LACTIC ACID BACTERIA, METHOD FOR ACQUIRING SAME, AND LACTIC-ACID-BACTERIA-CONTAINING FOOD OR BEVERAGE

TECHNICAL FIELD

The present invention relates to novel lactic acid bacteria, a method for acquiring the same, and a lactic-acid-bacteria-containing food or beverage that contains the lactic acid bacteria.

BACKGROUND ART

The optimal growth pH range for typical lactic acid bacteria is 6–7. It can be said that the growth boundary value in the acidic range is a pH of 4, and that the growth boundary value in the alkaline range is a pH of 8. However, there are also lactic acid bacteria that have exceptional acid resistance and that can grow even in harsh environments (pH: about 3) in which typical lactic acid bacteria do not readily grow (for example, refer to Patent Documents 1 and 2).

Additionally, there are also lactic acid bacteria that have exceptional halotolerance enabling growth even in environments having a high salt concentration such as miso and soy sauce (for example, refer to Patent Documents 3 and 4).

Although there are many lactic acid bacteria that can grow even in harsh environments in this manner, the presence of lactic acid bacteria that can satisfactorily grow in harsh environments having a low pH (high acidity) and a high sucrose concentration has not previously been reported, to the knowledge of the applicant.

Thus, considering that the presence of such lactic acid bacteria would make it possible to easily ferment, e.g., a

liquid obtained by mixing a plant and a sugar and pressing the resultant mixture, a beverage having the aforementioned liquid as a base, or an ingredient such as a jam or a fruit sauce that has a high acidity value and sucrose concentration and that is not readily fermented using typical lactic acid bacteria, and to provide a new fermented food or fermented beverage, the applicant pursued research regarding lactic acid bacteria that can satisfactorily grow in low-pH and high-sucrose environments as mentioned above, whereupon the applicant was successful in acquiring lactic acid bacteria that can satisfactorily grow in harsh environments having a low pH and a high sucrose concentration.

[Patent Documents]

[Patent Document 1] Japanese Laid-open Patent Publication No. 2009-225792

[Patent Document 2] WO 2013/001862

[Patent Document 3] Japanese Laid-open Patent Publication No. 2007-236344

[Patent Document 4] Japanese Laid-open Patent Publication No. 2018-42556

DISCLOSURE OF THE INVENTION

PROBLEM TO BE SOLVED BY THE INVENTION

An object of the present invention is to provide lactic acid bacteria that can satisfactorily grow in low-pH and high-sucrose environments.

MEANS FOR SOLVING THE PROBLEM

The main points of the present invention are described below with reference to the accompanying drawings.

~~The~~ A first aspect of the present invention relates to lactic acid bacteria characterized by being of a species that belongs to the genus *Lactobacillus* (*Lactobacillus* sp.) and that is deposited under accession no. NITE BP-03116 (identification indication: WR16-4).

~~The present invention relates to the lactic acid bacteria according to the first aspect, characterized by having a base sequence in the 16S rDNA region indicated by SEQ ID NO.: 1.~~

~~The~~ A second aspect of the present invention relates to the lactic acid bacteria according to the first aspect, characterized by ~~being acquired from a fermented plant extract~~exhibiting a higher proliferation rate in an environment having a pH lower than 6–7 and a high sucrose concentration obtained by sucrose being included alone than in an environment having a pH of 6–7.

~~The~~A third aspect of the present invention relates to the lactic acid bacteria according to any of the first to second ~~aspect~~aspects, characterized by ~~being acquired from~~having a ~~fermented plant extract~~base sequence in the 16S rDNA region indicated by SEQ ID NO.: 1.

~~The~~ A fourth aspect of the present invention relates to the lactic acid bacteria according to any of the first to third ~~aspect~~aspects, characterized ~~in that the fermented plant extract is~~by being acquired from a fermented ~~daikon~~plant extract.

~~The~~ A fifth aspect of the present invention relates to the lactic acid bacteria according to the fourth aspect,

characterized in that the fermented plant extract is a fermented daikon extract.

~~The~~ A sixth aspect of the present invention relates to the lactic acid bacteria according to the fifth aspect, characterized in that the fermented daikon extract is obtained by mixing daikon, caster sugar, and a fungus liquid and allowing the mixture to ferment.

~~The~~A seventh aspect of the present invention relates to the lactic acid bacteria according to the sixth aspect, characterized in that the ~~fermented daikon extract is obtained by mixing daikon, caster sugar, and a fungus liquid and allowing the mixture to ferment.~~

~~The present invention relates to the lactic acid bacteria according to the seventh aspect, characterized in that the~~ fungus liquid is composed of yeast.

~~The present invention relates to the lactic acid bacteria according to the eighth aspect, characterized in that the fungus liquid is composed of yeast.~~

~~The present invention relates to the lactic acid bacteria according to any of the first to tenth aspects, characterized by proliferating in low-pH environments having a pH of 3.5-5.5, and also in high-sucrose-concentration environments having a sucrose concentration of 30-60 wt%.~~

~~The present invention relates to the lactic acid bacteria according to any of the first to tenth aspects, characterized by proliferating in low-pH environments having a pH of 3.5-5.5, and also in high-fructose/glucose-concentration~~

~~environments in which the fructose concentration and glucose concentration are each 10-30 wt%.~~

~~The present invention relates to the lactic acid bacteria according to any of the first to tenth aspects, characterized by proliferating in low-pH environments having a pH of 3.5-5.5, and also in high-sucrose-concentration environments having a sucrose concentration of 30-60 wt%, and furthermore characterized by proliferating in low-pH environments having a pH of 3.5-5.5, and also in high-fructose/glucose-concentration environments in which the fructose concentration and glucose concentration are each 10-30 wt%.~~

~~The~~ An eighth aspect of the present invention relates to a method for acquiring lactic acid bacteria, the method being characterized in that a fermented plant extract is added to a wet-sterilized liquid culture medium to obtain a culture solution, the resultant culture solution is next subjected to enrichment culturing to obtain an enriched culture solution, the enriched culture solution is next poured together with a wet-sterilized isolation culture medium to produce a flat-plate culture medium, and bacteria are then extracted from a colony that emerges on the flat-plate culture medium to acquire lactic acid bacteria ~~that can proliferate~~exhibiting a higher proliferation rate in ~~low-~~an environment having a pH lower than 6-7 and a high-sucrose-concentration ~~environments~~obtained by sucrose being included alone than in an environment having a pH of 6-7.

~~The~~A ninth aspect of the present invention relates to the method for acquiring lactic acid bacteria according to the

~~fourteenth~~eighth aspect, characterized in that the fermented plant extract is a fermented daikon extract obtained by mixing daikon, caster sugar, and a fungus liquid and allowing the mixture to ferment.

~~The~~ A tenth aspect of the present invention relates to the method for acquiring lactic acid bacteria according to the ~~fifteenth~~ninth aspect, characterized in that the fungus liquid is composed of yeast.

~~The~~ A eleventh aspect of the present invention relates to the method for acquiring lactic acid bacteria according to any of the ~~fourteenth~~eighth to ~~sixteenth~~tenth aspects, characterized in that the liquid culture medium is obtained by adding an antibiotic substance to a mixture obtained by blending together a fermented mixed extract, which is obtained using a fermented vegetable extract and/or a fermented fruit extract, and a wildflower/caster sugar mixed liquid, which is obtained by mixing caster sugar and a wildflower stock liquid.

~~The~~A twelfth aspect of the present invention relates to the method for acquiring lactic acid bacteria according to the ~~seventeenth~~eleventh aspect, characterized in that the antibiotic substance is cycloheximide.

~~The~~ A thirteenth aspect of the present invention relates to a lactic-acid-bacteria-containing food or beverage characterized by containing the lactic acid bacteria according to any of the first to ~~tenth~~seventh aspects.

The fourteenth aspect of the present invention relates to ~~a~~the lactic-acid-bacteria-containing food or beverage

~~characterized by containing the lactic acid bacteria according to the eleventh aspect.~~

~~The present invention relates to a lactic-acid-bacteria-containing food or beverage characterized by containing the lactic acid bacteria according to the twelfth aspect.~~

~~The present invention relates to a lactic-acid-bacteria-containing food or beverage characterized by containing the lactic acid bacteria~~ according to the thirteenth aspect~~.~~

~~The present invention relates to the lactic-acid-bacteria-containing food or beverage according to the nineteenth aspect~~, characterized by being a fermented food or beverage.

~~The present invention relates to the lactic-acid-bacteria-containing food or beverage according to the twentieth aspect, characterized by being a fermented food or beverage.~~

~~The present invention relates to the lactic-acid-bacteria-containing food or beverage according to the twenty-first aspect, characterized by being a fermented food or beverage.~~

~~The present invention relates to the lactic-acid-bacteria-containing food or beverage according to the twenty-second aspect, characterized by being a fermented food or beverage.~~

EFFECT OF THE INVENTION

Because the lactic acid bacteria according to the present invention grow (proliferate) in a superior manner in low-pH and high-sucrose environments, it is possible to easily

ferment, e.g., a liquid obtained by mixing a plant and a sugar and pressing the resultant mixture, a beverage having the aforementioned liquid as a base, or an ingredient such as a jam or a fruit sauce that has a high acidity value and sucrose concentration and that is not readily fermented using typical lactic acid bacteria, and to provide a new fermented food or fermented beverage.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph showing results of experimentation on the pH resistance of *Lactobacillus acidophilus* JCM 1132^{T} in a present example;

FIG. 2 is a graph showing results of experimentation on the pH resistance of *Lactococcus lactis* subsp. *lactis* NBRC 12007 in the present example;

FIG. 3 is a graph showing results of experimentation on the pH resistance of *Leuconostoc mesenteroides* subsp. *dextranicum* NBRC 100495^{T} in the present example;

FIG. 4 is a graph showing results of experimentation on the pH resistance of a WR16-4 strain in the present example;

FIG. 5 is a graph showing results of experimentation on the sucrose resistance of *Lactobacillus acidophilus* JCM 1132^{T} in the present example;

FIG. 6 is a graph showing results of experimentation on the sucrose resistance of *Lactococcus lactis* subsp. *lactis* NBRC 12007 in the present example;

FIG. 7 is a graph showing results of experimentation on the sucrose resistance of *Leuconostoc mesenteroides* subsp. *dextranicum* NBRC 100495^{T} in the present example;

FIG. 8 is a graph showing results of experimentation on the sucrose resistance of the WR16-4 strain in the present example;

FIG. 9 is a table showing the composition of an MRS culture in experimentation on sucrose resistance in the present example;

FIG. 10 is a table showing the composition of an MRS culture in experimentation on fructose/glucose resistance in the present example;

FIG. 11 is a graph showing results of experimentation on the fructose/glucose resistance of the WR16-4 strain in the present example; and

FIG. 12 is a data list showing results of a homology search in a gene database (from the NCBI) according to the present invention.

BEST MODE FOR CARRYING OUT THE INVENTION

Preferred embodiments of the present invention are briefly described below with reference to the drawings while indicating the effects of the present invention.

The lactic acid bacteria according to the present invention are of a species that belongs to the genus *Lactobacillus* (*Lactobacillus* sp.) and that is deposited under accession no. NITE BP-03116 (identification indication: WR16-4).

As a result of conducting a homology search on the basis of sequence data obtained from the lactic acid bacteria according to the present invention (i.e., a 16S rDNA sequence) using the gene database of the National Center for

Biotechnology Information (NCBI), a homology value of 93% was found in the sequences having the highest homology with the aforementioned sequence (refer to FIG. 12). From this result, it can be said that the lactic acid bacteria according to the present invention are a new species of lactic acid bacteria (species belonging to the genus *Lactobacillus*) not seen in the prior art.

The lactic acid bacteria according to the present invention have a characteristic of not readily growing at a pH of 6–7, which is the optimal growth environment for typical lactic acid bacteria, and of satisfactorily growing in environments at a lower pH (e.g., a pH of 3.5–5.5).

Furthermore, the lactic acid bacteria according to the present invention have a characteristic of also satisfactorily growing in environments at a low pH (e.g., a pH of 5 or lower) and a high sucrose concentration (e.g., 30 wt% or greater), in which typical lactic acid bacteria do not readily grow.

Thus, because the lactic acid bacteria according to the present invention have a characteristic of proliferating in a superior manner in low-pH and high-sucrose environments, it is possible to easily ferment, e.g., a liquid obtained by mixing a plant and a sugar and pressing the resultant mixture, a beverage having the aforementioned liquid as a base, or an ingredient such as a jam or a fruit sauce that has a high acidity value and sucrose concentration and that is not readily fermented using typical lactic acid bacteria.

[Examples]

Specific examples of the present invention are described below with reference to the drawings.

The lactic acid bacteria according to the present example have the 16S rDNA base sequence indicated in SEQ ID NO.: 1, and are of a species that belongs to the genus *Lactobacillus* (*Lactobacillus* sp.) and that is deposited under accession no. NITE BP-03116 (identification indication: WR16-4).

The cell morphology of the lactic acid bacteria according to the present example is a short bacillary form, the lactic acid bacteria undergoing whitening while in a colony and producing an exopolysaccharide (viscous substance).

The lactic acid bacteria according to the present example (referred to below as the "WR16-4 strain") are described in greater detail below.

(Bacterial species of WR16-4 strain)

A microbe identification test was conducted on the basis of the 16S rDNA data pertaining to the WR16-4 strain to investigate the bacterial species of the WR16-4 strain. The microbe identification test was commissioned by FASMAC Co., Ltd.

As a result of the microbe identification test, it was found that the bacterial species having the highest homology value was *Lactobacillus kefiri* (ATCC=35441) (homology value: 93.14%).

(Novelty of WR16-4 strain)

A homology search in the NCBI gene database was conducted on the basis of the 16S rDNA data pertaining to the WR16-4 strain to investigate the novelty of this strain.

FIG. 12 is a list of data showing high homology with a sequence derived from a sample in the homology search. As shown in FIG. 12, even in strains having the highest homology with the WR16-4 strain, the homology value was about 93%. Typically, a species is judged to be new if there is no species having a homology value of 98.7% or greater therewith. Therefore, the WR16-4 strain can be said to be novel lactic acid bacteria (a new species thereof).

(Source from which WR16-4 strain is isolated)

A fermented plant extract, specifically a fermented daikon extract, is employed as a source from which the WR16-4 strain is isolated.

The fermented daikon extract serving as the isolation source is obtained by mixing daikon, caster sugar, and a fungus liquid and allowing the mixture to ferment. Specifically, the fermented daikon extract is obtained by mixing cut daikon and caster sugar in an amount equal to that of the daikon, causing yeast to ferment the resultant mixture, subsequently pressing the fermented mixture, and furthermore allowing the resultant liquid fraction to ferment and age. The plant serving as the isolation source may also be a vegetable other than daikon.

(Method for isolating WR16-4 strain)

When isolating the WR16-4 strain from the aforementioned fermented daikon extract, it is impossible to use a typical method for isolating lactic acid bacteria, but it is possible to use the following isolation method discovered by the applicant.

(1) 80 μL of the fermented daikon extract is added to 10 mL of a liquid culture medium that has been subjected to wet sterilization (for 15 minutes at 121°C), and the resultant liquid is incubated for six days at 25°C to obtain a culture solution.

(2) 80 μL of the resultant culture solution is added to 10 mL of a liquid culture medium, and the resultant liquid is again incubated for three to four days at 25°C to obtain a culture solution.

(3) Enrichment culturing in which the process in (2) above is repeated a number of times (eight times in the present example) is conducted to obtain an enriched culture solution.

(4) 1 mL of the resultant enriched culture solution is poured together with 15 mL of an isolation culture medium that has been subjected to wet sterilization (for 15 minutes at 121°C), after which the resultant mixture is solidified through cooling to produce a flat plate.

(5) The flat plate is incubated for ten days at 25°C.

(6) Bacteria are extracted from a colony that emerges on the flat plate to thereby obtain the WR16-4 strain.

The liquid culture medium described above is obtained by adding an antibiotic substance to a mixture obtained by blending together a fermented mixed extract, which is obtained using a fermented vegetable extract and/or a fermented fruit extract, and a wildflower/caster sugar mixed liquid, which is obtained by mixing caster sugar and a wildflower stock liquid. Specifically, the liquid culture medium is a mixed liquid in

which the fermented mixed extract, a wildflower/caster sugar mixed liquid obtained by mixing caster sugar with a stock liquid of a plurality of types of wildflowers in a ratio of 1:1, and 1 g/L of a cycloheximide solution are mixed in a ratio of 10:90:1.

The isolation culture medium described above is obtained by carrying out wet sterilization on a culture medium A and a culture medium B, which are indicated in the table below, and mixing the two culture media.

Table 1

Culture medium A		Culture medium B	
MRS broth	5.5 g	Sucrose	10 g
Fermented mixed extract	10 mL	Agar	1.4 g
Pure water	50 mL	Pure water	50 mL

In order to confirm the characteristics of the WR16-4 strain acquired through the aforementioned isolation method, experimentation on pH resistance and sucrose resistance through comparison with typical lactic acid bacteria was conducted using typical lactic acid bacteria.

Specifically, three species of lactic acid bacteria, including *Lactobacillus acidophilus* JCM 1132^{T} of the genus *Lactobacillus*, *Lactococcus lactis* subsp. *lactis* NBRC 12007 of the genus *Lactococcus*, and *Leuconostoc mesenteroides* subsp. *dextranicum* NBRC 100495^{T} of the genus *Leuconostoc*, were used as control lactic acid bacteria for the WR16-4 strain, these species belonging to well-known genera within lactic acid bacteria, and having an optimal growth pH range of 6-7, similarly to typical lactic acid bacteria.

(Preculturing of lactic acid bacteria)

In the experimentation on the two types of resistance indicated above, preculturing such as is described below was conducted on the lactic acid bacteria.

(1) The MRS culture medium indicated in FIG. 9 was adjusted to double the concentration thereof. In this adjustment, a 4N sodium hydroxide solution or 4N hydrochloric acid was added, whereby the culture medium used for the WR16-4 strain was adjusted to a pH of 4.5, and the culture medium used for the control was adjusted to a pH of 6.5.

(2) A 4 wt% sucrose solution or 4 wt% glucose solution was adjusted.

(3) Wet sterilization (for 20 minutes at 121°C) using an autoclave was carried out on each of the solutions in (1) and (2) above.

(4) The wet-sterilized solutions in (1) and (2) were cooled, after which the solutions were mixed inside a clean bench so as to reach a ratio of 1:1 (v/v), and a 2 wt% sucrose-containing MRS culture medium (pH: 4.5) or a 2 wt% glucose-containing MRS culture medium (pH: 6.5) was adjusted.

(5) Preculturing was conducted under anaerobic conditions for 24-48 hours at 30°C using the 2 wt% sucrose-containing MRS culture medium (pH: 4.5) for the WR16-4 strain and using the 2 wt% glucose-containing MRS culture medium (pH: 6.5) for the control.

(Experimentation on pH resistance)

Experimentation on the pH resistance of the precultured lactic acid bacteria was conducted as described below.

(1) The MRS culture medium indicated in FIG. 9 was adjusted to double the concentration thereof. In this adjustment, a 4N sodium hydroxide solution or 4N hydrochloric acid was added, whereby the culture medium was adjusted to a pH of 3, 3.5, 4, 4.5, 5, 5.5, 6, or 6.5.

(2) A 4 wt% sucrose solution was adjusted.

(3) Wet sterilization (for 20 minutes at 121°C) using an autoclave was carried out on each of the solutions in (1) and (2) above.

(4) The wet-sterilized solutions in (1) and (2) were cooled, after which the solutions were mixed inside a clean bench so as to reach a ratio of 1:1 (v/v), and a 2 wt% sucrose-containing MRS culture medium (pH: 3–6.5) was adjusted.

(5) The precultured lactic acid bacterial strains were centrifuged (for ten minutes at 15,000×g) and a supernatant was removed, after which the bacterial strains were again suspended in the culture medium adjusted in (3) such that the initial concentration A_{660nm} was approximately 0.05–0.10.

(6) The resultant suspensions were dispensed into a 96-hole microplate, three wells at a time (n=3), so as to reach 200 μL/well, after which the suspensions were cultured under anaerobic conditions at 30°C.

(7) The turbidity was measured over time using a microplate reader.

FIGS. 1–3 are graphs showing results of measuring the turbidity of the control lactic acid bacteria (i.e., *Lactobacillus acidophilus* JCM 1132^T (FIG. 1), *Lactococcus*

lactis subsp. *lactis* NBRC 12007 (FIG. 2), and *Leuconostoc mesenteroides* subsp. *dextranicum* NBRC 100495^{T} (FIG. 3)). FIG. 4 is a graph showing results of measuring the turbidity of the WR16-4 strain.

In the experimentation on pH resistance in which the 2 wt% sucrose was used as a carbon source, it was confirmed, as indicated in the results of measuring the turbidity of the lactic acid bacteria, that whereas the control lactic acid bacteria exhibited excellent growth in an acidic range having a pH of 5–6.5, which is close to neutral, the WR16-4 strain exhibited excellent growth in a range more acidic than that in which the aforementioned lactic acid bacteria exhibited excellent growth, specifically in a low-pH range having a pH of 3.5–5, and substantially did not grow in a range having a higher pH closer to neutral.

(Experimentation on sucrose resistance)

Experimentation on the sucrose resistance of the precultured lactic acid bacteria was conducted as described below.

(1) The MRS culture medium indicated in FIG. 9 to which sucrose was added was adjusted so as to reach a sucrose concentration of 5 wt%, 10 wt%, 20 wt%, 30 wt%, 40 wt%, 50 wt%, 60 wt%, or 70 wt%, after which 4N hydrochloric acid was added to each of the culture media to adjust the same so as to reach a pH of 4.5.

(2) Wet sterilization (for 20 minutes at 121°C) using an autoclave was carried out on each of the solutions in (1) above.

(3) The precultured lactic acid bacterial strains were centrifuged (for ten minutes at 15,000×g) and a supernatant was removed, after which the bacterial strains were again suspended in the culture medium adjusted in (2) such that the initial concentration A_{660nm} was approximately 0.05–0.10.

(4) The resultant suspensions were dispensed into a 96-hole microplate, three wells at a time (n=3), so as to reach 200 μL/well, after which the suspensions were cultured under anaerobic conditions at 30°C.

(5) The turbidity was measured over time using a microplate reader.

FIGS. 5–~~8~~7 are graphs showing results of measuring the turbidity of the control lactic acid bacteria (i.e., *Lactobacillus acidophilus* JCM 1132^T (FIG. 5), *Lactococcus lactis* subsp. *lactis* NBRC 12007 (FIG. 6), and *Leuconostoc mesenteroides* subsp. *dextranicum* NBRC 100495^T (FIG. 7)). FIG. 8 is a graph showing results of measuring the turbidity of the WR16-4 strain.

In the experimentation on sucrose resistance under acidic conditions with an initial pH of 4.5, it was confirmed, as indicated in the results of measuring the turbidity of the lactic acid bacteria, that whereas the control lactic acid bacteria exhibited a low growth state (low proliferation rate) despite confirmation of growth at a sucrose concentration of 20 wt% or less and were substantially incapable of growing in a high-sucrose environment at a sucrose concentration of 30 wt% or greater, the WR16-4 strain exhibited an excellent growth state up to a sucrose concentration of about 40 wt% and

grew even in a high-sucrose environment at a sucrose concentration of 60 wt%.

(Experimentation on fructose/glucose resistance)

Sucrose is a disaccharide in which single sugars fructose and glucose bond together. Because the WR16-4 strain was confirmed to be capable of growing in high-sucrose environments as described above, it was thought that this strain might also be capable of growing in high-fructose/glucose-concentration environments in which each of fructose and glucose is present in a single-sugar state, in the same manner as in the case of sucrose. Experimentation on fructose/glucose resistance was conducted as described below in the same manner as in the experimentation on sucrose resistance.

(1) The MRS culture medium indicated in FIG. 10 to which each of the sugars sucrose, fructose, and glucose was added alone was adjusted so as to reach a respective sugar concentration of 20 wt%, after which 4N hydrochloric acid was added to each of the culture media to adjust the same so as to reach a pH of 4.5.

(2) Separately, the MRS culture medium indicated in FIG. 10 to which fructose and glucose were added was adjusted so as to reach a fructose concentration and a glucose concentration of 10 wt% each (a total [sugar concentration] of 20 wt%), after which 4N hydrochloric acid was added to each of the culture media to adjust the same so as to reach a pH of 4.5.

(3) Wet sterilization (for 20 minutes at 121°C) using an autoclave was carried out on each of the solutions in (1) and (2) above.

(4) The precultured WR16-4 strain was centrifuged (for ten minutes at 15,000×g) and a supernatant was removed, after which the WR16-4 strain was again suspended in the adjusted culture medium such that the initial concentration A_{660nm} was approximately 0.05-0.10.

(5) The resultant suspensions were dispensed into a 96-hole microplate, three wells at a time (n=3), so as to reach 200 μL/well, after which the suspensions were cultured under anaerobic conditions at 30°C.

(6) The turbidity was measured over time using a microplate reader.

The results of measuring the turbidity in the experimentation on fructose/glucose resistance under acidic conditions having an initial pH of 4.5 are shown in FIG. 11.

As shown in FIG. 11, although the WR16-4 strain exhibited a low proliferation rate (low growth state) when either fructose or glucose was included alone, this strain exhibited a high proliferation rate (high growth state), in the same manner as in the case in which sucrose was included, when both fructose and glucose were included. From this result, it was confirmed that the WR16-4 strain has not only sucrose resistance but also fructose/glucose resistance.

Because sucrose is a disaccharide in which fructose and glucose bond together as mentioned previously, it is thought that the WR16-4 strain is capable of adequately growing even

in high-sugar-concentration environments in which, for example, each of fructose and glucose is included in an amount of 30 wt% each (a total sugar concentration of 60 wt%), in the same manner as in the case of sucrose.

(Applications of WR16-4 strain)

The WR16-4 strain can be used in lactic-acid-bacteria-containing foods and beverages, specifically in fermented foods and beverages.

Because the WR16-4 strain is lactic acid bacteria having the characteristic of proliferating in a superior manner in low-pH and high-sucrose environments as mentioned above, it is possible to easily ferment, e.g., a liquid obtained by mixing a plant and a sugar and pressing the resultant mixture, a beverage having the aforementioned liquid as a base, or an ingredient such as a jam or a fruit sauce that has a high acidity value and sucrose concentration and that is not readily fermented using typical lactic acid bacteria (i.e., an acidic and high-sugar-concentration extract), thereby making it possible to incorporate an organic acid into the extract and yielding effects such as imparting a sour flavor (reducing sweetness or imparting freshness) or preventing decay (without requiring a preservative material as an additive).

Additionally, because the strain WR16-4 is lactic acid bacteria having the characteristic of proliferating in a superior manner in low-pH and high-fructose/glucose environments, it is possible, in addition to the features described above, to use this strain in foods and beverages using a sugar that includes both fructose and glucose (such as

isomerized sugars, sugars obtained by mixing grape sugar and fruit sugar, etc.).

An example of a method for acquiring the aforementioned acidic and high-sugar-concentration extract is indicated below. The method for acquiring the extract is in no way limited to the method indicated below.

(1) A cut vegetable or fruit and caster sugar are mixed in equal proportions, a fungus liquid is added to the mixture, and the mixture is fermented.

(2) After fermentation, the mixture is pressed to recover a liquid fraction, and the recovered liquid is furthermore allowed to ferment and age to obtain a fermented liquid A.

(3) The fermented liquid A is added to a substance obtained by adding caster sugar to a vegetable stock liquid, and the resultant liquid is allowed to ferment and age to obtain a fermented liquid B.

The fermented liquids A and B are acidic and high-sugar-concentration extracts. The fermented liquids A and B are produced as beverages. Allowing these beverages to ferment using the WR16-4 strain makes it possible to impart a sour flavor, achieve a decay-preventing effect without adding a preservative material, and provide an unprecedented beverage.

Additionally, as indicated in the experimentation results above, because the WR16-4 strain has the characteristic of substantially not growing in environments having a pH of 6-7, which is the optimal pH environment for typical lactic acid bacteria, the WR16-4 strain does not grow until the optimum pH therefor is reached in a case in which, for example, an

ingredient having a high sucrose concentration and a pH close to neutral is to be fermented using both the WR16-4 strain and other microbes (e.g., typical lactic acid bacteria or yeast), wherefore the WR16-4 strain does not hinder the growth of the other microbes. Thus, the WR16-4 strain is useful when using composite fermentation by a plurality of species of microbes.

Due to the aforementioned characteristics, fermentation by WR16-4 strain can be stopped, without heating, by adjusting the pH of the ingredient so as to be close to neutral, and this strain is useful even when used in a fermented product that cannot be heated.

The present invention is in no way limited to the present example; the specific configuration of each configurational element of the present invention can be designed as appropriate.

[Accession No.]

NITE BP-03116

CLAIMS

1. Lactic acid bacteria characterized by being of a species that belongs to the genus *Lactobacillus* (*Lactobacillus* sp.) and that is deposited under accession no. NITE BP-03116 (identification indication: WR16-4).

~~2~~ 2. Lactic acid bacteria according to claim 1, characterized by exhibiting a higher proliferation rate in an environment having a pH lower than 6-7 and a high sucrose concentration obtained by sucrose being included alone than in an environment having a pH of 6-7.

3. The lactic acid bacteria according to claim 1, characterized by having a base sequence in the 16S rDNA region indicated by SEQ ID NO.: 1.

~~3~~4. The lactic acid bacteria according to claim ~~1, characterized by being acquired from a fermented plant extract.~~

~~4. The lactic acid bacteria according to claim~~ 2, characterized by having a base sequence in the 16S rDNA region indicated by SEQ ID NO.: 1.

5. The lactic acid bacteria according to any of claims 1 to 4, characterized by being acquired from a fermented plant extract.

~~5. The lactic acid bacteria according to claim 3, characterized in that the fermented plant extract is a fermented daikon extract.~~

6. The lactic acid bacteria according to claim ~~4~~5, characterized in that the fermented plant extract is a fermented daikon extract.

7. The lactic acid bacteria according to claim ~~5, characterized in that the fermented daikon extract is obtained by mixing daikon, caster sugar, and a fungus liquid and allowing the mixture to ferment.~~

~~8. The lactic acid bacteria according to claim~~ 6, characterized in that the fermented daikon extract is obtained by mixing daikon, caster sugar, and a fungus liquid and allowing the mixture to ferment.

~~9~~8. The lactic acid bacteria according to claim 7, characterized in that the fungus liquid is composed of yeast.

~~10. The lactic acid bacteria according to claim 8, characterized in that the fungus liquid is composed of yeast.~~

~~11. The lactic acid bacteria according to any of claims 1 to 10, characterized by proliferating in low-pH environments having a pH of 3.5-5.5, and also in high-sucrose-concentration environments having a sucrose concentration of 30-60 wt%.~~

~~12. The lactic acid bacteria according to any of claims 1 to 10, characterized by proliferating in low-pH environments having a pH of 3.5-5.5, and also in high-fructose/glucose-concentration environments in which the fructose concentration and glucose concentration are each 10-30 wt%.~~

~~13. The lactic acid bacteria according to any of claims 1 to 10, characterized by proliferating in low-pH environments having a pH of 3.5-5.5, and also in high-sucrose-concentration environments having a sucrose concentration of 30-60 wt%, and furthermore characterized by proliferating in low-pH environments having a pH of 3.5-5.5, and also in high-fructose/glucose-concentration environments in which the~~

~~fructose concentration and glucose concentration are each 10-30 wt%.~~

~~14~~ 9. A method for acquiring lactic acid bacteria, the method being characterized in that a fermented plant extract is added to a wet-sterilized liquid culture medium to obtain a culture solution, the resultant culture solution is next subjected to enrichment culturing to obtain an enriched culture solution, the enriched culture solution is next poured together with a wet-sterilized isolation culture medium to produce a flat-plate culture medium, and bacteria are then extracted from a colony that emerges on the flat-plate culture medium to acquire lactic acid bacteria ~~that can proliferate~~exhibiting a higher proliferation rate in ~~low-~~an environment having a pH lower than 6-7 and a high-sucrose-concentration ~~environments~~obtained by sucrose being included alone than in an environment having a pH of 6-7.

~~15~~10. The method for acquiring lactic acid bacteria according to claim ~~14~~9, characterized in that the fermented plant extract is a fermented daikon extract obtained by mixing daikon, caster sugar, and a fungus liquid and allowing the mixture to ferment.

~~16~~11. The method for acquiring lactic acid bacteria according to claim ~~15~~10, characterized in that the fungus liquid is composed of yeast.

~~17~~12. The method for acquiring lactic acid bacteria according to any of claims ~~14~~9 to ~~16~~11, characterized in that the liquid culture medium is obtained by adding an antibiotic substance to a mixture obtained by blending together a

fermented mixed extract, which is obtained using a fermented vegetable extract and/or a fermented fruit extract, and a wildflower/caster sugar mixed liquid, which is obtained by mixing caster sugar and a wildflower stock liquid.

~~18~~13. The method for acquiring lactic acid bacteria according to claim ~~17~~12, characterized in that the antibiotic substance is cycloheximide.

~~19~~14. A lactic-acid-bacteria-containing food or beverage characterized by containing the lactic acid bacteria according to any of claims 1 to ~~10~~4.

~~20~~15. A lactic-acid-bacteria-containing food or beverage characterized by containing the lactic acid bacteria according to ~~claim 11~~claims 5.

~~21~~16. A lactic-acid-bacteria-containing food or beverage characterized by containing the lactic acid bacteria according to ~~claim 12~~claims 6.

~~22~~17. A lactic-acid-bacteria-containing food or beverage characterized by containing the lactic acid bacteria according to ~~claim 13~~claims 7.

18. A lactic-acid-bacteria-containing food or beverage characterized by containing the lactic acid bacteria according to claims 8.

19. The lactic-acid-bacteria-containing food or beverage according to claim 14~~ 23. The lactic-acid-bacteria-containing food or beverage according to claim 19~~, characterized by being a fermented food or beverage.

~~24~~20. The lactic-acid-bacteria-containing food or beverage according to claim ~~20~~15, characterized by being a fermented food or beverage.

~~25~~21. The lactic-acid-bacteria-containing food or beverage according to claim ~~21~~16, characterized by being a fermented food or beverage.

~~26~~22. The lactic-acid-bacteria-containing food or beverage according to claim ~~22~~17, characterized by being a fermented food or beverage.

23. The lactic-acid-bacteria-containing food or beverage according to claim 18, characterized by being a fermented food or beverage.