

COMPOSITION FOR IMPROVING ORAL CAVITY ENVIRONMENT

CROSS-REFERENCE TO RELATED APPLICATION

[0001]

The present application enjoys the benefit of priority from the prior Japanese Patent Application No. 2016-59662 filed on March 24, 2016, the entire disclosure of which is incorporated herein by reference.

TECHNICAL FIELD

[0002]

The present invention relates to a composition for use in improving the oral cavity environment, and, more specifically, relates to a food composition for use in improving the oral cavity environment.

BACKGROUND ART

[0003]

In recent years, consumers are highly interested in oral care. Especially, with regard to commodity products targeting halitosis care, the market for oral refreshing confectioneries, in addition to dentifrices and mouth washes, is expanding. Typical oral refreshing confectioneries for suppression of halitosis include commodity products which contain a refreshing component such as mint flavor or menthol, provide temporary refreshing/invigorating feeling, and also provide a bodily perception as if halitosis is reduced.

[0004]

However, the bodily perception obtained by oral refreshing confectioneries described above is derived from transient invigorating feeling due to the refreshing component contained therein, and thus halitosis cannot be solved fundamentally. Even if oral bacteria can be topically or temporarily eliminated by using a conventional mouth wash comprising a bactericidal component, there is a risk that oral bacteria may be propagated/fixed from any other site, and, additionally, disturbance of stable bacterial flora by a bactericide may rather cause easy fixation of pathogenic bacteria (Non-Patent Document 1).

[0005]

On the other hand, it has been known that, in addition to pathogenic bacteria in the oral cavity such as halitosis-causing bacteria and periodontal disease-causing bacteria, bifidobacteria also live as oral indigenous bacteria (probiotic bacteria) in the oral cavity. However, the influence of the proliferation of bifidobacteria in the oral cavity on the oral cavity environment has not been reported so far.

Reference List

Non-Patent Documents

[0006]

Non-Patent Document 1: Tomotari Mitsuoka: Probiotics·Prebiotics·Biogenics, Japan Bifidus Foundation, 2011, 263-265

SUMMARY OF INVENTION

[0007]

An object of the present invention is to provide a novel composition for use in improving the oral cavity environment.

[0008]

The present inventors have found that a fermentation product of propionic acid bacterium promotes the proliferation of human oral cavity-derived Bifidobacterium bacteria; that the proliferation of Bifidobacterium bacteria in the oral cavity is actually promoted by feeding to a subject a tablet comprising the fermentation product of propionic acid bacterium; and further that the feeding of a tablet containing the fermentation product of propionic acid bacterium suppresses halitosis and reduces the number of halitosis-causing bacteria. The present invention is based on these findings.

[0009]

The present invention provides the following inventions.

[1] A composition for use in improving the oral cavity environment and an agent for improving the oral cavity environment (hereinafter referred to as the “composition and agent according to the present invention”), each comprising a fermentation product of propionic acid bacterium as an active ingredient.

[2] The composition and agent according to [1], wherein the propionic acid bacterium is *Propionibacterium freudenreichii*.

[3] The composition and agent according to [1] or [2], wherein the fermentation product is a whey fermentation product.

[4] The composition and agent according to any one of [1] to [3], wherein the oral cavity environment is improved by increasing Bifidobacterium bacteria.

[5] The composition and agent according to any one of [1] to [4], which is used for suppressing halitosis.

[6] The composition and agent according to any one of [1] to [5], which are each a food composition.

[7] The composition and agent according to any one of [1] to [6], which are in the form of a tablet.

[8] The composition and agent according to any one of [1] to [7], which are in the form of a package suitable for single feeding.

[9] The composition and agent according to any one of [1] to [8], which are in the form of a package suitable for multiple feeding.

[10] Use of a fermentation product of propionic acid bacterium for the manufacture of a composition for use in improving the oral cavity environment or a composition for use in suppressing halitosis.

[11] A method for improving the oral cavity environment and a method for suppressing halitosis, each comprising feeding or administering an effective amount of a fermentation product of propionic acid bacterium to a subject in need of improvement in oral cavity environment or suppression of halitosis.

[0010]

The present invention can provide a composition for use in improving the oral cavity environment and an agent for improving the oral cavity environment, each comprising a fermentation product of propionic acid bacterium as an active ingredient. The active ingredient of the composition and agent according to the present invention is a fermentation product by a natural microorganism conventionally used in the manufacture of a food, and the fermentation product can also be mass-produced because microorganisms are used. Therefore, the composition and agent according to the present invention can be said to be very attractive techniques from the viewpoints of safety and cost.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011]

Fig. 1 shows a change in number of bifidobacteria in the oral cavity between before and after feeding when a tablet containing a whey fermentation product of propionic acid bacterium is fed to a subject.

Fig. 2 shows changes in hydrogen sulfide concentration (A) and methyl mercaptan concentration (B) when a tablet containing a whey fermentation product of propionic acid bacterium is fed to a subject.

Fig. 3 shows a change in number of halitosis-causing bacteria, *Fusobacterium nucleatum*, when a tablet containing a whey fermentation product of propionic acid bacterium is fed to a subject.

DETAILED DESCRIPTION OF THE INVENTION

[0012]

The composition and agent according to the present invention each comprise a fermentation product of propionic acid bacterium as an active ingredient. The

fermentation product of propionic acid bacterium used in the present invention is obtained by culturing a propionic acid bacterium, and examples of nutrient sources for a medium used in the culturing step include dairy products, yeast extract, soy bean extract and enzyme-treated products thereof. A medium containing a proteolytic enzyme-treated product of dairy product is preferred as a culture medium. A medium containing a carbon source is preferred, and the carbon source in the present invention refers to a carbon source which can be utilized by propionic acid bacteria. Examples of the carbon source which can be utilized by propionic acid bacteria include lactose, glucose, lactic acid, glycerol, gluten and cellulose, and lactose is especially preferred. The carbon source content of the medium before the initiation of culture is preferably 4 to 8% by mass, more preferably 4 to 7% by mass, especially preferably 4 to 6.7% by mass. Examples of the medium containing lactose selected as the carbon source from among these substances include whey powder, casein, skim milk powder, whey protein concentrates having a lactose content reduced by dialysis treatment of whey, or whey protein isolates obtained by isolating a lactose content with higher purity. These substances can also be used as they are or through protease treatment, and the medium can be prepared by adding a peptone such as yeast extract or TrypticaseTM, an appropriate amount of saccharides such as a monosaccharide and/or a disaccharide which can serve as the carbon source that can be utilized by propionic acid bacteria, such as glucose, lactose or a lactase-treated product of lactose, lactic acid, glycerol, gluten, cellulose, a mineral such as whey mineral, and, optionally, an animal/plant food that abundantly comprises a mineral, such as oyster or ginger, or an extract thereof.

[0013]

Examples of the dairy products used as the nutrient source for the culture medium include raw milk, concentrated milk, whole milk powder, skim milk, skim milk powder, milk protein concentrates (MPC), casein, whey, concentrated whey, whey protein concentrates (WPC), whey protein isolates (WPI) and whey powder. Among these, whey, concentrated whey and whey protein concentrates are preferred. In the present invention, the fermentation product of propionic acid bacterium cultured by using a proteolytic enzyme-treated product of whey, concentrated whey or whey protein concentrate as the nutrient source for the medium is referred to as the “whey fermentation product of propionic acid bacterium.”

[0014]

Examples of the propionic acid bacteria which can be used in the present invention include *Propionibacterium*, *Propionimonas*, *Propionifera*, *Propionimicrobium* and *Propionivibrio* bacteria, but are not limited thereto, and preferred bacteria are bacteria belonging to the genus *Propionimicrobium*. Examples of *Propionibacterium* bacteria include propionic acid bacteria such as

Propionibacterium freudenreichii, *Propionibacterium thoenii*, *Propionibacterium acidipropionici*, *Propionibacterium jensenii*, *Propionibacterium avidum*, *Propionibacterium acnes*, *Propionibacterium lymphophilum* and *Propionibacterium granulosam*. Among these bacteria, *Propionibacterium freudenreichii* is more preferred, and, especially, *Propionibacterium freudenreichii* IFO12424, *Propionibacterium freudenreichii* ATCC6207 and *Propionibacterium freudenreichii* ET-3 strain (FERM BP-8115) are preferred.

[0015]

The *Propionibacterium freudenreichii* ET-3 strain was deposited on August 9, 2001 at the International Patent Organism Depository of the National Institute of Advanced Industrial Science and Technology (Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki, Japan) and then transferred to the international depository authority, and Accession No. FERM BP-8115 was given thereto. Since the National Institute of Technology and Evaluation (IPOD, NITE) was succeeded in the patent microorganism depository services from the International Patent Organism Depository of the National Institute of Advanced Industrial Science and Technology (IPOD, AIST), as set forth in Budapest Notification No. 282

(http://www.wipo.int/treaties/en/notifications/budapest/treaty_budapest_282.html), the *Propionibacterium freudenreichii* ET-3 strain has currently been deposited under Accession No. FERM BP-8115 at the National Institute of Technology and Evaluation (IPOD, NITE) (#120, 2-5-8, Kazusakamatari, Kisarazu, Chiba).

[0016]

The fermentation product of propionic acid bacterium can be manufactured, for example, in the following manner.

- After inoculation of a culture solution containing the precultured propionic acid bacterium into another culture solution (1 to 3 L), main culture is carried out.

- During culture at 30 to 40°C, the culture solution is sparged with nitrogen gas at 0.1 to 1 L/min., and anaerobic culture is carried out at a stirring speed of 50 to 200 rpm for 140 to 160 hours. At this time, after culture for 1 to 2 day(s) after the propionic acid bacterium inoculation, a saccharide which is an optional medium compositional component may be continuously added at 0.5 to 20 mL/hr.

- Further, the anaerobic culture described above is preferably followed by aerobic culture. At this time, oxygen gas is similarly supplied, in place of nitrogen gas, to carry out the aerobic culture for 110 to 130 hours.

- The propionic acid bacterium is cultured in this manner to prepare a culture solution containing the propionic acid bacterium. Further, optionally, an antioxidant such as ascorbic acid is added to the culture solution, and the mixture can be used as a fermentation product of propionic acid bacterium.

[0017]

The fermentation product of propionic acid bacterium, which is the active ingredient of the present invention, is obtained by culturing a propionic acid bacterium and includes a propionic acid bacterium and a culture thereof. The resultant fermentation product of propionic acid bacterium may be used as it is, but may be concentrated, diluted or dried as appropriate.

[0018]

The fermentation product of propionic acid bacterium can also be manufactured according to a known manufacturing method as described in WO 2003/016544, WO 2005/099725, WO 2009/107380, WO 2012/132913 or the like. Alternatively, a commercial product comprising a fermentation product of propionic acid bacterium (for example, B.G.S. POWDER manufactured by Meiji Co., Ltd.) may be used.

[0019]

In the composition and agent according to the present invention, the fermentation product of propionic acid bacterium can be used singly or in combination with any other component. The content (in terms of solid content) of the fermentation product of propionic acid bacterium in the composition and agent according to the present invention is not particularly limited so long as the effect of improving the oral cavity environment is exerted, and can be defined as 10 to 100% by mass, preferably 10 to 50% by mass, more preferably 10 to 20% by mass. In the present invention, the agent according to the present invention can be an agent consisting of the fermentation product of propionic acid bacterium, and the composition according to the present invention can be a composition comprising the fermentation product of propionic acid bacterium and any other component.

[0020]

The composition and agent according to the present invention may comprise a saccharide which can be utilized by bifidobacteria in the oral cavity, in addition to the fermentation product of propionic acid bacterium. Examples of such a saccharide include monosaccharides and disaccharides such as glucose, galactose, fructose, sucrose, maltose, melibiose and lactose and oligosaccharides which selectively proliferate bifidobacteria such as raffinose, lactulose, fructooligosaccharide, isomaltooligosaccharide and galactooligosaccharide, and palatinose. The content (in terms of solid content) of the saccharide which can be utilized by bifidobacteria in the oral cavity in the composition and agent according to the present invention is not particularly limited so long as the effect of improving the oral cavity environment is exerted, and can be defined as 0 to 80% by mass, preferably 0 to 60% by mass.

[0021]

The composition according to the present invention is used in improving the oral cavity environment, and can be used as an agent for improving the oral cavity environment. The phrase “improving the oral cavity environment” is used in the meaning including the improvement in bacterial flora in the oral cavity, and encompasses, for example, the reduction in total number of pathogenic bacteria in the oral cavity such as halitosis-causing bacteria and periodontal disease-related bacteria and/or suppression of proliferation of such bacteria, and increase in total number of so-called probiotic bacteria such as bifidobacteria in the oral cavity and/or promotion of proliferation of such bacteria.

[0022]

Examples of the halitosis-causing bacteria include *Fusobacterium* bacteria such as *Fusobacterium nucleatum* and *Veillonella* bacteria such as *Veillonella dispar*.

[0023]

Examples of the periodontal disease-related bacteria include *Porphyromonas* bacteria such as *Porphyromonas gingivalis*, *Prevotella* bacteria such as *Prevotella intermedia* and *Prevotella nigrescens*, *Treponema denticola*, *Tannella forsythensis*, and *Actinobacillus actinomycetemcomitans*.

[0024]

Examples of the bifidobacteria in the oral cavity include *Bifidobacterium dentium*, *Bifidobacterium adolescentis*, *Bifidobacterium longum* and *Bifidobacterium urinalis*.

[0025]

According to the composition and agent of the present invention, it is possible to reduce the total number of pathogenic bacteria in the oral cavity such as halitosis-causing bacteria and periodontal disease-related bacteria or suppress the proliferation of such bacteria, and thus to prevent or treat oral diseases such as halitosis and periodontal disease. Briefly, the composition and agent according to the present invention can be used to suppress halitosis and used as an agent for suppressing halitosis. Here, the “halitosis” refers to a halitosis component contained in exhalation and perceived by a human, and is a generic name of malodor emitted from the oral cavity together with the exhalation. Examples of typical components of the malodor include volatile sulfur-containing compounds such as hydrogen sulfide and methyl mercaptan.

[0026]

The form and shape of the composition and agent according to the present invention are not particularly limited so long as the effect of improving the oral cavity environment is exerted, and may be either a solid form or a liquid, semi-liquid, gel,

paste or powder form. Examples of the composition and agent according to the present invention include food compositions such as tablets (for example, troche and chewable tablets), gum, gummi, candies, tablet confectioneries, beverages, yogurt and jelly and oral care compositions such as edible films, dentifrices, mouth washes, gargles, denture cleaning agents, orally disintegrating tablets and gel. Tablets, gum, gummi and candies are preferred from the viewpoints of portability and retentivity in the oral cavity. The composition and agent according to the present invention can contain any other known additive as an optional component, in addition to the fermentation product of propionic acid bacterium, according to the dosage form thereof.

[0027]

When the composition and agent according to the present invention is provided as a tablet, they can be manufactured by mixing, in addition to a fermentation product of propionic acid bacterium, one or more components selected from a saccharide, an excipient, a sweetener, an acidulant, a perfume, a colorant, a preservative, a stabilizer, an emulsifier, a vegetable extract, a fruit juice powder and the like according to a conventional method.

[0028]

The fermentation product of propionic acid bacterium has an excellent effect of improving the oral cavity environment, and thus can also be provided in such a state that it is added in foods taken daily and food taken as supplements. Briefly, the composition and agent according to the present invention can be provided as a food composition. When the composition and agent according to the present invention are provided as a food composition, they can be manufactured according to common procedures for manufacturing the foods, except that a fermentation product of propionic acid bacterium which is an active ingredient is added.

[0029]

When the fermentation product of propionic acid bacterium which is the active ingredient of the present invention is provided as a food composition, the food composition can be prepared by adding the fermentation product of propionic acid bacterium itself in a food. The food composition thus obtained is a food comprising an effective amount of the fermentation product of propionic acid bacterium. The phrase "comprising an effective amount" of the fermentation product of propionic acid bacterium refers to a content of the fermentation product of propionic acid bacterium to be taken within a predetermined range, when a normally-eaten amount of individual foods is fed. An embodiment in which a user adds the fermentation product of propionic acid bacterium itself, which is the active ingredient of the present invention, or a composition comprising the fermentation product to a food (for example, a

beverage or yogurt) to prepare a food composition also falls within the scope of the present invention.

[0030]

The composition and agent according to the present invention can be provided as a composition and an agent each comprising a single intake amount of the fermentation product of propionic acid bacterium which is effective in improving the oral cavity environment. The single intake amount of the fermentation product of propionic acid bacterium which is effective in improving the oral cavity environment and the number of times (i.e., frequency) of feeding per day can be determined depending on the age, symptom and bodily perception of a user. As an example, the single intake amount of the fermentation product of propionic acid bacterium which is effective in improving the oral cavity environment is 100 mg to 1,000 mg per time, preferably 100 mg to 300 mg per time in terms of solid content. The number of times of feeding per day, which is effective in improving the oral cavity environment, is 1 to 10 times per day, preferably 1 to 5 times per day.

[0031]

When the composition and agent according to the present invention are provided as a composition comprising a single intake amount of the fermentation product of propionic acid bacterium, the composition is desirably provided in the form of a unit package so that an effective single intake amount thereof can be taken. When the composition and agent according to the present invention are provided in the form of a package, it is desirable to indicate the intake amount on the package so as to ensure taking of the effective single intake amount thereof, or to provide a document which indicates the intake amount together. The composition and agent according to the present invention can exert a more excellent effect of improving the oral cavity environment through continuous feeding. For example, when the effective daily intake amount is provided in multiple package, a plurality of packages for the effective daily intake amount can also be provided as a set, for convenient feeding.

[0032]

The packaging form for providing the composition and agent according to the present invention is not particularly limited so long as the form defines a constant amount, and include containers capable of containing them such as wrapping papers, wrapping sheets, bags, soft bags, paper containers, cans, bottles, capsules and plastic cases.

[0033]

Users themselves can determine the intake amount of the composition and agent of the present invention according to their bodily perception to take them. Hence, the composition and agent according to the present invention can be provided

preferably in a form in which the intake amount can be adjusted according to the users' preference, i.e., in a form suitable for multiple feeding, and examples of such a form include tablets, powder, gum, gummi and candies. Examples of the packaging form suitable for multiple feeding include plastic cases, cans, bottles and wrapping papers. For example, the composition and agent according to the present invention can be provided as a plurality of tablets containing an intake amount for multiple (for example, 10 to 15 times) feeding. The form of the tablets in this case is preferably a form in which one or more tablet(s) (for example, 1 to 10 tablet(s)) can provide a single intake amount. The tablets containing an intake amount for multiple (for example, 10 to 15 times) feeding can take any of the packaging forms as described above, and are preferably contained in a plastic case for convenient storage, portability and easy feeding.

[0034]

The composition and agent according to the present invention are preferably continuously fed for 3 days or more in order to exert the effect better, and the administration or feeding period is more preferably 1 to 2 week(s), especially preferably 1 to 4 week(s). The term "continuously" as used herein means continuation of daily feeding in a determined amount. When the composition and agent according to the present invention are provided in a packaged form, an effective intake amount for a certain period (for example, 1 week) may be provided as a set for continuous feeding.

[0035]

According to another aspect of the present invention, there is provided a method for improving the oral cavity environment and a method for suppressing halitosis, each comprising feeding or administering an effective amount of a fermentation product of propionic acid bacterium to a subject in need of improvement of oral cavity environment or suppression of halitosis. The method for improving the oral cavity environment and method for suppressing halitosis according to the present invention can be carried out according to the descriptions concerning the composition and agent according to the present invention. The subject for feeding or administration is a mammal including a human, and is a human or non-human mammal.

[0036]

According to still another aspect of the present invention, there is provided use of a fermentation product of propionic acid bacterium for the manufacture of a composition for use in improving the oral cavity environment or a composition for suppressing halitosis. The present invention further provides use of a fermentation product of propionic acid bacterium for the manufacture of an agent for improving the

oral cavity environment or an agent for suppressing halitosis. The present invention further provides a fermentation product of propionic acid bacterium for use in improving the oral cavity environment or suppressing halitosis. The present invention further provides use of a fermentation product of propionic acid bacterium as an agent for improving the oral cavity environment or an agent for suppressing halitosis. These uses according to the present invention can be carried out according to the descriptions concerning the composition and agent of the present invention.

EXAMPLE

[0037]

Hereinafter, the present invention will be described in more detail by way of the following examples, but is not limited thereto.

[0038]

Example 1: Confirmation of effect of whey fermentation product of propionic acid bacterium on promoting proliferation of Bifidobacterium bacteria derived from human oral cavity

(1) Preparation of whey fermentation product of propionic acid bacterium

A whey fermentation product of propionic acid bacterium was prepared according to Example 1 of WO 2012/132913. Specifically, a *Propionibacterium freudenreichii* ET-3 strain (FERM BP-8115) was activated and precultured at 30°C under anaerobic conditions for 48 hours and further cultured at 30°C under anaerobic conditions for 72 hours. The resultant culture was centrifuged (conditions for centrifugation: 8,000 rpm, 4°C, 10 min.), and a culture supernatant was recovered and subjected to sterilization through a filter, thereby obtaining a sterilized culture supernatant. The culture supernatant was used as a whey fermentation product of propionic acid bacterium. It should be noted that the whey fermentation product of propionic acid bacterium has been confirmed to comprise 1,4-dihydroxy-2-naphthoic acid (DHNA) as the main active ingredient.

[0039]

(2) Test method

The fermentation product of propionic acid bacterium was added to 5 mL of an MN1 medium so that the amount of DHNA arrived at 25 or 50 ng/mL, and further various human oral cavity-derived *Bifidobacterium* bacteria or control bacteria (*Fusobacterium nucleatum* and *Porphyromonas gingivalis*), as indicated in Table 1, were each inoculated in an amount of 10 to 30 µL into the medium and cultured at 37°C. After culture for 15 to 21 hours, the absorbance was measured by using a spectrophotometer (manufactured by Beckman).

[0040]

The MN1 medium was prepared by dissolving 2 g of TrypticaseTM peptone, 0.75 g of PhytonTM Peptone, 1.25 g of yeast extract, 0.5 g of K₂HPO₄, 0.75 g of KH₂PO₄, 0.125 g of MgCl₂·6H₂O, 0.125 g of L-cysteine·hydrochloride, 2.5 mg of FeSO₄·7H₂O and 20 g of glucose in 1,000 mL of pure water, adjusting the pH of the solution to pH 6.5 with hydrochloric acid or sodium hydroxide and then subjecting it to autoclave treatment at 121°C for 15 minutes.

[0041]

The content of DHNA in the whey fermentation product was measured by using a high-speed liquid chromatograph (HPLC-UV) analyzer equipped with an ultraviolet spectrophotometric detector under the following conditions for HPLC analysis.

[0042]

<Conditions for HPLC analysis>

Detection wavelength: UV 254 nm

Column: CAPCELL PAK C18 MGII (i.d. 2.0×100 mm, 3 µm; manufactured by Shiseido Company, Limited)

Column temperature: 45°C

Mobile phase: Used was a solution obtained by adding 50 mg of dithiothreitol to 1 L of a liquid mixture containing methanol (for high-speed liquid chromatograph), purified water and acetic acid (mixing ratio (volume) = 50:49:1) for dissolution.

Flow rate: 0.2 mL/min.

Amount of sample solution to be injected: 5 µL

[0043]

(3) Results

Table 1 indicates the results.

[Table 1]

Table 1: Evaluation of effect of fermentation product of propionic acid bacteria on promoting proliferation of Bifidobacterium bacteria in human oral cavity

Bacterial species	Strain	Origin	Δ O.D. 550 nm (DHNA 25 ng/mL)	Δ O.D. 550 nm (DHNA 50 ng/mL)
<i>Bifidobacterium longum</i>	OLB6376	Healthy adult oral cavity	1.729	1.564
<i>Bifidobacterium adolescentis</i>	OLB6410	Healthy adult oral cavity	1.323	1.517
<i>Bifidobacterium dentium</i>	OLB6381	Reference strain (JCM1195T)	0.480	0.711

<i>Bifidobacterium adolescentis</i>	OLB6398	Healthy adult oral cavity	0.480	0.530
<i>Fusobacterium nucleatum</i>	OB7134	Reference strain (JCM8532T)	0.161	0.169
<i>Porphyromonas gingivalis</i>	OB7124	Reference strain (ATCC33277)	-0.053	-0.062

[0044]

From the results indicated in Table 1, it was found that the addition of the fermentation product of propionic acid bacterium promoted the proliferation of various *Bifidobacterium* bacteria derived from the human oral cavity. On the other hand, it was found that the proliferation of *Fusobacterium nucleatum* known as a halitosis-causing bacterium and *Porphyromonas gingivalis* known as a periodontal disease bacterium was not promoted. From the above results, it was suggested that the fermentation product of propionic acid bacterium does not promote the proliferation of pathogenic bacteria in the oral cavity and has the effect of promoting the proliferation of *Bifidobacterium* bacteria in the oral cavity.

[0045]

It should be noted that the present inventors have confirmed that, when *Bifidobacterium* bacteria in the oral cavity is cocultured with *Porphyromonas gingivalis* which is known as a periodontal disease bacterium, *Bifidobacterium* bacteria is proliferated, thereby suppressing the proliferation of *Porphyromonas gingivalis* (data not shown). Therefore, it is considered that the fermentation product of propionic acid bacterium promotes the proliferation of *Bifidobacterium* bacteria in the oral cavity, thereby making it possible to suppress the proliferation of pathogenic bacteria in the oral cavity such as *Porphyromonas gingivalis*.

[0046]

Example 2: Feeding test for whey fermentation product of propionic acid bacterium

(1) Test food

A tablet containing a powder comprising as the main ingredient a whey fermentation product of propionic acid bacterium was prepared according to an ordinary method and used as a test food. The tablet contained 95 mg of the whey fermentation product (prepared according to Example 1, item (1)) and 308 mg of glucose, in terms of solid content, per tablet (500 mg).

[0047]

(2) Test method

Seven adult men in their twenties to fifties were defined as subjects and took 3 tablets of the test food daily by sucking without biting (the intraoral retention time: approximately 3 to 5 minutes) for 2 weeks. Each one tablet was fed in the morning, in

the afternoon and at night (after 6PM), i.e., fed at three timings, in total. The feeding initiation date was defined as Day 1, and an examination on bacteria in saliva was conducted on the day before the feeding initiation date (Day 0), Day 7 of feeding and the feeding termination date (Day 14).

[0048]

The examination on bacteria involved collecting saliva immediately after rising and counting the number of Bifidobacterium bacteria in the saliva by a real-time PCR method.

[0049]

As for the obtained results, a Wilcoxon signed rank test was conducted on the number of bacteria before feeding (Day 0) and the number of bacteria after feeding (Days 7 and 14), and it was determined that there was a significant difference when the level of significance (p value) was less than 5% and that there was a significant difference tendency when the level of significance was less than 10%.

[0050]

(3) Results

The results are shown in Fig. 1. From the results shown in Fig. 1, it was found that the number of Bifidobacterium bacteria in saliva (in the oral cavity) increased upon feeding of the whey fermentation product. From the above results, it was confirmed that the proliferation of Bifidobacterium bacteria in the oral cavity could be promoted upon feeding of the whey fermentation product.

[0051]

Example 3: Evaluation of effect of feeding of tablet containing fermentation product of propionic acid bacterium on the improvement of the oral cavity environment

In this Example, the influence of the whey fermentation product of propionic acid bacterium on the oral cavity environment was evaluated.

[0052]

(1) Test food

A tablet containing a powder comprising as the main ingredient a whey fermentation product of propionic acid bacterium was prepared according to an ordinary method and used as a test food. The tablet contained 95 mg of the whey fermentation product (prepared according to Example 1, item (1)), in terms of solid content, per tablet (900mg).

[0053]

(2) Test method

Nine adult men in their twenties to fifties were defined as subjects and took 3 tablets of the test food daily by sucking without biting (the intraoral retention time: approximately 3 to 5 minutes) for 4 weeks. Each one tablet was fed in the morning, in

the afternoon and at night (after 6PM), i.e., fed at three timings, in total. The feeding initiation date was defined as Day 1, and halitosis measurement and an examination on bacteria in saliva were conducted on the day before the feeding initiation date (Day 0), Day 14 of feeding and the feeding termination date (Day 28).

[0054]

The halitosis measurement was conducted by measuring the concentrations of volatile sulfur compounds (hydrogen sulfide and methyl mercaptan) as a halitosis-causing substances in an oral gas by means of OralChroma (manufactured by Abilit-medical-and-environmental).

[0055]

The examination on bacteria involved collecting saliva immediately after rising and counting the number of *Fusobacterium nucleatum* known as halitosis-causing bacteria by a real-time PCR method.

[0056]

As for the obtained results, a Wilcoxon signed rank test was conducted on the number of bacteria before feeding (Day 0) and the number of bacteria after feeding (Days 14 and 28), and it was determined that there was a significant difference when the level of significance (p value) was less than 5% and that there was a significant difference tendency when the level of significance was less than 10%.

[0057]

(3) Results

The results of the halitosis measurement are shown in Fig. 2. From the results shown in Fig. 2, it was found that the concentrations of hydrogen sulfide and methyl mercaptan were significantly suppressed upon 4-week feeding of the tablets containing the whey fermentation product of propionic acid bacterium. From the above results, it was confirmed that halitosis was suppressed upon feeding of the whey fermentation product of propionic acid bacterium.

[0058]

The results of the examination on bacteria are shown in Fig. 3. From the results shown in Fig. 3, it was found that the number of *Fusobacterium nucleatum* in saliva (in the oral cavity) decreased upon 4-week feeding of the tablets containing the whey fermentation product of propionic acid bacterium.

[0059]

From the above results, it was confirmed that the oral cavity environment can be improved upon feeding of the fermentation product of propionic acid bacterium.

CLAIMS

1. Use of a composition comprising an effective amount of a whey fermentation product of a propionic acid bacterium as an active ingredient in suppressing halitosis in a subject in need thereof, wherein the whey fermentation product comprises 1,4-dihydroxy-2-naphthoic acid (DHNA).
2. The use according to claim 1, wherein the propionic acid bacterium is *Propionibacterium freudenreichii*.
3. The use according to claim 1 or 2, wherein the composition is a food composition.
4. The use according to any one of claims 1 to 3, wherein the composition is in the form of a tablet.
5. The use according to any one of claims 1 to 4, wherein the composition is in the form of a package suitable for single feeding.
6. The use according to any one of claims 1 to 4, wherein the composition is in the form of a package suitable for multiple feeding.
7. A method for suppressing halitosis, comprising feeding or orally administering an effective amount of a whey fermentation product of a propionic acid bacterium to a subject in need of suppressing halitosis, wherein the whey fermentation product comprises 1,4-dihydroxy-2-naphthoic acid (DHNA).
8. Use of a whey fermentation product of a propionic acid bacterium in preparing a composition or an agent comprising the whey fermentation product as an active ingredient for use in suppressing halitosis in a subject in need thereof, wherein the whey fermentation product comprises 1,4-dihydroxy-2-naphthoic acid (DHNA).
9. The use according to claim 8, wherein the composition or agent is in the form of a tablet.
10. The use according to claim 8 or 9, wherein the composition or agent is in the form of a package suitable for single feeding.

11. The use according to claim 8 or 9, wherein the composition or agent is in the form of a package suitable for multiple feeding.