

Description

Title of Invention: LACTIC ACID BACTERIA SUPPRESSING PURINE ABSORPTION AND USE THEREOF

Technical Field

[0001]

The present invention relates to lactic acid bacteria suppressing purine absorption 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 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 acquiring 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 (Kokai) 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 2008/129802

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

Summary of Invention

Technical Problem

[0005]

An object of the present invention is to provide lactic acid bacteria suppressing purine absorption and use thereof.

Solution to Problem

[0006]

In order to solve the above-described problem, as a result of their extensive research, the present inventors found that certain bacterial species of the genus *Lactobacillus* have a

high ability to convert purine bases into purine nucleotides, and completed the present invention.

[0007]

That is, the present invention includes the following.

[1] A method of screening for lactic acid bacteria having an enhanced ability to convert a purine base into a purine nucleotide, comprising culturing lactic acid bacteria in a solution containing adenine, 5-phospho-D-ribose-1-diphosphate, and Mg^{2+} , and comparing the resulting activity of converting adenine to adenylic acid as an index of the ability with that of *Lactobacillus gasseri* ATCC 33323 strain.

[2] The method according to [1] above, further comprising measuring 5'-nucleotidase activity, and selecting lactic acid bacteria having a reduced 5'-nucleotidase activity compared to *Lactobacillus gasseri* ATCC 33323 strain.

[3] The method according to [1] or [2] above, wherein the lactic acid bacteria are *Lactobacillus* bacteria.

[4] The method according to any of [1] to [3] above, wherein the solution is a buffer solution.

[0008]

[5] A conversion agent for converting a purine base into a purine nucleotide, comprising the lactic acid bacteria obtainable by the method according to any of [1] to [4] above as an active component.

[6] The conversion agent according to [5] above, which is for use in converting adenine to adenylic acid, or converting guanine to guanylic acid.

[7] The conversion agent according to [5] or [6] above, wherein the lactic acid bacteria are *Lactobacillus gasseri* OLL2959 strain (Accession No: NITE BP-224).

[0009]

[8] A food or beverage product or a pharmaceutical preparation comprising the conversion agent according to any of [5] to [7] above.

[9] The food or beverage product or the pharmaceutical preparation according to [8] above, which is for use in reducing a serum uric acid level.

[10] The food or beverage product or the pharmaceutical preparation according to [9] above, wherein reducing the serum uric acid level involves promoting conversion of adenine to adenylic acid and promoting conversion of guanine to guanylic acid in an intestinal tract.

[11] The food or beverage product or the pharmaceutical preparation according to [9] or [10] above, which is for use in administration to a human subject having a serum uric acid level of 6 to 8 mg/dL.

[12] The food or beverage product or the pharmaceutical preparation according to any of [8] to [11] above, comprising 1×10^8 to 10^{10} cfu of the above-described lactic acid bacteria per dose.

[0010]

[13] A method of producing a purine nucleotide from a purine base, comprising reacting the conversion agent according to any of [5] to [7] above with the purine base in the presence of 5-phospho-D-ribose-1-diphosphate and Mg^{2+} .

[14] The method according to [13] above, which is for producing adenylic acid from adenine, or for producing guanylic acid from guanine.

Advantageous Effects of Invention

[0011]

Through the use of the lactic acid bacteria or the conversion agent according to the present invention a purine base can be efficiently converted into a purine nucleotide, thereby reducing the serum uric acid level in a subject to which the lactic acid bacteria or the conversion agent is administered.

The present specification includes the contents of Japanese Patent Application Nos. 2014-134973, 2014-234050, and 2015-064201, from which the present application claims priority.

Brief Description of Drawings

[0012]

[Figure 1] Figure 1 is a graph showing changes with time in the conversion rate from adenine to AMP (AMP/adenine conversion rate) obtained with *Lactobacillus gasseri* OLL2959 strain.

[Figure 2] Figure 2 is a graph showing changes with time in the conversion rate of guanine into GMP (GMP/guanine conversion rate) obtained with *Lactobacillus gasseri* OLL2959 strain.

[Figure 3] Figure 3 is a graph showing purine nucleosidase activity of *Lactobacillus gasseri* OLL2959 strain. The left bar shows the conversion rate from adenosine to adenine, the central bar shows the conversion rate from inosine to hypoxanthine, and the right bar shows the conversion rate from guanosine to guanine.

[Figure 4] Figure 4 is a graph showing 5'-nucleotidase activity of *Lactobacillus gasseri* OLL2959 strain. The left bar shows the conversion rate from AMP to adenosine, the central bar shows the conversion rate from IMP to inosine, and the right bar shows the conversion rate from GMP to guanosine.

[Figure 5] Figure 5 is a graph showing changes with time in the serum uric acid level in a human subject who has continuously ingested *Lactobacillus gasseri* OLL2959 strain.

[Figure 6] Figure 6 shows a change rate of the serum uric acid level in each subject after the ingestion of a food product for the study (from the pre-ingestion examination to the examination at week 8).

[Figure 7] Figure 7 is a graph showing a purine uptake ability of *Lactobacillus gasseri* OLL2959 strain.

[Figure 8] Figure 8 is a graph showing proliferative capacity of *Lactobacillus gasseri* OLL2959 strain in the presence of purines.

[Figure 9] Figure 9 shows adenine uptake abilities of a plurality of lactic acid bacterial strains.

[Figure 10] Figure 10 is a graph showing proliferative capacities of a plurality of lactic acid bacterial strains in the presence of adenine.

[Figure 11] Figure 11 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 12] Figure 12 is a graph showing the results of comparing adenine uptake abilities between species of lactic acid bacterial strains.

[Figure 13] Figure 13 is a graph showing the results of comparing proliferative capacities of the lactic acid bacterial strains in the presence of adenine between species of lactic acid bacterial strains.

Description of Embodiments

[0013]

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).

[0014]

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.

[0015]

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).

[0016]

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.

[0017]

In the present invention, lactic acid bacteria having a high ability to convert a purine base into a nucleotide can be acquired by selecting such lactic acid bacteria, using as an index of the ability an activity of converting adenine to adenylic acid. The present invention relates to a screening method by contacting adenine as a substrate with lactic acid bacteria in a solution and screening for lactic acid bacteria having an enhanced ability to convert a purine base into a nucleotide, using as an index an activity of converting adenine to adenylic acid in the solution which is generated by culturing them.

[0018]

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 *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*, and *Lactobacillus reuteri*; and are particularly preferably *Lactobacillus gasseri*. The lactic acid bacteria are preferably used in the screening after being cultured in a suitable medium (e.g., MRS medium) and having its concentration being adjusted.

[0019]

In the screening method of the present invention, the lactic acid bacteria are cultured in a solution containing adenine, 5-phospho-D-ribose-1-diphosphate, and Mg^{2+} . This is because the reaction of converting adenine to adenylic acid (AMP-based salvage activity) is catalyzed by adenine phosphoribosyltransferase (APRT), using as substrates adenine and Mg^{2+} -PRPP formed by 5-phospho-D-ribose-1-diphosphate (PRPP; also referred to as phosphoribosyl pyrophosphate) in the presence of Mg^{2+} . PRPP may be blended into the solution by addition into the solution in the form of any salt of PRPP (any salt, for example, a sodium salt such as 5-phospho-D-ribose-1-diphosphate pentasodium salt, a potassium salt, or a calcium salt). Mg^{2+} can also be blended into the solution by addition into the solution in the form of any salt thereof (e.g., $MgCl_2$). The solution for use in the screening is preferably a buffer solution, although it may be any solution as long as the lactic acid bacteria can survive in the solution and the solution does not inhibit enzyme activities of the lactic acid bacteria. Examples of buffer solutions include, but are not limited to, phosphate buffer, Tris buffer, Tris-HCl buffer, and HEPES buffer. The pH of the solution is, but not particularly limited to, typically preferably pH 6 to 8.

[0020]

The concentrations of adenine, 5-phospho-D-ribose-1-diphosphate (PRPP), Mg^{2+} , and the lactic acid bacteria in the solution (reaction solution) for use in the screening may be any levels suitable for the reaction of converting adenine to adenylic acid by the lactic acid bacteria. For example, the solution may contain 0.1 mM to 1 mM (preferably, 0.1 mM to 0.3 mM) of adenine, 1 to 15 mM (preferably, 1 mM to 5 mM) of PRPP, and 4 mM to 40 mM

(preferably, 4 mM to 15 mM) of Mg^{2+} , as final concentrations at the beginning of the reaction. The reaction solution may also contain 5×10^8 cfu/ml to 5×10^{10} cfu/ml of the lactic acid bacteria, for example, 5×10^9 cfu/ml of the lactic acid bacteria, although the concentration of the lactic acid bacteria is not limited thereto. The above-described solution may additionally contain any substances that do not substantially affect the reaction of converting adenine to adenylic acid by the lactic acid bacteria.

[0021]

In the screening, the lactic acid bacteria are preferably suspended and cultured in the solution containing adenine, 5-phospho-D-ribose-1-diphosphate (PRPP), and Mg^{2+} . The lactic acid bacteria are preferably cultured by incubation at a temperature suitable for culturing the lactic acid bacteria, generally at 20 to 50°C, and preferably 30 to 40°C, for example, at approximately 37°C. The lactic acid bacteria can be suitably cultured by, for example, incubation in a water bath with shaking. The lactic acid bacteria are cultured for a certain time, typically for at least 15 minutes or more, preferably for 30 minutes or more, and more preferably 60 minutes or more.

[0022]

After culturing the lactic acid bacteria for a certain time in the above-described solution, the reaction of converting adenine to adenylic acid is stopped. This conversion reaction is stopped preferably by the addition of TFA (trifluoroacetic acid) to the solution. For example, an equivalent amount of 5% TFA may be added to the solution. In order to examine a time course of the conversion reaction, the reaction is preferably stopped while gradually varying the length of the culture time of the lactic acid bacteria.

[0023]

Adenine and adenylic acid in the reaction solution in which the conversion reaction has been stopped are subsequently quantified. Other purines (e.g., purine nucleosides) in the reaction solution may also be quantified. This quantification may be performed by any measurement method, for example, a HPLC method. Preferably, a supernatant of the reaction solution is collected and filtered, and the filtrate is subjected to measurement for quantitative determination.

[0024]

Measurement by the HPLC method can be performed, for example, under the following conditions:

- Mobile phase: A, 20 mM phosphate buffer solution (pH 7.5)
B, 40 mM phosphate buffer solution (pH 7.5)/acetonitrile (1:1)
- Column: SHISEIDO CAPCELL PAK C18 MG2 (2.0 mm id x 150 mm)
- Flow rate: 0.2 mL/minute
- Temperature: 40°C
- Injection volume: 5 µl
- Detection wavelength: 254 nm (UV)
- Gradient A/B (minutes): 100/0 (0 minutes)-100/0 (5 minutes)-80/20 (20 minutes) [%]

In the case of quantification by the HPLC method, the amounts of adenine and adenylic acid in the reaction solution can be calculated as relative values through measurement of the areas under the peaks in the HPLC chart. The conversion rate of adenine to adenylic acid (AMP) at each time point when the reaction is stopped can be calculated by the following formula:

Conversion rate (%) = (Area under the peak of AMP for the test sample – Area under the peak of AMP for the control sample) / Area under the peak of adenine at a time point of zero minutes for the test sample – Area under the peak of adenine at a time point of zero minutes for the control sample)

[0025]

The "control" used herein was similarly measured for a solution prepared using an equivalent amount of the buffer solution, instead of adding adenine as the substrate.

[0026]

In the screening method of the present invention, the conversion rate of adenine to adenylic acid (AMP) can be used as the value indicating the activity of converting adenine to adenylic acid. That is, in the screening method of the present invention, whether or not the lactic acid bacteria have an activity of converting adenine to adenylic acid (AMP), and the conversion activity level can be determined using the conversion rate of adenine to adenylic

acid (AMP) as an index of the activity. For example, according to the present invention, the lactic acid bacteria can be determined to have an activity of converting adenine to adenylic acid (AMP) if the conversion rate of adenine to adenylic acid (AMP) reaches a maximum of 30% or more as measured with time. In the screening method of the present invention, lactic acid bacteria having a conversion rate of adenine to adenylic acid (AMP) of preferably a maximum of 40% or more, more preferably 50% or more, still more preferably 70% or more, and particularly preferably 90% or more as measured with time, are preferably selected. In a preferred embodiment of the screening method of the present invention, when the conversion rate of adenine to adenylic acid (AMP) increases, for example, to not less than 5 times, preferably not less than 7 times, more preferably not less than 10 times, still more preferably not less than 13 times, and particularly preferably not less than 20 times higher than that obtained with, for example, ATCC 33323 strain, which is a *Lactobacillus gasseri* reference strain, by the time point when the conversion rate of adenine to adenylic acid (AMP) reaches a maximum level (preferably a plateau) as measured with time, the lactic acid bacteria can be determined to have "an enhanced ability to convert a purine base into a purine nucleotide", as having a significantly enhanced ability to convert adenine to adenylic acid.

[0027]

The lactic acid bacteria thus selected may have not only a high ability to convert adenine to adenylic acid (AMP-based salvage activity), but also a high ability to convert other purine bases into purine nucleotides by the salvage activity. Specifically, the lactic acid bacteria may have an enhanced ability to convert guanine to guanylic acid. Therefore, it is also preferred that the lactic acid bacteria selected as having an enhanced ability to convert adenine to adenylic acid be similarly examined for activities of converting other purine bases into purine nucleotides. Specifically, the lactic acid bacteria may be cultured in a solution containing guanine, 5-phospho-D-ribose-1-diphosphate, and Mg^{2+} and examined as to whether its ability to convert guanine to guanylic acid has been enhanced, using as an index the thus generated activity of converting guanine to guanylic acid. The measurement of the activity of converting guanine to guanylic acid and the calculation of the conversion rate can be performed in the same manner as that described above for the conversion of adenine to

adenylic acid, except that guanine is used instead of adenine, and guanylic acid is used instead of adenylic acid. In the present invention, when the conversion rate of guanine to guanylic acid (GMP) increases, for example, to not less than 5 times, preferably not less than 7 times, more preferably not less than 10 times, still more preferably not less than 13 times, and particularly preferably not less than 20 times higher than that obtained with, for example, ATCC 33323 strain, which is a *Lactobacillus gasseri* reference strain, by the time point when the conversion rate of guanine to guanylic acid (GMP) reaches a maximum level (preferably a plateau) as measured with time, the lactic acid bacteria can be determined to have a significantly enhanced ability to convert guanine to guanylic acid as well.

[0028]

In the present invention, lactic acid bacteria having an enhanced ability to convert a purine base into a purine nucleotide can be screened for in this manner. The lactic acid bacteria obtainable by the screening method of the present invention have a high ability to convert a purine base into a purine nucleotide, particularly, a high ability to convert adenine to adenylic acid and a high ability to convert guanine to guanylic acid.

[0029]

The lactic acid bacteria obtainable by the screening method of the present invention preferably have an activity of converting a purine base into a purine nucleotide (salvage activity) higher than an activity of degrading the purine nucleotide into a purine nucleoside. The screening method of the present invention therefore may further comprise steps of measuring an activity of degrading a purine nucleotide as a substrate into a purine nucleoside, and selecting lactic acid bacteria exhibiting a reduction of the activity. Specifically, using a purine nucleotide (e.g., adenylic acid) as a substrate, 5'-nucleotidase (5'-NT) activity that catalyzes the degradation of purine nucleotides into purine nucleosides in the lactic acid bacteria may be measured.

[0030]

5'-nucleotidase (5'-NT) activity of the lactic acid bacteria can be measured by a conventional method. For example, the lactic acid bacteria are cultured in a solution containing a purine nucleotide (e.g., adenylic acid) and Mg^{2+} . Mg^{2+} can be blended into the

solution by addition into the solution in the form of any salt thereof (e.g., MgCl_2). The solution for use in the screening is preferably a buffer solution, although it may be any solution as long as the lactic acid bacteria can survive in the solution and the solution does not inhibit enzyme activities of the lactic acid bacteria. Examples of buffer solutions include, but are not limited to, phosphate buffer, Tris buffer, Tris-HCl buffer, and HEPES buffer. The pH of the solution is, but not particularly limited to, typically preferably pH 6 to 8. As a control, ATCC 33323 strain, which is a *Lactobacillus gasseri* reference strain, is preferably used.

[0031]

The concentrations of the purine nucleotide, Mg^{2+} , and the lactic acid bacteria in the reaction solution may be any levels suitable for the reaction of converting the purine nucleotide into the purine nucleoside by ATCC 33323 strain. For example, the reaction solution may contain 0.1 mM to 1 mM (preferably 0.1 mM to 0.3 mM) of the purine nucleotide and 4 mM to 40 mM (preferably 4 mM to 15 mM) of Mg^{2+} , as final concentrations at the beginning of the reaction. The reaction solution may also contain 5×10^8 cfu/ml to 5×10^{10} cfu/ml of the lactic acid bacteria, for example, 5×10^9 cfu/ml of the lactic acid bacteria, although the concentration of the lactic acid bacteria is not particularly limited thereto. The above-described solution may additionally contain any substances that do not substantially affect the reaction of converting the purine nucleotide into the purine nucleoside by the lactic acid bacteria. The culture conditions for the lactic acid bacteria may be the same conditions as those in the above-described test of converting adenine to adenylic acid.

[0032]

The measurement of the activity of converting a purine nucleotide into a purine nucleoside (i.e., 5'-nucleotidase activity) and the calculation of the conversion rate can be performed in the same manner as the above-described test for the conversion rate of adenine to adenylic acid, except that a purine nucleotide (e.g., adenylic acid) is used instead of adenine, and a purine nucleoside (e.g., adenosine when adenylic acid is used as the substrate) is used instead of adenylic acid. The reaction time is, but not particularly limited to, preferably set longer (e.g., 120 minutes) than the reaction time for converting adenine to adenylic acid. In the present invention, when the conversion rate of a purine nucleotide to a purine nucleoside,

(as a preferred example, the conversion rate of adenylic acid to adenosine) decreases to, for example, not more than 1/5, preferably not more than 1/7, more preferably not more than 1/10, still more preferably not more than 1/13, and particularly preferably not more than 1/20 of that obtained with, for example, ATCC 33323 strain, which is a *Lactobacillus gasseri* reference strain, the lactic acid bacteria can be determined to have significantly decreased 5'-nucleotidase activity. In the lactic acid bacteria having significantly decreased 5'-nucleotidase activity, the conversion of a purine base into a purine nucleotide is more predominant than the conversion of the purine nucleotide into a purine nucleoside. This further leads to a decrease in purine nucleosides which are absorbed from the intestinal tract within the body of a mammal to promote uric acid production.

[0033]

The present invention also provides a conversion agent for converting a purine base into a purine nucleotide, which comprises, as an active component, the lactic acid bacteria that can be obtained by the above-described screening method, and have a high ability to convert a purine base into a purine nucleotide. This conversion agent is also preferably used, for example, for converting adenine to adenylic acid or for converting guanine to guanylic acid. With the use of this conversion agent, the purine base as the substrate can be efficiently converted into the purine nucleotide in an *in vivo* or *in vitro* reaction system.

[0034]

A preferred example of the lactic acid bacteria having a high ability to convert a purine base into a purine nucleotide and having decreased 5'-nucleotidase activity as described above includes *Lactobacillus gasseri* OLL2959 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.

[0035]

Lactobacillus gasseri OLL2959 strain has a high ability to convert a purine base into a purine nucleotide (salvage activity), while having a low ability to convert the purine nucleotide into a purine nucleoside. It is therefore believed that when *Lactobacillus gasseri* OLL2959 strain is administered to a human, the recycle of the purine nucleotide from the purine by the microorganism is more predominant than the absorption/metabolism of the purine base or purine nucleoside by a human, which contributes to a decrease in the amount of uric acid production.

[0036]

The present invention also provides a food or beverage product or a pharmaceutical preparation comprising the above-described conversion agent for converting a purine base into a purine nucleotide. The conversion agent of the present invention may be a drug or composition containing cells of the lactic acid bacteria of the present invention having an ability to convert a purine base into a purine nucleotide, or the conversion agent may be a fermented product or culture prepared with the lactic acid bacteria, or a concentrate thereof, or a drug or composition containing the same. The lactic acid bacteria according to the present invention contained in the conversion agent of the present invention may be killed cells or treated cells so long as they show their enzyme activities, but are preferably viable cells. The conversion agent of the present invention is capable of imparting an activity of converting a purine base into a purine nucleotide in a living body (typically in the intestinal tract) to a food or beverage product or a pharmaceutical preparation. The food or beverage product or the pharmaceutical preparation containing the conversion agent of the present invention can promote, through the lactic acid bacteria and the enzyme activities thereof, the conversion of a purine base into a purine nucleotide, for example, promote the conversion of adenine to adenylic acid and promote the conversion of guanine to guanylic acid, in the intestinal tract in a subject to which the food or beverage product or the pharmaceutical preparation is administered, thereby effectively reducing the serum uric acid level. That is, the food or beverage product or the pharmaceutical preparation of the present invention may be for use in reducing the serum uric acid level, along with promoting the conversion of adenine to adenylic

acid and promoting the conversion of guanine to guanylic acid. The food or beverage product or the pharmaceutical preparation containing the conversion agent of the present invention can be preferably used, for example, for use in preventing, treating, or ameliorating of gout or hyperuricemia, or alleviating the symptoms of gout or hyperuricemia.

[0037]

As used herein, the "food or beverage product" includes, but is not particularly limited to, beverages, food products, and functional foods. The type of the food or beverage product containing the conversion agent of the present invention is not particularly limited. Examples of beverages containing the conversion agent of the present invention include beverages such as 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, or vegetable juices such as tomato or carrot), alcoholic beverages (beer, low-malt beer, wine, etc.), carbonated beverages, soft drinks, and water-based beverages. 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 yogurt such as set type yogurt or soft yogurt, confectionery, and cheese, and an example of a particularly preferred food product includes yogurt, for example, set type yogurt or soft yogurt. For production methods and the like of various food products, reference may be made to existing guidebooks.

[0038]

Fermented milk such as yogurt containing the lactic acid bacteria having an ability to convert a purine base into a purine nucleotide may be also produced by, for example, adding the lactic acid bacteria of the present invention having an ability to convert a purine base into a purine nucleotide, 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 an ability to convert a purine base into a purine nucleotide. Alternatively, a dairy product or fermented milk may be produced using a starter obtained by mixing the above-described starter and the lactic acid bacteria of the present invention having a high ability to convert a purine base into a purine nucleotide. The dairy product or fermented milk using the starter can be produced in accordance with a conventional method. For example, plain yogurt can be produced by, for example, mixing the 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 of the present invention having a high ability to convert a purine base into a purine nucleotide, in the production 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 production of yogurt. Furthermore, the present invention preferably provides a method of enhancing the ability to convert a purine base into a purine nucleotide for a dairy product or fermented milk such as yogurt or cheese, the method using as an active component the lactic acid bacteria of the present invention having a high ability to convert a purine base into a purine nucleotide. The present invention particularly preferably provides a method of enhancing the ability to convert a purine base into a purine nucleotide in yogurt.

[0039]

Functional foods are particularly preferred as the food or beverage product containing the conversion agent of 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) 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.

[0040]

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.

[0041]

The functional foods of the present invention are useful for reducing the serum uric acid level. The functional foods 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 promoting the conversion of a purine base into a purine nucleotide. The functional foods of the present invention can be preferably used for, for example, reducing the serum uric acid level along with promoting the conversion of adenine to adenylic acid and promoting the conversion of guanine to guanylic acid.

[0042]

The food or beverage product of the present invention such as a functional food (in Japan, preferably a food for a specified health use or a qualified food for a specified health use) may be 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 of the present invention having a high ability to convert a purine base into a purine nucleotide, in the production of a functional food (which preferably comprises adding (blending) the lactic acid bacteria into the functional food or raw materials thereof), and particularly preferably provides use of the lactic acid bacteria in the production of a food for a specified health use. Furthermore, the present invention preferably provides a method of enhancing the ability to convert a purine base into a purine nucleotide for a functional food, the method using as an active component the lactic acid bacteria of the present invention having a high ability to convert a purine base into a purine nucleotide. The present invention particularly preferably

provides a method of enhancing the ability to convert a purine base into a purine nucleotide for a food for a specified health use.

[0043]

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).

[0044]

The food or beverage product of the present invention may contain any food components which is not particularly limited. The food or beverage product of the present invention may contain, in addition to the lactic acid bacteria or conversion agent of the present invention, 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 food products containing large amounts of these components.

[0045]

The pharmaceutical preparation (pharmaceutical composition) containing the conversion agent of the present invention may contain, in addition to the lactic acid bacteria or conversion agent of the present invention, pharmaceutically acceptable carriers or additives. Examples of usable 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. The carriers or additives can be selected as appropriate or in combination, in accordance with the dosage form of the preparation. The pharmaceutical preparation of the present invention may additionally contain other pharmacological components.

[0046]

The 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.

[0047]

The dose of the food or beverage product or the pharmaceutical preparation of the present invention is not particularly limited and 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, administration route, number of doses, and the like. For example, the food or beverage product or the pharmaceutical preparation of the present invention is administered preferably in an amount of 1×10^5 to 1×10^{11} cfu, more preferably 1×10^8 to 1×10^{10} cfu, and

still more preferably 1×10^9 to 1×10^{10} cfu, and for example, preferably 4×10^9 to 6×10^{10} cfu, of the lactic acid bacteria of the present invention, per dose. The food or beverage product or the pharmaceutical preparation of the present invention preferably contains such an amount of the lactic acid bacteria of the present invention per dose. The food or beverage product or the pharmaceutical preparation of the present invention is preferably administered to the subject once or more a day, preferably twice or more, and typically twice a day. The food or beverage product or the pharmaceutical preparation of the present invention is preferably administered continuously, and is more preferably administered every day, for example. The food or beverage product or the pharmaceutical preparation of the present invention is preferably administered for at least a week, and preferably for 2 weeks or more, for example, for 4 weeks or more. The food or beverage product or the pharmaceutical preparation of the present invention is preferably orally administered. In the present invention, the "administration" includes both the "ingestion" of the food or beverage product and the "administration" of the pharmaceutical preparation. In the present invention, the "oral administration" also includes administration via tube feeding through a nasal tube, a gastric tube, or the like.

[0048]

The subject to which 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. In particular, the subject is preferably a human subject, more preferably a human subject with gout and/or hyperuricemia, and still more preferably, but is not limited to, 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 is preferably a human subject with mild to borderline hyperuricemia having a serum uric acid level of 6 to 8 mg/dL. In another embodiment, the subject is preferably a human subject having a serum uric acid level of 7 mg/dL or more, and preferably 7 to 11 mg/dL (in one example, between 7.6 mg/dL and 9.5 mg/dL), and among the above, the subject is more preferably a human subject suffering from hyperuricemia and gout. Moreover, a subject showing a correlation between the serum uric acid level and the amount of purines ingested from foods prior to the measurement of the

serum uric acid level (e.g., the total amount of purines ingested for 3 days) is more preferred as the subject for administration (feeding). The food or beverage product or the pharmaceutical preparation of the present invention is suitable for administration to a subject in which the activity of converting a purine base into a purine nucleotide (salvage activity), for example, activity of converting adenine to adenylic acid, and particularly adenine phosphoribosyltransferase activity that plays the role of converting adenine to adenylic acid, have been decreased.

[0049]

The present invention also provides a method of producing a purine nucleotide from a purine base, by reacting the conversion agent of the present invention with the purine base in the presence of 5-phospho-D-ribose-1-diphosphate and Mg^{2+} . The method may be implemented *in vivo* or *in vitro*. In the case of implementation *in vivo*, the method using the conversion agent of the present invention may use the food or beverage product containing the conversion agent of the present invention and may not include medical practice. In the case of implementation *in vitro*, the method of the present invention is a method of synthesizing (producing) a purine nucleotide using a purine base in a solution as the substrate. Specifically, the method of the present invention is also preferably a method of producing adenylic acid from adenine. The method of the present invention may be a method of producing guanylic acid from guanine. The conversion of the purine base by the lactic acid bacteria in the presence of 5-phospho-D-ribose-1-diphosphate and Mg^{2+} can be performed in the same manner as the above-described test for the conversion rate of adenine to adenylic acid.

[0050]

Furthermore, the present inventors found that some species of lactic acid bacteria such as *Lactobacillus gasseri* bacteria, for example, *Lactobacillus gasseri* OLL2959 strain, have a purine uptake ability and high proliferative capacity in the presence of purines, and there is 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 suppresses purine absorption, thus contributing to a decrease in the serum uric acid level.

Based on this finding, a purine capturing effect of the lactic acid bacteria can also be utilized to reduce the serum uric acid level.

[0051]

The present invention therefore enables efficient acquisition (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. There is provided a method of screening lactic acid bacteria comprising measuring an amount of uptake of purines by lactic acid bacteria in a medium containing purines and acquiring (selecting) lactic acid bacteria having the purine capturing effect, using as an index the amount of uptake of purines. More specifically, according to the present invention, lactic acid bacteria can be screened by culturing lactic acid bacteria in a medium containing purines, measuring, preferably over time, an amount of uptake of purines into the cells, and acquiring (selecting) lactic acid bacteria having the purine capturing effect using as an index the amount of uptake of purines. The lactic acid bacteria having the purine capturing effect thus obtained 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, cells of the lactic acid bacteria 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 are to be excreted out of the body together with the lactic acid bacteria. According to the present invention, therefore, screening for lactic acid bacteria having the effect of reducing the serum uric acid level can be performed by culturing lactic acid bacteria in a medium containing 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 acquiring (selecting) the obtained lactic acid bacteria having the purine capturing effect as the lactic acid bacteria having the effect of reducing the serum uric acid level.

[0052]

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, in addition to the above-described selection using the purine uptake ability as an index, enhancement of the proliferative capacity of the selected lactic acid bacteria in the presence of purines may be examined, and the enhancement of the proliferative capacity may be used as an index, thereby enabling more accurate acquisition (selection) of the lactic acid bacteria having the purine capturing effect. That is, according to the present invention, lactic acid bacteria can be screened by measuring an amount of proliferation of lactic acid bacteria having a purine uptake ability in a medium containing purines and acquiring (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, according to the present invention, screening for lactic acid bacteria having the effect of reducing the serum uric acid level can be performed by culturing lactic acid bacteria in a medium containing 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 acquiring (selecting) the obtained 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 methods, 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.

[0053]

While the lactic acid bacteria to be subjected to the screening method of the present invention are not particularly limited, they are preferably *Lactobacillus* bacteria. Examples of *Lactobacillus* bacteria are the same as those illustrated above, and *Lactobacillus gasseri* bacteria are particularly preferred. 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 5 shows an example of a particularly preferred minimal medium.

[0054]

While the lactic acid bacteria to be subjected to the screening method of the present invention are not particularly limited, they are preferably *Lactobacillus* bacteria. Examples of *Lactobacillus* bacteria are the same as those illustrated above, and *Lactobacillus gasseri* bacteria are particularly preferred. Any strain of these lactic acid bacteria 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 the lactic acid bacteria used, for example, *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 5 shows an example of a particularly preferred minimal medium.

[0055]

Purines to be incorporated into the medium may be purine bases, purine nucleosides, and/or purine nucleotides. 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. Purines may be labeled with a radioisotope.

[0056]

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 enables quantitative detection, 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 assessed by, for example, culturing lactic acid bacteria in a medium containing 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, the lactic acid bacteria can be determined to have an ability to take in purines into the cells (purine uptake ability). In the present invention, the lactic acid bacteria thus determined to have a purine uptake ability can be acquired (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 acquired (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.

[0057]

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 containing 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 has significantly increased 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. 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 e.g., the proliferative capacity thereof in the presence of purines can be measured.

[0058]

In the measurement described above, preferably, the lactic acid bacteria are inoculated at 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.

[0059]

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 thus-selected lactic acid bacteria 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).

[0060]

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.

[0061]

Furthermore, using the lactic acid bacteria having the purine capturing effect that can be obtained by the above-described screening method, an agent for capturing purines, and preferably an agent for capturing purines for use in oral administration, comprising the lactic acid bacteria as an active component, can be provided. The agent for capturing purines may contain carriers or additives acceptable for oral administration, in addition to the lactic acid bacteria having the purine capturing effect, which are preferably *Lactobacillus gasseri* bacteria. The agent for capturing purines may be a drug or composition containing cells of the lactic acid bacteria having the purine capturing effect, 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 containing the same. The lactic acid bacteria having the purine capturing effect contained in the agent for capturing purines are preferably viable cells. As described above, the agent for capturing purines 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.

[0062]

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.

[0063]

The agent for capturing purines can also be used in combination with a food or beverage product or a pharmaceutical preparation. Thus, there is also provided an agent for capturing purines for use in combination with a food or beverage product or a pharmaceutical preparation.

[0064]

Using the above-described agent for capturing purines, a food or beverage product or a pharmaceutical preparation comprising the above-described agent for capturing purines can also be provided. This food or beverage product or the pharmaceutical preparation provides 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 and beverage products and pharmaceutical preparations can therefore be used for use in reducing purines in the intestinal tract. The "purines in the intestinal tract" herein 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 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 containing the agent for capturing purines can be preferably used, for example, for preventing, treating, ameliorating, or alleviating the symptoms of gout or hyperuricemia.

[0065]

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 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, or vegetable juices such as tomato or carrot), alcoholic beverages (beer, low-malt beer, wine, etc.), carbonated beverages, soft drinks, and water-based beverages. 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 yogurt such as set type yogurt or soft yogurt, confectionery, and cheese, and an example of a particularly preferred beverage includes yogurt, for example, set type yogurt or soft yogurt. For production methods and the like of various food products, reference may be made to existing guidebooks.

[0066]

Fermented milk such as yogurt containing the lactic acid bacteria having the purine capturing effect may also 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 the starter into milk or a dairy product cooled after heating, mixing, homogenization, and pasteurization treatment, and subsequently fermenting and cooling it. In a preferred aspect, there is provided use of the lactic acid bacteria having the purine capturing effect in the production 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). In a particularly preferred aspect, there is provided use of the lactic acid bacteria in the production of yogurt. Furthermore, there is also provided a method of reducing purines based on the purine capturing effect of the lactic acid bacteria, in 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.

[0067]

Functional foods are particularly preferred as the food or beverage product. Types, preferred examples and the like of the functional foods are the same as those described for the functional foods in connection with the above-described food or beverage product in which the above-mentioned conversion agent is used.

[0068]

This functional food is particularly useful for reducing the serum uric acid level by reducing purines in the intestinal tract. The functional food 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.

[0069]

The food or beverage product such as this functional food (in Japan, preferably a food for a specified health use, a qualified food for a specified health use, or a food with a function claim) 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. There is also provided use of the lactic acid bacteria having the purine capturing effect in the production of such functional foods (which preferably comprises adding (blending) the lactic acid bacteria to the functional food or raw materials thereof).

[0070]

The dosage form or shape of this functional food is the same as that described for the functional food in connection with the above-described food or beverage product in which the above-mentioned conversion agent is used.

[0071]

Food components other than the above-described lactic acid bacteria contained in the food or beverage product are also the same as those described in connection with the above-described food or beverage product in which the above-mentioned conversion agent is used.

[0072]

The above-described functional food containing the lactic acid bacteria having the purine capturing effect or the agent for capturing purines may also contain carriers or additives acceptable for oral administration, in addition to the lactic acid bacteria or the agent for capturing purines. 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.

[0073]

Furthermore, the pharmaceutical preparation (pharmaceutical composition) containing the lactic acid bacteria having the purine capturing effect or the agent for capturing purines 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 and additives are the same as those described in connection with the above-described pharmaceutical preparation in which the above-mentioned conversion agent is used. 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 may additionally contain other pharmacological components.

[0074]

The above-described pharmaceutical preparation is preferably orally administered. The pharmaceutical preparation 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.

[0075]

The dose (amount of ingestion) of the lactic acid bacteria having the purine capturing effect, the agent for capturing purines, or the food or beverage product or the pharmaceutical preparation 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. While the dose of the lactic acid bacteria having the purine capturing effect, the agent for capturing purines, or the food or beverage product or the pharmaceutical preparation is not particularly limited, the dose is an amount such that the lactic acid bacteria (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. The agent for capturing purines, or the food or beverage product or the pharmaceutical preparation contains the lactic acid bacteria having the purine capturing effect 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.

[0076]

In one embodiment, the lactic acid bacteria having the purine capturing effect, the agent for capturing purines, or the food or beverage product or the pharmaceutical preparation 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 having the purine capturing effect, the agent for capturing purines, or the food or beverage product or the pharmaceutical preparation may be administered to a subject continuously, and may be administered every day, for example. In this case, the lactic acid bacteria having the purine capturing effect, the agent for capturing purines, or the food or beverage product or the pharmaceutical preparation 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 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.

[0077]

In another embodiment, the lactic acid bacteria having the purine capturing effect, the agent for capturing purines, or the food or beverage product or the pharmaceutical preparation may be administered in a single dose. When the agent for capturing purines, or the food or beverage product or the pharmaceutical preparation 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 having the purine capturing effect, per dose. The lactic acid bacteria having the purine capturing effect, the agent for capturing purines, or the food or beverage product or the pharmaceutical preparation are (is) preferably orally administered (orally ingested).

[0078]

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" 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, there is also provided an oral preparation that can be used for such an oral administration. In a preferred embodiment, therefore, there is also provided an oral preparation comprising the lactic acid bacteria having the purine capturing effect or the agent for capturing purines, for use in reducing purines in the intestinal tract and reducing the serum uric acid level.

[0079]

The subject to which the lactic acid bacteria having the purine capturing effect, the agent for capturing purines, or the food or beverage product or the pharmaceutical preparation is administered is a mammal including a human, livestock, a pet, and an laboratory (test) animal. The subject is preferably a human subject, more preferably a human subject with gout and/or hyperuricemia, and still more preferably, but is not limited to, 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 is preferably a human subject with mild to borderline hyperuricemia having a serum uric acid level of 6 to 8 mg/dL. In another embodiment, the subject is preferably a human subject having a serum uric acid level of 7 mg/dL or more, and preferably 7 to 11 mg/dL (in one example, between 7.6 mg/dL and 9.5 mg/dL), and among the above, the subject is more preferably a human subject suffering from hyperuricemia and gout. Moreover, a subject showing a correlation between the serum uric acid level and the amount of purines ingested from foods prior to the measurement of the serum uric acid level (e.g., the total amount of purines ingested for 3 days) is more preferred as the subject for administration (feeding).

[0080]

That is, the following aspects are also provided.

[a1] A method of screening lactic acid bacteria comprising measuring an amount of uptake of purines by lactic acid bacteria in a medium containing the purines, and selecting lactic acid bacteria having a purine capturing effect using, as an index, the amount of uptake of purines.

[a2] The method according to [a1], wherein the purines are at least one selected from the group consisting of adenine, adenosine, and adenylic acid.

[a3] The method according to [a1] or [a2], wherein the purines are adenine.

[a4] The method according to any of [a1] to [a3], wherein the purines are labeled with a radioisotope.

[a5] The method according to any of [a1] to [a4], 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.

[a6] The method according to any of [a1] to [a5], wherein the lactic acid bacteria are *Lactobacillus gasseri* bacteria.

[0081]

[a7] A lactic acid bacterium having a purine capturing effect obtainable by the method according to any of [a1] to [a6].

[a8] 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 [a1] to [a6].

[a9] The agent for capturing purines according to [a8], which is for use in reducing a serum uric acid level.

[a10] The agent for capturing purines according to [a8] or [a9], wherein the lactic acid bacteria are *Lactobacillus gasseri* OLL2959 strain (Accession No: NITE AP-224).

[0082]

[a11] A food or beverage product or pharmaceutical preparation comprising the agent for capturing purines according to any of [a8] to [a10].

[a12] The food or beverage product or pharmaceutical preparation according to [a11], which is for use in reducing purines in an intestinal tract.

[a13] The food or beverage product or pharmaceutical preparation according to [a11] or [a12], which is for use in administration to a human subject having a serum uric acid level of 6 to 8 mg/dL.

[a14] The food or beverage product or pharmaceutical preparation according to any of [a11] to [a13], comprising 1×10^8 to 1×10^{10} cfu of said lactic acid bacteria per dose.

Examples

[0083]

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.

[0084]

[Example 1]

(1) Preparation of lactic acid bacteria

Lactobacillus gasseri OLL2959 strain and *Lactobacillus gasseri* ATCC 33323 strain were used as lactic acid bacteria. ATCC 33323 strain, which is a *Lactobacillus gasseri* reference strain, is available from ATCC (American Type Culture Collection) under Catalog No: ATCC 33323, or available from Japan Collection of Microorganisms (RIKEN BRC-JCM), RIKEN BioResource Center (RIKEN BRC) under Catalog No: JCM 1131T.

[0085]

The lactic acid bacteria *Lactobacillus gasseri* OLL2959 strain and *Lactobacillus gasseri* ATCC 33323 strain were each inoculated into MRS medium in two Falcon tubes and cultured at 37°C for 16 to 20 hours. The cultures were each centrifuged at 4°C and 6,000 rpm for 10 minutes and harvested, and then the obtained lactic acid bacteria were combined. The lactic acid bacteria were washed twice in the following manner: 10 mL of a buffer solution was added thereto, and the lactic acid bacteria were suspended, subsequently centrifuged at 4°C and 6,000 rpm for 10 minutes, and harvested. A buffer solution was then added to prepare a cell suspension (viable cells) diluted to approximately 1×10^{10} cfu/mL and the cell suspension was used below.

[0086]

(2) Measurement of salvage activity of converting adenine to adenylic acid (AMP)

The lactic acid bacteria prepared in (1) were measured for their activity of converting adenine to adenylic acid (AMP), using adenine as a substrate.

[0087]

For each of *Lactobacillus gasseri* OLL2959 strain and ATCC 33323 strain, a reaction solution (100 μ L each) was prepared to have the composition shown below. The lactic acid bacteria were suspended into a solution (buffer solution) containing MgCl₂, PRPP, adenine,

and Tris-HCl after the preparation. The concentration of lactic acid bacteria in the reaction solution was approximately 5×10^9 cfu/mL.

[0088]

[Table 1]

	80mM MgCl ₂	30mM PRPP	2mM Adenine	100mM Tris-HCl (pH 7.5)	Lactic Acid Bacteria
Control (without Substrate)	10 v/v%	10 v/v%	0 v/v%	30 v/v%	50 v/v%
Test (Lactic Acid Bacteria+Substrate)	10 v/v%	10 v/v%	10 v/v%	20 v/v%	50 v/v%

30 mM PRPP: Prepared with 5-Phospho-D-Ribose-1-Diphosphate Pentasodium Salt (SIGMA)

[0089]

After the preparation of the reaction solution, the lactic acid bacteria were cultured by incubation in a water bath with shaking at 37°C, and the conversion reaction was stopped by the addition of an equivalent amount of 5% TFA (trifluoroacetic acid) at time points of 0 minutes, 15 minutes, 30 minutes, and 60 minutes.

[0090]

The reaction solution after the stoppage of the reaction was centrifuged at 4°C and 15,000 rpm for 10 minutes. Supernatant was collected, and centrifuged again at 4°C and 15,000 rpm for 10 minutes, using a centrifugal filter unit, 0.22-μm Ultrafree-MC (micon) for microfiltration.

[0091]

The resulting filtrate was subjected to HPLC analysis. The HPLC analytical conditions used were as follows:

- Mobile phase: A, 20 mM phosphate buffer (pH 7.5)
B, 40 mM phosphate buffer (pH 7.5)/acetonitrile (1:1)
- Column: SHISEIDO CAPCELL PAK C18 MG2 (2.0 mm id x 150 mm)
- Flow rate: 0.2 mL/min
- Temperature: 40°C

- Injection volume: 5 µl
- Detection wavelength: 254 nm (UV)
- Gradient A/B (minutes): 100/0 (0 minutes)-100/0 (5 minutes)-80/20 (20 minutes) [%]

Adenine and AMP in the reaction solution were quantified by measuring the area under the peak of the HPLC chart. The conversion rate of adenine to AMP at each time point when the reaction was stopped was calculated by the following formula:

Conversion rate (%) = (Area under the peak of AMP for the test sample - Area under the peak of AMP for the control sample) / (Area under the peak of adenine at the time point of zero minutes for the test sample - Area under the peak of adenine at the time point of zero minutes for the control sample)

[0092]

Changes with time in the obtained conversion rate are shown in Table 2 and Figure 1. When ATCC 33323 strain was used for the reaction, very little activity of converting adenine to AMP was shown. On the other hand, when OLL2959 strain was used, the amount of adenine decreased with an rapid increase in the amount of AMP, and most of adenine was converted into AMP after 60 minutes. As shown in Table 2 and Figure 1, OLL2959 strain showed an activity of converting adenine to AMP dramatically higher than that of ATCC 33323 strain.

[0093]

[Table 2]

Lactic Acid Bacterial Strain	Conversion Rate from Adenine into AMP (%)			
	Reaction Time (min)			
	0	15	30	60
ATCC 33323	-0.8	0.7	-1.3	0.3
OLL2959	-0.4	91.7	108.2	106.9

[0094]

These results revealed that *Lactobacillus gasseri* OLL2959 strain has a very high ability to convert adenine into AMP. That is, *Lactobacillus gasseri* OLL2959 strain was

considered to have high adenine phosphoribosyltransferase (APRT) activity that catalyzes the conversion of adenine into AMP (salvage pathway).

[0095]

(3) Measurement of activity of converting guanine into guanosine (GMP)

The salvage pathway includes, in addition to the pathway for converting adenine to AMP, a pathway for converting guanine to guanylic acid (GMP), a pathway for converting hypoxanthine into inosinic acid (IMP), and a pathway for converting xanthine into xanthylic acid (XMP). In mammals such as humans and in lactic acid bacteria, the enzyme APRT that catalyzes the conversion of adenine into AMP is considered to be also involved in the conversion of guanine into GMP. On the other hand, in mammals and lactic acid bacteria, hypoxanthine-guanine phosphoribosyltransferase is involved in the conversion of hypoxanthine into IMP and the conversion of xanthine into XMP. In lactic acid bacteria, xanthine phosphoribosyltransferase is known to be also involved in the conversion of xanthine into XMP and the conversion of guanine into GMP.

[0096]

An activity of converting guanine into GMP was therefore also measured. In this measurement, the activity of converting guanine into GMP was measured as described in (2) above, except that guanine was used instead of adenine, and GMP was used instead of AMP. The results are shown in Table 3 and Figure 2. It was shown that while ATCC 33323 strain had a very low activity of converting guanine into GMP, OLL2959 strain also had a relatively high ability to convert guanine into GMP (Figure 2).

[0097]

[Table 3]

Conversion Rate from Guanine to GMP (%)				
Lactic Acid Bacterial Strain	Reaction Time (min)			
	0	15	30	60
ATCC 33323	2.7	2.4	1.0	0.5
OLL2959	5.3	7.9	9.1	14.2

[0098]

[Example 2]

(1) Measurement of purine nucleosidase activity

As shown in Patent Literature 1, *Lactobacillus gasseri* OLL2959 strain has a high ability to degrade purine nucleosides such as inosine and guanosine. Thus, purine nucleosidase activity was measured as the activity of degrading a purine nucleoside into a purine base.

[0099]

Specifically, in an adenosine solution prepared using 100 mM phosphate buffer (pH 7.0) to have a final concentration of 0.2 mM, OLL2959 strain cells were suspended at approximately 5×10^9 cfu/ml to prepare a reaction solution. The reaction solution was allowed to react at 37°C for 120 minutes, and adenosine and adenine were measured by HPLC analysis as the areas. As a comparative control, ATCC 33323 strain was tested in the same manner as described above. The measurement results were expressed as the conversion rate (by comparison of the areas) of adenosine (purine nucleoside) into adenine (purine base). This conversion rate was calculated as described in Example 1 (2).

[0100]

Moreover, the same test was performed as described above except that inosine and guanosine were each used instead of adenosine. The results are shown in Figure 3. *Lactobacillus gasseri* OLL2959 strain had significantly higher conversion rates from all the purine nucleosides into the purine bases, i.e., had higher purine nucleosidase activity, compared with *Lactobacillus gasseri* ATCC 33323 strain.

[0101]

(2) Measurement of 5'-nucleotidase (5'-NT) activity

A purine nucleotide converted from a purine base by the salvage activity is converted into a nucleoside by 5'-nucleotidase activity. Thus, 5'-nucleotidase activity was measured for *Lactobacillus gasseri* OLL2959 strain.

[0102]

OLL2959 strain cells were suspended in a solution prepared using 25 mM Tris-HCl buffer (pH 7.5) to have a final concentration of 5 mM MgCl₂ and 0.2 mM AMP, at

approximately 5×10^9 cfu/ml to prepare a reaction solution (Test sample). As a control sample, a reaction solution was prepared using the above-described buffer instead of 0.2 mM AMP. The reaction solution was allowed to react at 37°C for 120 minutes, and AMP and adenosine were measured by HPLC analysis as the areas. As a comparative control, ATCC 33323 strain was tested in the same manner as described above. The measurement results were expressed as the conversion rate (by comparison of the areas) from AMP (purine nucleotide) to adenosine (nucleoside). This conversion rate was calculated as described in Example 1 (2).

[0103]

Moreover, the same test was performed as described above except that IMP and GMP were each used instead of AMP. The results are shown in Table 4 and Figure 4. *Lactobacillus gasseri* OLL2959 strain had significantly lower conversion rates from all the purine nucleotides into the purine nucleosides, compared with *Lactobacillus gasseri* ATCC 33323 strain. That is, *Lactobacillus gasseri* OLL2959 strain had significantly low 5'-nucleotidase activity.

[0104]

[Table 4]

Conversion Rate (%) from Purine Nucleotide to Purine Nucleoside (%)			
Lactic Acid Bacterial Strain	AMP→Adenosine	IMP→Inosine	GMP→Guanosine
ATCC 33323	77.1	82.9	97.5
OLL2959	2.7	4.4	1.5

[0105]

From the measurement results of 5'-nucleotidase activity, as well as the measurement results of the salvage activity in Example 1 and the measurement results of purine nucleosidase activity in Example 2 (1) described above, it was shown that *Lactobacillus gasseri* OLL2959 strain had a generally high purine metabolic activity, and the metabolic activity is particularly biased toward active production and accumulation of nucleotides that can be utilized as energy.

[0106]

[Example 3]

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).

[0107]

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 that does not contain *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 1×10^8 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.

[0108]

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. 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 were analyzed statistically using two-way repeated measures ANOVA. The results are shown in Figure 5.

[0109]

As shown in Figure 5, the active group had significantly lower 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.

[0110]

[Example 4]

In this example, patients with hyperuricemia and gout who were undergoing outpatient care, as subjects, were fed *Lactobacillus gasseri* OLL2959 strain and effects thereof upon the serum uric acid level were evaluated. A randomized placebo-controlled double blind parallel-group comparative study was performed.

[0111]

(1) Production of food products for the study

A food product to be ingested by the subjects for the study (a test food product or a control food product) was produced as follows. As the test food product containing *Lactobacillus gasseri* OLL2959 strain, yogurt was prepared containing the two bacterial species, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, as a yogurt starter, and *Lactobacillus gasseri* OLL2959 strain (8.5×10^7 cfu/mL) as an active component, and then bottled beverages filled with 100 g of the yogurt per bottle were produced.

[0112]

As the control food product not containing *Lactobacillus gasseri* OLL2959 strain, yogurt containing the two bacterial species, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, as a yogurt starter, and not containing *Lactobacillus gasseri* OLL2959 strain, was prepared in the same manner, and then bottled beverages filled with 100 g of the yogurt per bottle were produced. The yogurt in each of the food products was prepared through combining *Lactobacillus gasseri* OLL2959 strain (only for the test food product), the above-described yogurt starter, a dairy product, a stabilizer (pectin), a flavor, water, and the like as ingredients.

[0113]

After the production of the food products for the study, the bacterial number was determined for randomly-selected one cup of the food product per each product. Specifically, the yogurt as the food product for the study was inoculated on BL medium and aerobically cultured for 3 days, and then the number of colonies on the medium was counted to confirm that the bacterial number met the above-described level.

[0114]

(2) Ingestion Test

Patients (subjects) of 20 years old and above with hyperuricemia and gout who were taking antihyperuricemic drugs and consented to participate in the study received a preliminary examination including the measurement of serum uric acid level and discontinued the antihyperuricemic drugs for 4 weeks.

[0115]

After 4 weeks of drug discontinuation the subjects received a pre-ingestion examination including the measurement of serum uric acid level. A diet survey was also conducted on the subjects for 3 days prior to the pre-ingestion examination.

[0116]

Subjects having a serum uric acid level over 7 mg/dL after the drug discontinued were selected, and the subjects were assigned into a test food product ingestion group and a control food product ingestion group, based on serum uric acid levels after the drug discontinued, so as not to produce a significant difference in age and serum uric acid levels measured in the pre-ingestion examination. No significant difference was also found between the groups in measured values of height, body weight, BMI, systolic blood pressure phase, diastolic blood pressure phase, and pulse.

[0117]

According to this assignment, after the pre-ingestion examination, the subjects ingested two bottles per day of the food product for the study (the test food product containing 8.5×10^7 cfu/mL of OLL2959 strain or the control food product not containing OLL2959 strain) for 8 weeks (study food product ingestion period). The subjects still discontinued the drug for the study food product ingestion period. After 8 weeks from the beginning of ingestion, examination including the measurement of serum uric acid level was performed (week 8 examination). A diet survey was also conducted for 3 days prior to the week 8 examination.

[0118]

The diet survey was conducted on all the subjects for 3 days prior to each of the pre-ingestion examination (week 0) and the week 8 examination. The subjects were instructed to report the contents of breakfast, lunch, dinner, and snacks between meals for the 3 days, by writing them down in diaries and/or taking the pictures of them. A national registered

dietitian calculated the intakes of energy, proteins, lipids, carbohydrates, and salt, based on the reported meal contents. The amount of purines was also calculated based on the report from each subject.

[0119]

A population excluding subjects who dropped out during the ingestion period and subjects who ingested 90% or less of the food product for the study, as well as subjects not showing a correlation between the serum uric acid level and the purine intake (17 subjects; the test food product ingestion group of 9 subjects and the control food product ingestion group of 8 subjects (serum uric acid level after the drug discontinuation: 7.6 mg/dL to 9.5 mg/dL)) was subjected to differential analysis. The results up to the 8-week examination revealed that the test food product ingestion group showed a tendency not to experience a substantial increase in serum uric acid level even during the drug discontinuation (Figure 6), and the test food product ingestion group showed a significant decrease in serum uric acid level at $p < 0.05$, compared to the control food product ingestion group ($p = 0.0342$, Mann-Whitney U test).

[0120]

The foregoing results showed that the ingestion of *Lactobacillus gasseri* OLL2959 strain suppresses an increase in serum uric acid level due to food-derived purines in patients with hyperuricemia and gout as well.

[0121]

[Example 5] Test of evaluating purine uptake ability

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

[0122]

Lactobacillus gasseri OLL2959 strain was deposited on March 31, 2006 (original deposit date) in the National Institute of Technology and Evaluation, Patent Microorganisms Depository (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 7×10^8 cfu/ml) was used below.

[0123]

To 0.1 mL of a minimal medium (DM medium; Table 5), 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 1.4×10^6 cfu) and anaerobically cultured at 37°C for 30 minutes.

[0124]

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 7. In Figure 7, 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 case of 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.

[0125]

The foregoing 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 7).

[0126]

[Table 5]

Minimal Medium Composition

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

[0127]

[Example 6] Test of evaluating proliferative capacity in the presence of purines

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

[0128]

To 1 mL of the DM medium (Table 5), adenylic acid (AMP), adenosine, or adenine, as purines, was added to a final concentration of 400 μ M, and then the culture of *Lactobacillus gasseri* OLL2959 strain prepared in Example 5 was inoculated at 4 wt% (0.04 mL: 1.6 to 2.8×10^7 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 for comparison, *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 8.

[0129]

The foregoing 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 8).

[0130]

[Example 7] Comparative test of adenine uptake ability and proliferative capacity in the presence of adenine

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.

[0131]

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 6).

[0132]

[Table 6]

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

[0133]

Adenine uptake abilities were evaluated as described in Example 5, except that adenine (^{14}C -adenine) only was used as purines labeled with the radioisotope ^{14}C . The results are shown in Figure 9. It was found that *Lactobacillus gasseri* P14054ME002 strain also had a high adenine uptake ability (Figure 9), 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 9).

[0134]

For evaluation of proliferative capacities in the presence of adenine, adenine was added to 1 mL of the DM medium (Table 5) to a final concentration of 400 μM , and then any of the culture of *Lactobacillus gasseri* OLL2959 strain prepared in Example 5 and the cultures of P14054ME001 strain and P14054ME002 strain prepared in the same manner as that described in Example 5 was inoculated at 4 wt% (0.04 mL: 1.6 to 2.8×10^7 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 10. 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.

[0135]

The foregoing 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.

[0136]

[Example 8] Purine uptake ability of *Lactobacillus gasseri* bacteria (animal tests)

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.

[0137]

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 after the fasting were measured. 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.

[0138]

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 (1×10^{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 $\mu\text{Ci/body}$ of ^{14}C -AMP was administered. The volume of dose was 2 mL/body in all the individuals (all the groups).

[0139]

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. Immediately after the completion of these tests, the rats were sacrificed through the inhalation of carbon dioxide.

[0140]

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 11. As shown in Figure 11, 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.

[0141]

[Example 9] Comparative test between species of 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 containing adenine labeled with a radioisotope (RI) (^{14}C -adenine), and

influences of the species of 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).

[0142]

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 7).

[0143]

[Table 7]

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

[0144]

In the comparative test of adenine uptake ability, initially, a medium for this test was prepared by adding ¹⁴C-adenine to the minimal medium (Table 5) 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.

[0145]

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 12. The radioactivity (vertical axis) in Figure 12 is expressed as the number of decay of the radioactive substance per minute (disintegrations per minute; dpm).

[0146]

As shown in Figure 12, 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.

[0147]

The foregoing 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.

[0148]

(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 the species of lactic acid bacterial strains upon proliferative capacity of the cells were compared.

[0149]

Adenine was added to the minimal medium (Table 5) 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 13.

[0150]

As shown in Figure 13, 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.

[0151]

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

Industrial Applicability

[0152]

The lactic acid bacteria of the present invention can efficiently convert a purine base as a substrate into a purine nucleotide *in vivo* or *in vitro*. Use of the conversion agent comprising the lactic acid bacteria of the present invention for converting a purine base into a purine nucleotide can promote the conversion of the purine base into the purine nucleotide in the intestinal tract even in a subject having a high serum uric acid level or a subject having reduced salvage activity, thereby reducing the serum uric acid level. Therefore, the conversion agent comprising the lactic acid bacteria of the present invention for converting a purine base into a purine nucleotide, as well as the food or beverage product and the pharmaceutical preparation containing the same, are effective for, for example, prevention and/or treatment of gout and hyperuricemia.

Claims

[Claim 1]

A method of screening for lactic acid bacteria having an enhanced ability to convert a purine base into a purine nucleotide, comprising culturing lactic acid bacteria in a solution containing adenine, 5-phospho-D-ribose-1-diphosphate, and Mg^{2+} , and comparing the resulting activity of converting adenine to adenylic acid as an index of the ability with that of *Lactobacillus gasseri* ATCC 33323 strain.

[Claim 2]

The method according to claim 1, further comprising measuring 5'-nucleotidase activity, and selecting lactic acid bacteria having a reduced 5'-nucleotidase activity compared to *Lactobacillus gasseri* ATCC 33323 strain.

[Claim 3]

The method according to claim 1 or 2, wherein the lactic acid bacteria are *Lactobacillus* bacteria.

[Claim 4]

The method according to any one of claims 1 to 3, wherein the solution is a buffer solution.

[Claim 5]

A conversion agent for converting a purine base into a purine nucleotide, comprising lactic acid bacteria obtainable by the method according to any one of claims 1 to 4 as an active component.

[Claim 6]

The conversion agent according to claim 5, which is for use in converting adenine to adenylic acid, or converting guanine to guanylic acid.

[Claim 7]

The conversion agent according to claim 5 or 6, wherein the lactic acid bacteria are *Lactobacillus gasseri* OLL2959 strain (Accession No: NITE BP-224).

[Claim 8]

A food or beverage product or a pharmaceutical preparation comprising the conversion agent according to any one of claims 5 to 7.

[Claim 9]

The food or beverage product or the pharmaceutical preparation according to claim 8, which is for use in reducing a serum uric acid level.

[Claim 10]

The food or beverage product or the pharmaceutical preparation according to claim 9, wherein reducing the serum uric acid level involves promoting conversion of adenine to adenylic acid and promoting conversion of guanine to guanylic acid in an intestinal tract.

[Claim 11]

The food or beverage product or the pharmaceutical preparation according to claim 9 or 10, which is for use in administration to a human subject having a serum uric acid level of 6 to 8 mg/dL.

[Claim 12]

The food or beverage product or the pharmaceutical preparation according to any one of claims 8 to 11, comprising 1×10^8 to 10^{10} cfu of the lactic acid bacteria per dose.

[Claim 13]

A method of producing a purine nucleotide from a purine base, comprising reacting the conversion agent according to any one of claims 5 to 7 with the purine base in the presence of 5-phospho-D-ribose-1-diphosphate and Mg^{2+} .

[Claim 14]

The method according to claim 13, which is for producing adenylic acid from adenine, or for producing guanylic acid from guanine.