

COMPOSITION FOR INHIBITING REDUCTION OF LACTIC ACID
BACTERIUM OF THE GENUS *LACTOBACILLUS* IN THE INTESTINE

CROSS-REFERENCE TO RELATED APPLICATIONS

5 [0001]

This application is based upon and claims the benefit of
priority from Japanese Patent Application No. 2016-202465, filed
on October 14, 2016; the entire contents of which are
incorporated herein by reference.

10

BACKGROUND OF THE INVENTION

[0002]

Field of the Invention

15 The present invention relates to a composition for inhibiting
reduction of a lactic acid bacterium of the genus *Lactobacillus* in
the intestine in a subject to whom an antimicrobial agent is
administered.

[0003]

Background Art

20 Many antimicrobial agents have been used for the purpose
of treatment of infection, prevention of bacterial infection, and the
like. Harmful effects of the use of antimicrobial agents include
deterioration of the intestinal environment due to intake of
antimicrobial agents. This is said to be due to the fact that intake
25 of antimicrobial agents reduces not only pathogenic
microorganisms but also useful bacteria such as bifidobacteria and
lactic acid bacteria, which are so-called beneficial bacteria, and
adversely affects the intestinal flora, namely the ecosystem of
intestinal bacteria. As a result, symptoms such as constipation,
30 diarrhea, nausea, and headache occur. As measures against such
harmful effects, simultaneous intake of live beneficial bacteria
themselves (probiotics) and/or simultaneous intake of components
that increase beneficial bacteria (prebiotics) are recommended
when antimicrobial agents are taken. Representative examples of
35 prebiotics include growth factors for intestinal beneficial bacteria,
such as oligosaccharides and dietary fibers, and supplements of

beneficial bacteria themselves, such as lactic acid bacteria and bifidobacteria, in the form of capsule.

[0004]

Among beneficial bacteria, *Lactobacillus* bacteria, one of
5 lactic acid bacteria, themselves have been widely used as bacterial
cells for probiotics. However, it has been reported that lactic acid
bacteria of the genus *Lactobacillus* are Gram-positive bacilli and
sensitive to antimicrobial agents, and thus they are markedly
reduced by administration of antimicrobial agents (for example,
10 Non Patent Document 1). Therefore, inhibiting reduction of lactic
acid bacteria of the genus *Lactobacillus* in the intestine in a subject
to whom an antimicrobial agent was administered is an important
issue.

[0005]

15 Meanwhile, recently a bifidogenic growth stimulator (BGS)
has been reported as a new component for prebiotics (Non Patent
Document 2). Specifically, Non Patent Document 2 has reported
that the supernatant filtrate of a culture of *Propionibacterium*
freudenreichii specifically proliferated bifidobacteria. However, it
20 has been also reported that a bifidogenic growth stimulator did not
substantially exert the growth-stimulating effect on intestinal
bacteria other than bifidobacteria including lactic acid bacteria (see
Table 1 of Non Patent Document 2). In addition, an intestinal
flora-improving effect in young livestock using a feed composition
25 containing a bifidogenic growth stimulator has been reported
(Patent Document 1). Specifically, Patent Document 1 has
reported that, when Profec (trade mark), a fermented whey
product by propionic acid bacteria, was taken by young livestock,
intestinal bifidobacteria were significantly increased and the
30 occupancy to the total number of bacteria was markedly increased.
On the other hand, it has been shown that lactic acid bacteria of
the genus *Lactobacillus* were not significantly increased (see Table
1 of Example 1 of Patent Document 1). Thus, there has been no
report on a component that significantly proliferates lactic acid
35 bacteria of the genus *Lactobacillus* in the intestine. Also, there
have been no reports on an attempt to inhibit reduction of lactic

acid bacteria of the genus *Lactobacillus* by antimicrobial agents or its effective method or composition.

Prior Art Document

5 Patent Document

[0006]

Patent Document 1: JP 4073922 B1

Non Patent Documents

[0007]

10 Non Patent Document 1: Annual report of the Yakult
Institute for Microbiological Research, No. 30, 1-6, 2012

Non Patent Document 2: Bioscience Microflora Vol.18(2),
73-80, 1999

15 SUMMARY OF THE INVENTION

[0008]

An object of the present invention is to provide a
composition capable of inhibiting reduction of lactic acid bacteria of
the genus *Lactobacillus* in the intestine in a subject to whom an
20 antimicrobial agent is administered and an inhibition method
thereof.

[0009]

The present inventors have recently found that, when
patients took a fermented whey product by propionic acid bacteria
25 before or during administration of an antimicrobial agent, it was
possible to significantly inhibit reduction of lactic acid bacteria of
the genus *Lactobacillus* in the intestine, compared with patients
who did not take a fermented whey product by the propionic acid
bacteria, in a clinical study in humans. The present invention is
30 based on the findings.

[0010]

According to the present invention, the following inventions
are provided.

(1) A composition for inhibiting reduction of lactic acid bacteria of
35 the genus *Lactobacillus* in the intestine in a subject to whom an
antimicrobial agent is administered, including a fermented whey

product by a propionic acid bacterium.

(2) The composition according to (1), wherein the propionic acid bacterium is *Propionibacterium freudenreichii*.

- (3) The composition according to (1) or (2), wherein the
5 antimicrobial agent is at least one selected from the group
consisting of a penicillin antimicrobial agent, a cephem
antimicrobial agent, a cephalosporin antimicrobial agent, a
carbapenem antimicrobial agent, a β -lactamase inhibitor, an
oxazolidinone antimicrobial agent, a new quilonone antimicrobial
10 agent, and a macrolide antimicrobial agent.
- (4) The composition according to any one of (1) to (3), wherein a
dose per day is 0.15 to 200 mg/kg body weight in terms of a dry
mass of a fermented whey product by the propionic acid bacterium.
- (5) The composition according to any one of (1) to (4), which is a
15 food composition or a pharmaceutical composition.
- (6) The composition according to any one of (1) to (5), which is in
a form of powder, liquid, or tablet.
- (7) Use of a fermented whey product by a propionic acid bacterium
for producing a composition for inhibiting reduction of lactic acid
20 bacteria of the genus *Lactobacillus* in the intestine in a subject to
whom an antimicrobial agent is administered.
- (8) A method for inhibiting reduction of lactic acid bacteria of the
genus *Lactobacillus* in the intestine in a subject to whom an
antimicrobial agent is administered, the method comprising
25 allowing the subject to take an effective amount of a fermented
whey product by a propionic acid bacterium.
- (9) The method according to (8), which is a method for improving
a disease or condition related to reduction of lactic acid bacteria of
the genus *Lactobacillus* in the intestine caused by administration of
30 an antimicrobial agent.
- (10) A fermented whey product by a propionic acid bacterium for
inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus*
in the intestine in a subject to whom an antimicrobial agent is
administered.
- 35 (11) Use of a fermented whey product by a propionic acid
bacterium for inhibiting reduction of lactic acid bacteria of the

genus *Lactobacillus* in the intestine in a subject to whom an antimicrobial agent is administered.

[0011]

According to the present invention, using a fermented whey product by a propionic acid bacterium, it is possible to effectively inhibit reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine in a subject to whom an antimicrobial agent is administered.

10 BRIEF DESCRIPTION OF THE DRAWINGS

[0012]

FIG. 1 is a graph showing changes in the total number of bacteria and the number of *Lactobacillus* bacteria in feces of tube feeding patients to whom an antimicrobial agent is administered (control group) (study of the control group). *; $p < 0.05$ (comparison with 0 day after administration).

FIG. 2 is a graph showing changes in the total number of bacteria and the number of *Lactobacillus* bacteria in feces of tube feeding patients to whom an antimicrobial agent is administered (composition treatment group) (study of the composition treatment group). *; $p < 0.05$ (comparison with 0 day after administration).

FIG. 3 is a graph comparing the total number of bacteria and the number of *Lactobacillus* bacteria in feces of the control group with those of the composition treatment group. *; $p < 0.05$.

FIG. 4 is a graph comparing the number of bifidobacteria in feces of the control group with that of the composition treatment group.

30 DETAILED DESCRIPTION OF THE INVENTION

[0013]

Composition for Inhibiting Reduction of Lactic Acid Bacteria of the Genus *Lactobacillus* in the Intestine

A composition of the present invention is a composition for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine in a subject to whom an antimicrobial agent is

administered, which comprises a fermented whey product by a propionic acid bacterium. It is a surprising fact that, when a fermented whey product by a propionic acid bacterium was used as an active ingredient, it was possible to significantly inhibit reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine in a subject before, during, or after administration of an antimicrobial agent although reduction of intestinal bifidobacteria was not inhibited.

[0014]

10 “Subject to whom an antimicrobial agent is administered” means a subject to whom an antimicrobial agent will be administered (before administration), a subject to whom an antimicrobial agent is being administered (during administration), or a subject to whom an antimicrobial agent was administered
15 (after administration), preferably a subject in whom lactic acid bacteria of the genus *Lactobacillus* in the intestine will be reduced, are being reduced, or were reduced by an antimicrobial agent. The subject is preferably a human, and may be other than a human (for example, livestock such as a horse and cattle, a rodent,
20 a pet such as a dog and a cat, and an ornamental animal kept in zoos, etc.). Such embodiments are also included in the present invention.

[0015]

 An antimicrobial agent of the present invention is not
25 particularly limited as long as it is an antimicrobial agent to which lactic acid bacteria of the genus *Lactobacillus* are sensitive, and preferably an antibiotic to which lactic acid bacteria of the genus *Lactobacillus* are sensitive. More specific examples thereof include a penicillin antimicrobial agent (e.g., amoxicillin (AMPC), ampicillin
30 (ABPC)), a cephem antimicrobial agent (e.g., cefpirome (CPR)), a cephalosporin antimicrobial agent (e.g., cefrazidime (CAZ)), a carbapenem antimicrobial agent (e.g., meropenem (MEPM)), a β -lactamase inhibitor, an oxazolidinone antimicrobial agent (e.g., linezolid (LZD)), a new quilonone antimicrobial agent (e.g.,
35 levofloxacin (LVFX), oazufloxacin (PZFX)), and a macrolide antimicrobial agent (e.g., azithromycin (AZM)), as well as a

lincomycin antimicrobial agent, an aminoglycoside antimicrobial agent, a tetracycline antimicrobial agent, a chloramphenicol antimicrobial agent, a peptide antimicrobial agent, a sulfa agent, an antituberculous agent, a quinolone antimicrobial agent, an antiviral agent, and an antifungal agent. The antimicrobial agent is preferably a penicillin antimicrobial agent (e.g., amoxicillin (AMPC), ampicillin (ABPC)), a cephem antimicrobial agent (e.g., cefpirome (CPR)), a cephalosporin antimicrobial agent (e.g., cefazidime (CAZ)), a carbapenem antimicrobial agent (e.g., meropenem (MEPM)), a β -lactamase inhibitor, an oxazolidinone antimicrobial agent (e.g., linezolid (LZD)), a new quilonone antimicrobial agent (e.g., levofloxacin (LVFX), oazufloxacin (PZFX)), and a macrolide antimicrobial agent (e.g., azithromycin (AZM)).

15 [0016]

A method for administration of the antimicrobial agent is not particularly limited as long as lactic acid bacteria of the genus *Lactobacillus* in the intestine show sensitivity, and the method can be appropriately selected from, for example, oral intake (or oral administration), enteral administration, percutaneous endoscopic gastrostomy, subcutaneous administration, intravenous administration, and the like, according to the subject to whom an antimicrobial agent is administered and the application (e.g., for treatment or for prevention). A method for using the antimicrobial agent can be appropriately selected by a person skilled in the art according to the disease and condition of the subject and the application, and can be appropriately selected by a person skilled in the art in accordance with the method mentioned in, for example, the JAID/JAS Guide to Clinical Management of Infectious Diseases 2014 (edited by the Committee for Preparation of the JAID/JAS Guide to/Guideline for Clinical Management of Infectious Diseases).

25 [0017]

Lactic acid bacteria of the genus *Lactobacillus* are Gram-positive bacilli, and are known as one of beneficial bacteria. Preferable specific examples of the lactic acid bacteria of the genus *Lactobacillus* include *Lactobacillus bulgaricus*, *L. johnsonii*, *L. casei*,

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L. paracasei, *L. gasseri*, *L. helveticus*, *L. rhamnosus*, *L. acidophilus*,
L. salivarius, *L. pentosus*, *L. plantarum*, *L. brevis*, *L. reuteri*, *L.*
sporogenes from the results of the clinical study in humans. Lactic
acid bacteria of the genus *Lactobacillus* are known to have
5 bioactivities such as, for example, an effect to regulate the
functions of the intestine (e.g., an effect to regulate the functions
of the intestine for diarrhea, constipation, and irritable bowel
syndrome), an immunostimulating effect, an effect to eradicate
Helicobacter pylori, an effect to inhibit allergy such as atopy,
10 pollinosis, and asthma, an effect to improve irritable bowel
syndrome (IBS), an effect to improve lipid metabolism or visceral
fat accumulation, an effect to inhibit risks of infection such as
respiratory diseases, an effect to improve inflammatory bowel
disease, an effect to improve endometriosis, an effect to suppress
15 cancers, an effect to improve stress and QOL, an effect to improve
diabetes mellitus, and an effect to improve oxidative stress. It is
desirable that a certain number of lactic acid bacteria of the genus
Lactobacillus exist in the intestine because of having the above
bioactivities.

20 [0018]

Lactic acid bacteria of the genus *Lactobacillus* in the
intestine mean lactic acid bacteria of the genus *Lactobacillus*
contained in the intestinal flora, and preferably are lactic acid
bacteria having a function to keep the intestinal environment in a
25 good balance. The number of lactic acid bacteria of the genus
Lactobacillus in the intestine is preferably $\text{Log}10^4$ or more, more
preferably $\text{Log}10^5$ or more, still more preferably $\text{Log}10^6$ or more,
and yet more preferably $\text{Log}10^7$ or more per 1 g of human feces.
[0019]

30 A fermented whey product by the propionic acid bacterium
used for the present invention is obtained by culturing the
propionic acid bacterium using a medium containing whey.
[0020]

A Propionic acid bacterium is Gram-positive anaerobes
35 bacterium belonging to the genus *Propionibacterium* and means a
microorganism that anaerobically produces propionic acid from

saccharides. They have been utilized as a starter for Emmental cheese from long ago, and the safety thereof has been confirmed. Examples of the propionic acid bacteria used for the present invention include *Propionibacterium freudenreichii*, *P. thoenii*, *P.*
 5 *acidipropionici*, *P. jensenii*, *P. avidum*, *P. acnes*, *P. lymphophilum*, and *P. granulosam*, and preferably *Propionibacterium freudenreichii*. These may be used alone, or two or more may be used in combination.

The propionic acid bacterium is a more preferably
 10 bifidogenic growth stimulator (BGS)-producing bacterium belonging to *Propionibacterium freudenreichii*, and examples thereof include *Propionibacterium freudenreichii* ET-3 (deposit number: FERM-BP-8115), *Propionibacterium freudenreichii* ATCC 6207, *Propionibacterium freudenreichii* ATCC 8262, *Propionibacterium*
 15 *freudenreichii* IFO 12424, *Propionibacterium freudenreichii* IFO 12426, and *Propionibacterium freudenreichii* IFO 12391, and still more preferably *Propionibacterium freudenreichii* ET-3 (deposit number: FERM-BP-8115).

[0021]

20 Whey is also called whey, and means a transparent, yellow-green, water-soluble component obtained by removing fat, casein, fat-soluble vitamins, etc., from milk. Examples of the whey used for the present invention include a stock solution of whey (sweet whey, acid whey, etc.), a concentrate thereof, a dried
 25 product thereof (whey powder), a frozen product thereof, etc., and a reconstituted solution thereof, and desalted whey, a whey protein concentrate (WPC), a whey protein isolate (WPI), α -lactalbumin (α -La), β -lactoglobulin (β -Lg), immunoglobulin, lactoferrin, etc., and a reconstituted solution thereof, and a whey protein
 30 degradation product thereof. The whey is preferably selected from a stock solution of whey, a whey powder, WPC, WPI, and a reconstituted solution thereof, and a protease degradation product thereof. These may be used alone, or two or more may be used in combination.

35 [0022]

The whey is contained at preferably 1 to 5% by mass, and

more preferably 1.5 to 4.0% by mass as an amount of protein in a medium. The sugar is contained at 1 to 4% by mass, and more preferably 1.5 to 3.0% by mass in a medium. A medium in which the amount of whey added (amount of whey contained) is adjusted so that the composition of the medium is such composition can be used. The sugar is preferably a monosaccharide in which glucose or lactose is treated with lactase. The medium may contain components other than whey as long as it is a medium in which Gram-positive anaerobes bacteria can be cultured. Here, the amount of proteins can be measured and calculated by the Kjeldahl method and the Lowry method. In the case of the Kjeldahl method, nitrogen contained in various proteins is measured, and the value is multiplied by a nitrogen-protein conversion factor (usually 6.25), thus enabling calculation of the amount of proteins.

[0023]

The medium used for the present invention is preferably a medium having pH of 5 to 8 (suitably 5.5 to 7.5) consisted of a protease degradation product of a 10% by mass reconstituted whey powder solution. The protease is not particularly limited as long as it can moderately degrade a whey protein. As the medium, it is possible to use, for example, a 10% by mass reconstituted whey powder solution degraded with Protease Amano A (Amano Pharmaceutical Co., Ltd.) at 50°C and pH of 7.0.

[0024]

The propionic acid bacterium can be cultured aerobically or anaerobically (e.g., under pressure of N₂ gas (0.5 kg/cm²)) in accordance with an ordinary method except that a medium containing whey is used. Usually, it is only necessary to prepare the temperature of medium from 20 to 40°C and take propionic acid bacteria as a starter such that the pH will be about 6 to 8 and the viable cell count will be 10⁷ to 10⁸ cfu/mL to perform liquid culture for 3 to 4 days. The concentration of propionic acid bacteria contained in the culture thus obtained reaches a concentration 5-fold higher than that of the starter.

[0025]

As the fermented whey product by the propionic acid

bacterium of the present invention, it is possible to use at least one of a culture obtained from culture of the propionic acid bacterium, isolated bacterium itself, a culture solution (culture supernatant), or a mixture thereof. The fermented whey product by the
5 propionic acid bacterium may be an extract, a lyophilized product, or a diluted product thereof. Furthermore, the fermented whey product by the propionic acid bacterium may be subjected to pasteurization treatment. Examples of the pasteurization process include low-temperature long-time pasteurization,
10 high-temperature long-time pasteurization, high-temperature short-time pasteurization, and ultrahigh-temperature pasteurization.

[0026]

According to one preferred embodiment of the present
15 invention, as the fermented whey product by the propionic acid bacterium of the present invention, it is possible to use a fermented whey product by the propionic acid bacterium obtained by culturing *Propionibacterium freudenreichii* ET-3 (deposit number: FERM BP-8115) with a 10% by mass reconstituted whey
20 powder solution, and more preferably, it is possible to use a fermented whey product by the propionic acid bacterium obtained by culturing *Propionibacterium freudenreichii* ET-3 at 35°C and pH of 6.0 for 75 hours in a medium consisted of a degradation product degraded with Protease Amano A (Amano Pharmaceutical Co., Ltd.)
25 of a 10% by mass reconstituted whey powder solution at 50°C and pH of 7.0. Such fermented whey product by the propionic acid bacterium may be synthesized or commercially available products may be used. Examples of the commercially available products include Profec (trade mark) (manufactured by Meiji Co., Ltd.) as a
30 raw material, which is approved as an involved component of foods for specified health use having a growth-stimulating effect on particularly bifidobacteria mainly among intestinal bacteria.

[0027]

According to one embodiment of the present invention, the
35 fermented whey product by the propionic acid bacterium of the present invention may contain a bifidogenic growth stimulator

(BGS). Examples of the bifidogenic growth stimulator include 1,4-dihydroxy-2-naphthoic acid (DHNA), 2-amino-3-carboxy-1,4-naphthoquinone (ACNQ), and an analog thereof, and the analog includes 1,4-naphthoquinone, 2-methyl-1,4-naphthoquinone, 4-amino-2-methyl-1-naphthol, and 2-amino-3-chloro-1,4-naphthoquinone. According to a preferred embodiment of the present invention, the fermented whey product by the propionic acid bacterium of the present invention contains 1,4-dihydroxy-2-naphthoic acid.

10 [0028]

The fermented whey product by the propionic acid bacterium used for the present invention itself can be used as the composition for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine.

15 [0029]

The composition for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine of the present invention may contain other orally acceptable components other than the fermented whey product by the propionic acid bacterium. The other components are not particularly limited as long as they do not interfere with the effect of the present invention, and examples thereof include additives such as excipients, stabilizers, antiseptics, wetting agents, emulsifiers, lubricants, sweeteners, colorants, fragrances, buffers, antioxidants, and pH adjusters.

25 According to a preferred embodiment of the present invention, the composition for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine of the present invention contains functional sugars (e.g., oligosaccharides and dietary fibers) serving as feeds for lactic acid bacteria of the genus *Lactobacillus*. By containing functional sugars serving as feeds for lactic acid bacteria of the genus *Lactobacillus*, the composition for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine becomes feeds for lactic acid bacteria of the genus *Lactobacillus* remaining in the intestine, thus enabling stimulation of growth.

35 [0030]

The form of the composition for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine of the present invention is not particularly limited, and may be any of solid (powder, tablet, etc.), liquid (drink form, etc.), and semiliquid, and preferably powder, tablet, or liquid. Examples of commercially available products in such form include Meiji B.G.S. POWDER (manufactured by Meiji Co., Ltd.), which is a powder, Onakakatsuryoku Tablet (manufactured by Meiji Co., Ltd.), which is a tablet, and YH-Flore (manufactured by Meiji Co., Ltd.), which is a liquid.

[0031]

According to a preferred embodiment of the present invention, the composition for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine of the present invention contains 1,4-dihydroxy-2-naphthoic acid, proteins, carbohydrates (e.g., sugars, dietary fibers), and in some cases, vitamins and/or minerals (e.g., sodium, calcium, phosphorus, magnesium, potassium). According to a preferred embodiment, 0.01 to 1000 μg , more preferably 0.1 to 100 μg , and still more preferably 0.5 to 50 μg of 1,4-dihydroxy-2-naphthoic acid is contained in 1.5 g of the composition for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine of the present invention.

[0032]

When the composition for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine of the present invention is taken, it is possible to effectively inhibit reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine in a subject to whom an antimicrobial agent is administered.

As mentioned in Example 1 of Examples, the effect to inhibit reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine can be evaluated in a comparative study in which the number of *Lactobacillus* bacteria in the intestine in a subject to whom an antimicrobial agent is administered is determined and comparison is performed between the group in which the composition of the present invention is taken and the group in

which the composition of the present invention is not taken, using the number of bacteria as an index. In the comparative study, it is considered that the composition of the present invention has a potent activity to inhibit reduction of lactic acid bacteria of the genus *Lactobacillus*, when the number of *Lactobacillus* bacteria is significantly higher in the group in which the composition of the present invention is taken than that in the group in which the composition of the present invention is not taken, and when the number of *Lactobacillus* bacteria is increased compared with before administration of an antimicrobial agent.

[0033]

The composition for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine of the present invention contains a fermented whey product by the propionic acid bacterium, and thus is compatible with various food materials and can be safely and easily taken.

[0034]

According to one embodiment of the present invention, the composition for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine of the present invention is preferably simultaneously or sequentially administered with live *Lactobacillus* bacteria. When the composition for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine of the present invention is simultaneously or sequentially taken with live *Lactobacillus* bacteria as probiotics, the number of lactic acid bacteria of the genus *Lactobacillus* in the intestine in a subject to whom an antimicrobial agent is administered can be increased, and the balance of the intestinal flora can be made better.

[0035]

According to one embodiment of the present invention, the composition for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine of the present invention is preferably composed of a form of daily intake unit so that a fermented whey product by the propionic acid bacteria is an effective amount for inhibiting reduction of the lactic acid bacteria

of the genus *Lactobacillus* in the intestine in a subject to whom an antimicrobial agent is administered. Regarding the above unit in the composition for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine of the present invention, the
5 daily intake (dose) is desirably 0.15 to 200 mg/kg body weight, preferably 0.65 to 120 mg/kg body weight, more preferably 1.15 to 70 mg/kg body weight, and still more preferably 1.5 to 20 mg/kg body weight in terms of the dry mass of the fermented whey product by the propionic acid bacteria. Here, the representative
10 body weight of a subject is estimated to be 60 kg.
[0036]

Furthermore, the composition for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine of the present invention is preferably provided in a form of being
15 packaged in one intake unit. Examples of a unit packaging form per one intake include a form specifying a constant amount using packs, containers, etc., and the surface thereof may have a label of components for one intake and a label of application, such as inhibition of reduction of lactic acid bacteria of the genus
20 *Lactobacillus* in the intestine. Suitable examples of the unit packaging form include supplements, pharmaceutical preparations, etc.
[0037]

A method for allowing a subject to take the composition for
25 inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine of the present invention (or a method for administering the composition for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine of the present invention to a subject) is not particularly limited as long as it does
30 not interfere with the effect of the present invention, and can be appropriately selected from oral intake (or oral administration), tube administration, enteral administration, percutaneous endoscopic gastrostomy, and the like, according to the subject and the application.

35 [0038]

The composition for inhibiting reduction of lactic acid

bacteria of the genus *Lactobacillus* in the intestine of the present invention may be taken by a subject before administration of an antimicrobial agent, may be taken by a subject during administration of an antimicrobial agent, or may be taken by a subject after administration of an antimicrobial agent. Preferably, the composition of the present invention is taken by a subject before administration of an antimicrobial agent. Considering that the balance of the intestinal flora is more improved by a fermented whey product by propionic acid bacteria when the period of intake of the composition of the present invention is longer, the period before administration of an antimicrobial agent is preferably 1 day, more preferably 3 days, and still more preferably 5 days before administration. When the composition of the present invention is taken by a subject during administration, the composition of the present invention may be simultaneously administered with an antimicrobial agent, or may be sequentially administered preferably within 72 hours and more preferably within 24 hours to a subject. [0039]

A plan for intake of the composition for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine of the present invention is not particularly limited, and can be appropriately set by a person skilled in the art according to the age, sex, symptom, and condition of the subject. For example, one oral intake unit may be administered 1 to 10 times daily, preferably 1 to 3 times daily, and more preferably once to a subject. The composition for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine of the present invention can be taken together with a diet. [0040]

According to one embodiment of the present invention, the composition for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine of the present invention can be used alone, and as long as a function to inhibit reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine in a subject to whom an antimicrobial agent is administered is exerted, it can also be contained as a raw material (material) or an additive in

various compositions for oral intake (for oral administration) such as foods and medical products, and it is possible to obtain a composition (e.g., a pharmaceutical composition and a food composition) having an effect to inhibit reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine in a subject to whom an antimicrobial agent is administered. Since the composition for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine of the present invention does not impair testes even after added to a diet, it can be easily taken by being added to an ordinary diet or added to a beverage.

[0041]

According to a preferred embodiment of the present invention, a food for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine in a subject to whom an antimicrobial agent is administered including a fermented whey product by the propionic acid bacterium is provided.

The form of "food" (food composition) is not particularly limited as long as it is a form that can be orally taken, such as solution, suspension, emulsion, powder, and solid molded product. Specific examples thereof include instant foods such as instant noodles, retort pouch foods, canned foods, microwave foods, instant soups and miso soups, and freeze dried foods; beverages such as soft drinks, fruit juice beverages, vegetable beverages, soymilk beverages, coffee beverages, tea beverages, powder beverages, concentrated beverages, and alcohol beverages; flour products such as breads, pastas, noodles, cake mix, and bread crumbs; confectionery such as candies, caramels, chewing gums, chocolates, cookies, biscuits, cakes, pies, snacks, crackers, Japanese-style confectionery, and dessert confectionery; seasonings such as sauces, tomato processed seasonings, flavor seasonings, cooked mix, sauces, dressings, soups, and curry and stew premix; oils and fats such as processed oils and fats, butter, margarine, and mayonnaise; dairy products such as milk beverages, fermented milk (yogurt, etc.), lactic acid bacteria beverages, natural cheeses, process cheeses, ice creams, and creams; processed agricultural products such as agricultural

canned foods, jams and marmalades, and cereals; frozen foods, and liquid diets.

[0042]

Foods also include foods classified as health foods,
5 functional foods, dietary supplements, foods for specified health use, foods for sick persons, infant formulas, powdered milk for expectant mothers or nursing mothers, or foods with a label stating that they are used for inhibition of reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine in a subject to whom an
10 antimicrobial agent is administered.

[0043]

According to another embodiment of the present invention, a medical product for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine in a subject to whom an
15 antimicrobial agent is administered including a fermented whey product by the propionic acid bacteria is provided. "Medical product" (pharmaceutical composition) is an agent prepared as an oral preparation or a parenteral preparation in accordance with an ordinary method by combining additives that are acceptable for
20 formulation. When the medical product is an oral preparation, it can be in the form of solid preparation, such as tablet, powder, fine granule, granule, capsule, pill, and controlled-release agent, and liquid preparation such as solution, suspension, and emulsion. When the medical product is a parenteral preparation, it can be in
25 the form of injection and suppository. In terms of easiness of intake by (administration to) a patient, the medical product is preferably an oral preparation. Here, examples of the additives that are acceptable for formulation include excipients, stabilizers, antiseptics, wetting agents, emulsifiers, lubricants, sweeteners,
30 colorants, fragrances, buffers, antioxidants, and pH adjusters. The medical product of the present invention can be used for treatment or prevention of diseases requiring inhibition of reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine in a subject to whom an antimicrobial agent is administered, specifically,
35 symptoms of adverse drug reactions due to administration of an antimicrobial agent, such as constipation, diarrhea, nausea, and

headache.

[0044]

The composition for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine of the present invention can be easily produced by mixing a fermented whey product by the propionic acid bacteria with other orally acceptable components as desired. Therefore, according to another embodiment of the present invention, a method for producing a composition for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine in a subject to whom an antimicrobial agent is administered, wherein an effective amount of a fermented whey product by propionic acid bacteria is contained in the composition, is provided.

[0045]

According to another embodiment of the present invention, a method for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine in a subject to whom an antimicrobial agent is administered, including allowing the subject to take an effective amount of a fermented whey product by propionic acid bacteria, is provided. Here, according to another preferred embodiment of the present invention, the method for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine excludes medical interventions for humans. Here, "medical interventions for humans" mean interventions to allow humans to take (to administer medical products to humans) that require prescriptions by a physician, etc. In the above embodiment, the subject is preferably a healthy individual.

[0046]

The method for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine of the present invention may be used for improving a disease or condition related to reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine in a subject to whom an antimicrobial agent is administered. Therefore, the method for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine of the present invention is a method for improving a disease or condition

related to reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine caused by administration of an antimicrobial agent, and more preferably a method for improving constipation, diarrhea, nausea, and associated headache. Here,
5 "improving" includes not only treating an established disease or condition but also preventing a disease or condition that may be established in the future.

[0047]

10 In the inhibition method, an effective amount of a fermented whey product by the propionic acid bacteria can be the same as one intake unit in the composition mentioned above.

[0048]

15 According to one embodiment of the present invention, use of a fermented whey product by the propionic acid bacteria for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine in a subject to whom an antimicrobial agent is administered is provided. According to one preferred embodiment of the present invention, use of the present invention is non-therapeutic use.

20 [0049]

According to one embodiment of the present invention, use of a fermented whey product by the propionic acid bacteria for producing a composition for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine in a subject to
25 whom an antimicrobial agent is administered is provided. According to one preferred embodiment of the present invention, the use is use for producing a food or medical product for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus*.

[0050]

30 According to one embodiment of the present invention, a fermented whey product by the propionic acid bacteria for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine in a subject to whom an antimicrobial agent is administered is provided.

35

EXAMPLES

[0051]

The present invention will be described in detail below by way of Examples, but the present invention is not limited to these Examples.

5 [0052]

Example 1: Preparation of Composition (Preparation of Fermented Whey Product by the Propionic Acid)

As a starter, *Propionibacterium freudenreichii* ET-3 (deposit number: FERM BP-8115) was taken so that the viable cell count
10 was 10^7 to 10^8 cfu/mL in a medium consisted of a degradation product obtained by degrading a 10% by mass reconstituted whey powder solution with Protease Amano A (Amano Pharmaceutical Co., Ltd.) at 50°C and pH of 7.0, and cultured at 35°C and pH of 6.0 for 75 hours. The culture supernatant thus obtained was
15 pasteurized and lyophilized. To 0.2 g of the lyophilized product, glucose and dietary fiber-containing dextrin were added to make a preparation with a total amount of 1.5 g. The composition contained 0.03 g of proteins, 0 g of lipids, 0.6 g of sugars as carbohydrates, 0.8 g of dietary fibers, 2.6 mg of sodium, 1.0 mg of
20 calcium, 1.3 mg of phosphorus, 0.2 mg of magnesium, and 15.8 mg of potassium.

[0053]

The preparation thus obtained was used as a composition of the present invention. The preparation was individually
25 aluminum-packaged in a unit of 1.5 g.

[0054]

Example 2: Clinical Study

(1) Study Subjects

The clinical study was conducted using two groups divided
30 into the control group and the composition treatment group. All study subjects in both groups are patients with severe diseases (patients with cerebral hemorrhage, trauma, or cerebral infarction) who were transferred to the Osaka Mishima Emergency Critical Care Center. The subjects were managed by tube feeding during
35 hospitalization in principle, and a tube was inserted from the nasal cavity and placed in the stomach. In principle, administration of

an antimicrobial agent was initiated within 1 weeks after initiation of the study. An antimicrobial agent selected from the antimicrobial agents listed in Table 1 below was administered according to the disease and condition of the patient in accordance with the method mentioned in the JAID/JAS Guide to Clinical Management of Infectious Diseases 2014 (edited by the Committee for Preparation of the JAID/JAS Guide to/Guideline for Clinical Management of Infectious Diseases).

[0055]

10 [Table 1]

Type of antimicrobial agents
SBT/ABPC, CAZ, MEPM, ABPC, CAZ, TAZ/PIPC, CEZ, CAZ · LZD, PZFX, AZM, CPR, PCG, oral LVFX, LVFX, CAZ·TEIC

SBT: sulbactam, ABPC: ampicillin, CEZ: cefazolin, CAZ: ceftazidime, MEPM: meropenem, TAZ: tazobactam, PIPC: piperacillin, LZD: linezolid, PZFX: pazufloxacin, AZM: azithromycin, PCG: penicillin G, CPR: cefpirome, LVFX: levofloxacin

[0056]

Control Group and Composition Treatment Group

15 In the control group, a liquid diet was administered for 2 weeks. As the liquid diet, at least one type selected from two types of Meibalance HP or MEIN (Meiji Co., Ltd.) was used. These do not contain a component that affects intestinal bacterial flora, particularly a component that reduces lactic acid bacteria of the genus *Lactobacillus*. Meanwhile, in the composition treatment group, a liquid diet to which the composition obtained in Example 1 (2 packets/day (3 g/day)) was added was administered for 2 weeks. The control group included 46 subjects and the composition treatment group included 14 subjects. Since 1 subject in the control group did not receive an antimicrobial agent during the 2-week study period, analysis was performed by excluding this 1 subject. Therefore, the number of subjects included in the analysis was 45 in the control group and 14 in the composition treatment group.

30 [0057]

The results of the patient characteristics and the number of days of administration of an antimicrobial agent in the control group and the composition treatment group analyzed are shown in Table 2 below.

5 [0058]

[Table 2]

Table 2 Patient characteristics and the number of days of administration of an antimicrobial agent

	Control group	Composition treatment group	
Number of subjects	45	14	-
Sex ratio (male/female)	24/21	7/7	N.S.
APACHE II* ¹	19.7±7.7	19.8±6.1	N.S
Number of days of administration of an antimicrobial agent (day)	11.5±3.2	11.8±2.9	N.S

*1: Acute Physiology and Chronic Health Evaluation (APACHE) II score

10

This is a prognosis prediction method consisting of APACHE scores and a part that calculates the expected hospital mortality for objectively evaluating the severity of the condition of patients. The higher the score is, the higher the severity is determined to be.

15

[0059]

As shown in Table 2, the number of days of administration of an antimicrobial agent was 11.5 ± 3.2 days in the control group and 11.8 ± 2.9 days in the composition treatment group, showing almost no difference between both groups. The sex ratio and APACHE II also showed almost no difference.

20

[0060]

(2) Analysis of Bacterial Flora in Feces

Collection of Feces

25

In both of the control group and the composition treatment group, feces were collected before initiation of the study (administration of a liquid diet) (0 day), 3 days after initiation of the study, 7 days after initiation of the study (1 week), and 14

days after initiation of the study (2 weeks).

[0061]

Analysis of Bacterial Flora in Feces

The collected feces were immediately stored at -20°C or less
 5 after collection. From feces of patients, bacterial flora DNA in
 feces was extracted using Multi-Beads Shocker (Yasui Kikai
 Corporation) according to the method of QIAamp DNA stool Mini Kit
 (QIAGEN). Regarding real time PCR reaction, the number of lactic
 acid bacteria of the genus *Lactobacillus*, the number of
 10 bifidobacteria, and the total number of bacteria were determined
 with ABI 7300 Real Time PCR System (Applied Biosystems), using
 QuantiTect SYBR Green RT-PCR (QIAGEN) and the primers
 mentioned in Table 3 below at 95°C for 15 seconds -> (94°C for 15
 seconds -> annealing temperature for each primer for 30 seconds
 15 -> 72°C for 30 seconds) × 30 to 55 cycles. The number of
 bacteria was expressed as a logarithmic value of the number of
 bacteria per 1 g of feces.

[0062]

[Table 3]

Real time PCR primer

Target organism		Sequence (5' to 3')	Annealing temperature (°C)
<i>Lactobacillus</i>	Forward	CTTGTACACACCGCCCGTCA	57
	Reverse	CTCAAACTAAACAAAGTTTC	
<i>Bifidobacterium</i>	Forward	CTCCTGGAAACGGGTGG	60
	Reverse	GGTGTTCCTCCCGATATCTACA	
Total bacteria	Forward	ACTCCTACGGGAGGCAGCAGT	60
	Reverse	GTATTACCGCGGCTGCTGGCAC	

20

[0063]

Before-and-after comparison in each group was analyzed by
 paired t-test. Analysis between two groups of the control group
 25 and the composition treatment group was evaluated by comparing
 the study results of the control group with those of the composition
 treatment group, and was performed by Students' t-test (equal
 variance) or Welch test (unequal variance).

[0064]

Results are shown in FIGS. 1 to 4.

FIG. 1 is a graph showing changes in the total number of bacteria and the number of *Lactobacillus* bacteria in feces in the control group. In the control group in FIG. 1, both of the total number of bacteria and the number of *Lactobacillus* bacteria were significantly reduced from 3 days after initiation of the study compared with at the time of initiation of the study.

FIG. 2 shows changes in the total number of bacteria and the number of *Lactobacillus* bacteria in feces in the composition treatment group. The composition treatment group in FIG. 2 showed no significant reduction in the number of *Lactobacillus* bacteria during the study period compared with before initiation of the study. On the other hand, the total number of bacteria was significantly reduced 7 days after initiation of the study compared with before initiation of the study, but no significant differences were observed 3 days and 14 days after initiation of the study.

FIG. 3 compares the total number of bacteria and the number of *Lactobacillus* bacteria in feces in the control group with those in the composition treatment group. The number of *Lactobacillus* bacteria in FIG. 3 showed almost no difference between the control group and the composition treatment group at the time of initiation of the study, but from 3 days after initiation of the study, the number of *Lactobacillus* bacteria was significantly reduced in the control group compared with the composition treatment group. On the other hand, there was no significant change in the total number of bacteria during the study period between both groups (FIG. 3). Thus, it was confirmed that administration of an antimicrobial agent reduces the number of *Lactobacillus* bacteria in the intestine, while administration of the composition of the present invention before or during administration of an antimicrobial agent inhibits reduction in the number of *Lactobacillus* bacteria in the intestine.

FIG. 4 is a graph comparing the number of bifidobacteria in feces in the control group with that in the composition treatment group. The number of bifidobacteria in FIG. 4 was significantly

- reduced from 3 days after initiation of the study compared with at the time of initiation of the study in both of the control group and the composition treatment group. Comparison of the number of bifidobacteria in the control group with that in the composition
- 5 treatment group showed no significant change between both groups.

CLAIMS

1. A composition for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine in a subject to whom an antimicrobial agent is administered, comprising a fermented whey product by a propionic acid bacterium.
2. The composition according to claim 1, wherein the propionic acid bacterium is *Propionibacterium freudenreichii*.
3. The composition according to claim 1 or 2, wherein the antimicrobial agent is at least one selected from the group consisting of a penicillin antimicrobial agent, a cephem antimicrobial agent, a cephalosporin antimicrobial agent, a carbapenem antimicrobial agent, a β -lactamase inhibitor, an oxazolidinone antimicrobial agent, a new quilonone antimicrobial agent, and a macrolide antimicrobial agent.
4. The composition according to any one of claims 1 to 3, wherein a dose per day is 0.15 to 200 mg/kg body weight in terms of a dry mass of a fermented whey product by the propionic acid bacterium.
5. The composition according to any one of claims 1 to 4, which is a food composition or a pharmaceutical composition.
6. The composition according to any one of claims 1 to 5, which is in a form of powder, liquid, or tablet.
7. Use of a fermented whey product by a propionic acid bacterium for producing a composition for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine in a subject to whom an antimicrobial agent is administered.
8. A method for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine in a subject to whom an

antimicrobial agent is administered, the method comprising allowing the subject to take an effective amount of a fermented whey product by a propionic acid bacterium.

9. The method according to claim 8, which is a method for improving a disease or condition related to reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine caused by administration of an antimicrobial agent.

10. A fermented whey product by a propionic acid bacterium for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine in a subject to whom an antimicrobial agent is administered.

11. Use of a fermented whey product by a propionic acid bacterium for inhibiting reduction of lactic acid bacteria of the genus *Lactobacillus* in the intestine in a subject to whom an antimicrobial agent is administered.