16 and 17 (long) disappeared as the heating temperature was increased. Since a product utilizing a heat-treated lactic acid bacterium EF-2001 strain needs a certain thermal history, disappearance of the band of the primers for detecting a long sequence shows the result meaning that a certain thermal history is being given, which is very useful. Meanwhile, for the primer set of SEQ ID NOs: 16 and 18 (short), a band upon electrophoresis could be observed even when heating was performed at 110°C.

10 [0060]

<Example 5: Measurement of the TNF- α production amount>

The change in the TNF- α production amount of a lactic acid bacterium EF-2001 strain due to heat treatment was confirmed using a lactic acid bacterium EF-2001 strain as a test bacterial strain. An untreated bacterium (live bacterium) and a lactic acid bacterium EF-2001 strain which was heat-treated at 110°C were used.

[0061]

First, RAW264.7 cells were used to induce TNF- α . 100 μ L of RAW264.7 cell solution adjusted to 1.0×10^6 cells/mL per 1 well was dispensed into 96 wells, and the solution was cultured for 24 hours in a 5% CO₂ thermostatic vessel at 37°C. The culture supernatant was removed and the cultured solution was washed with an FBS-free RPMI-1640 medium, followed by adding 100 μ L of sample solution and reacting the mixture thereof for 6 hours in a 5% CO₂ thermostatic vessel at 37°C. The sample was prepared by preparing 1 mg/mL of EF-2001 solution using FBS-free RPMI-1640 Medium with L-glutamine and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture (R8758-500ML; SIGMA) and adjusting the solution by appropriately diluting it.

30 [0062]

After 6 hours, the supernatant was collected, and the product thereof was stored at -80°C until the TNF- α measurement. After the sample was appropriately diluted, the

concentration of TNF- α which was induced and produced was measured according to the protocol of "Mouse TNF- α Quantikine ELISA Kit". Figure 5 shows the result thereof.

[0063]

5 As shown in Figure 5, it was confirmed that addition of heat treatment (110°C) to the lactic acid bacterium EF-2001 strain resulted in an increase in the TNF- α production amount, which means that a product utilizing a heat-treated lactic acid bacterium EF-2001 strain has an increased TNF- α production amount. In combination with the results shown in Example 4 that a band of primers for detecting a long sequence disappears and a band of primers for detecting a short sequence remains as a result of giving a certain thermal history, the above result showed a possibility that confirming that a band of primers for detecting a long sequence disappears and a band of primers for detecting a short sequence remains may be an indicator as to whether a lactic acid bacterium EF-2001 strain with an increased TNF- α production amount has been obtained.

20 [0064]

(Note)

As described above, the present disclosure is exemplified by the use of its preferred embodiments. However, it is understood that the scope of the present disclosure should be interpreted solely based on the Claims. It is also understood that any patent, any patent application, and any references cited herein should be incorporated herein by reference in the same manner as the contents are specifically described herein. The present application claims priority to Japanese Patent Application No. 2021-21102 filed on February 12, 2021 with the Japan Patent Office, the content of which being incorporated by reference in the same manner as if the entirety thereof constitutes the content of the present application.

35 [Industrial Applicability]

[0065]

Since the method of the present invention can rapidly, simply, and specifically detect a lactic acid bacterium *Enterococcus faecalis* EF-2001 strain, said method can be expected to be widely applied to estimating of a gene involved in various activities of an EF-2001 strain, development of functional food or supplements using the gene, and the like.

[Sequence Listing Free Text]

10 [0066]

SEQ ID NO: 1: Forward primer in contig 1

SEQ ID NO: 2: Reverse primer (long) in contig 1

SEQ ID NO: 3: Reverse primer (short) in contig 1

SEQ ID NO: 4: Forward primer in contig 4

15 SEQ ID NO: 5: Reverse primer (long) in contig 4

SEQ ID NO: 6: Reverse primer (short) in contig 4

SEQ ID NO: 7: Forward primer in contig 7

SEQ ID NO: 8: Reverse primer (long) in contig 7

SEQ ID NO: 9: Reverse primer (short) in contig 7

20 SEQ ID NO: 10: Forward primer in contig 11

SEQ ID NO: 11: Reverse primer (long) in contig 11

SEQ ID NO: 12: Reverse primer (short) in contig 11

SEQ ID NO: 13: Forward primer in contig 13

SEQ ID NO: 14: Reverse primer (long) in contig 13

25 SEQ ID NO: 15: Reverse primer (short) in contig 13

SEQ ID NO: 16: Forward primer in contig 16

SEQ ID NO: 17: Reverse primer (long) in contig 16

SEQ ID NO: 18: Reverse primer (short) in contig 16

SEQ ID NO: 19: Forward primer in contig 22

30 SEQ ID NO: 20: Reverse primer (long) in contig 22

SEQ ID NO: 21: Reverse primer (short) in contig 22

SEQ ID NO: 22: Forward primer in contig 25

SEQ ID NO: 23: Reverse primer (long) in contig 25

SEQ ID NO: 24: Reverse primer (short) in contig 25

35 SEQ ID NO: 25: Forward primer in contig 31

SEQ ID NO: 26: Reverse primer (long) in contig 31
 SEQ ID NO: 27: Reverse primer (short) in contig 31
 SEQ ID NO: 28: Forward primer in contig 43
 SEQ ID NO: 29: Reverse primer (long) in contig 43
 5 SEQ ID NO: 30: Reverse primer (short) in contig 43
 SEQ ID NO: 31: Amplification product (long) of contig 1
 SEQ ID NO: 32: Amplification product (short) of contig 1
 SEQ ID NO: 33: Amplification product (long) of contig 4
 SEQ ID NO: 34: Amplification product (short) of contig 4
 10 SEQ ID NO: 35: Amplification product (long) of contig 7
 SEQ ID NO: 36: Amplification product (short) of contig 7
 SEQ ID NO: 37: Amplification product (long) of contig 11
 SEQ ID NO: 38: Amplification product (short) of contig 11
 SEQ ID NO: 39: Amplification product (long) of contig 13
 15 SEQ ID NO: 40: Amplification product (short) of contig 13
 SEQ ID NO: 41: Amplification product (long) of contig 16
 SEQ ID NO: 42: Amplification product (short) of contig 16
 SEQ ID NO: 43: Amplification product (long) of contig 22
 SEQ ID NO: 44: Amplification product (short) of contig 22
 20 SEQ ID NO: 45: Amplification product (long) of contig 25
 SEQ ID NO: 46: Amplification product (short) of contig 25
 SEQ ID NO: 47: Amplification product (long) of contig 31
 SEQ ID NO: 48: Amplification product (short) of contig 31
 SEQ ID NO: 49: Amplification product (long) of contig 43
 25 SEQ ID NO: 50: Amplification product (short) of contig 43
 SEQ ID NO: 51: Contig 1
 SEQ ID NO: 52: Contig 4
 SEQ ID NO: 53: Contig 7
 SEQ ID NO: 54: Contig 11
 30 SEQ ID NO: 55: Contig 13
 SEQ ID NO: 56: Contig 16
 SEQ ID NO: 57: Contig 22
 SEQ ID NO: 58: Contig 25
 SEQ ID NO: 59: Contig 31
 35 SEQ ID NO: 60: Contig 43

SEQ ID NO: 61: Forward primer of a positive control

SEQ ID NO: 62: Reverse primer of a positive control

[Claims]

[Claim 1]

A primer set for specifically detecting a nucleic acid derived from a lactic acid bacterium *Enterococcus faecalis* EF-2001 strain, the primer set comprising:

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 1 and an oligonucleotide comprising the base sequence of SEQ ID NO: 2 or 3;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 4 and an oligonucleotide comprising the base sequence of SEQ ID NO: 5 or 6;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 7 and an oligonucleotide comprising the base sequence of SEQ ID NO: 8 or 9;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 10 and an oligonucleotide comprising the base sequence of SEQ ID NO: 11 or 12;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 13 and an oligonucleotide comprising the base sequence of SEQ ID NO: 14 or 15;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 16 and an oligonucleotide comprising the base sequence of SEQ ID NO: 17 or 18;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 19 and an oligonucleotide comprising the base sequence of SEQ ID NO: 20 or 21;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 22 and an oligonucleotide comprising the base sequence of SEQ ID NO: 23 or 24;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 25 and an oligonucleotide comprising the base sequence of SEQ ID NO: 26 or 27; or

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 28 and an oligonucleotide comprising the base sequence of SEQ ID NO: 29 or 30.

[Claim 2]

A primer set for specifically detecting a nucleic acid derived from a lactic acid bacterium *Enterococcus faecalis* EF-2001 strain, the primer set selected from the group consisting of

(a) a primer set (1) for amplifying a part of the base sequence of SEQ ID NO: 51:

a forward primer CGAAAAGGATGTAGTCAGCGG (SEQ ID NO: 1); and

a reverse primer CCGAAGGCGAAACAGAGGAT (SEQ ID NO: 2),

(b) a primer set (2) for amplifying a part of the base sequence of SEQ ID NO: 51:

a forward primer CGAAAAGGATGTAGTCAGCGG (SEQ ID NO: 1); and

a reverse primer CCCAGACATAATCGCATGGC (SEQ ID NO: 3),

(c) a primer set (3) for amplifying a part of the base sequence of SEQ ID NO: 52:

a forward primer GCGGCTGCACAATTTATTGC (SEQ ID NO: 4); and

a reverse primer AGAATACTTGGGCGGTCGTG (SEQ ID NO: 5),

(d) a primer set (4) for amplifying a part of the base sequence of SEQ ID NO: 52:

a forward primer GCGGCTGCACAATTTATTGC (SEQ ID NO: 4); and

a reverse primer AATTCAGCTTCGCTAGATAAGGC (SEQ ID NO: 6),

(e) a primer set (5) for amplifying a part of the base sequence of SEQ ID NO: 53:

a forward primer CGCGTATGACTTGCAATCGA (SEQ ID NO: 7); and

a reverse primer AGGATTGTTTGACGGTGCAA (SEQ ID NO: 8),

(f) a primer set (6) for amplifying a part of the base sequence of SEQ ID NO: 53:

a forward primer CGCGTATGACTTGCAATCGA (SEQ ID NO: 7); and

a reverse primer ACATGAGATAGTTGGGGTAGACA (SEQ ID NO: 9),

(g) a primer set (7) for amplifying a part of the base sequence of SEQ ID NO: 54:

5 a forward primer CTTCAGAGAGCTGGGCGAAG (SEQ ID NO: 10); and

a reverse primer TACTTTTCTAGCTGCCCCGCC (SEQ ID NO: 11),

10 (h) a primer set (8) for amplifying a part of the base sequence of SEQ ID NO: 54:

a forward primer CTTCAGAGAGCTGGGCGAAG (SEQ ID NO: 10); and

a reverse primer GGGTTGTAGCCCTACCCGAT (SEQ ID NO: 12),

15 (i) a primer set (9) for amplifying a part of the base sequence of SEQ ID NO: 55:

a forward primer CGTAACGTGACATTGCGGAC (SEQ ID NO: 13); and

20 a reverse primer ATGCCAGTACGTCGCGTTAA (SEQ ID NO: 14),

(j) a primer set (10) for amplifying a part of the base sequence of SEQ ID NO: 55:

a forward primer CGTAACGTGACATTGCGGAC (SEQ ID NO: 13); and

25 a reverse primer CGATTGTCAACTAATTGTGCCGA (SEQ ID NO: 15),

(k) a primer set (11) for amplifying a part of the base sequence of SEQ ID NO: 56:

30 a forward primer CATGGCTTGCCGTTTCACAA (SEQ ID NO: 16); and

a reverse primer ACCGCAACAACCTACATACTACCA (SEQ ID NO: 17),

(l) a primer set (12) for amplifying a part of the base sequence of SEQ ID NO: 56:

35 a forward primer CATGGCTTGCCGTTTCACAA (SEQ ID NO:

16); and

a reverse primer ACCAAAAGGAACGCTACCAGT (SEQ ID NO: 18),

(m) a primer set (13) for amplifying a part of the base sequence of SEQ ID NO: 57:

a forward primer TCAGCATAATCCCCAGACGT (SEQ ID NO: 19); and

a reverse primer AATGAACGCCCTTCAGCAGA (SEQ ID NO: 20),

(n) a primer set (14) for amplifying a part of the base sequence of SEQ ID NO: 57:

a forward primer TCAGCATAATCCCCAGACGT (SEQ ID NO: 19); and

a reverse primer GGCTCCTCTACCTGAACAACT (SEQ ID NO: 21),

(o) a primer set (15) for amplifying a part of the base sequence of SEQ ID NO: 58:

a forward primer GCGTTCAAACCTGTTCTGGTGT (SEQ ID NO: 22); and

a reverse primer TACAAGGCTTGCGAGGTAGC (SEQ ID NO: 23),

(p) a primer set (16) for amplifying a part of the base sequence of SEQ ID NO: 58:

a forward primer GCGTTCAAACCTGTTCTGGTGT (SEQ ID NO: 22); and

a reverse primer GCTGCAATGGAAAGCAAATCG (SEQ ID NO: 24),

(q) a primer set (17) for amplifying a part of the base sequence of SEQ ID NO: 59:

a forward primer AGGCATATGGGTCATCTGCT (SEQ ID NO: 25); and

a reverse primer GAGCATCACAGAGCCTCGAA (SEQ ID NO: 26),

(r) a primer set (18) for amplifying a part of the base sequence of SEQ ID NO: 59:

a forward primer AGGCATATGGGTCATCTGCT (SEQ ID NO: 25); and

a reverse primer AGAGATTTTTCAGTATTGCTGGGT (SEQ ID NO: 27),

5 (s) a primer set (19) for amplifying a part of the base sequence of SEQ ID NO: 60:

a forward primer CGTTGGGTGTGCAGAAATGG (SEQ ID NO: 28); and

10 a reverse primer TGTACCGTCAACCTCGTTTCG (SEQ ID NO: 29), or

(t) a primer set (20) for amplifying a part of the base sequence of SEQ ID NO: 60:

a forward primer CGTTGGGTGTGCAGAAATGG (SEQ ID NO: 28); and

15 a reverse primer AACGGGTTGCGACTCTTTTT (SEQ ID NO: 30).

[Claim 3]

A kit for detecting a lactic acid bacterium *Enterococcus faecalis* EF-2001 strain, characterized by comprising the
20 primer set of claim 1 or 2.

[Claim 4]

A method for specifically detecting a nucleic acid derived from a lactic acid bacterium *Enterococcus faecalis* EF-2001 strain, the method comprising:

25 the step of amplifying a nucleic acid fragment using the primer set of claim 1 or 2; and

the step of detecting a nucleic acid fragment obtained in the amplification step.

[Claim 5]

30 A lactic acid bacterium composition comprising a nucleic acid fragment,

wherein the nucleic acid fragment is not amplified by one or more primer sets selected from the group consisting of:

35 a pair of an oligonucleotide comprising the base sequence

of SEQ ID NO: 1 and an oligonucleotide comprising the base sequence of SEQ ID NO: 2;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 4 and an oligonucleotide comprising the base sequence of SEQ ID NO: 5;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 7 and an oligonucleotide comprising the base sequence of SEQ ID NO: 8;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 10 and an oligonucleotide comprising the base sequence of SEQ ID NO: 11;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 13 and an oligonucleotide comprising the base sequence of SEQ ID NO: 14;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 16 and an oligonucleotide comprising the base sequence of SEQ ID NO: 17;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 19 and an oligonucleotide comprising the base sequence of SEQ ID NO: 20;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 22 and an oligonucleotide comprising the base sequence of SEQ ID NO: 23;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 25 and an oligonucleotide comprising the base sequence of SEQ ID NO: 26; and/or

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 28 and an oligonucleotide comprising the base sequence of SEQ ID NO: 29, and

wherein the nucleic acid fragment is amplified by one or more primer sets selected from the group consisting of:

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 1 and an oligonucleotide comprising the base sequence of SEQ ID NO: 3;

a pair of an oligonucleotide comprising the base sequence

of SEQ ID NO: 4 and an oligonucleotide comprising the base sequence of SEQ ID NO: 6;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 7 and an oligonucleotide comprising the base sequence of SEQ ID NO: 9;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 10 and an oligonucleotide comprising the base sequence of SEQ ID NO: 12;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 13 and an oligonucleotide comprising the base sequence of SEQ ID NO: 15;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 16 and an oligonucleotide comprising the base sequence of SEQ ID NO: 18;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 19 and an oligonucleotide comprising the base sequence of SEQ ID NO: 21;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 22 and an oligonucleotide comprising the base sequence of SEQ ID NO: 24;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 25 and an oligonucleotide comprising the base sequence of SEQ ID NO: 27; and/or

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 28 and an oligonucleotide comprising the base sequence of SEQ ID NO: 30.

[Claim 6]

The composition of claim 5, comprising a nucleic acid fragment which is not amplified by a primer set of an oligonucleotide comprising the base sequence of SEQ ID NO: 16 and an oligonucleotide comprising the base sequence of SEQ ID NO: 17 and is amplified by a primer set of an oligonucleotide comprising the base sequence of SEQ ID NO: 16 and an oligonucleotide comprising the base sequence of SEQ ID NO: 18.

[Claim 7]

The composition of claim 5 or 6, comprising a heat-treated lactic acid bacterium product resulting from subjecting a lactic acid bacterium *Enterococcus faecalis* EF-2001 strain to heat treatment at at least about 70°C.

[Claim 8]

The composition of any one of claims 5 to 7, comprising a heat-treated lactic acid bacterium product resulting from subjecting a lactic acid bacterium *Enterococcus faecalis* EF-2001 strain to heat treatment at at least about 90°C.

[Claim 9]

A method for manufacturing a lactic acid bacterium composition, the method comprising:

the step of heating a lactic acid bacterium *Enterococcus faecalis* EF-2001 strain to obtain a plurality of lots of a heat-treated lactic acid bacterium product;

the step of extracting a nucleic acid fragment in one of the lots of the heat-treated lactic acid bacterium product;

the step of amplifying the nucleic acid fragment using at least one of the primer sets of claim 1 or 2;

the step of detecting a nucleic acid fragment obtained in the amplification step; and

the step of selecting a lot of the heat-treated lactic acid bacterium product in which the nucleic acid fragment has been detected.

[Claim 10]

The method of claim 9, wherein the heating is heating at at least about 70°C.

[Claim 11]

The method of claim 9 or 10, wherein the heating is heating at at least about 90°C.

[Claim 12]

The method of any one of claims 9 to 11, wherein the nucleic acid fragment is amplified by one or more primer sets selected from the group consisting of:

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 1 and an oligonucleotide comprising the base sequence of SEQ ID NO: 3;

5 a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 4 and an oligonucleotide comprising the base sequence of SEQ ID NO: 6;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 7 and an oligonucleotide comprising the base sequence of SEQ ID NO: 9;

10 a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 10 and an oligonucleotide comprising the base sequence of SEQ ID NO: 12;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 13 and an oligonucleotide comprising the base
15 sequence of SEQ ID NO: 15;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 16 and an oligonucleotide comprising the base sequence of SEQ ID NO: 18;

20 a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 19 and an oligonucleotide comprising the base sequence of SEQ ID NO: 21;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 22 and an oligonucleotide comprising the base sequence of SEQ ID NO: 24;

25 a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 25 and an oligonucleotide comprising the base sequence of SEQ ID NO: 27; and/or

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 28 and an oligonucleotide comprising the base
30 sequence of SEQ ID NO: 30, and

wherein the nucleic acid fragment is not amplified by one or more primer sets selected from the group consisting of:

a pair of an oligonucleotide comprising the base sequence
35 of SEQ ID NO: 1 and an oligonucleotide comprising the base

sequence of SEQ ID NO: 2;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 4 and an oligonucleotide comprising the base sequence of SEQ ID NO: 5;

5 a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 7 and an oligonucleotide comprising the base sequence of SEQ ID NO: 8;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 10 and an oligonucleotide comprising the base
10 sequence of SEQ ID NO: 11;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 13 and an oligonucleotide comprising the base sequence of SEQ ID NO: 14;

a pair of an oligonucleotide comprising the base sequence
15 of SEQ ID NO: 16 and an oligonucleotide comprising the base sequence of SEQ ID NO: 17;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 19 and an oligonucleotide comprising the base sequence of SEQ ID NO: 20;

20 a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 22 and an oligonucleotide comprising the base sequence of SEQ ID NO: 23;

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 25 and an oligonucleotide comprising the base
25 sequence of SEQ ID NO: 26; and/or

a pair of an oligonucleotide comprising the base sequence of SEQ ID NO: 28 and an oligonucleotide comprising the base sequence of SEQ ID NO: 29.

[Claim 13]

30 A polynucleotide characterized by being amplified by a primer set for detecting a lactic acid bacterium *Enterococcus faecalis* EF-2001 strain, wherein the polynucleotide is:

the polynucleotide set forth in SEQ ID NO: 31 which is amplified by a primer set consisting of a pair of an
35 oligonucleotide comprising the base sequence of SEQ ID NO:

1 and an oligonucleotide comprising the base sequence of SEQ ID NO: 2;

the polynucleotide set forth in SEQ ID NO: 32 which is amplified by a primer set consisting of a pair of an
5 oligonucleotide comprising the base sequence of SEQ ID NO: 1 and an oligonucleotide comprising the base sequence of SEQ ID NO: 3;

the polynucleotide set forth in SEQ ID NO: 33 which is amplified by a primer set consisting of a pair of an
10 oligonucleotide comprising the base sequence of SEQ ID NO: 4 and an oligonucleotide comprising the base sequence of SEQ ID NO: 5;

the polynucleotide set forth in SEQ ID NO: 34 which is amplified by a primer set consisting of a pair of an
15 oligonucleotide comprising the base sequence of SEQ ID NO: 4 and an oligonucleotide comprising the base sequence of SEQ ID NO: 6;

the polynucleotide set forth in SEQ ID NO: 35 which is amplified by a primer set consisting of a pair of an
20 oligonucleotide comprising the base sequence of SEQ ID NO: 7 and an oligonucleotide comprising the base sequence of SEQ ID NO: 8;

the polynucleotide set forth in SEQ ID NO: 36 which is amplified by a primer set consisting of a pair of an
25 oligonucleotide comprising the base sequence of SEQ ID NO: 7 and an oligonucleotide comprising the base sequence of SEQ ID NO: 9;

the polynucleotide set forth in SEQ ID NO: 37 which is amplified by a primer set consisting of a pair of an
30 oligonucleotide comprising the base sequence of SEQ ID NO: 10 and an oligonucleotide comprising the base sequence of SEQ ID NO: 11;

the polynucleotide set forth in SEQ ID NO: 38 which is amplified by a primer set consisting of a pair of an
35 oligonucleotide comprising the base sequence of SEQ ID NO:

10 and an oligonucleotide comprising the base sequence of
SEQ ID NO: 12;

the polynucleotide set forth in SEQ ID NO: 39 which is
amplified by a primer set consisting of a pair of an
5 oligonucleotide comprising the base sequence of SEQ ID NO:
13 and an oligonucleotide comprising the base sequence of
SEQ ID NO: 14;

the polynucleotide set forth in SEQ ID NO: 40 which is
amplified by a primer set consisting of a pair of an
10 oligonucleotide comprising the base sequence of SEQ ID NO:
13 and an oligonucleotide comprising the base sequence of
SEQ ID NO: 15;

the polynucleotide set forth in SEQ ID NO: 41 which is
amplified by a primer set consisting of a pair of an
15 oligonucleotide comprising the base sequence of SEQ ID NO:
16 and an oligonucleotide comprising the base sequence of
SEQ ID NO: 17;

the polynucleotide set forth in SEQ ID NO: 42 which is
amplified by a primer set consisting of a pair of an
20 oligonucleotide comprising the base sequence of SEQ ID NO:
16 and an oligonucleotide comprising the base sequence of
SEQ ID NO: 18;

the polynucleotide set forth in SEQ ID NO: 43 which is
amplified by a primer set consisting of a pair of an
25 oligonucleotide comprising the base sequence of SEQ ID NO:
19 and an oligonucleotide comprising the base sequence of
SEQ ID NO: 20;

the polynucleotide set forth in SEQ ID NO: 44 which is
amplified by a primer set consisting of a pair of an
30 oligonucleotide comprising the base sequence of SEQ ID NO:
19 and an oligonucleotide comprising the base sequence of
SEQ ID NO: 21;

the polynucleotide set forth in SEQ ID NO: 45 which is
amplified by a primer set consisting of a pair of an
35 oligonucleotide comprising the base sequence of SEQ ID NO:

22 and an oligonucleotide comprising the base sequence of SEQ ID NO: 23;

the polynucleotide set forth in SEQ ID NO: 46 which is amplified by a primer set consisting of a pair of an
5 oligonucleotide comprising the base sequence of SEQ ID NO: 22 and an oligonucleotide comprising the base sequence of SEQ ID NO: 24;

the polynucleotide set forth in SEQ ID NO: 47 which is amplified by a primer set consisting of a pair of an
10 oligonucleotide comprising the base sequence of SEQ ID NO: 25 and an oligonucleotide comprising the base sequence of SEQ ID NO: 26;

the polynucleotide set forth in SEQ ID NO: 48 which is amplified by a primer set consisting of a pair of an
15 oligonucleotide comprising the base sequence of SEQ ID NO: 25 and an oligonucleotide comprising the base sequence of SEQ ID NO: 27;

the polynucleotide set forth in SEQ ID NO: 49 which is amplified by a primer set consisting of a pair of an
20 oligonucleotide comprising the base sequence of SEQ ID NO: 28 and an oligonucleotide comprising the base sequence of SEQ ID NO: 29; or

the polynucleotide set forth in SEQ ID NO: 50 which is amplified by a primer set consisting of a pair of an
25 oligonucleotide comprising the base sequence of SEQ ID NO: 28 and an oligonucleotide comprising the base sequence of SEQ ID NO: 30.