Description

Title of Invention: LACTIC ACID BACTERIA HAVING PURINE UPTAKE ABILITY AND USE THEREOF

Technical Field

[0001]

The present invention relates to lactic acid bacteria having purine uptake ability and use thereof.

Background Art

[0002]

In recent years in Japan, along with dietary changes, the number of patients with gout or hyperuricemic individuals is annually increasing. Hyperuricemia involves decreased excretion of uric acid or production of excess uric acid, and an increase in serum uric acid level frequently induces gout, which develops acute arthritis with severe pain. Currently in Japan, the number of patients with gout is estimated to be one million, and the number of hyperuricemic individuals is estimated to be 10 million. At present, hyperuricemia is prevented or treated mainly by controlling the serum uric acid level through a combination of diet therapy, exercise therapy, and medication. In diet therapy, the intake of dietary purines that are ultimately degraded into uric acid is reduced by restricting the intake of calories. It is not necessarily easy, however, to continue strict restrictions on the intake of calories. Therefore, a more effective therapeutic method for gout or hyperuricemia is desired. With regard to gout or hyperuricemia, the development of food products effective for the prevention thereof or alleviation of the symptoms is also demanded.

[0003]

Microorganisms and fermented products showing efficacy in reducing the serum uric acid level in hyperuricemia have been reported (Patent Literatures 1 to 5). Patent Literature 1, for example, discloses that lactic acid bacteria have a high ability to degrade purine

nucleosides into purine bases. Patent Literatures 4 and 5, for example, disclose that lactic acid bacteria have an ability to degrade purines. These conventional microorganisms and fermented products having the effect of reducing the serum uric acid level have been believed to suppress purine absorption or promote purine excretion, by promoting the conversion of purine nucleosides into purine bases in the intestinal tract, that is, converting the purine nucleosides, which are readily absorbed from the intestinal tract, into the purine bases, which are poorly absorbed from the intestinal tract. However, few reports are available on human test results, and no efficient method of obtaining lactic acid bacteria having the effect of reducing the serum uric acid level is known.

Citation List

Patent Literature

[0004]

Patent Literature 1: JP Patent Publication No. 2008-005834 A

Patent Literature 2: International Publication No. WO 2011/102310

Patent Literature 3: International Publication No. WO 2004/112809

Patent Literature 4: International Publication No. WO 2009/069704

Patent Literature 5: JP Patent Publication No. 2013-048636 A

Summary of Invention

Technical Problem

[0005]

An object of the present invention is to provide an efficient method of obtaining (or selecting) lactic acid bacteria having the effect of reducing the serum uric acid level. Another object of the present invention is to provide a lactic acid bacterium having a purine uptake ability and use thereof.

Solution to Problem

[0006]

As a result of their extensive research to solve the above-described problems, the present inventors found that there are strains of lactic acid bacteria such as *Lactobacillus gasseri* bacteria having a purine uptake ability and high proliferative capacity in the presence of purines, and such lactic acid bacteria have a correlation between the purine uptake ability and the proliferative capacity in the presence of purines, and also found that the administration (ingestion) of such lactic acid bacteria can reduce the serum uric acid level, and thus, completed the present invention.

[0007]

That is, the present invention includes the following.

[1] A method of screening lactic acid bacteria comprising measuring an amount of uptake of purines by lactic acid bacteria in a medium comprising the purines, and selecting lactic acid bacteria having a purine capturing effect, using, as an index, the amount of uptake of purines.

[2] The method according to [1] above, wherein the purines in the medium are purine bases.

[3] The method according to [1] or [2] above, wherein the purines in the medium are labeled with a radioisotope.

[4] The method according to any of [1] to [3] above, comprising measuring an amount of proliferation of said lactic acid bacteria in the medium comprising the purines, and selecting lactic acid bacteria having a purine capturing effect, using, as an index, the amount of proliferation together with said amount of uptake of purines.

[5] The method according to any of [1] to [4] above, wherein the lactic acid bacteria are *Lactobacillus gasseri* bacteria.

[6] A lactic acid bacterium having a purine capturing effect obtainable by the method according to any of [1] to [5] above.

[7] An agent for capturing purines comprising as an active component the lactic acid bacteria having a purine capturing effect obtainable by the method according to any of [1] to [5] above.

[8] The agent for capturing purines according to [7] above, which is for use in reducing a serum uric acid level.

[9] The agent for capturing purines according to [7] or [8] above, wherein the lactic acid bacteria are *Lactobacillus gasseri* OLL2959 strain (Accession No: NITE BP-224).

[10] A food or beverage product or pharmaceutical preparation comprising the agent for capturing purines according to any of [7] to [9] above.

[11] The food or beverage product or pharmaceutical preparation according to [10] above, which is for use in reducing purines in an intestinal tract.

[12] The food or beverage product or the pharmaceutical preparation according to [10] or [11] above, which is for use in administration to a human subject having a serum uric acid level of 6 to 8 mg/dL.

[13] The food or beverage product or the pharmaceutical preparation according to any of [10] to [12] above, comprising 1 x 10^8 to 1 x 10^{10} cfu of the above-described lactic acid bacteria per dose.

Advantageous Effects of Invention

[0008]

According to the present invention, the lactic acid bacteria having the purine capturing effect and the effect of reducing the serum uric acid level can be efficiently obtained (selected). Through the use of the lactic acid bacteria or the capturing agent according to the present invention, purines can be efficiently captured.

The present specification includes the contents of Japanese Patent Application Nos. 2014-234050 and 2015-064201, from which the present application claims priority.

Brief Description of Drawings

[0009]

[Figure 1] Figure 1 is a graph showing a purine uptake ability of *Lactobacillus gasseri* OLL2959 strain.

[Figure 2] Figure 2 is a graph showing proliferative capacity of *Lactobacillus gasseri* OLL2959 strain in the presence of purines.

[Figure 3] Figure 3 shows adenine uptake abilities of a plurality of lactic acid bacterial strains.

[Figure 4] Figure 4 is a graph showing proliferative capacities of a plurality of lactic acid bacterial strains in the presence of adenine.

[Figure 5] Figure 5 is a graph showing changes over time in the serum uric acid level in a human subject to which *Lactobacillus gasseri* OLL2959 strain was continuously orally administered (orally ingested).

[Figure 6] Figure 6 is a graph showing a purine uptake ability of *Lactobacillus gasseri* OLL2959 strain in an animal to which *Lactobacillus gasseri* OLL2959 strain and purines were concurrently administered.

[Figure 7] Figure 7 is a graph showing the results of comparing adenine uptake abilities between lactic acid bacterial strains.

[Figure 8] Figure 8 is a graph showing the results of comparing proliferative capacities of the lactic acid bacterial strains in the presence of adenine between lactic acid bacterial strains.

[Figure 9] Figure 9 is a graph showing changes over time in turbidity (OD650) and the amount of nucleic acids in *Lactobacillus gasseri* OLL2959 strain.

[Figure 10] Figure 10 is a graph showing changes over time in the amount of the nucleic acids and the radioactivity in the nucleic acids from *Lactobacillus gasseri* OLL2959 strain.

[Figure 11] Figure 11 indicates graphs each showing the effect of reducing IMP absorption by OLL2959 strain in animals to which *Lactobacillus gasseri* OLL2959 strain and ^{14}C-IMP were administered. Radioactivity (dpm): mean ± SD. * $p<0.1$. A: 15 minutes after the administration, B: 30 minutes after the administration. Each bar shows, from the left, the results for the negative group (negative control group), the IMP group (IMP-treated group), and the IMP+OLL2959 group (IMP+OLL2959 strain-treated group).

[Figure 12] Figure 12 indicates graphs each showing the effect of reducing IMP absorption by OLL2959 strain in animals to which *Lactobacillus gasseri* OLL2959 strain and ^{14}C-IMP were administered. Radioactivity (dpm): mean ± SD. * $p<0.1$. A: 45 minutes after the administration, B: 60 minutes after the administration. Each bar shows, from the left, the

results for the negative group (negative control group), the IMP group (IMP-treated group), and the IMP+OLL2959 group (IMP+OLL2959 strain-treated group).

[Figure 13] Figure 13 indicates graphs each showing the effect of reducing hypoxanthine absorption by OLL2959 strain in animals to which *Lactobacillus gasseri* OLL2959 strain and ^{14}C-hypoxanthine were administered. Radioactivity (dpm): mean ± SD. ## $p<0.01$. A: 15 minutes after the administration, B: 30 minutes after the administration. Each bar shows, from the left, the results for the negative group (negative control group), the hypoxanthine group (hypoxanthine-treated group), and the hypoxanthine+OLL2959 group (hypoxanthine+OLL2959 strain-treated group).

[Figure 14] Figure 14 indicates graphs each showing the effect of reducing hypoxanthine absorption by OLL2959 strain in animals to which *Lactobacillus gasseri* OLL2959 strain and ^{14}C-hypoxanthine were administered. Radioactivity (dpm): mean ± SD. ## $p<0.01$, # $p<0.05$. A: 45 minutes after the administration, B: 60 minutes after the administration. Each bar shows, from the left, the results for the negative group (negative control group), the hypoxanthine group (hypoxanthine-treated group), and the hypoxanthine+OLL2959 group (hypoxanthine+OLL2959 strain-treated group).

[Figure 15] Figure 15 is a graph showing the hypoxanthine uptake ability (capturing effect) and inosine uptake ability (capturing effect) of *Lactobacillus gasseri* OLL2959 strain.

[Figure 16] Figure 16 is a graph showing the survival rate of *Lactobacillus gasseri* OLL2959 strain in yogurt.

Description of Embodiments

[0010]

The present invention will be hereinafter described in detail.

The "purines" refer collectively to substances having a purine skeleton and are classified into purine bases, purine nucleosides, and purine nucleotides. Purines serve various functions mainly in cells in living bodies and play the role of, for example, transmitting genetic information as constituents of nucleic acids. Examples of major purine bases include adenine, guanine, hypoxanthine, and xanthine. Purine nucleosides are

compounds in which sugar is bound to purine bases, and examples thereof include adenosine, guanosine, inosine, and xanthosine in which ribose is bound to a purine base; and deoxyadenosine, deoxyguanosine, deoxyinosine, and deoxyxanthosine in which deoxyribose is bound to a purine base. Purine nucleotides are compounds in which phosphate is bound to purine nucleosides, and examples thereof include adenylic acid (AMP), guanylic acid (GMP), inosinic acid (IMP), and xanthylic acid (XMP).

[0011]

Purines are fed into the body from food, as dietary purines, through intestinal absorption, or are biosynthesized *de novo* from amino acids or the like via the *de novo* pathway. Purines are also biosynthesized via the salvage pathway by which purine bases produced by degradation of purine nucleotides are recycled to synthesize purine nucleotides.

[0012]

In the case of a human, purine nucleotides are ultimately metabolized into uric acid. For example, adenylic acid is metabolized into adenosine by 5'-nucleotidase (5'-NT) activity, and adenosine is metabolized into hypoxanthine through inosine. Hypoxanthine is converted into xanthine by xanthine dehydrogenase (XDH) and xanthine oxidase (XO) activities. Guanylic acid is converted into guanosine by 5'-nucleotidase activity and further converted into guanine by purine nucleoside phosphorylase (PNP) activity. Guanine is converted into xanthine by guanine deaminase (GDA). Xanthine is metabolized into uric acid by xanthine dehydrogenase (XDH) and xanthine oxidase (XO) activities. On the other hand, the purine nucleosides (adenosine, inosine, xanthosine, and guanosine) are converted into the purine bases (adenine, hypoxanthine, xanthine, and guanine), respectively, by purine nucleoside phosphorylase (PNP) activity. Adenine, guanine, hypoxanthine, and xanthine are mostly recycled for biosynthesis of adenylic acid, guanylic acid, inosinic acid, and xanthylic acid, respectively, by salvage enzyme activity (salvage pathway).

[0013]

Lactic acid bacteria have a purine metabolic pathway similar to that of a human; however, the purine metabolic pathway differs in some ways from the human metabolic pathway. For example, most of lactic acid bacteria metabolize purine nucleosides ultimately

into bases. Moreover, in the case of *Lactobacillus gasseri* bacteria, purine nucleosides are converted into purine bases by purine nucleosidase.

[0014]

The present invention enables efficient obtainment (selection) of lactic acid bacteria having the purine capturing effect by selecting (screening for) the lactic acid bacteria using, as an index, an ability to take in purines into the cells. The present invention relates to a method of screening lactic acid bacteria comprising measuring an amount of uptake of purines by lactic acid bacteria in a medium comprising purines and obtaining (selecting) lactic acid bacteria having the purine capturing effect, using as an index the amount of uptake of purines. More specifically, the present invention relates to a method of screening lactic acid bacteria comprising culturing lactic acid bacteria in a medium comprising purines, measuring, preferably over time, an amount of uptake of purines into the cells, and obtaining (selecting) lactic acid bacteria having the purine capturing effect using as an index the amount of uptake of purines. The thus obtained lactic acid bacteria having the purine capturing effect highly probable to have the effect of reducing the serum uric acid level. Herein, the fact that lactic acid bacteria have a large amount of uptake of purines, that is, the lactic acid bacterial cells have the purine capturing effect, means that purines in a living body, particularly in the intestinal tract, are captured in large amounts by the lactic acid bacteria and removed from the environment in the intestinal tract, thus leading to suppression of purine absorption from the intestinal tract. Lactic acid bacteria are known to be excreted without being absorbed from the gastrointestinal tract, and hence, purines captured by the lactic acid bacteria escape being absorbed from the intestinal tract and are to be excreted out of the body together with the lactic acid bacteria. The present invention, therefore, also relates to a method of screening lactic acid bacteria having the effect of reducing the serum uric acid level comprising culturing lactic acid bacteria in a medium comprising purines, measuring an amount of uptake of purines into the cells, selecting lactic acid bacteria having the purine capturing effect using the amount of uptake of purines as an index, and obtaining (selecting) the resulting lactic acid bacteria having the purine capturing effect as the lactic acid bacteria having the effect of reducing the serum uric acid level.

[0015]

In the present invention, the lactic acid bacteria having a purine uptake ability show high proliferative capacity in the presence of purines, as is correlated with the high purine uptake ability. In the present invention, therefore, more accurate obtainment (selection) of the lactic acid bacteria having the purine capturing effect can be made by, in addition to the above-described selection using the purine uptake ability as an index, examining enhancement of the proliferative capacity of the selected lactic acid bacteria in the presence of purines. That is, the present invention also relates to a method of screening lactic acid bacteria comprising measuring an amount of proliferation of lactic acid bacteria having a purine uptake ability in a medium comprising purines and obtaining (selecting) lactic acid bacteria having the purine capturing effect using as an index the amount of proliferation together with the amount of uptake of purines measured as described above. Furthermore, the present invention also relates to a method of screening lactic acid bacteria having the effect of reducing the serum uric acid level comprising culturing lactic acid bacteria in a medium comprising purines, measuring an amount of proliferation of the bacterial cells over time, selecting lactic acid bacteria having the purine capturing effect using as an index the amount of proliferation together with the amount of uptake of purines measured as described above, and obtaining (selecting) the resulting lactic acid bacteria having the purine capturing effect as lactic acid bacteria having the effect of reducing the serum uric acid level. However, in the present invention, the measurement of the amount of proliferation in the presence of purines and the selection using the amount of proliferation as an index may be or not may be performed. Purines (for example, purine bases) taken in in large amounts by lactic acid bacteria are used for nucleic acid synthesis required for proliferation, which provides high proliferative capacity of lactic acid bacteria.

[0016]

The lactic acid bacteria to be subjected to the screening method of the present invention are, but not particularly limited to, preferably *Lactobacillus* bacteria. The *Lactobacillus* bacteria include strains of *Lactobacillus gasseri*, *Lactobacillus delbrueckii subsp. burgalicus*, *Lactobacillus delbrueckii subsp. lactis*, *Lactobacillus paracasei subsp. paracasei*,

Lactobacillus acidophilus, *Lactobacillus helveticus*, *Lactobacillus helveticus subsp. jugurti*, *Lactobacillus crispatus*, *Lactobacillus amylovorus*, *Lactobacillus gallinarum*, *Lactobacillus oris*, *Lactobacillus casei subsp. rhamnosus*, *Lactobacillus johnsonii*, *Lactobacillus fermentum*, *Lactobacillus brevis*, *Lactobacillus plantarum*, *Lactobacillus reuteri and the like*; and are particularly preferably *Lactobacillus gasseri bacteria*. Any strain of the lactic acid bacteria to be subjected to the screening method of the present invention is preferably used in the screening after being cultured in a suitable medium (e.g., MRS medium) and having its concentration being adjusted. While the medium used in the screening is any medium that allows proliferation of *Lactobacillus gasseri* bacteria, it is preferably a minimal medium or a minimal medium-based medium which has been supplemented with purines, or in which some components have been replaced with purines. Table 1 shows an example of a particularly preferred minimal medium.

[0017]

Purines to be incorporated into the medium may be purine bases, purine nucleosides, and/or purine nucleotides. In one preferred embodiment, the purines to be incorporated into the medium are purine bases. Examples of purine bases include, but are not limited to, adenine, guanine, hypoxanthine, and xanthine, and adenine is particularly preferred. Examples of purine nucleosides include, but are not limited to, adenosine, guanosine, inosine, and xanthosine, and adenosine is particularly preferred. Examples of purine nucleotides include, but are not limited to, adenylic acid (AMP), guanylic acid (GMP), inosinic acid (IMP), and xanthylic acid (XMP), and adenylic acid is particularly preferred. In another embodiment, preferred examples of purine bases, purine nucleosides, and purine nucleotides are hypoxanthine, inosine (IMP), and inosinic acid, respectively, and hypoxanthine is particularly preferred.

[0018]

For measurement of an amount of uptake of purines by lactic acid bacteria, a medium can be preferably used in which purines labeled with a labeling substance that is quantitatively detectable, for example, purines labeled with a radioisotope or fluorescent substance are used as some or all of the purines contained in the medium. A preferred radioisotope is ^{14}C, for

example. The amount of uptake of purines by lactic acid bacteria can be measured or determined by, for example, culturing lactic acid bacteria in a medium comprising purines, stopping the reaction through addition of TFA (trifluoroacetic acid) or the like after a certain time of culture, quantifying the labeled purines in the cultured cells based on a detection of activity of the labeling substance, and comparing it with the activity of the cells at the beginning of culture. If the amount of uptake of purines by the lactic acid bacteria has significantly increased compared to that at the beginning of culture (at a time point of 0 minutes after the beginning of culture), the lactic acid bacteria can be determined to have an ability to take in purines into the cells (purine uptake ability). If the amount of uptake of purines by the lactic acid bacteria has markedly increased compared to that at the beginning of culture (at a time point of 0 minutes after the beginning of culture), the lactic acid bacteria can be determined to have a high ability to take in purines into the cells (high purine uptake ability). Alternatively, if the amount of uptake of purines by the lactic acid bacteria has significantly increased compared to that by *Lactobacillus gasseri* JCM1130 strain, the lactic acid bacteria can be further determined to have a high ability to take in purines into the cells (high purine uptake ability). In the present invention, the lactic acid bacteria thus determined to have a purine uptake ability can be obtained (selected) as lactic acid bacteria having the purine capturing effect. The lactic acid bacteria selected as the lactic acid bacteria having the purine capturing effect can then be obtained (selected) as lactic acid bacteria having the effect of reducing the serum uric acid level, or a candidate thereof. The period of culture of lactic acid bacteria preferably lasts until any time point in the lag phase or logarithmic phase of the growth curve. For example, lactic acid bacteria can be cultured until after 30 minutes and 60 minutes from the beginning of culture, and then the purine uptake ability or the like can be measured. In this measurement, generally, radioactivity of the radioisotope-labeled purines may be measured using a liquid scintillation counter.

[0019]

The amount of proliferation of lactic acid bacteria in the presence of purines can be measured or determined by, for example, culturing lactic acid bacteria in a medium comprising purines, measuring turbidities (typically absorbance at 650 nm) of the medium at

the beginning of culture and after culture for a certain time, and calculating the difference between them. If an increase level in the turbidity of the medium when culturing in the presence of purines shows a significantly increased level compared to that of the medium when culturing in the absence of purines, the lactic acid bacteria used in the culture can be determined to show enhanced proliferative capacity in the presence of purines. Alternatively, if an increase level in the turbidity of the medium when culturing in the presence of purines shows a significantly increased level compared to that with *Lactobacillus gasseri* JCM1130 strain, the lactic acid bacteria can be further determined to show significantly enhanced proliferative capacity in the presence of the purines. The fact that lactic acid bacteria have a purine uptake ability and show enhanced proliferative capacity in the presence of purines means that the lactic acid bacteria are highly capable of assimilating purines. That is, this supports that the lactic acid bacteria are highly capable of capturing purines, and hence supports the possibility that the lactic acid bacteria have the effect of reducing the serum uric acid level. The period of culture of lactic acid bacteria preferably lasts until any time point in the logarithmic phase of the growth curve. For example, lactic acid bacteria can be cultured until after 4 hours and 6 hours from the beginning of culture, and the proliferative capacity thereof and the like in the presence of purines can be measured.

[0020]

In the measurement described above, preferably, the lactic acid bacteria are inoculated at 1.0×10^6 to 1.0×10^{11} cfu, for example, 0.8×10^7 to 3×10^7 cfu per 1 mL of medium and cultured. While the culture conditions for the lactic acid bacteria are not particularly limited, the lactic acid bacteria are preferably anaerobically cultured at 30 to 39°C, preferably 36 to 38°C.

[0021]

In the present invention, it is also preferred that the lactic acid bacteria selected as described above be further tested as to whether they have the effect of reducing the serum uric acid level, for example, in accordance with the method described in the Examples described below. For example, it can be determined whether or not the lactic acid bacteria selected as described above have the effect of reducing the serum uric acid level, by administering the

lactic acid bacteria selected above to a subject in single dose or multiple doses, measuring the serum uric acid level, and examining the presence or absence of a change in the serum uric acid level (decrease in the serum uric acid level).

[0022]

The lactic acid bacteria selected as described above have a purine uptake ability and preferably, high proliferative capacity in the presence of purines, and, that is, have a high purine capturing effect. Such lactic acid bacteria typically have the effect of reducing the serum uric acid level. The lactic acid bacteria selected as described above can exert a purine uptake ability and high proliferative capacity (i.e., a high ability to assimilate purines) in the presence of purines in a living body (typically in the intestinal tract), and consequently capture and reduce a large amount of purines in the living body (typically in the intestinal tract), and reduce the amount of purine absorption, thereby reducing the serum uric acid level. Examples of purines that can be taken in, i.e., captured, by the selected lactic acid bacteria include purine bases, purine nucleosides, and/or purine nucleotides. The purines that can be taken in by the selected lactic acid bacteria are not necessarily limited to purines incorporated into the medium in the screening. Examples of purine bases include, but are not limited to, adenine, guanine, hypoxanthine, and xanthine. Examples of purine nucleosides include, but are not limited to, adenosine, guanosine, inosine, and xanthosine. Examples of purine nucleotides include, but are not limited to, adenylic acid (AMP), guanylic acid (GMP), inosinic acid (IMP), and xanthylic acid (XMP). In one embodiment, the purines that can be taken in by the selected lactic acid bacteria comprise at least one selected from the group consisting of adenine, adenosine, adenylic acid, hypoxanthine, inosine, and inosinic acid, and preferably all of them.

[0023]

The present invention also provides lactic acid bacteria having a purine capturing effect, selected by the above-described screening method. Such lactic acid bacteria typically have the effect of reducing the serum uric acid level.

[0024]

Furthermore, the present invention also provides an agent for capturing purines, and preferably an agent for capturing purines for use in oral administration, comprising, as an active component, the lactic acid bacteria having the purine capturing effect that are obtainable by the above-described screening method (hereinafter also referred to as the lactic acid bacteria of the present invention). The agent for capturing purines of the present invention may comprise carriers or additives acceptable for oral administration, in addition to *Lactobacillus gasseri* bacteria of the present invention. The agent for capturing purines of the present invention may be a drug or composition comprising cells of the lactic acid bacteria of the present invention, or the agent for capturing purines may be a fermented product or culture produced using the bacteria, or a concentrate or dried product thereof, or a drug or composition comprising any of them. The lactic acid bacteria according to the present invention contained in the agent for capturing purines of the present invention are preferably viable cells. As described above, the agent for capturing purines of the present invention has the effect of reducing purines in the intestinal tract through the uptake of purines by the lactic acid bacteria, and therefore can be preferably used for reducing purines in the intestinal tract, and hence, for reducing the serum uric acid level. Further, the lactic acid bacteria of the present invention or the agent for capturing purines of the present invention takes in purines as a tasty component in fish or meat, for example, purine nucleotides such as inosinic acid (IMP) and adenylic acid (AMP), into the cells, and reduces the absorption of such purines into the body from the intestinal tract. Thus, the lactic acid bacteria of the present invention or the agent for capturing purines of the present invention can also be used for reducing the absorption of such a tasty component from the intestinal tract. The present invention also provides an agent for reducing the absorption of a tasty component (herein, a purine or a purine nucleotide as a tasty component in fish or meat, such as inosinic acid), comprising the lactic acid bacteria of the present invention or the agent for capturing purines of the present invention. This agent for reducing the absorption of a tasty component is also preferably for use in oral administration.

[0025]

Preferred examples of the lactic acid bacteria having the purine capturing effect as described above include, but are not limited to, *Lactobacillus gasseri* bacteria such as *Lactobacillus gasseri* OLL2959 strain and *Lactobacillus gasseri* P14054ME002 strain. *Lactobacillus gasseri* OLL2959 strain is homolactic fermentative and lacks an ability to produce a gas. *Lactobacillus gasseri* OLL2959 strain was deposited on March 31, 2006 (original deposit date) in the National Institute of Technology and Evaluation, Patent Microorganisms Depositary (NPMD) (#122, 2-5-8 Kazusakamatari, Kisarazu-shi, Chiba 292-0818, Japan) under Accession No: NITE P-224, and was subsequently transferred to a deposit under the Budapest Treaty (international deposit) on November 21, 2007, under Accession No: NITE BP-224 into which the original accession number was changed. Alternatively, in another embodiment, the lactic acid bacteria having the purine capturing effect as described above may be lactic acid bacteria or *Lactobacillus gasseri* bacteria except for *Lactobacillus gasseri* OLL2959 strain.

[0026]

In the present invention, the agent for capturing purines according to the present invention is also intended for use in combination with a food or beverage product or a pharmaceutical preparation. The present invention therefore also provides an agent for capturing purines according to the present invention, for use in combination with a food or beverage product or a pharmaceutical preparation.

[0027]

The present invention also provides a food or beverage product or a pharmaceutical preparation comprising the agent for capturing purines according to the present invention. The food or beverage product or the pharmaceutical preparation of the present invention can provide a reduction of purines in the intestinal tract and an effective decrease in the serum uric acid level by causing active uptake of purines into the cells and assimilation thereof in a subject to which it is administered (or by which it is ingested). The food or beverage product or the pharmaceutical preparation of the present invention may therefore be used for reducing purines in the intestinal tract. The "purines in the intestinal tract" of the present invention do not include purines carried by the bacteria (e.g., lactic acid bacteria), fungi, viruses, subject's

cells, and the like present in the intestinal tract. The food and beverage products and pharmaceutical preparations of the present invention may also be used for use in reducing the serum uric acid level based on a decrease in purines in the intestinal tract. The food or beverage product or the pharmaceutical preparation comprising the agent for capturing purines of the present invention can be preferably used, for example, for preventing, treating, ameliorating, or alleviating the symptoms of gout or hyperuricemia.

[0028]

The "food or beverage product" herein includes, but is not particularly limited to, beverages, food products, and functional foods. The type of the food or beverage product according to the present invention is not particularly limited, and examples of beverages include fermented milk (yogurt drinks, etc.), lactic acid bacteria beverages, milk beverages (coffee-flavored milk, fruit-flavored milk, etc.), tea-based beverages (green tea, black tea, oolong tea, etc.), fruit/vegetable-based beverages (beverages containing fruit juices such as orange, apple, or grape juice, or vegetable juices such as tomato or carrot juice), alcoholic beverages (beer, sparkling alcoholic drink, wine, etc.), carbonated beverages, soft drinks, and water. Examples of preferred beverages include yogurt drinks, lactic acid bacteria beverages, milk beverages, and water-based beverages, and an example of a particularly preferred beverage includes a yogurt drink. For production methods and the like of various beverages, reference may be made to existing guidebooks such as "Latest Soft Drinks" (2003) (Korin Corporation), for example. Examples of food products include fermented milk (set type yogurt, soft yogurt, cheese, etc.), dairy products, confectionery, and instant foods. Examples of preferred food products include set type yogurt or soft yogurt and confectionery, and an example of a particularly preferred beverage includes set type yogurt or soft yogurt. For production methods and the like of various food products, reference may be made to existing guidebooks.

[0029]

Fermented milk such as yogurt comprising the lactic acid bacteria having the purine capturing effect may be produced by, for example, adding the lactic acid bacteria having the purine capturing effect to a dairy product or fermented milk produced using a starter that may

contain other microorganisms such as lactic acid bacteria that may or may not have the purine capturing effect. The dairy product or fermented milk using the starter can be produced in accordance with a conventional method. For example, yogurt can be produced by mixing a starter into milk or a dairy product cooled after heating, mixing, homogenization, and pasteurization treatment, and subsequently fermenting and cooling it. The present invention preferably provides use of the lactic acid bacteria having the purine capturing effect in the manufacture of a dairy product or fermented milk such as yogurt or cheese (which preferably comprises adding (blending) the lactic acid bacteria to the dairy product or fermented milk, or raw materials thereof), and particularly preferably provides use of the lactic acid bacteria in the manufacture of yogurt. Furthermore, the present invention also provides a method of reducing purines based on the purine capturing effect of the lactic acid bacteria, in the manufacture of fermented milk or a dairy product such as yogurt or cheese, the method using as an active component the lactic acid bacteria having the purine capturing effect. The lactic acid bacteria having the purine capturing effect according to the present invention (for example, *Lactobacillus gasseri* OLL2959 strain) can exhibit good survivability in fermented milk or a dairy product such as yogurt.

[0030]

Functional foods are particularly preferred as the food or beverage product according to the present invention. The "functional foods" of the present invention refer to foods having certain functions for the body and encompass so-called health foods in general, for example, foods with health claims including foods for specified health uses (including qualified foods for specified health uses) in Japan and foods with nutrient function claims, foods with function claims, foods for specified dietary uses, nutritional supplements, health supplements, supplements (e.g., in various dosage forms such as tablets, coated tablets, sugar-coated tablets, capsules, and liquids), and foods for beauty (e.g., weight loss foods). The functional foods of the present invention also encompass health foods to which health claims based on the food standards by Codex (Joint FAO/WHO Codex Alimentarius Commission) are applied.

[0031]

More specific preferred examples as the functional foods of the present invention include foods for specified dietary uses such as foods for sick persons, dry milk for pregnant women and lactating mothers, modified dry milk for infants, foods for the elderly, and nursing care foods.

[0032]

This functional food of the present invention is particularly useful for reducing the serum uric acid level by reducing purines in the intestinal tract. The functional food of the present invention can be preferably used for reducing the serum uric acid level, particularly for reducing the serum uric acid level along with a decrease in purines in the intestinal tract through the uptake of purines by the lactic acid bacteria and promoted proliferation of the lactic acid bacteria, and the resulting decrease in purine absorption in the intestinal tract.

[0033]

The food or beverage product of the present invention such as a functional food (preferably a food for a specified health use or a qualified food for a specified health use) may be for use in reducing purines in the intestinal tract, or for use in reducing the serum uric acid level or suppressing or alleviating an increase in the serum uric acid level, and may include a description or indication thereof. The present invention preferably provides use of the lactic acid bacteria having the purine capturing effect in the manufacture of such functional foods (which preferably comprises adding (blending) the lactic acid bacteria to the functional food or raw materials thereof).

[0034]

The functional foods of the present invention may be solid preparations such as tablets, granules, powders, pills, and capsules; liquid preparations such as liquids, suspensions, and syrups; gels, or pastes or may be in the form of general food or beverage products (e.g., beverages, yogurt, and confectionery).

[0035]

The food or beverage product of the present invention may comprise any food components which are not particularly limited. The food or beverage product of the present invention may comprise water, proteins, sugars, lipids, vitamins, minerals, organic acids,

organic bases, fruit juices, flavors, and the like. Examples of proteins include animal and vegetable proteins such as whole milk powder, skimmed milk powder, partially skimmed milk powder, casein, whey powder, whey protein, whey protein concentrate, whey protein isolate, α-casein, β-casein, κ-casein, β-lactoglobulin, α-lactalbumin, lactoferrin, soybean protein, hen egg protein, and meat proteins, and hydrolysates thereof; and various milk-derived components such as butter, whey mineral, cream, whey, non-protein nitrogen, sialic acids, phospholipids, and lactose. Examples of sugars include general saccharides, modified starches (dextrin, soluble starch, British starch, oxidized starch, starch ester, starch ether, etc.), and dietary fiber. Examples of lipids include animal fats and oils such as lard and fish oil, as well as fractionated oil, hydrogenated oil, and interesterified oil thereof; vegetable fats and oils such as palm oil, safflower oil, corn oil, rapeseed oil, and coconut oil, as well as fractionated oil, hydrogenated oil, and interesterified oil thereof. Examples of vitamins include vitamin A, carotenes, vitamin B group, vitamin C, vitamin D group, vitamin E, vitamin K group, vitamin P, vitamin Q, niacin, nicotinic acid, pantothenic acid, biotin, inositol, choline, and folic acid; and examples of minerals include calcium, potassium, magnesium, sodium, copper, iron, manganese, zinc, selenium, and whey mineral. Examples of organic acids include malic acid, citric acid, lactic acid, and tartaric acid. These components can be used alone or in combinations of two or more and may be added using synthetic products and/or foods containing large amounts of these components.

[0036]

The above-described functional food comprising the lactic acid bacteria or the agent for capturing purines of the present invention may also contain carriers or additives acceptable for oral administration, in addition to the lactic acid bacteria or the agent for capturing purines of the present invention. Examples of carriers include water, organic solvents acceptable for oral administration, collagen, polyvinyl alcohols, polyvinylpyrrolidone, carboxyvinyl polymer, sodium arginine, water-soluble dextran, water-soluble dextrin, sodium carboxymethyl starch, pectin, xanthan gum, gum arabic, casein, gelatin, agar, glycerin, propylene glycol, polyethylene glycol, vaseline, paraffin, stearyl alcohol, stearic acid, human serum albumin, mannitol, sorbitol, lactose, and surfactants acceptable for oral administration. Examples of

additives include binders, excipients, lubricants, disintegrators, wetting agents, stabilizers, buffer agents, flavoring agents, preservatives, and coloring agents. These carriers or additives can be used alone or in combinations of two or more and can be used as appropriate, in accordance with the dosage form of the preparation. The functional food of the present invention may additionally contain other functional components.

[0037]

Furthermore, the pharmaceutical preparation (or pharmaceutical composition) comprising the lactic acid bacteria or the agent for capturing purines of the present invention may contain, in addition to the lactic acid bacteria or the agent for capturing purines of the present invention, pharmaceutically acceptable carriers or additives, particularly, carriers or additives acceptable for oral administration. Examples of carriers include water, pharmaceutically acceptable organic solvents, collagen, polyvinyl alcohols, polyvinylpyrrolidone, carboxyvinyl polymer, sodium arginine, water-soluble dextran, water-soluble dextrin, sodium carboxymethyl starch, pectin, xanthan gum, gum arabic, casein, gelatin, agar, glycerin, propylene glycol, polyethylene glycol, vaseline, paraffin, stearyl alcohol, stearic acid, human serum albumin, mannitol, sorbitol, lactose, pharmaceutically acceptable surfactants, and artificial cell structures such as liposomes. Examples of additives include binders, excipients, lubricants, disintegrators, wetting agents, stabilizers, buffer agents, flavoring agents, preservatives, and coloring agents. These carriers or additives can be used alone or in combinations of two or more and can be used as appropriate, in accordance with the dosage form of the preparation. This pharmaceutical preparation of the present invention may additionally contain other pharmacological components.

[0038]

The above-described pharmaceutical preparation of the present invention is preferably orally administered. The pharmaceutical preparation of the present invention may take any dosage form, for example, a solid preparation such as a tablet, a granule, a powder, a pill, or a capsule, a gel, or a liquid preparation such as a liquid, a suspension, or a syrup.

[0039]

The dose (amount of ingestion) of the lactic acid bacteria, the agent for capturing purines, or the food or beverage product or the pharmaceutical preparation of the present invention may be changed over a wide range at a discretion of a skilled artisan, in consideration of the age and body weight of the subject for administration (feeding), administration route, number of doses, and the like. Regarding the dose of the lactic acid bacteria, the agent for capturing purines, or the food or beverage product or the pharmaceutical preparation of the present invention, the dose is an amount such that the lactic acid bacteria of the present invention (e.g., *Lactobacillus gasseri* bacteria) are administered, for example, preferably in an amount of 1×10^5 to 1×10^{11} cfu, more preferably in an amount of 1×10^8 to 1×10^{10} cfu, still more preferably in an amount of 1×10^9 to 1×10^{10} cfu, and particularly preferably in an amount of 4×10^9 to 6×10^{10} cfu, per dose, although the dose is not particularly limited thereto. The agent for capturing purines, or the food or beverage product or the pharmaceutical preparation of the present invention comprises the lactic acid bacteria of the present invention preferably in an amount of 1×10^5 to 1×10^{11} cfu, more preferably in an amount of 1×10^8 to 1×10^{10} cfu, still more preferably in an amount of 1×10^9 to 1×10^{10} cfu, and particularly preferably in an amount of, for example, 4×10^9 to 6×10^{10} cfu, per dose.

[0040]

In one embodiment of the present invention, the lactic acid bacteria, the agent for capturing purines, or the food or beverage product or the pharmaceutical preparation of the present invention are/is administered to a subject (or ingested by a consumer) once or more a day, preferably twice or more a day, and more preferably twice a day. The lactic acid bacteria, the agent for capturing purines, or the food or beverage product or the pharmaceutical preparation of the present invention may be administered to a subject continuously, and may be administered every day, for example. In this case, the lactic acid bacteria, the agent for capturing purines, or the food or beverage product or the pharmaceutical preparation of the present invention are/is administered to a subject for at least a week, preferably 2 weeks or more, and more preferably 4 weeks or more. When the agent for capturing purines, or the food or beverage product or the pharmaceutical preparation of the present invention is continuously administered to a subject, it is administered preferably in an amount of 1×10^5 to

1×10^{11} cfu, more preferably in an amount of 1×10^{8} to 1×10^{10} cfu, still more preferably in an amount of 1×10^{9} to 1×10^{10} cfu, and particularly preferably in an amount of, for example, 4×10^{9} to 6×10^{10} cfu of the lactic acid bacteria of the present invention, per dose.

[0041]

In another embodiment of the present invention, the lactic acid bacteria, the agent for capturing purines, or the food or beverage product or the pharmaceutical preparation of the present invention may be administered in a single dose. When the agent for capturing purines, or the food or beverage product or the pharmaceutical preparation of the present invention is administered to a subject in a single dose, it is administered preferably in an amount of 1×10^{5} to 1×10^{11} cfu, more preferably in an amount of 1×10^{8} to 1×10^{10} cfu, still more preferably in an amount of 1×10^{9} to 1×10^{10} cfu, and particularly preferably in an amount of, for example, 4×10^{9} to 6×10^{10} cfu of the lactic acid bacteria of the present invention per dose. The lactic acid bacteria, the agent for capturing purines, or the food or beverage product or the pharmaceutical preparation of the present invention are/is preferably orally administered (orally ingested).

[0042]

The "administration" herein includes both the "ingestion" used for food and beverage products in general and the "administration" used for pharmaceutical preparations. The "oral administration" in the present invention includes, in addition to the administration or ingestion through the mouth, but also administration via tube feeding through a nasal tube, a gastric tube, or the like. Thus, the present invention also provides an oral preparation that can be used for such an oral administration. In a preferred embodiment of the present invention, therefore, there is also provided an oral preparation comprising the lactic acid bacteria or the agent for capturing purines of the present invention, for use in reducing purines in the intestinal tract and reducing the serum uric acid level.

[0043]

The subject to which the lactic acid bacteria, the agent for capturing purines, or the food or beverage product or the pharmaceutical preparation of the present invention is administered is a mammal including a human, livestock, a pet, and a laboratory (test) animal.

The subject is preferably a human subject, more preferably a human subject with gout and/or hyperuricemia, and still more preferably a human subject having a serum uric acid level of 6 mg/dL or more, for example, 6 to 10 mg/dL. In one embodiment, the subject for administration is even more preferably a human subject with mild to borderline hyperuricemia having a serum uric acid level of 6 to 8 mg/dL. The present invention also provides a method for reducing purines in the intestinal tract and reducing the serum uric acid level comprising administering to the above-described subject (feeding the subject with) the lactic acid bacteria of the present invention, or the agent for capturing purines or the food or beverage product or the pharmaceutical preparation of the present invention comprising an effective amount of the lactic acid bacteria of the present invention, as described above, whereby causing capture of purines into the cells of the lactic acid bacteria. The present invention also provides a method for capturing purines into the cells of the lactic acid bacteria comprising contacting the lactic acid bacteria or the agent for capturing purines of the present invention with purines. The present invention also provides use of the lactic acid bacteria or the agent for capturing purines of the present invention, for imparting a purine capturing effect to a food or beverage product, a pharmaceutical preparation, or other drugs. The present invention also provides use of the lactic acid bacteria, the agent for capturing purines, the food or beverage product, or the pharmaceutical preparation of the present invention, for reducing the risk of the onset of gout or hyperuricemia.

Examples

[0044]

The present invention will be hereinafter described more specifically with reference to Examples. However, the technical scope of the present invention shall not be limited to these Examples.

[0045]

[Example 1] Test of evaluating purine uptake ability

In this Example, using purines labeled with a radioisotope (RI), a purine uptake ability of *Lactobacillus gasseri* OLL2959 strain was evaluated.

[0046]

Lactobacillus gasseri OLL2959 strain was deposited on March 31, 2006 (original deposit date) in the National Institute of Technology and Evaluation, Patent Microorganisms Depositary (NPMD) (#122, 2-5-8 Kazusakamatari, Kisarazu-shi, Chiba 292-0818, Japan) under Accession No. NITE P-224, and was subsequently transferred to a deposit under the Budapest Treaty (international deposit) on November 21, 2007 under Accession No. NITE BP-224 into which the original accession number was changed. *Lactobacillus gasseri* OLL2959 strain was inoculated into MRS medium (Lactobacilli MRS Broth, Difco Co., Ltd.) and cultured at 37°C for 16 to 20 hours, and the resulting culture (4 to 7 x 10^8 cfu/ml) was used below.

[0047]

To 0.1 mL of a minimal medium (DM medium; Table 1), adenylic acid (AMP), adenosine, or adenine labeled with radioisotope ^{14}C (^{14}C-AMP, ^{14}C-adenosine, or ^{14}C-adenine, respectively) was added to a final concentration of 20 μM, and then the above-prepared culture of *Lactobacillus gasseri* OLL2959 strain was inoculated at 2 wt% (0.002 mL: 0.8 to 1.4 x 10^6 cfu) and anaerobically cultured at 37°C for 30 minutes.

[0048]

A TFA solution (trifluoroacetic acid, 5%) was subsequently added to these media, and then the cells were washed with physiological saline, after which radioactivity was measured using a liquid scintillation counter (manufactured by Aloka Co., Ltd., LSC-6100). As a control (0 minutes), the TFA solution (5%) was added immediately after the preparation of the sample, and then the cells were washed with physiological saline, after which radioactivity was measured as described above. The results are shown in Figure 1. In Figure 1, the units of radioactivity (vertical axis) expressing the amount of ^{14}C-labeled purines in the cells are disintegrations per minute (dpm), which represent the number of decay of the radioactive substance per minute. It was confirmed that in the culture using DM medium for 60 minutes, the viable cell count did not significantly change between the beginning of the test and the end of the test.

[0049]

The results showed that *Lactobacillus gasseri* OLL2959 strain had an ability to take in adenylic acid (AMP), adenosine, and adenine, which are purines, into the cells (purine uptake ability), and particularly had a high ability to take in adenine into the cells (purine uptake ability) (Figure 1).

[0050]

[Table 1]

Minimal Medium Composition

Components	Final Concentration (/L)
D (+) Glucose	10 g
Potassium hydrogen phosphate	3.1 g
Diammonium hydrogen citrate	2 g
Potassium dihydrogen phosphate	1.5 g
Potassium acetate	10 g
Calcium lactate pentahydrate	1 g
Tween-80	1 g
Magnesium sulfate heptahydrate	500 mg
Sodium chloride	20 mg
Manganese sulfate hydrate	20 mg
Cobalt sulfate heptahydrate	500 mg
Biotin	1 mg
Cyanocobalamin	0.02 mg
Folic acid	0.2 mg
Para-aminobenzoic acid	0.2 mg
Nicotinic acid	10 mg
Calcium pantothenate	10 mg
Riboflavin	10 mg
Pyridoxal hydrochloride	10 mg
Myo-inositol	10 mg
Ascorbic acid	0.5 mg
DL-aminobutyric acid	100 mg
L-phenylalanine	100 mg
L-serine	100 mg
L-threonine	100 mg
L-cysteine hydrochloride monohydrate	100 mg
L-asparagine monohydrate	100 mg
L-isoleucine	100 mg
L-methionine	100 mg
L-tyrosine	100 mg
L-tryptophan	100 mg
L-valine	100 mg
DL-alanine	200 mg
Glycine	200 mg
L-histidine hydrochloride monohydrate	200 mg
L-lysine hydrochloride	200 mg
L-proline	200 mg
L-arginine	200 mg
L-leucine	200 mg
L-aspartic acid	300 mg
L-glutamic acid	300 mg
Glutamine	100 mg
Thymine	100 mg
Cytidine	100 mg
2'-deoxyuridine	100 mg

[0051]

[Example 2] Test of evaluating proliferative capacity in the presence of purines

In the present example, *Lactobacillus gasseri* OLL2959 strain was cultured in the presence of purines, and its proliferative capacity in the presence of the purines was evaluated.

[0052]

To 1 mL of the DM medium (Table 1), adenylic acid (AMP), adenosine, or adenine, as purines, was added to a final concentration of 400 μM, and then the culture of *Lactobacillus gasseri* OLL2959 strain prepared in Example 1 was inoculated at 4 wt% (0.04 mL: 1.6 to 2.8 x 10^7 cfu) and anaerobically cultured at 37°C. After 0 hours, 4 hours, and 6 hours from the beginning of this culture, turbidities (absorbance at 650 nm) of the medium were measured. As a control, *Lactobacillus gasseri* OLL2959 strain was cultured in the same manner except that the purines were not added to the minimal medium, and turbidities of the medium were measured. The results are shown in Figure 2.

[0053]

The results showed that the proliferative capacity of *Lactobacillus gasseri* OLL2959 strain is enhanced in the presence of adenylic acid (AMP), adenosine, or adenine, and particularly the proliferative capacity is further enhanced in the presence of adenine (Figure 2).

[0054]

[Example 3] Comparative test of adenine uptake ability and proliferative capacity in the presence of adenine

In this Example, *Lactobacillus gasseri* OLL2959 strain and other *Lactobacillus gasseri* strains were cultured in the presence of adenine, and adenine uptake abilities and proliferative capacities in the presence of adenine of the respective strains were compared.

[0055]

Lactobacillus gasseri P14054ME001 strain and P14054ME002 strain were used as the other *Lactobacillus gasseri* strains. When *Lactobacillus gasseri* P14054ME001 strain and P14054ME002 strain were cultured in MRS medium (Lactobacilli MRS Broth, Difco, Co.,

Ltd.) not containing purines for 20 hours, the proliferative capacity of each strain was equivalent to that of *Lactobacillus gasseri* OLL2959 strain (Table 2).

[0056]

[Table 2]

	cfu/ml
OLL2959	6.0E+08
P14054ME001	6.8E+08
P14054ME002	5.1E+08

[0057]

The evaluation test of the adenine uptake abilities was performed as described in Example 1, except that adenine (^{14}C-adenine) only was used as the purines labeled with the radioisotope ^{14}C. The results are shown in Figure 3. It was found that *Lactobacillus gasseri* P14054ME002 strain also had a high adenine uptake ability (Figure 3), although *Lactobacillus gasseri* P14054ME002 strain did not have an adenine uptake ability as high as that of *Lactobacillus gasseri* OLL2959 strain. It was found that *Lactobacillus gasseri* P14054ME001 strain had an adenine uptake ability lower than those of *Lactobacillus gasseri* OLL2959 strain and P14054ME002 strain (Figure 3).

[0058]

For evaluation of proliferative capacities in the presence of adenine, adenine was added to 1 mL of the DM medium (Table 1) to a final concentration of 400 μM, and then either of the culture of *Lactobacillus gasseri* OLL2959 strain prepared in Example 1 and the cultures of P14054ME001 strain and P14054ME002 strain prepared in the same manner as that described in Example 1 was inoculated at 4 wt% (0.04 mL: 1.6 to 2.8 x 10^7 cfu) and anaerobically cultured at 37°C. After 0 hours, 4 hours, and 6 hours from the beginning of this culture, turbidities (absorbance at 650 nm) of the medium were measured. The results are shown in Figure 4. As with *Lactobacillus gasseri* OLL2959 strain, *Lactobacillus gasseri* P14054ME001 strain and P14054ME002 strain also showed enhanced proliferative capacity in the presence of adenine. Moreover, *Lactobacillus gasseri* OLL2959 strain showed a degree of enhancement of proliferative capacity much higher than those of *Lactobacillus gasseri* P14054ME001 strain and P14054ME002 strain. *Lactobacillus gasseri* P14054ME002 strain

had a degree of enhancement of proliferative capacity higher than that of *Lactobacillus gasseri* P14054 ME001 strain.

[0059]

These results showed that in *Lactobacillus gasseri* bacteria, enhancement of the proliferative capacity in the presence of adenine is correlated with the degree of adenine uptake ability. Moreover, some species of lactic acid bacteria were shown to have a high ability to assimilate adenine.

[0060]

[Example 4] Evaluation test of the effect of *Lactobacillus gasseri* OLL2959 strain to reduce the serum uric acid level

Human subjects suspected to have mild to borderline hyperuricemia were continuously fed *Lactobacillus gasseri* OLL2959 strain, and its influence upon the uric acid level was analyzed using a placebo-controlled double blind comparative study (test using human subjects).

[0061]

Fourteen adult males of 35 years old and above (average age: 44.3 years old) who had a uric acid level of 6 to 8 mg/dL in an examination prior to the beginning of the test were assigned into the two groups, i.e., a placebo group and an active group, so as not to produce a significant difference in uric acid levels and age. The placebo group ingested two cups (85 g/cup) per day of yogurt not containing *Lactobacillus gasseri* OLL2959 strain for 4 weeks. The active group ingested, for 4 weeks, two cups (85 g/cup) per day of yogurt in which 1 x 10^8 cfu/g of *Lactobacillus gasseri* OLL2959 strain was added to the same yogurt as that given to the placebo group. The two cups per day of yogurt were fed after any two of breakfast, lunch, and dinner.

[0062]

Each subject had his blood tested at the beginning of the test (prior to ingestion of the test food), and after 2 weeks and 4 weeks (test food ingestion period), and the serum uric acid level was measured using a conventional method. An amount of change in serum uric acid level at each time point compared to the serum uric acid level at the beginning of the test was

calculated, and the time course of the amount of change in serum uric acid level during the test period was analyzed statistically using two-way repeated measures ANOVA. The results are shown in Figure 5.

[0063]

As shown in Figure 5, the active group had significantly low serum uric acid levels compared to the placebo group (p=0.042). That is, *Lactobacillus gasseri* OLL2959 strain was shown to have the effect of reducing the serum uric acid level.

[0064]

[Example 5] Single-dose test using *Lactobacillus gasseri* bacteria (animal test)

Unlike humans, rats have uricase, which is an enzyme that degrades uric acid, and thus, it is necessary to administer to them potassium oxonate, which is an uricase inhibitor, in order to elevate the serum uric acid level. Thus, 0.5 g/kg of potassium oxonate is forcefully administered orally to Wistar rats (male) after 16 hours of fasting. Sixty minutes after the administration of potassium oxonate, to a lactic acid bacteria group are forcefully administered orally dried yeast (suspended in water for injection) for a purine load and *Lactobacillus gasseri* OLL2959 strain (suspended in physiological saline). To a negative group are administered water for injection instead of dried yeast, and physiological saline instead of *Lactobacillus gasseri* bacteria. To a control group are administered physiological saline instead of *Lactobacillus gasseri* bacteria, together with dried yeast.

[0065]

After the forceful oral administration, blood is collected over time (after 30 minutes, 60 minutes, 90 minutes, 120 minutes, and 150 minutes), the blood uric acid level in each of the blood samples is measured using a conventional method, and the time course of the blood uric acid level is examined. If the time course of the blood uric acid level is lower in the lactic acid bacteria group than in the control group, it is demonstrated that the amount of purine absorption from the intestinal tract is generally reduced by the purine uptake effect of *Lactobacillus gasseri* OLL2959 strain even in a single dose.

[0066]

[Example 6] Purine uptake ability of *Lactobacillus gasseri* bacteria (animal test)

It was considered that if the purine uptake ability of lactic acid bacteria is high, concurrent administration (ingestion) of the lactic acid bacteria and purines into (by) an animal subject suppresses purine absorption in the subject, compared to the case where the subject is fed purines alone. Thus, an animal experiment was performed using the following procedure, in order to test the purine uptake ability of *Lactobacillus gasseri* bacteria.

[0067]

Initially, fourteen 8-week-old Wistar rats (male, 190 to 210 g) were purchased and acclimated for one week. These rats were fasted for about 16 hours from the day before the test, and body weights were measured after the fasting. Based on the body weights after the fasting, rats were divided into the three groups in total, i.e., a negative group (physiological saline-treated group), an AMP (radioisotope ^{14}C-AMP)-treated group, and an AMP+OLL2959 strain (radioisotope ^{14}C-AMP and OLL2959 strain)-treated group, by random sampling, using a grouping program (The negative group only included four rats, and each of the other groups included five rats). All these rats in an unanesthetized state were placed in a holder, the tail veins were nicked with a scalpel, and 60 μL of the blood effused from the tail veins was collected with hematocrit tubes. This blood was used as blood samples collected at a time point of 0 minutes prior to the administration of test substances. To these collected blood samples, an equivalent amount of 2 mg/mL EDTA-2Na solution (EDTA-2Na dissolved in physiological saline) was added.

[0068]

Test substances were then forcefully orally administered. As the test substances here, physiological saline was used for the negative group, adenylic acid labeled with radioisotope ^{14}C (^{14}C-AMP: 57.6 mCi/mmol, 0.1 mCi/ml) was used for the AMP-treated group, and ^{14}C-AMP and *Lactobacillus gasseri* OLL2959 strain (1 x 10^{10} cfu/body) were used for the AMP+OLL2959 strain-treated group. ^{14}C-AMP and OLL2959 strain were diluted with physiological saline (Otsuka Pharmaceutical Co., Ltd.) and used. To the AMP group and the AMP+OLL2959 group, 10 μCi/body of ^{14}C-AMP was administered. The volume of dose was 2 mL/body in all the individuals (all the groups).

[0069]

After 15, 30, 45, 60, 90, 120, and 180 minutes from the administration of the test substances, all these rats in an unanesthetized state were placed in a holder, the tail veins were nicked with a scalpel, and 60 μL of the blood effused from the tail veins was collected with hematocrit tubes. To these collected blood samples, an equivalent amount of 2 mg/mL EDTA-2Na solution (EDTA-2Na dissolved in physiological saline) was added. After the completion of these tests, the rats were immediately sacrificed by carbon dioxide inhalation.

[0070]

Radioactivity in these collected blood samples was measured using a liquid scintillation counter (manufactured by Aloka Co., Ltd., LSC-6100). The results are shown in Figure 6.

As shown in Figure 6, significant differences were seen in the amount of purine absorption, after 30, 45, and 60 minutes from the administration, i.e., when peaks of the blood concentrations were reached (* $p<0.05$, ** $p<0.01$; t-test). These results showed that the amount of purine absorption from the intestinal tract can be suppressed through the ingestion of *Lactobacillus gasseri* OLL2959 strain.

[0071]

[Example 7] Single-dose test using *Lactobacillus gasseri* bacteria (test using human subjects)

Healthy males of 20 years old and above ingest 498 mg of a purine preparation (a mixture of 5'-adenylic acid, disodium 5'-inosinate, and disodium 5'-guanylate) once, blood is collected after 30 minutes, 60 minutes, 120 minutes, and 150 minutes from the ingestion, and the time course of the blood uric acid level is examined. Ten subjects showing a similar time course are selected as the subjects to be tested in this Example. In this test, the selected subjects ingest 112 mL of yogurt containing *Lactobacillus gasseri* OLL2959 strain (active group; containing 8.5×10^7 cfu/mL of *Lactobacillus gasseri* OLL2959 strain) or yogurt not containing the bacteria (placebo group). Blood is collected after 30 minutes, 60 minutes, 120 minutes, and 150 minutes from the ingestion, the blood uric acid level in each of the blood samples is measured using a conventional method, the time course of the blood uric acid level is examined, and a crossover test is performed. If an increase in uric acid level is suppressed more in the active group than in the placebo group, it is demonstrated that the amount of

purine absorption from the intestinal tract is reduced by the purine uptake effect of *Lactobacillus gasseri* OLL2959 strain in a single dose.

[0072]

[Example 8] Comparative test between lactic acid bacterial strains

(1) Comparative test of adenine uptake ability

Lactobacillus gasseri OLL2959 strain and *Lactobacillus gasseri* JCM1130 strain were cultured in a medium comprising adenine labeled with a radioisotope (RI) (^{14}C-adenine), and influences of the lactic acid bacterial strains upon adenine uptake ability were compared. *Lactobacillus gasseri* JCM1130 strain is available under JCM1130 from Japan Collection of Microorganisms, RIKEN BioResource Center (RIKEN BRC JCM; Tsukuba City, Ibaragi Prefecture, Japan).

Each of *Lactobacillus gasseri* OLL2959 strain and *Lactobacillus gasseri* JCM1130 strain was cultured using MRS medium, and proliferative capacity thereof was evaluated in advance. That is, each of *Lactobacillus gasseri* OLL2959 strain and *Lactobacillus gasseri* JCM1130 strain was anaerobically cultured at 37°C for 20 hours using MRS medium. After 20 hours of the anaerobic culture, the bacterial number of *Lactobacillus gasseri* JCM1130 strain was not less than 2.5 times higher than that of *Lactobacillus gasseri* OLL2959 strain. This revealed that when *Lactobacillus gasseri* OLL2959 strain and *Lactobacillus gasseri* JCM1130 strain were cultured in the same medium, *Lactobacillus gasseri* JCM1130 strain basically had higher proliferative capacity compared with the other (Table 3).

[0073]

[Table 3]

	cfu/ml
OLL2959	5.2E+08
JCM1130	1.4E+09

[0074]

In the comparative test of adenine uptake ability, initially, a medium for this test was prepared by adding ^{14}C-adenine to the minimal medium (Table 1) to a final concentration of 20 μM. Then, each of *Lactobacillus gasseri* OLL2959 strain and *Lactobacillus gasseri*

JCM1130 strain was cultured using MRS medium, and these cultures were inoculated at 2 wt% into the medium for this test and anaerobically cultured at 37°C. Each of these cultures was adjusted to give an equivalent bacterial number, using MRS medium.

[0075]

At the beginning (0 minutes) of the culture, and after 30 or 60 minutes from the beginning of the culture, a 5% TFA solution was added to stop the culture, and subsequently the cells were washed with physiological saline, after which radioactivity thereof was measured using a liquid scintillation counter (manufactured by Aloka Co., Ltd., LSC-6100). The results are shown in Figure 7. The radioactivity (vertical axis) in Figure 7 is expressed as the number of decay of the radioactive substance per minute (disintegrations per minute; dpm).

[0076]

As shown in Figure 7, both *Lactobacillus gasseri* OLL2959 strain and *Lactobacillus gasseri* JCM1130 strain exerted an adenine uptake ability, but after both 30 and 60 minutes from the beginning of culture, *Lactobacillus gasseri* OLL2959 strain took in a higher amount of adenine and showed a significant difference in the amount of adenine uptake ($p<0.05$, t-test), compared to *Lactobacillus gasseri* JCM1130 strain.

The results showed that *Lactobacillus gasseri* OLL2959 strain is capable of taking in a significantly higher amount of purines, compared to *Lactobacillus gasseri* JCM1130 strain having high proliferative capacity in MRS medium.

[0077]

(2) Comparative test of proliferative capacity in the presence of adenine

Lactobacillus gasseri OLL2959 strain and *Lactobacillus gasseri* JCM1130 strain were cultured in the presence of adenine, and influences of lactic acid bacterial strains upon proliferative capacity of the cells were compared.

Adenine was added to the minimal medium (Table 1) to a final concentration of 400 μM, and a medium for this test was prepared. Then, each of *Lactobacillus gasseri* OLL2959 strain and *Lactobacillus gasseri* JCM1130 strain was cultured using MRS medium, and these cultures were inoculated at 4 wt% into the medium for this test and anaerobically cultured at

37°C. At the beginning (0 hours) of the culture, and after 4 and 6 hours from the beginning of the culture, turbidities (absorbance at 650 nm) were measured. The results are shown in Figure 8.

[0078]

As shown in Figure 8, it was revealed that both *Lactobacillus gasseri* OLL2959 strain and *Lactobacillus gasseri* JCM1130 strain had enhanced proliferative capacity in the presence of adenine, but the degree of enhancement of proliferative capacity of *Lactobacillus gasseri* OLL2959 strain was significantly higher than that of *Lactobacillus gasseri* JCM1130 strain having high proliferative capacity in MRS medium ($p<0.05$, t-test). It was therefore revealed that in *Lactobacillus gasseri* OLL2959 strain, the proliferative capacity in the presence of purines is particularly greatly enhanced.

[0079]

[Example 9] Utilization of Purines for Nucleic Acid Synthesis

A test was performed to verify how *Lactobacillus gasseri* OLL2959 strain utilizes adenine in the process of proliferation after the uptake of adenine.

[0080]

Cells of *Lactobacillus gasseri* OLL2959 strain in culture were collected, adjusted to 1.0 x 10^{10} (1.0E+10) cfu/ml, and inoculated at 6% into a minimal medium comprising adenine at a final concentration of 400 μM. Then, ^{14}C-adenine was added, the medium was collected after 0, 1, 2, 3, and 4 hours, and the turbidity (OD at 650 nm) was measured. Furthermore, the cells were collected by centrifugation of the medium at 3,000 rpm for 10 minutes at 4°C and washed with distilled water twice, and subsequently, nucleic acids were extracted from the cells, using a DNA extraction kit ISOPLANT II (Nippon Gene). The concentration of the extracted nucleic acids were measured using a spectrophotometer. Then, the extracted nucleic acids were entirely added into a vial containing a liquid scintillation cocktail, and radioactivity was measured using a liquid scintillation counter.

[0081]

As a result, the turbidity increased in a culture time-dependent manner (OLL2959 strain proliferated), and the amount of the nucleic acids (DNA concentration) also increased in a

culture time-dependent manner (Figure 9). Moreover, because the radioactivity from the nucleic acids also increased as the amount of the nucleic acids increased (Figure 10), it was demonstrated that the OLL2959 strain takes in adenine and utilizes adenine for the synthesis of the nucleic acids required for cell proliferation.

[0082]

[Example 10] Evaluation of the hypoxanthine and IMP (inosinic acid) uptake ability of *Lactobacillus gasseri* bacteria (animal tests)

Twenty-eight Wistar rats (8-week-old) were purchased and acclimated for approximately one week. The rats were fasted for about 16 hours from the day before the test, and body weights after the fasting were measured. Based on the body weights after the fasting, the rats were divided into the three groups in total, i.e., a negative control (physiological saline) group, an IMP (radioisotope ^{14}C-IMP)-treated group, and an IMP+OLL2959 strain (radioisotope ^{14}C-IMP and OLL2959 strain)-treated group (the negative control group only included eight rats, and each of the other groups included ten rats).

[0083]

In all of the cases, 60 μL of blood was collected via the tail vein of the rats in an unanesthetized state. This blood was used as blood samples collected at a time point of 0 minutes prior to the administration of the test substances. These collected blood samples were blended with an equivalent amount of 2 mg/mL EDTA-2Na solution (EDTA-2Na dissolved in physiological saline) and cooled on ice.

[0084]

The test substances were then forcefully administered orally to the rats. To the IMP-treated group was administered 2 mL/body of ^{14}C-IMP diluted with physiological saline. To the IMP+OLL2959 strain-treated group was administered 2 mL/body of a mixture prepared by mixing ^{14}C-IMP and OLL2959 strain before the administration. To these groups, ^{14}C-IMP was administered at 10 μCi/body. To the negative control group was administered 2 mL/body of physiological saline.

[0085]

In all of the cases, at time points of 15 minutes, 30 minutes, 45 minutes, 60 minutes, 90 minutes, 120 minutes, and 180 minutes after the administration of the test substances, 60 μL of blood was collected via the tail vein of the rats in an unanesthetized state. The collected blood samples were blended with an equivalent amount of 2 mg/mL EDTA-2Na solution and cooled on ice.

[0086]

Radioactivity in the collected blood samples was measured using a liquid scintillation counter.

[0087]

Furthermore, an animal test was similarly performed using ^{14}C-hypoxanthine instead of ^{14}C-IMP. In this test using ^{14}C-hypoxanthine, the three groups in total, i.e., a negative control (physiological saline-treated) group, a hypoxanthine-treated group, and a hypoxanthine+OLL2959 strain-treated group (the negative control group only included four rats, and each of the other groups included five rats), were used.

[0088]

The results are shown in Figures 11 to 14. As shown in Figures 11 and 12, around the time points when the blood concentrations of the test substances reached peak values, the amount of IMP absorption significantly decreased in the rats to which a single dose of *Lactobacillus gasseri* OLL2959 strain was administered (* $p<0.1$, the hypoxanthine-treated group vs. the hypoxanthine+OLL2959 strain-treated group; Student's t-test). Likewise, as shown in Figures 13 and 14, the amount of hypoxanthine absorption significantly decreased in the rats to which a single dose of *Lactobacillus gasseri* OLL2959 strain was administered (## $p<0.01$, # $p<0.05$, the hypoxanthine-treated group vs. the hypoxanthine+OLL2959 strain-treated group; Student's t-test). These results further revealed the effect of reducing the purine absorption by the administration of *Lactobacillus gasseri* OLL2959 strain in a single dose. Since IMP is also a tasty component in fish or meat, the confirmation of the effect of reducing the absorption of IMP or its base, hypoxanthine, is of great significance.

[0089]

[Example 11] Evaluation of the purine uptake ability *in vitro*

^{14}C-hypoxanthine or ^{14}C-inosine was added at 1 μCi/ml to *Lactobacillus gasseri* OLL2959 strain adjusted with PBS to approximately 1.0 x 10^8 cfu/ml, and the resulting mixture was incubated at 37°C for 15 minutes or 30 minutes. The cells after the incubation were collected, and radioactivity was measured using a liquid scintillation counter.

[0090]

The results are shown in Figure 15. It was shown that *Lactobacillus gasseri* OLL2959 strain takes in (captures) both inosine and hypoxanthine into the cells *in vitro* (Figure 15). The amount of uptake of hypoxanthine, which is a purine base, was greater than the amount of uptake of inosine, which is a nucleoside. This result was the same as that obtained for the relationship between adenosine and adenine.

[0091]

[Example 12] Evaluation of the survivability of OLL2959 strain in yogurt

Lactobacillus gasseri OLL2959 strain was added at a concentration of 2.5 x 10^8 ± 0.1 x 10^8 cfu/ml to a yogurt drink or physiological saline (day 0), and the viable cell count was determined at day 7, day 21, and day 28. The survival rate at each of the time points was expressed as the ratio (%) of the viable cell count relative to the viable cell count at day 0.

[0092]

As a result, the survival rate of *Lactobacillus gasseri* OLL2959 strain added into the yogurt drink was 72.6% at day 7, 58.5% at day 21, and 61.0% at day 28 (Figure 16). On the other hand, the survival rate of *Lactobacillus gasseri* OLL2959 strain added into physiological saline was 10.6% at day 7, and 0.0% at day 21 and day 28 (Figure 16).

[0093]

These results revealed that the survivability of *Lactobacillus gasseri* OLL2959 strain is higher in yogurt than in physiological saline.

Industrial Applicability

[0094]

According to the present invention, lactic acid bacteria having a purine capturing effect, which have a high purine uptake ability and high proliferative capacity in the presence of

purines, can be efficiently obtained. Such lactic acid bacteria can reduce the amount of absorption of purines in the intestinal tract by reducing the amount of purines in the intestinal tract of the subject to which the lactic acid bacteria are administered, resulting in a decrease in the serum uric acid level. The present invention is therefore also useful for the development of an oral preparation for use in reducing purines in the intestinal tract and reducing the serum uric acid level.

[0095]

All the publications, patents, and patent applications cited in the present specification shall be incorporated by reference in their entirety herein.

Claims

1. A method of screening lactic acid bacteria comprising measuring an amount of uptake of purines by lactic acid bacteria in a medium comprising the purines, and selecting lactic acid bacteria having a purine capturing effect, using, as an index, the amount of uptake of purines.

2. The method according to claim 1, wherein the purines in the medium are purine bases.

3. The method according to claim 1 or 2, wherein the purines in the medium are labeled with a radioisotope.

4. The method according to any one of claims 1 to 3, comprising measuring an amount of proliferation of the lactic acid bacteria in the medium comprising the purines, and selecting lactic acid bacteria having a purine capturing effect, using, as an index, the amount of proliferation together with the amount of uptake of purines.

5. The method according to any one of claims 1 to 4, wherein the lactic acid bacteria are *Lactobacillus gasseri* bacteria.

6. A lactic acid bacterium having a purine capturing effect obtainable by the method according to any one of claims 1 to 5.

7. An agent for capturing purines comprising as an active component the lactic acid bacteria having a purine capturing effect obtainable by the method according to any one of claims 1 to 5.

8. The agent for capturing purines according to claim 7, which is for use in reducing a serum uric acid level.

9. The agent for capturing purines according to claim 7 or 8, wherein the lactic acid bacteria are *Lactobacillus gasseri* OLL2959 strain (Accession No: NITE BP-224).

10. A food or beverage product or pharmaceutical preparation comprising the agent for capturing purines according to any one of claims 7 to 9.

11. The food or beverage product or pharmaceutical preparation according to claim 9, which is for use in reducing purines in the intestinal tract.

12. The food or beverage product or pharmaceutical preparation according to claim 10 or 11, which is for use in administration to a human subject having a serum uric acid level of 6 to 8 mg/dL.

13. The food or beverage product or pharmaceutical preparation according to any one of claims 10 to 12, comprising 1×10^8 to 1×10^{10} cfu of the lactic acid bacteria per dose.