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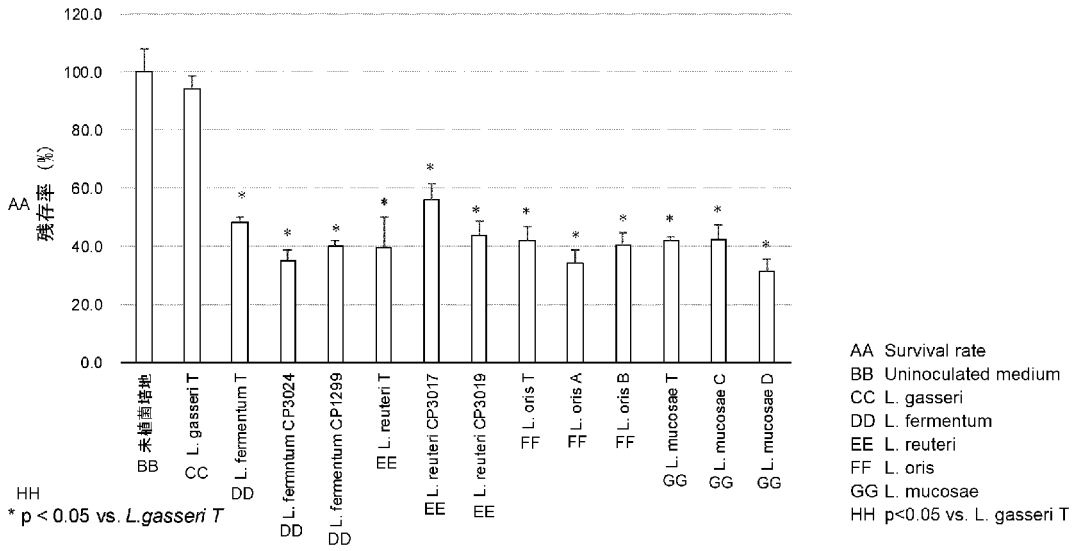
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(54) **Title:** METHOD FOR MANUFACTURING PLANT-MILK FERMENTED LIQUID

(54) 発明の名称： 植物性ミルク発酵液の製造方法



(57) **Abstract:** Provided is a method for manufacturing a plant-milk fermented liquid having reduced benzaldehyde. This method for manufacturing a plant-milk fermented liquid includes bringing plant milk into contact with a lactic acid bacterium including at least one type of bacterium selected from the group consisting of Lactobacillus fermentum, Lactobacillus reuteri, Lactobacillus oris, and Lactobacillus mucosae.

(57) 要約：ベンズアルデヒドが低減された植物性ミルク発酵液の製造方法が提供される。ラクトバチルス・ファーメンタム、ラクトバチルス・ロイテリ、ラクトバチルス・オリス及びラクトバチルス・ムコサエからなる群から選択される少なくとも１種を含む乳酸菌と、植物性ミルクとを接触させることを含む植物性ミルク発酵液の製造方法である。

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— 規則13の2に基づいて明細書とは別に提出された、寄託された生物材料に関する表示 (規則13の2.4(d)(i)及び48.2(a)(viii))

DESCRIPTION

METHOD FOR PRODUCING PLANT-BASED MILK-FERMENTED LIQUID

TECHNICAL FIELD

[0001] The present invention relates to a method for producing a plant-based milk-fermented liquid.

BACKGROUND ART

[0002] Plant-based milk is a product prepared by size reduction and liquefaction of a plant material such as rice, wheat, or soybeans (see, for example, J. Food Sci. Technol (September 2016) 53(9): 3408-3423). Examples of plant-based milk include rice milk, which is a food prepared by saccharifying rice with enzyme, malted rice(koji), and/or the like, seasoning the saccharified rice by addition of a vegetable oil and/or the like, and then making the resulting product into the form of a milk.

[0003] In general, plant-based milk contains aldehydes as flavor components. Aldehydes may be favorable flavor components in beverages having plant flavors. However, in beverages having fermented-milk-like flavors, aldehydes are known to be a cause of unpleasant odors due to unfavorable plant odors. For example, benzaldehyde, which is an aromatic aldehyde, is known to have, for example, an almond- or almond-jelly-like aroma. Therefore, it gives a

favorable aroma for beverages for which, for example, an almond- or almond-jelly-like aroma is expected. However, benzaldehyde gives an off-flavor in cases where a fermented-milk-like flavor is expected. Examples of methods of reducing the amount of aldehydes contained in plant-based milk include the following methods.

[0004] For example, JP H9-205999 A describes a method in which the lactic acid bacterium *Leuconostoc mesenteroides* or the lactic acid bacterium *Lactobacillus brevis* is brought into contact with a food containing medium-chain aldehydes while preventing the growth of the lactic acid bacterium, to cause reduction of the medium-chain aldehydes to alcohols. Further, JP 2020-17 A describes a lactic acid fermented beverage obtained by subjecting a plant-based milk obtained using a rice material to lactic acid fermentation using the lactic acid bacterium *Lactobacillus sakei* strain No. 7 or the lactic acid bacterium *Lactobacillus sakei* strain No. 16, and describes that the beverage contained reduced amounts of diacetyl and hexanal.

SUMMARY OF THE INVENTION

PROBLEMS TO BE SOLVED BY THE INVENTION

[0005] An object of the present invention is to provide a method for producing a plant-based milk-fermented liquid in which the amount of benzaldehyde is reduced.

MEANS FOR SOLVING PROBLEMS

[0006] The present invention provides a method for producing a plant-based milk-fermented liquid, the method including bringing a plant-based milk into contact with a lactic acid bacterium/bacteria including at least one selected from the group consisting of *Lactobacillus fermentum*, *Lactobacillus reuteri*, *Lactobacillus oris*, and *Lactobacillus mucosae*.

[0007] In an embodiment, the plant-based milk in the production method may be derived from a cereal. In an embodiment, the plant-based milk in the production method may be derived from at least one selected from the group consisting of rice and barley. In an embodiment, the rice from which the plant-based milk is derived in the production method may be brown rice or white rice. In an embodiment, the plant-based milk in the production method may be a saccharified plant-based milk. In one embodiment, the lactic acid bacterium/bacteria in the production method may include at least one selected from the group consisting of *Lactobacillus fermentum* and *Lactobacillus reuteri*. In an embodiment, the contact between the lactic acid bacterium/bacteria and the plant-based milk in the production method may involve fermentation of the plant-based milk by the lactic acid bacterium/bacteria.

[0008] The present invention also provides a method for reducing the amount of benzaldehyde in a plant-based milk, the method including bringing a plant-based milk containing benzaldehyde into contact with a lactic acid bacterium/bacteria including at least one selected from the group consisting of *Lactobacillus fermentum*, *Lactobacillus reuteri*, *Lactobacillus oris*, and *Lactobacillus mucosae*.

[0009] In an embodiment, the plant-based milk in the reducing method may be derived from a cereal. In an embodiment, the plant-based milk in the reducing method may be derived from at least one selected from the group consisting of rice and barley. In an embodiment, the rice from which the plant-based milk is derived in the reducing method may be brown rice or white rice. In an embodiment, the plant-based milk in the reducing method may be a saccharified plant-based milk. In an embodiment, the lactic acid bacterium/bacteria in the reducing method may include at least one selected from the group consisting of *Lactobacillus fermentum* and *Lactobacillus reuteri*. In an embodiment, the contact between the lactic acid bacterium/bacteria and the plant-based milk in the reducing method may involve fermentation of the plant-based milk by the lactic acid bacterium/bacteria.

ADVANTAGEOUS EFFECTS OF THE INVENTION

[0010] According to the present invention, a method for producing a plant-based milk-fermented liquid in which the amount of benzaldehyde is reduced may be provided.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] Fig. 1 is a graph showing the abilities of lactic acid bacteria to reduce the amount of benzaldehyde at low concentration.

Fig. 2 is a graph showing the abilities of lactic acid bacteria to reduce the amount of benzaldehyde at high concentration.

Fig. 3 is a graph showing the abilities of lactic acid bacteria to reduce the amount of benzaldehyde contained in a plant-based milk derived from brown rice.

Fig. 4 is a graph showing the abilities of lactic acid bacteria to reduce the amount of benzaldehyde contained in a plant-based milk derived from white rice.

Fig. 5 is a graph showing the abilities of lactic acid bacteria to reduce the amount of benzaldehyde contained in a plant-based milk derived from barley.

MODE FOR CARRYING OUT THE INVENTION

[0012] In the present specification, the term "step" includes not only an independent step, but also a step that is not clearly distinguishable from another step, as long

as the desired purpose of the step can be achieved. Unless otherwise specified, when a plurality of substances corresponding to a certain component is present in a composition, the content of the component in the composition means the total amount of the plurality of substances present in the composition. The upper limit and lower limit of a numerical range described in the present description may be an arbitrary combination of values selected from those exemplified for the numerical range. Embodiments of the present invention are described below in detail. The embodiments described below, however, are merely examples of methods of producing a plant-based milk-fermented liquid for realization of the technological thought of the present invention. Therefore, the present invention is not limited to the methods of producing a plant-based milk-fermented liquid described below.

[0013] Method for Producing Plant-based milk-fermented liquid

The method for producing a plant-based milk-fermented liquid includes bringing a plant-based milk into contact with a lactic acid bacterium/bacteria including at least one selected from the group consisting of *Lactobacillus fermentum*, *Lactobacillus reuteri*, *Lactobacillus oris*, and *Lactobacillus mucosae*.

[0014] By bringing a particular lactic acid

bacterium/bacteria into contact with a plant-based milk, the content of benzaldehyde in the plant-based milk may be effectively reduced to reduce the plant odor derived from the benzaldehyde, to enable production of a plant-based milk-fermented liquid having an excellent yogurt-like flavor. The plant-based milk-fermented liquid produced has an excellent balance between sweetness and sourness, and an improved mellow taste as a fermented milk. This may be thought to be due to, for example, the excellent activity of the particular lactic acid bacterium/bacteria to reduce the amount of benzaldehyde contained in the plant-based milk.

[0015] In the method for producing a plant-based milk-fermented liquid, the content of benzaldehyde in a plant-based milk may be reduced by bringing a particular lactic acid bacterium/bacteria into contact with the plant-based milk. In other words, the method for producing a plant-based milk-fermented liquid may be a method for reducing the benzaldehyde content in a plant-based milk.

[0016] Lactic Acid Bacterial Strains

In the method for producing a plant-based milk-fermented liquid, a particular lactic acid bacterium/bacteria is/are used. The particular lactic acid bacterium/bacteria include(s) at least one selected from the group consisting of *Lactobacillus fermentum*,

Lactobacillus reuteri, *Lactobacillus oris*, and *Lactobacillus mucosae*. The *Lactobacillus fermentum* used in the production method is not limited as long as it is an available bacterial strain, and may include the type strain *Lactobacillus fermentum* T. The *Lactobacillus fermentum* may include other bacterial strains such as the CP1299 strain and the CP3024 strain. The *Lactobacillus reuteri* is not limited as long as it is an available bacterial strain, and may include the type strain *Lactobacillus reuteri* T. The *Lactobacillus reuteri* may include other bacterial strains such as the CP3017 strain and the CP3019 strain. The *Lactobacillus oris* is not limited as long as it is an available bacterial strain. It may include the type strain *Lactobacillus oris* T, or may include bacterial strains other than the type strain. The *Lactobacillus mucosae* is not limited as long as it is an available bacterial strain. It may include the type strain *Lactobacillus mucosae* T, or may include bacterial strains other than the type strain.

[0017] The *Lactobacillus fermentum* CP1299 strain is a lactic acid bacterium belonging to *Lactobacillus fermentum*, and has been internationally deposited with Patent Microorganisms Depositary, NITE National Institute of Technology and Evaluation (Room 122, 2-5-8 Kazusakamatari, Kisarazu-shi, Chiba, 292-0818 Japan), which is an international depository institution in accordance with the

Budapest Treaty, under the accession number: NITE BP-1512 as of January 18, 2013. The *Lactobacillus fermentum* CP3024 strain is a lactic acid bacterium belonging to *Lactobacillus fermentum*, and has been internationally deposited under the accession number: NITE BP-03428 as of March 1, 2021. The *Lactobacillus reuteri* CP3017 strain is a lactic acid bacterium belonging to *Lactobacillus reuteri*, and has been internationally deposited under the accession number: NITE BP-03426 as of March 1, 2021. The *Lactobacillus reuteri* CP3019 strain is a lactic acid bacterium belonging to *Lactobacillus reuteri*, and has been internationally deposited under the accession number: NITE BP-03427 as of March 1, 2021.

[0018] The particular lactic acid bacterium/bacteria preferably include(s) at least one selected from the group consisting of *Lactobacillus fermentum* and *Lactobacillus reuteri* from the viewpoint of safety in the eating experience, more preferably includes(s) at least *Lactobacillus fermentum*.

[0019] In the method for producing a plant-based milk-fermented liquid, the particular lactic acid bacterium/bacteria may be used in combination with other lactic acid bacteria, bifidobacteria, and the like. Examples of the lactic acid bacteria other than the particular lactic acid bacterium/bacteria include the genus

Lactobacillus, such as *Lactobacillus gasseri*, *Lactobacillus fructivorans*, *Lactobacillus casei*, *Lactobacillus delbrueckii*, *Lactobacillus bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus brevis*, *Lactobacillus plantarum*, *Lactobacillus lactis*, *Lactobacillus acidophilus*, *Lactobacillus amylovorus*, *Lactobacillus crispatus*, *Lactobacillus gallinarum*, *Lactobacillus jensenii*, *Lactobacillus johnsonii*, *Lactobacillus kefirgranum*, *Lactobacillus mali*, *Lactobacillus paracasei*, *Lactobacillus rhamnosus*, *Lactobacillus salivarius*, and *Lactobacillus tolerans*; the genus *Brucella*, such as *Brucella adolescentis*, *Brucella angulatum*, *Brucella animalis*, *Brucella bifidum*, *Brucella breve*, *Brucella catenulatum*, *Brucella dentium*, *Brucella gallicum*, *Brucella infantis*, *Brucella longum*, *Brucella pseudocatenulatum*, *Brucella pseudolongum*, *Brucella suis*, and *Brucella thermophilum*; the genus *Streptococcus*, such as *Streptococcus thermophilus*, *Streptococcus lactis*, *Streptococcus cremoris*, *Streptococcus faecalis*, and *Streptococcus faecium*; the genus *Leuconostoc*, such as *Leuconostoc mesenteroides*, *Leuconostoc dextranicum*, *Leuconostoc cremoris*, and *Leuconostoc oenos*; and the genus *Pediococcus*, such as *Pediococcus cerevisiae*, *Pediococcus acidilactici*, and *Pediococcus halophilus*. Examples of the bifidobacteria include *Bifidobacterium longum*, *Bifidobacterium bifidum*, *Bifidobacterium breve*,

Bifidobacterium adolescentis, *Bifidobacterium infantis*, and *Bifidobacterium animalis*.

[0020] In cases where the lactic acid bacterium/bacteria used in the method for producing a plant-based milk-fermented liquid include(s) at least one lactic acid bacterium other than the particular lactic acid bacterium/bacteria, and at least one bifidobacterium, its/their content ratio may be not more than 50%, preferably not more than 10%, or less than 5% with respect to the total of the lactic acid bacterium and/or bifidobacterium. The content ratio of the lactic acid bacterium and/or bifidobacterium may be determined from the number of bacterial cells of the at least one lactic acid bacterium and/or bifidobacterium used. Alternatively, in cases where a preculture is used as the at least one lactic acid bacterium and/or bifidobacterium, the content ratio may be determined based on the volume of the preculture.

[0021] Plant-based milk

The plant-based milk may be a product prepared by size reduction and liquefaction of a plant material by physical crushing, and/or saccharification using an enzyme, malted rice, and/or the like. The plant-based milk may be a product prepared by liquefaction of a plant material by a known method, or may be a product selected from commercially available products as appropriate. Examples

of the plant material include cereals such as rice, barley, wheat, oats, rye, soybeans, and peanuts; nuts and seeds such as almonds, coconuts, cashew nuts, macadamia nuts, and walnuts; and vegetables such as carrot, potato, sweet potato, cassava, and tomato. In particular, the plant material preferably contains a cereal since a cereal contains a large amount of carbohydrates. The plant material more preferably contains at least one selected from the group consisting of at least rice and barley, still more preferably contains at least rice or barley.

[0022] In cases where rice is used as the plant material, the rice may be at least one selected from the group consisting of unhulled rice, brown rice (including germinated brown rice), polished rice, red bran (akanuka), middle bran (chunuka), white bran (shironuka), and higher white bran (joshironuka). Examples of the polished rice include those with different degrees of polishing, such as 30%-polished rice, 50%-polished rice, 70%-polished rice, and white rice, and the polished rice may contain residual germs. The rice as the plant material is preferably at least one selected from the group consisting of brown rice, polished rice, middle bran, and higher white bran, more preferably at least one selected from the group consisting of brown rice, polished rice, and higher white bran. The rice is still more preferably brown rice or white rice.

The rice as the plant material may be pulverized to a predetermined grain size by a known method, or the pulverization may be omitted to leave the rice as it is. The rice may also be in a state where it has been saccharified by a microorganism or an enzyme treatment. Depending on the starch contained, rice may be classified into non-glutinous rice, glutinous rice, and the like. Any of these may be used. In cases where the plant material contains rice, plant materials other than rice may also be contained. The plant material may contain, in addition to rice, for example, other cereals such as barley, wheat, oats, rye, soybeans, and peanuts; and nuts and seeds such as almonds, coconuts, cashew nuts, macadamia nuts, and walnuts. The content of the other cereals and the like in the plant material containing rice may be, for example, not more than 30% by mass, not more than 20% by mass, or not more than 10% by mass. The lower limit of the content of the other cereals and the like in the plant material containing rice may be, for example, not less than 0.1% by mass or not less than 1% by mass.

[0023] In cases where barley is used as the plant material, it may be at least one selected from the group consisting of two-rowed barley, four-rowed barley, six-rowed barley, naked barley, and wild barley, or may be at least one selected from the group consisting of two-rowed

barley and six-rowed barley. The barley as the plant material may be pulverized to a predetermined grain size by a known method, or the pulverization may be omitted to leave the barley as it is. The barley may also be in a state where it has been saccharified by a microorganism, an enzyme treatment, and/or the like. In cases where the plant material contains barley, the plant material may also contain plant materials other than barley. The plant material may contain, in addition to barley, for example, other cereals such as rice, wheat, oats, rye, soybeans, and peanuts; and nuts and seeds such as almonds, coconuts, cashew nuts, macadamia nuts, and walnuts. The content of the other cereals and the like in the plant material containing barley may be, for example, not more than 30% by mass, not more than 20% by mass, or not more than 10 % by mass. The lower limit of the content of the other cereals in the plant material containing barley may be, for example, not less than 0.1% by mass or not less than 1% by mass.

[0024] The plant-based milk may be, for example, a product prepared by performing saccharification treatment in which, for example, starch contained in the plant material (preferably a cereal) is hydrolyzed by the use of an appropriate enzyme. In other words, the plant-based milk may be a saccharified plant-based milk, and is

preferably a plant-based milk prepared, from the viewpoint of increasing the monosaccharide content, by subjecting a plant-based milk that uses rice as a plant material to saccharification treatment. The plant-based milk is more preferably a plant-based milk prepared by subjecting a plant-based milk that uses brown rice or white rice as a plant material to saccharification treatment. As the enzyme in the saccharification treatment, for example, α-amylase (EC 3.2.1.1) may be used. The amount of the enzyme added may be, for example, 0.01% to 1% with respect to the mass of the plant material. The conditions of the hydrolysis reaction may be appropriately selected in accordance with, for example, the enzyme and the plant material used. The reaction temperature may be, for example, 40°C to 100°C, preferably 50°C to 70°C. The reaction time may be, for example, 1 hour to 24 hours, preferably 2 hours to 12 hours.

[0025] In the preparation of the plant-based milk by saccharification treatment, hydrolysis with α-amylase may be further followed by saccharification with glucoamylase (EC 3.2.1.3) or the like. The plant-based milk may be a product obtained by saccharification treatment in which malted rice or the like is allowed to act on the plant material.

[0026] The plant-based milk prepared by the

saccharification treatment contains various saccharides including monosaccharides such as glucose, disaccharides such as maltose, trisaccharides such as maltotriose, and tetra- and higher saccharides. The saccharide composition of the plant-based milk is preferably a glucose-rich composition since, while glucose is degraded by a lactic acid bacterium in the later-described fermentation step, glucose eventually becomes the main source of sweetness. The content of monosaccharides such as glucose may be, for example, not less than 30% by mass, preferably not less than 50% by mass, more preferably not less than 70% with respect to the total saccharides contained in the plant-based milk.

[0027] The plant-based milk may be in the form of, for example, a milk-like emulsion. After the size reduction and liquefaction of the plant material, the plant-based milk may be seasoned with a vegetable oil, an animal oil, salt, sugar, amino acid, a flavor, a thickener, a thickening agent, an emulsifier, a pH-adjusting agent, and/or the like.

[0028] Aldehydes

Plant-based milk contains benzaldehyde derived from a plant material. Plant-based milk contains not only benzaldehyde, but also aldehydes other than benzaldehyde, derived from a plant material. The other aldehydes may

have, for example, a plant-odor-like flavor. Examples of the other aldehydes include aliphatic aldehydes such as short-chain aliphatic aldehydes, medium-chain aliphatic aldehydes, and long-chain aliphatic aldehydes; and aromatic aldehydes. Short-chain aliphatic aldehydes have not more than 5 carbon atoms; medium-chain aliphatic aldehydes have 6 to 12 carbon atoms; and long-chain aliphatic aldehydes have not less than 13 carbon atoms. Specific examples of the aliphatic aldehydes include acetaldehyde, malondialdehyde, butanal, 2-methylpropanal, pentanal, 2-methylbutanal, 3-methylbutanal, hexanal, crotonaldehyde, 4-hydroxy-2-hexenal, 4-hydroxy-2-nonenal, 2,4-nonadienal, heptanal, octanal, nonanal, decanal, 2,4-decadienal, and tetradecanal. Specific examples of the aromatic aldehydes include anisaldehyde and cuminaldehyde.

[0029] The content of benzaldehyde in the plant-based milk may be, for example, 10 ng/ml to 3000 ng/ml, may be preferably 10 ng/ml to 500 ng/ml, or is more preferably 10 ng/ml to 200 ng/ml. The contents of benzaldehyde and other aldehydes in the plant-based milk may be measured by, for example, high performance liquid chromatography-mass spectrometry (such as LC-MS/MS).

[0030] By the method for producing a plant-based milk-fermented liquid, the content of benzaldehyde is reduced. At the same time, the contents of other aldehydes may be

reduced. The aldehydes whose contents are to be reduced may be aliphatic aldehydes and aromatic aldehydes, or may be at least one selected from a group consisting of short-chain aliphatic aldehydes, medium-chain aliphatic aldehydes, and aromatic aldehydes, or may be at least one selected from a group consisting of acetaldehyde, malondialdehyde, butanal, 2-methylpropanal, pentanal, 2-methylbutanal, 3-methylbutanal, hexanal, crotonaldehyde, 4-hydroxy-2-hexenal, 4-hydroxy-2-nonenal, 2,4-nonadienal, heptanal, octanal, nonanal, decanal, and 2,4-decadienal.

[0031] The method for producing a plant-based milk-fermented liquid includes a step of bringing a particular lactic acid bacterium/bacteria into contact with a plant-based milk. The step of bringing a particular lactic acid bacterium/bacteria into contact with a plant-based milk may be a fermentation step of adding the particular lactic acid bacterium/bacteria to the plant-based milk and carrying out lactic acid fermentation. More specifically, by maintaining a plant-based milk at a predetermined fermentation temperature, adding a predetermined amount of a culture liquid of a lactic acid bacterium/bacteria thereto, and then carrying out lactic acid fermentation for a predetermined fermentation time, a desired plant-based milk-fermented liquid may be obtained.

[0032] The lactic acid bacterium/bacteria used for the

contact with the plant-based milk may be a preculture preliminarily obtained by culturing in an appropriate medium, or may be a frozen product obtained by mixing the preculture with a cryoprotective agent and freezing the resulting mixture, or may be a powder obtained by freeze-drying the preculture. The medium used for the preparation of the preculture may be selected as appropriate from media normally used for culture of lactic acid bacteria. Examples of the medium include MRS Medium (manufactured by BD Japan). Regarding the culture conditions, the culture may be carried out, for example, under static or anaerobic conditions at 30°C to 40°C for 12 hours to 32 hours.

[0033] The amount of the preculture of the lactic acid bacterium/bacteria added to the plant-based milk may be, for example, 0.1% by volume to 30% by volume, preferably 0.5% by volume to 20% by volume, more preferably 1% by volume to 10% by volume, with respect to the liquid volume of the plant-based milk.

[0034] The fermentation temperature and the fermentation time may be appropriately selected in accordance with the culture conditions, the lactic acid bacterial strain(s) added, and the like. The fermentation temperature may be, for example, 10°C to 45°C, preferably 20°C to 40°C, more preferably 30°C to 40°C. The fermentation time may be, for example, 1 hour to 72 hours, preferably 6 hours to 48

hours, more preferably 12 hours to 32 hours.

[0035] In the method for producing a plant-based milk-fermented liquid, the benzaldehyde content in the plant-based milk is reduced by the fermentation step. The residual ratio (%), obtained by dividing the benzaldehyde content in the resulting plant-based milk-fermented liquid by the benzaldehyde content in a plant-based milk obtained by the same process except that no lactic acid bacterium is brought into contact, is, for example, not more than 85%, preferably not more than 70%, more preferably not more than 60%, still more preferably not more than 50%. The lower limit of the residual ratio (%) is not limited, and may be, for example, not less than 1%, or may be preferably not less than 5%.

[0036] The lactic acid bacterium/bacteria contained in the plant-based milk-fermented liquid obtained in the fermentation step may be in the state of viable cells as they are, or may be killed through high-temperature treatment or the like. The high-temperature treatment may be carried out, for example, by treating the resulting plant-based milk-fermented liquid for a predetermined time at a temperature of as high as not less than 63°C.

[0037] The plant-based milk-fermented liquid obtained by the production method may be provided as a lactic acid fermented beverage as it is, or the plant-based milk-

fermented liquid may be subjected to a predetermined post-treatment to provide a lactic acid fermented beverage or lactic acid fermented food in a desired form. Examples of the post-treatment include a concentration step for making the plant-based milk-fermented liquid into the form of a syrup; a step of adding a desired additive(s) (such as a flavor); a step of mixing with a carbonated beverage, a fruit juice beverage, an alcoholic beverage, or the like; and a food processing step.

[0038] Examples of the additives in the post-treatment step include sugar alcohols such as sorbitol, erythritol, maltitol, and xylitol; high-intensity sweeteners such as aspartame, stevioside, sucralose, and acesulfame K; organic acids such as citric acid, tartaric acid, malic acid, succinic acid, and lactic acid; vitamins such as L-ascorbic acid, dl-α-tocopherol, vitamin Bs, nicotinamide, and calcium pantothenate; surfactants such as glycerin fatty acid esters, polyglycerin fatty acid esters, sucrose fatty acid esters, sorbitan fatty acid esters, and propylene glycol fatty acid esters; thickening agents such as gum arabic, carrageenan, pectin, and agar; stabilizers such as casein and gelatin; amino acids; minerals such as calcium salts; additives such as sodium ascorbate, sodium erythorbate, glycerin, and propylene glycol; dyes; flavors; and preservatives.

[0039] Examples of the lactic acid fermented beverage obtained by the post-treatment step include carbonated beverages, fruit juice beverages, alcoholic beverages, syrups, and fruit-flavored beverages. To the lactic acid fermented beverage, other beverages such as soy milk may be added as long as the effect of the present invention is not impaired. Examples of the lactic acid fermented food obtained by the post-treatment step include cold confectionery such as jelly, yogurt, pudding, and ice cream; candy; soft candy; gum; and jam.

EXAMPLES

[0040] The present invention is described below more concretely by way of Examples. However, the present invention is not limited to these Examples.

[0041] Reference Example 1

As lactic acid bacteria, the bacterial strains shown in Table 1 were provided. All bacterial strains provided were type strains. Using a medium prepared by adding 0.05% by mass cysteine hydrochloride to MRS Medium (manufactured by BD Japan), each lactic acid bacterium was cultured in an environment at 37°C under anaerobic conditions for 16 hours to prepare a preculture of the lactic acid bacterium. Subsequently, 0.05% by mass cysteine hydrochloride and 0.05% by volume benzaldehyde were added to MRS Medium

(manufactured by BD Japan), and the preculture of the lactic acid bacterium was added thereto at 5% by volume, followed by performing culture in an environment at 37°C under anaerobic conditions for 16 hours. Subsequently, the bacterial cells were removed by centrifuging the culture, to obtain a culture supernatant. The concentration of benzaldehyde contained in the culture supernatant was quantified by a modification of the LC-MS/MS method described in Drug Test. Analysis, 8, 458-464 (2016). Details of the quantification method are described later. The residual ratio (%) of benzaldehyde was calculated by dividing the benzaldehyde concentration in each culture supernatant by the average benzaldehyde concentration in the uninoculated medium, and multiplying the resulting value by 100. The sample data number (n number) was set to 3. The results are shown in Table 1 and Fig. 1.

[0042] [Table 1]

	Strain name	Residual ratio (%)	
		Average	Standard deviation
uninoculated medium	-	100.0	7.8
L. acidophilus T	JCM1132	92.3	1.6
L. amylovorus T	JCM1126	50.8	9.5
L. brevis T	JCM1059	3.3	1.3
L. bulgaricus T	JCM1002	113.5	5.3
L. casei T	JCM1134	43.5	5.7
L. crispatus T	JCM1185	56.0	1.3
L. delbrueckii T	JCM1012	66.0	4.8
L. fermentum T	JCM1173	1.5	0.6
L. fructivorans T	JCM1117	4.9	0.5
L. gallinarum T	JCM2011	84.7	4.4
L. gasseri T	JCM1131	90.8	6.1
L. helveticus T	JCM1120	107.4	5.7

	Strain name	Residual ratio (%)	
		Average	Standard deviation
L. jensenii T	JCM15953	74.0	1.0
L. johnsonii T	JCM2012	74.1	0.6
L. kefirgranum T	JCM8572	69.5	3.7
L. lactis T	JCM1248	41.1	0.5
L. mali T	JCM1116	39.6	3.6
L. mucosae T	JCM12515	2.6	0.3
L. oris T	JCM11028	2.5	0.2
L. paracasei T	JCM8130	92.0	7.3
L. plantarum T	JCM1149	42.9	1.6
L. reuteri T	JCM1112	2.1	0.3
L. rhamnosus T	JCM1136	52.0	1.2
L. salivarius T	JCM1231	100.0	3.1
L. tolerans T	JCM1171	103.4	7.2

[0043] It could be confirmed that the ability to reduce the amount of benzaldehyde varies among the lactic acid bacterial species.

[0044] Reference Example 2

The ability of each lactic acid bacterium to reduce the amount of benzaldehyde was evaluated in the same manner as in Reference Example 1 except that the bacterial strains shown in Table 2 were used, and that the concentration of the benzaldehyde added was 0.2% by volume. The results are shown in Table 2 and Fig. 2. For statistical processing, the Student t-test was used. The test was carried out based on comparison with the residual ratio in the case of *Lactobacillus brevis* T. A significant difference was assumed at a level of $P < 0.05$. Each of the *Lactobacillus mucosae* A strain, the *Lactobacillus mucosae* B strain, the *Lactobacillus oris* C strain, and the *Lactobacillus oris* D strain is a human-derived lactic acid bacterial strain. The sample data number (n number) was set to 3.

[0045] [Table 2]

	Strain name	Residual ratio (%)		
		Average	Standard deviation	p-value (vs. *L. brevis T*)
uninoculated medium	-	100.0	6.5	0.002
L. gasseri T	JCM1131	79.4	2.6	0.004
L. fructivorans T	JCM1117	80.8	18.1	0.249
L. brevis T	JCM1059	63.4	2.9	
L. fermentum T	JCM1173	7.8	1.3	0.000
L. fermentum CP1299	CP1299	5.5	0.6	0.000
L. fermentum CP3024	CP3024	5.6	0.2	0.000
L. reuteri T	JCM1112	4.8	0.8	0.000
L. reuteri CP3017	CP3017	4.7	0.1	0.000
L. reuteri CP3019	CP3019	5.2	0.6	0.000
L. mucosae T	JCM12515	1.1	0.1	0.000
L. mucosae A	-	2.5	0.2	0.000
L. mucosae B	-	2.7	0.8	0.000
L. oris T	JCM11028	4.7	0.6	0.000
L. oris C	-	4.5	0.9	0.000
L. oris D	-	4.5	2.4	0.000

[0046] Under the high-concentration conditions, particular bacterial species were found to show higher benzaldehyde-reducing abilities compared to *Lactobacillus brevis* T. Under the low-concentration conditions, *Lactobacillus fructivorans* T was found to show a high ability to reduce the amount of benzaldehyde similarly to particular bacterial species. However, under the high-concentration conditions, the ability of this species to reduce the amount of benzaldehyde was equivalent to that of *Lactobacillus brevis* T. It can thus be seen that the

ability to reduce the amount of benzaldehyde varies among the bacterial species and bacterial strains.

[0047] Example 1

As lactic acid bacteria, the bacterial strains shown in Table 3 were provided. Using MRS Medium (manufactured by BD Japan) supplemented with 0.05% by mass cysteine hydrochloride, each lactic acid bacterium was cultured in an environment at 37°C under anaerobic conditions for 16 hours, to prepare a preculture of each lactic acid bacterial strain. Concentrated rice milk (manufactured by Kikkoman Corporation; raw material: processed brown rice) was diluted 1.67 times with pure water, and sterilized at 95°C for 15 minutes, to obtain a brown rice milk medium. To the brown rice milk medium, the preculture of the lactic acid bacterium was added at 5% by volume, and static culture was performed in an environment at 37°C for 16 hours, to obtain a culture as a plant-based milk-fermented liquid. The concentration of benzaldehyde contained in the resulting culture was quantified by a modification of the LC-MS/MS method described in Drug Test. Analysis, 8, 458-464 (2016). Details of the quantification method are described later. The residual ratio (%) of benzaldehyde was calculated by dividing the benzaldehyde concentration in each culture by the average benzaldehyde concentration in the uninoculated medium, and multiplying the resulting

value by 100. The results are shown in Table 3 and Fig. 3. For statistical processing, the Student t-test was used. The test was carried out based on comparison with the residual ratio in the case of *Lactobacillus gasseri* T. The sample data number (n number) was set to 3. A significant difference was assumed at a level of $P < 0.05$.

[0048] [Table 3]

	Strain name	Residual ratio (%)		
		Average	Standard deviation	p-value (vs. *L. gasseri T*)
uninoculated medium	-	100.0	8.1	0.413
L. gasseri T	JCM1131	94.1	4.2	
L. fermentum T	JCM1173	48.4	1.7	0.000
L. fermntum CP3024	CP3024	35.2	3.4	0.000
L. fermentum CP1299	CP1299	40.0	2.0	0.000
L. reuteri T	JCM1112	39.6	10.2	0.002
L. reuteri CP3017	CP3017	56.0	5.7	0.002
L. reuteri CP3019	CP3019	43.6	4.9	0.000
L. oris T	JCM11028	42.0	4.7	0.000
L. oris A	-	34.3	4.5	0.000
L. oris B	-	40.6	4.0	0.000
L. mucosae T	JCM12515	42.1	1.3	0.000
L. mucosae C	-	42.4	5.1	0.000
L. mucosae D	-	31.4	4.1	0.000

[0049] It could be confirmed that the amount of benzaldehyde was significantly reduced by particular bacterial species compared to the case of the control, *Lactobacillus gasseri* T.

[0050] Example 2

As lactic acid bacteria, the bacterial strains shown in Table 4 were provided. Using MRS Medium (manufactured by BD Japan) supplemented with 0.05% by mass cysteine hydrochloride, each lactic acid bacterium was cultured in an environment at 37°C under anaerobic conditions for 16 hours, to prepare a preculture of each lactic acid bacterial strain. A commercially available confectionery rice flour (Tomizawa Shoten; material: non-glutinous rice) and pure water were mixed together at a weight ratio of 3:7, and amylase (manufactured by Novozymes, BAN480L) was added thereto at 0.03% by volume. Thereafter, the mixture was allowed to react at 60°C for 6 hours, and then stirred, followed by sterilization at 95°C for 15 minutes to provide a white-rice milk medium. To the white-rice milk medium, the preculture of the lactic acid bacterium was added at 5% by volume, and static culture was performed in an environment at 37°C for 16 hours, to obtain a culture as a plant-based milk-fermented liquid. The concentration of benzaldehyde contained in the resulting culture was quantified by a modification of the LC-MS/MS method described in Drug Test. Analysis, 8, 458-464 (2016). Details of the quantification method are described later. The residual ratio (%) of benzaldehyde was calculated by dividing the benzaldehyde concentration in each culture by

the average benzaldehyde concentration in the uninoculated medium, and multiplying the resulting value by 100. The results are shown in Table 4 and Fig. 4. For statistical processing, the Student t-test was used. The test was carried out based on comparison with the residual ratio in the case of *Lactobacillus gasseri* T. The sample data number (n number) was set to 3. A significant difference was assumed at a level of $P < 0.05$.

[0051] [Table 4]

	Strain name	Residual ratio (%)		
		Average	Standard deviation	p-value (vs. *L. gasseri T*)
uninoculated medium	-	100.0	8.1	0.002
L. gasseri T	JCM1131	143.1	2.3	
L. fermentum T	JCM1173	42.7	4.8	0.000
L. fermntum CP3024	CP3024	35.8	5.4	0.000
L. fermentum CP1299	CP1299	57.5	8.1	0.000
L. reuteri T	JCM1112	82.9	2.0	0.000
L. reuteri CP3017	CP3017	65.1	1.9	0.000
L. reuteri CP3019	CP3019	49.6	2.2	0.000
L. oris T	JCM11028	42.7	8.3	0.000
L. oris A	-	47.8	6.4	0.000
L. oris B	-	47.8	0.4	0.000
L. mucosae T	JCM12515	44.0	4.9	0.000
L. mucosae C	-	63.6	14.5	0.002
L. mucosae D	-	60.7	1.9	0.000

[0052] It could be confirmed that the amount of benzaldehyde was significantly reduced by particular

bacterial species compared to the case of the control, *Lactobacillus gasseri* T.

[0053] Example 3

As lactic acid bacteria, the bacterial strains shown in Table 5 were provided. Using MRS Medium (manufactured by BD Japan) supplemented with 0.05% by mass cysteine hydrochloride, each lactic acid bacterium was cultured in an environment at 37°C under anaerobic conditions for 16 hours, to prepare a preculture of each lactic acid bacterial strain. A commercially available barley flour (Oomugi-club; material: whole grain flour) and pure water were mixed together at a weight ratio of 2:8, and amylase (manufactured by Novozymes, BAN480L) was added thereto at 0.02% by volume. Thereafter, the mixture was allowed to react with stirring at 60°C for 6 hours, and then sterilized at 95°C 15 minutes, to provide a barley milk medium. To the barley milk medium, the preculture of the lactic acid bacterium was added at 5% by volume, and static culture was performed in an environment at 37°C for 16 hours, to obtain a culture as a plant-based milk-fermented liquid. The concentration of benzaldehyde contained in the resulting culture was quantified by a modification of the LC-MS/MS method described in Drug Test. Analysis, 8, 458-464 (2016). Details of the quantification method are described later. The residual ratio (%) of benzaldehyde

was calculated by dividing the benzaldehyde concentration in each culture by the average benzaldehyde concentration in the uninoculated medium, and multiplying the resulting value by 100. The results are shown in Table 5 and Fig. 5. For statistical processing, the Student t-test was used. The test was carried out based on comparison with the residual ratio in the case of *Lactobacillus gasseri* T. The sample data number (n number) was set to 3. A significant difference was assumed at a level of $P < 0.05$.

[0054] [Table 5]

	Strain name	Residual ratio (%)		
		Average	Standard deviation	p-value (vs. *L. gasseri T*)
uninoculated medium	—	100.0	1.0	0.798
L. gasseri T	JCM1131	98.0	10.6	
L. fermentum T	JCM1173	36.5	12.3	0.006
L. fermentum CP3024	CP3024	32.8	9.4	0.003
L. fermentum CP1299	CP1299	40.3	6.8	0.003
L. reuteri T	JCM1112	63.3	8.3	0.022
L. reuteri CP3017	CP3017	65.2	6.5	0.020
L. reuteri CP3019	CP3019	56.3	8.3	0.012

[0055] It could be confirmed that the amount of benzaldehyde was significantly reduced by particular bacterial species compared to the case of the control, *Lactobacillus gasseri* T.

[0056] Method of Quantifying Benzaldehyde

Hexanal d-12 (manufactured by Sigma-Aldrich) was used

alone as an internal standard. An internal standard solution was prepared by dissolving hexanal d-12 at a concentration of 25 ng/μl in 50 % by volume aqueous ethanol solution. After mixing 40 μl of the internal standard solution, 80 μl of a solution prepared by dissolving dibutylhydroxytoluene (manufactured by Tokyo Chemical Industry Co., Ltd.) at a concentration of 1 mg/ml in 100 % by volume ethanol, 440 μl of 100 % by volume ethanol, and 440 μl of a culture supernatant or culture for which benzaldehyde was to be quantified, the resulting mixture was centrifuged at an acceleration of 3700 g for 10 minutes, followed by collecting 750 μl of the supernatant. After mixing 325 μl of this supernatant with 325 μl of a solution prepared by dissolving 2,4-dinitrophenylhydrazine hydrochloride (manufactured by Tokyo Chemical Industry Co., Ltd.) at a concentration of 0.05 mol/l in the mixed solvent "acetonitrile: acetic acid: pure water = 80:10:10 (volume ratio)", the resulting mixture was allowed to react in an environment at 60°C for 2 hours. Thereafter, the resulting reaction product was mixed with 1 ml of pure water, and then liquid-liquid extraction was carried out a total of four times using 500 μl of hexane. The hexane solution obtained by the liquid-liquid extraction was dried and solidified using a centrifugal evaporator, and the dried and solidified product was dissolved in 1 ml of the mixed

solvent "0.03 % by volume aqueous acetic acid solution: acetonitrile = 60:40 (volume ratio)". The resulting solution was subjected to high-performance liquid chromatography (HPLC) as a derivatized sample.

[0057] As a column and a guard column for the HPLC, an Atlantis T3 C18 column (3 μm, 2.1×150 mm), manufactured by Waters, and an Atlantis guard column T3 C18 (3 μm, 2.1×10 mm), manufactured by Waters, were used according to the method described in Drug Test. Analysis, 8, 458-464 (2016). For a modified LC-MS/MS equipment, the Nextera HPLC system (communication bus module: CBM-20A; pump: LC-30AD; autosampler: SIL-30AC; degasser: DGU-20A5R; column oven: CTO-20AC), manufactured by Shimadzu Corporation, was used as a high-performance liquid chromatography (HPLC) system, and Sciex Triple quad 6500+, manufactured by AB SCIEX, was used as a tandem mass spectrometry (MS/MS) system.

[0058] The following modified conditions were used for the LC-MS/MS. As mobile phase A, 0.03 % by volume aqueous acetic acid solution was used. As mobile phase B, 100 % by volume acetonitrile was used. At the beginning, "mobile phase A: B = 60:40 (volume ratio)" was used as the initial concentration. Two minutes later, the concentration was set to "mobile phase A: B = 52:48 (volume ratio), and this condition was maintained for 37 minutes. Four minutes thereafter, the concentration was set to "mobile phase A: B

= 0:100 (volume ratio), and this condition was maintained for 8 minutes. The sample injection volume for the HPLC was 1 μl. Modified conditions commonly employed for the compounds were as shown in Table 6 below, and modified conditions specifically employed for each compound were as shown in Table 7 below.

[0059] [Table 6]

Curtain gas (psi)	Collision activated dissociation gas (psi)	Ion spray voltage floating (volts)	Temperture (°C)	Ion source gas1 (psi)	Ion source gas2 (psi)	Entrance potential (volts)
30	8	-4500	500	30	70	-10

[0060] [Table 7]

	Declustering potential (volts)	Collision energy (volts)	Collision cell exit potential (volts)	Elution time (min)	Precusor ion (m/z)	Product ion (m/z)
Benzaldehyde	-35	-20	-13	31.3	284.8	162.8
Hexanal d-12	-5	-20	-1	45.6	291.0	163.1
Hexanal	-35	-32	-11	45.7	278.9	152.0
2-Methylpropanal	-40	-16	-11	25.5	251.0	162.8
3-Methylbutanal	-5	-26	-11	37.2	264.9	152.1
2,4-Decadienal	-40	-26	-19	47.7	331.0	180.9
2-Methylbutanal	-25	-18	-13	38.6	265.0	100.9
2,4-Nonadienal	-5	-24	-9	47.2	317.0	181.1

[0061] Based on the above conditions, a quantitative method was established in the multiple reaction monitoring mode using hexanal d-12 as an internal standard.

Benzaldehyde (manufactured by Tokyo Chemical Industry Co., Ltd.), hexanal (manufactured by Tokyo Chemical Industry Co., Ltd.), 2-methylpropanal (manufactured by Tokyo Chemical Industry Co., Ltd.), 3-methylbutanal (manufactured by Tokyo Chemical Industry Co., Ltd.), 2,4-decadienal (manufactured by Tokyo Chemical Industry Co., Ltd.), 2-methylbutanal (manufactured by Tokyo Chemical Industry Co., Ltd.), and 2,4-nonadienal (manufactured by Tokyo Chemical Industry Co., Ltd.) were derivatized under the conditions described above, to prepare calibration curves for the aldehydes. Thereafter, the benzaldehyde contained in the culture or culture supernatant was derivatized under the above conditions, and the concentration of the benzaldehyde contained in the culture supernatant (Reference Examples 1 and 2) or in the culture (Examples 1 to 3) was quantified.

[0062] The disclosure of Japanese patent application No. 2021-052283 (filing date: March 25, 2021) is incorporated herein by reference in its entirety. All documents, patent applications, and technical standards cited in the present description are incorporated herein by reference to the same extent as in cases where the individual documents, patent applications, and technical standards are specifically and individually described to be incorporated by reference.

Accession Numbers

[0063] NITE BP-1512

NITE BP-03426

NITE BP-03427

NITE BP-03428

CLAIMS

[Claim 1] A method for producing a plant-based milk-fermented liquid, the method comprising bringing a plant-based milk into contact with a lactic acid bacterium/bacteria including at least one selected from the group consisting of *Lactobacillus fermentum*, *Lactobacillus reuteri*, *Lactobacillus oris*, and *Lactobacillus mucosae*.

[Claim 2] The method for producing a plant-based milk-fermented liquid according to claim 1, wherein the plant-based milk is derived from a cereal.

[Claim 3] The method for producing a plant-based milk-fermented liquid according to claim 1 or 2, wherein the plant-based milk is derived from at least one selected from the group consisting of rice and barley.

[Claim 4] The method for producing a plant-based milk-fermented liquid according to claim 3, wherein the rice is brown rice or white rice.

[Claim 5] The method for producing a plant-based milk-fermented liquid according to any one of claims 1 to 4, wherein the plant-based milk is a saccharified plant-based milk.

[Claim 6] The method for producing a plant-based milk-fermented liquid according to any one of claims 1 to 5, wherein the lactic acid bacterium/bacteria include(s) at least one selected from the group consisting of

Lactobacillus fermentum and *Lactobacillus reuteri*.

[Claim 7] The method for producing a plant-based milk-fermented liquid according to any one of claims 1 to 6, wherein the contact between the lactic acid bacterium/bacteria and the plant-based milk involves fermentation of the plant-based milk by the lactic acid bacterium/bacteria.

[Claim 8] A method for reducing an amount of benzaldehyde in a plant-based milk, the method comprising bringing a plant-based milk containing benzaldehyde into contact with a lactic acid bacterium/bacteria including at least one selected from the group consisting of *Lactobacillus fermentum*, *Lactobacillus reuteri*, *Lactobacillus oris*, and *Lactobacillus mucosae*.

[Claim 9] The method for reducing the amount of benzaldehyde in a plant-based milk according to claim 8, wherein the plant-based milk is derived from a cereal.

[Claim 10] The method for reducing the amount of benzaldehyde in a plant-based milk according to claim 8 or 9, wherein the plant-based milk is derived from at least one selected from the group consisting of rice and barley.

[Claim 11] The method for reducing the amount of benzaldehyde in a plant-based milk according to claim 10, wherein the rice is brown rice or white rice.

[Claim 12] The method for reducing the amount of

benzaldehyde in a plant-based milk according to any one of claims 8 to 11, wherein the plant-based milk is a saccharified plant-based milk.

[Claim 13] The method for reducing the amount of benzaldehyde in a plant-based milk according to any one of claims 8 to 12, wherein the lactic acid bacterium/bacteria include(s) at least one selected from the group consisting of *Lactobacillus fermentum* and *Lactobacillus reuteri*.

[Claim 14] The method for reducing the amount of benzaldehyde in a plant-based milk according to any one of claims 8 to 13, wherein the contact between the lactic acid bacterium/bacteria and the plant-based milk involves fermentation of the plant-based milk by the lactic acid bacterium/bacteria.

DRAWINGS

Fig. 1

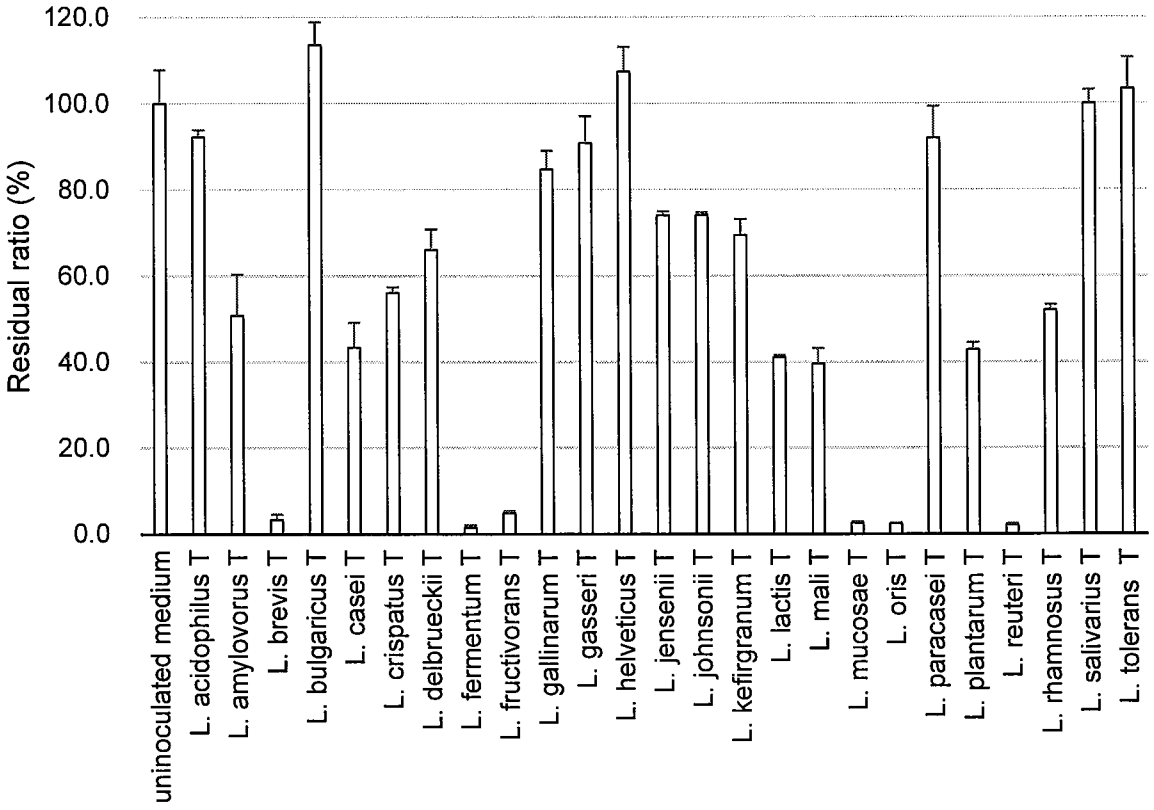
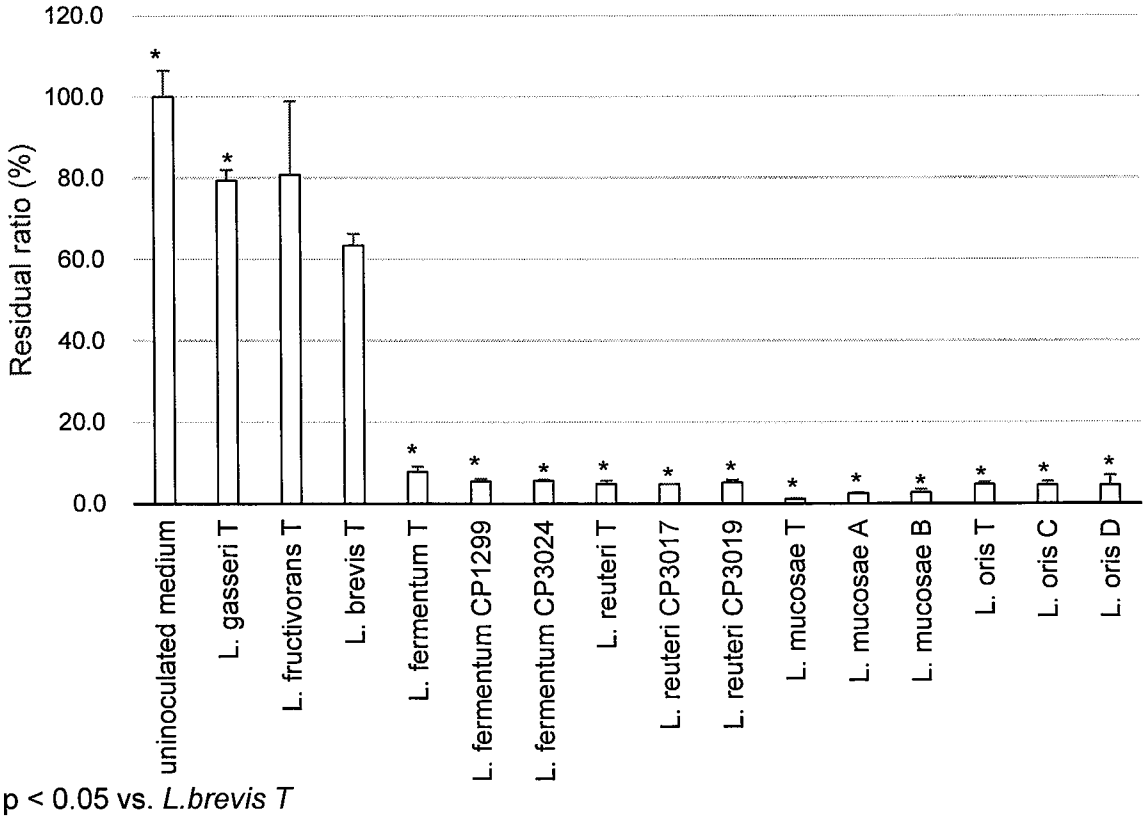
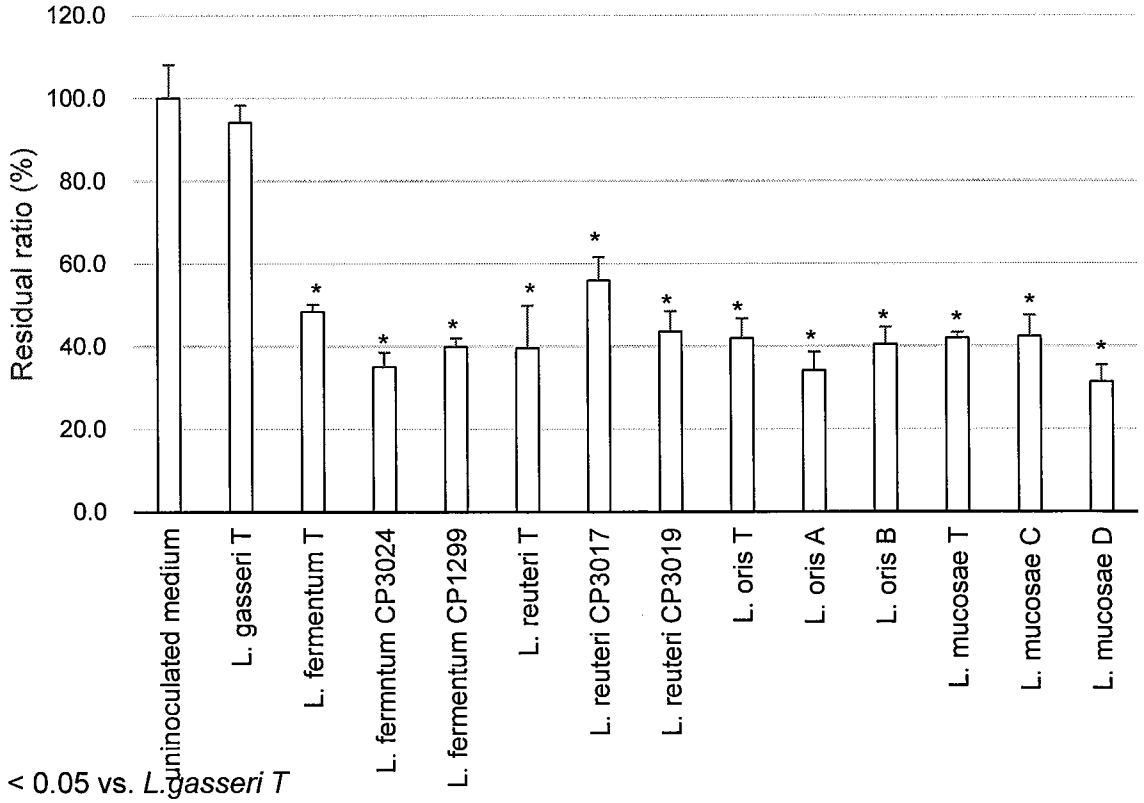


Fig. 2



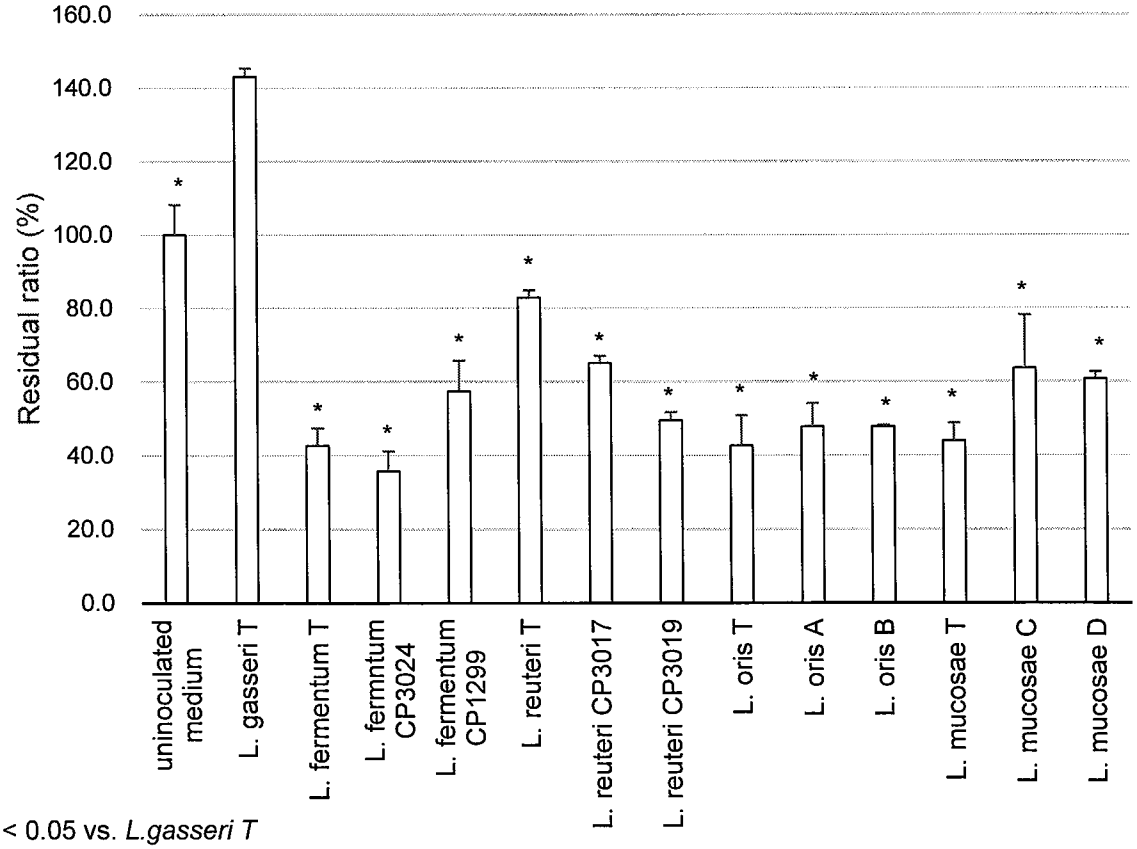
* $p < 0.05$ vs. *L.brevis T*

Fig. 3



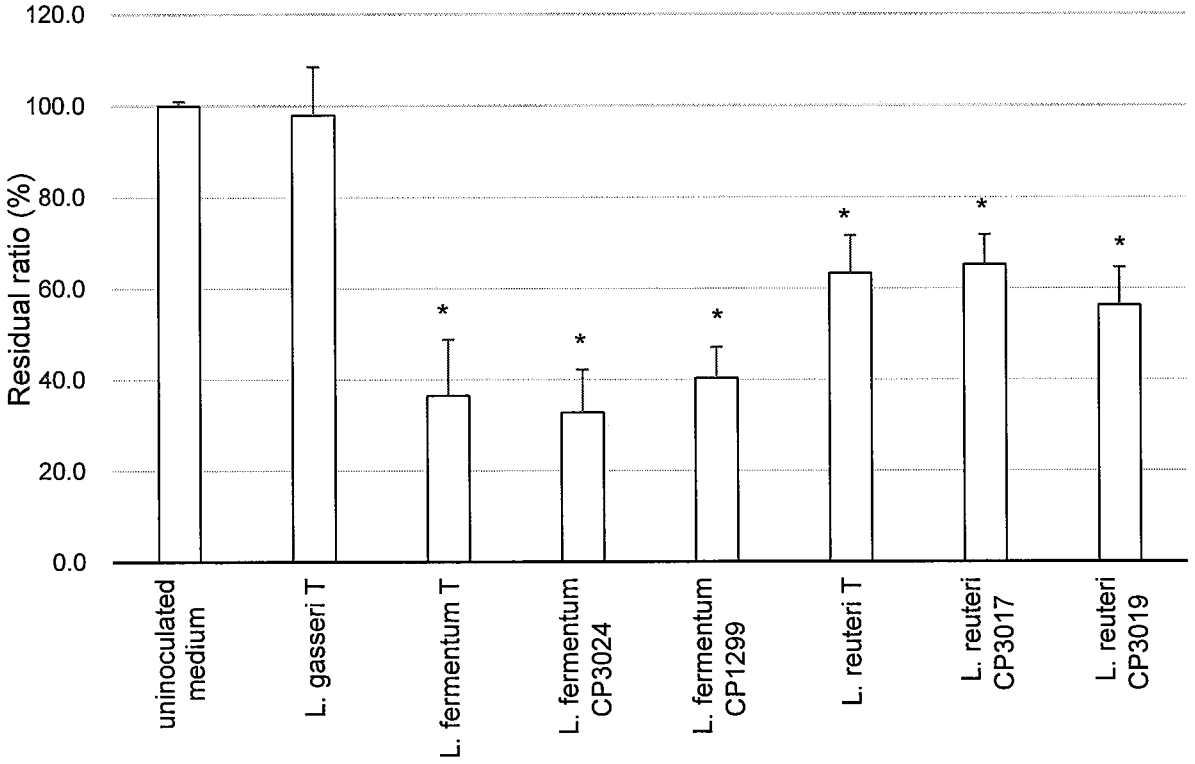
* p < 0.05 vs. *L.gasseri T*

Fig. 4



* p < 0.05 vs. *L.gasseri T*

Fig. 5



* $p < 0.05$ vs. *L. gasseri T*