

Specification

**[Title] Fermented milk showing suppressed increase in acidity and
method for producing same**

[Technical Field]

[0001]

The present invention relates to a fermented milk wherein an increase in acidity is suppressed, and a production method thereof. In particular, the present invention relates to useful thermophilus bacteria obtained using a novel screening method for thermophilus bacteria (*Streptococcus thermophilus*), and a fermented milk wherein an increase in acidity is suppressed by using said thermophilus bacteria, and a production method thereof.

[Background Art]

[0002]

In many of the fermented milk produced using lactic acid-producing bacteria and even in their final products, live lactic acid-producing bacteria are contained. The final products are usually refrigerated in order to maintain flavor of the fermented milk. However, even during refrigerated storage, its acidity increases with gradual progression of fermentation due to live lactic acid-producing bacteria, and therefore it is difficult to maintain a constant quality of the fermented milk for a long period of time. Such phenomenon is referred to as post-acidification or after-acidification, and is distinguished from the acid generation that is indispensable for the production of fermented milk.

[0003]

As a method for producing fermented milk wherein an increase acidity is suppressed during refrigerated storage, a method in

which activity of lactic acid bacteria in the fermented milk is decreased by pressure treatment after fermentation has been proposed (Patent Document 1).

In addition, methods for producing fermented milk wherein an acidity increase during storage is suppressed using a mutant strain of *Streptococcus thermophilus* have been proposed (Patent Documents 2 and 3).

[0004]

Furthermore, a method for producing fermented milk wherein an acidity increase during storage is suppressed by co-using an acid-generation suppressing strain of *Lactobacillus bulgaricus* and a viscous-substance producing strain of *Streptococcus thermophilus* as the starter lactic acid bacteria, has been proposed (Patent Document 4).

Incidentally, *Streptococcus thermophilus* OLS3290 that can be used in the present invention has already been isolated, and is known for the use in the production of frozen yogurt (Patent Document 5).

[Prior Art Documents]

[Patent Documents]

[0005]

Patent Document 1: JP A 04-075555

Patent Document 2: JP A 2000-270844

Patent Document 3: JP A 2005-021050

Patent Document 4: JP A 07-236416

Patent Document 5: JP B 4416127

[Summary of the Invention]

[Problems to Be Solved by the Invention]

[0006]

However, according to investigations of the present

inventors, in the invention of Patent Document 1, high-pressure treatment of entire product after the end of fermentation is necessary, so that the method is not practical. In the invention of Patent Document 2, it is necessary to use yeast extract, etc. in order to promote fermentation and to suppress delay of fermentation time, and the acidity achieved by conventional fermentation time is also low. Furthermore, in the invention of Patent Document 3, fermentation time to achieve sufficient lactic-acid acidity is 8-10 h (Fig. 2 of Patent Document 3), which is very long and not practical. In the invention of Patent Document 4, fermented milk is produced using a lactic acid bacteria starter that has been prepared using yeast extract.

[0007]

As described above, while various methods have been proposed in order to suppress post-acidification, a production method of fermented milk wherein an increase in acidity during refrigerated storage is suppressed, and wherein no additives to promote fermentation or to suppress acidity are required and no special production steps are required in usual fermentation time, has not yet been established. In addition, in conventional production methods of fermented milk wherein an increase in acidity during refrigerated storage is suppressed, in many cases fermentation time is long and preferred aroma of fermented milk is not rich.

[0008]

Accordingly, an object of the present invention is to solve the problems of the prior art and to provide a fermented milk wherein an increase in acidity during refrigerated storage is suppressed, by means of usual production steps with a conventional level of fermentation time, without using additives to promote

fermentation or to suppress acidity, as well as to provide a production method thereof.

[Means for Solving the Problems]

[0009]

The present inventors have found that, during intensive study to solve the above problems, it is possible to suppress an increase in acidity during refrigerated storage without delaying the fermentation, by using an acid-sensitive lactic acid-producing strain to produce fermented milk; and the inventors have already found an excellent strain of Bulgaria bacteria (*Lactobacillus delbrueckii* subsp. *bulgaricus*) (JP A 2013-116332). Furthermore, as a result of further investigation to find excellent thermophilus bacteria (*Streptococcus thermophilus*), the inventors have completed the present invention.

[0010]

Namely, the present invention relates to the following.

[1] A lactic acid bacteria starter comprising:

(a) a *Lactobacillus delbrueckii* subsp. *bulgaricus* strain having the following mycological properties: when a skim milk medium wherein its pH is adjusted to 4.2-4.3 by adding lactic acid is inoculated with said bacteria strain and the medium is maintained at 35-47°C, 15 h or longer time is required until the pH value is decreased by 0.2, and

(b) a *Streptococcus thermophilus* strain having the following mycological properties: when a skim milk medium is inoculated with said bacteria strain, cultured at 37-45°C for 12-24 h and then cooled at 1-10°C, aggregates are not generated.

[2] The lactic acid bacteria starter according to the above [1], wherein the *Lactobacillus delbrueckii* subsp. *bulgaricus* strain

is *Lactobacillus delbrueckii* subsp. *bulgaricus* OLL1171 (NITE BP-01569).

[3] The lactic acid bacteria starter according to the above [1] or [2], wherein the *Streptococcus thermophilus* strain is one or more kinds selected from the group consisting of *Streptococcus thermophilus* OLS3615 (NITE BP-01696), *Streptococcus thermophilus* OLS3078 (NITE BP-01697), and *Streptococcus thermophilus* OLS3290 (FERM BP-19638).

[4] The lactic acid bacteria starter according to any one of the above [1] to [3], which is for the production of fermented milk wherein an increase in acidity is suppressed.

[5] *Streptococcus thermophilus* OLS3615 (NITE BP-01696).

[6] *Streptococcus thermophilus* OLS3078 (NITE BP-01697).

[7] A fermented milk comprising *Streptococcus thermophilus* OLS3615 (NITE BP-01696) or *Streptococcus thermophilus* OLS3078 (NITE BP-01697).

[8] A fermented milk comprising:

(a) *Lactobacillus delbrueckii* subsp. *bulgaricus* OLL1171 (NITE BP-01569), and

(b) one or more kinds selected from the group consisting of *Streptococcus thermophilus* OLS3615 (NITE BP-01696), *Streptococcus thermophilus* OLS3078 (NITE BP-01697) and *Streptococcus thermophilus* OLS3290 (FERM BP-19638).

[0011]

[9] A method for producing fermented milk, comprising a step of adding the lactic acid bacteria starter according to any one of the above [1] to [4] to a raw material mix.

[10] The production method according to the above [9], comprising a step of fermentation at 35-45°C for any of 3-5 h.

[11] The production method according to the above [9] or [10],

wherein the fermentation step is terminated when the acidity becomes 0.6 or more and 1.4 or less.

[12] The production method according to any one of the above [9] to [11], comprising a step of deoxygenation of the raw material mix.

[13] A fermented milk produced by the production method according to any one of the above [9] to [12].

[14] The fermented milk according to any one of the above [7], [8] or [13], wherein pH is 4.1-4.7.

[15] The fermented milk according to any one of the above [7], [8], [13] or [14], which is subjected to refrigerated storage.

[16] The fermented milk according to the above [15], wherein an increase in acidity is suppressed.

[17] The fermented milk according to any one of the above [7], [8] or [13] to [16], wherein the acidity is 0.6-1.4.

[0012]

[18] A method for screening a *Streptococcus thermophilus* strain for the production of a fermented milk wherein an acidity increase is suppressed, comprising:

(a) a step of inoculating a candidate *Streptococcus thermophilus* strain into a skim milk medium,

(b) a step of culturing and then cooling the skim milk medium inoculated with the candidate *Streptococcus thermophilus* strain in the above (a),

(c) a step of confirming the generation of aggregates in the cooled skim milk medium in the above (b), and

(d) a step of determining the candidate *Streptococcus thermophilus* strain with which no aggregates are confirmed to be generated, as a *Streptococcus thermophilus* strain for the production of a fermented milk wherein an acidity increase is

suppressed.

[19] The screening method according to the above [18], wherein the amount of skim milk powder is any of 8-12 wt% and the amount of water is any of 92-88 wt%, relative to 100 wt% of the skim milk medium.

[20] The screening method according to the above [19], wherein yeast extract is mixed in the skim milk medium at any of 0.05-0.15 wt% relative to 100 wt% of the skim milk medium.

[21] The screening method according to the above [20], wherein the candidate *Streptococcus thermophilus* strain is inoculated at any of 1-3 wt% relative to 100 wt% of the skim milk medium.

[22] The screening method according to any one of the above [18] to [21], wherein the culturing in (b) is performed at 37-45°C for any of 12-24 h.

[23] The screening method according to any one of the above [18] to [22], wherein the cooling in (b) is performed at 1-10°C.

[Advantageous Effects of the Invention]

[0013]

According to the present invention, thermophilus bacteria (*Streptococcus thermophilus*) suitable as a starter can be efficiently and accurately screened without using a raw material mix, by means of a simple indicator of having or not having aggregability for a skim milk medium (which may be mixed with yeast extract).

Thermophilus bacteria selected by the screening method of the present invention can produce savory fermented milk without aggregation of raw material mix in the production of fermented milk. In particular, by using the thermophilus bacteria alone or by using them in combination with Bulgaria bacteria, it is possible to provide fermented milk wherein an increase in acidity

during refrigerated storage is suppressed.

[0014]

In the provision of the fermented milk of the present invention, the use of additives is not required for the promotion of fermentation or for the suppression of an acidity increase, and no special production steps are required in the conventional level of fermentation time. In particular, *Streptococcus thermophilus*, which has no aggregability for skim milk media, even when used in combination with Bulgarian bacteria capable of suppressing an acidity increase, can produce suitable fermented milk without inhibiting the action of Bulgarian bacteria of suppressing an acidity increase.

[0015]

In addition, the present invention can provide optimal thermophilus bacteria as a mixed starter, when used in combination with Bulgaria bacteria that are excellent as a lactic acid-producing strain having sensitivity to acid, such as *Lactobacillus delbrueckii* subsp. *bulgaricus* OLL1171 (NITE BP-01569) (hereinafter also referred to as OLL1171 strain). In particular, it is possible to suppress an increase in acidity and a decrease in pH of fermented milk over time during refrigerated storage, while maintaining fresh flavor and the number of live lactic acid-producing bacteria in the fermented milk for a long period of time, and to suppress changes in the flavor of the fermented milk after production during distribution and storage compared to those in conventional products.

Furthermore, the present invention can realize improvement in the texture (body feel = high viscosity and fillingness) while maintaining good flavor, especially in soft type yogurt.

[Brief Description of Drawings]

[0016]

[Fig. 1] Figure 1 is a diagram showing visual evaluation of smoothness of fermented milk (aggregability: generation of aggregates) (left: ◎ (no aggregation observed), right: X (aggregation observed)).

[Fig. 2] Figure 2 is a diagram showing comparison of flavor of fermented milk after refrigerated (low temperature) storage: fermented milk (plain yogurt) prepared using mixed starters of the thermophilus bacteria of the present invention (candidate strain: 6, candidate strain: 7, candidate strain: 19) and OLL1771 strain, and fermented milk (plain yogurt) prepared using a mixed starter of strain Y and strain B (Comparative example).

[Fig. 3] Figure 3 is a diagram showing comparison of flavor of fermented milk immediately after production: fermented milk (plain yogurt) prepared using a mixed starter of the thermophilus bacteria of the present invention (candidate strain: 19) and OLL1771 strain, and fermented milk (plain yogurt) prepared using a mixed starter of strain Y and strain B (Comparative example).

[Fig. 4] Figure 4 is a diagram showing comparison of flavor of fermented milk immediately after production: fermented milk (plain yogurt) prepared using a mixed starter of the thermophilus bacteria of the present invention (candidate strain: 6) and OLL1771 strain, and fermented milk (plain yogurt) prepared using a mixed starter of strain Y and strain B (Comparative example).

[Fig. 5] Figure 5 is a diagram showing comparison of flavor of fermented milk after refrigerated storage: fermented milk (plain yogurt) prepared using a mixed starter of the thermophilus bacteria of the present invention (candidate strain: 19) and OLL1771 strain, and fermented milk (plain yogurt) prepared using a mixed starter of strain Y and strain B (Comparative example).

[Fig. 6] Figure 6 is a diagram showing comparison of flavor of fermented milk after refrigerated storage: fermented milk (plain yogurt) prepared using a mixed starter of the thermophilus bacteria of the present invention (candidate strain: 6) and OLL1771 strain, and fermented milk (plain yogurt) prepared using a mixed starter of strain Y and strain B (Comparative example).

[Fig. 7] Figure 7 is a diagram showing comparison of flavor of fermented milk immediately after production: fermented milk (plain yogurt) prepared using a mixed starter of the thermophilus bacteria of the present invention (candidate strain: 19) and OLL1771 strain, and fermented milk (fruits yogurt) prepared using a mixed starter of strain Y and strain B (Comparative example).

[Fig. 8] Figure 8 is a diagram showing comparison of flavor of fermented milk after refrigerated storage: fermented milk (plain yogurt) prepared using a mixed starter of the thermophilus bacteria of the present invention (candidate strain: 19) and OLL1771 strain, and fermented milk (fruits yogurt) prepared using a mixed starter of strain Y and strain B (Comparative example).

[Modes for Carrying out the Invention]

[0017]

The "fermented milk" in the present specification refers to milk that has been fermented, and includes the "fermented milk," "lactic acid bacteria beverage," "milk beverage," and "natural cheese" etc. defined under the Ministerial Ordinance on Milk and Milk Products Concerning Compositional Standards, etc. (Ministerial Ordinance on Milk, etc.). For example, fermented milk refers to the "fermented milk" defined under the Ministerial Ordinance on Milk, etc., that is, those obtained by fermenting milk such as raw milk, cow's milk, special milk, raw goat's milk, pasteurized goat's milk, raw sheep's milk, composition modified

milk, low fat milk, skim milk or processed milk, or milk, etc. containing an equal or greater amount of milk solids-not-fat compared thereto, with lactic acid bacteria or yeasts, and then forming a solid (hard type), a paste (soft type) or a liquid (drink type), or frozen products thereof.

[0018]

Typical examples of fermented milk include yogurt. In the international standard defined by Food and Agriculture Organization (FAO)/World Health Organization (WHO), the following definition is made: "a product called yogurt is made from dairy products such as milk and skim milk powder by lactic acid fermentation action of both *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, and large amounts of these two types of bacteria are present in the final product." As used herein, "yogurt" is intended to include the yogurt defined above by FAO/WHO.

[0019]

Since yogurt contains a large amount of live lactic acid-producing bacteria, by using acid-sensitive lactic acid-producing bacteria of the present invention in the production of yogurt, the effect of suppressing an acidity increase during refrigerated storage of the fermented milk is clearly observed. Accordingly, in the present invention, suitable fermented milk includes yogurt, and lactic acid bacteria beverages, milk beverages and cheeses (natural cheese, processed cheese) containing live bacteria. In particular, yogurt that is stored under refrigeration, such as plain yogurt, hard yogurt (set type yogurt), soft yogurt, and yogurt drinks are suitable as fermented milk.

[0020]

Hereinafter, the present invention will be described in detail focusing mainly on yogurt as the fermented milk. However, the present invention is not limited to such embodiments, and intends to include embodiments such as lactic acid bacteria beverages, milk drinks and cheeses. As for yogurt and other embodiments, those skilled in the art will be able to appropriately make modifications based on the description herein.

[0021]

As used herein, "raw material mix" refers to a liquid containing milk components such as raw milk, whole milk, skim milk and whey. Here, the raw milk refers to animal milk such as cow milk. The raw material mix may contain, in addition to milk components such as raw milk, whole milk, skim milk and whey, the processed products thereof (such as whole milk powder, concentrated whole milk, skim milk powder, concentrated skim milk, condensed milk, whey powder, butter milk, butter, cream, cheese, whey protein concentrate (WPC), whey protein isolate (WPI), α -lactalbumin (α -La), β -lactoglobulin (β -Lg), etc.).

[0022]

Incidentally, the raw material mix may contain, in addition to the milk components, soymilk, sugar, saccharides, sweeteners, flavors, fruit juices, fruit pulp, vitamins, minerals, food products such as oils and fats, food ingredients and food additives, etc. In addition, the raw material mix may contain, if necessary, stabilizers, thickeners, and gelling agents, etc. such as pectin, soy polysaccharides, CMC (carboxymethylcellulose), agar, gelatin, carrageenan, and gums.

[0023]

In the present invention, preferably, the raw material mix only contains those which are commonly used in the production of

fermented milk, such as the above-mentioned food products, food ingredients, food additives and stabilizers, thickeners, and gelling agents. In particular, from the viewpoint of texture and flavor of fermented milk, it is desirable not to add (blend) components having fermentation-promoting effect and components having acidity-increase inhibitory effect, such as "yeast extract" and "chitosan" in fermented milk, or to add them in a trace amount that does not exhibit such effects even if they are added in fermented milk.

[0024]

In a preferred embodiment of the present invention, in the raw material mix, the amount of yeast extract added is, for example 0.01 wt% or less, preferably 0.005 wt% or less, more preferably 0.001 wt% or less, furthermore preferably 0.0005 wt% or less, particularly preferably 0.0001% or less, and most preferably, no yeast extract is contained. Also in a preferred embodiment of the present invention, in the fermented milk, the amount of yeast extract added is, for example 0.01 wt% or less, preferably 0.005 wt% or less, more preferably 0.001 wt% or less, furthermore preferably 0.0005 wt% or less, particularly preferably 0.0001 wt% or less, and most preferably, no yeast extract is contained.

[0025]

Furthermore, in a preferred embodiment of the present invention, in the raw material mix, the amount of chitosan added is, for example less than 0.01 wt%, preferably less than 0.005 wt%, more preferably less than 0.001 wt%, furthermore preferably less than 0.0005 wt%, particularly preferably less than 0.0001 wt%, and most preferably, no chitosan is contained. Also in a preferred embodiment of the present invention, in the fermented milk, the amount of chitosan added is, for example less than 0.01

wt%, preferably less than 0.005 wt%, more preferably less than 0.001 wt%, furthermore preferably less than 0.0005 wt%, particularly preferably less than 0.0001 wt%, and most preferably, no chitosan is contained.

[0026]

As used herein, expression such as "comprising lactic acid-producing bacteria," and "comprising *Streptococcus thermophilus* OLS3615 (accession number: NITE BP-01696)," "comprising *Streptococcus thermophilus* OLS3078 (accession number: NITE BP-01697)," "comprising *Streptococcus thermophilus* OLS3290 (accession number: FERM BP-19638)," "comprising *Lactobacillus delbrueckii* subsp. *bulgaricus* OLL1171 strain (accession number: NITE BP-01569)," means that fermented milk comprises said bacteria (said microorganisms), preferably comprises live cells of said bacteria. In addition to the cases wherein the obtained fermented milk comprises lactic acid-producing bacteria by using said bacteria as starter bacteria, it also includes cases wherein the fermented milk comprises lactic acid-producing bacteria by adding said bacteria before fermentation, during fermentation and after fermentation, in addition to lactic acid-producing bacteria used as the starter bacteria. As used herein, the term "starter bacteria" means microorganisms that ferment, and "starter" means a culture of starter bacteria.

[0027]

As used herein, "acidity" means a measured value in accordance with "5. Method of measuring acidity of milk and dairy products" in Milk-related laws and regulations (Dairy Group Hygienic Association, March 2004), p. 56, and the details thereof are as follows. That is, in the present specification, "acidity"

is a value of acidity measured as follows: "10 ml of a sample is diluted by adding the same amount of water without carbon dioxide gas, to which 0.5 ml of phenolphthalein solution as an indicator is added, and the sample is titrated using 0.1 mol/L sodium hydroxide solution for 30 s with a limit being set at the point where rose-pink color disappears; a percentage amount of lactic acid per 100 g of the sample is calculated from the obtained titer, and this value is determined to be the acidity. One ml of 0.1 mol/L sodium hydroxide solution corresponds to 9 mg of lactic acid. As the indicator, 1 g of phenolphthalein is dissolved in 50% ethanol to make an amount of 100 ml."

[0028]

In general, when the fermented milk to be shipped as a final product is yogurt, acidity of the fresh product (at the end of fermentation) is approximately 0.6-0.8%. Since fermented milk typically may be refrigerated for about 1 to 2 weeks (7-14 days), when the fermented milk is actually consumed, the acidity of the refrigerated products (after refrigerated storage) can rise to approximately 0.9-1.2%. In this case, from the viewpoint of texture and flavor (in particular, sourness) of the fermented milk, the acidity of the fermented milk should be, for example, 0.6-1.1%, preferably 0.7-0.9%, and more preferably about 0.75-0.85%. In the present invention, since an increase in acidity during refrigerated storage of fermented milk is suppressed, the acidity of fresh products of fermented milk is, for example, 0.6-0.9%, preferably 0.65-0.85%, more preferably 0.7-0.8%; and after refrigerated storage for about 1-2 weeks, the acidity of the refrigerated products of fermented milk is, for example, 0.7-1.1%, preferably 0.75-1.0%, and more preferably 0.75-0.9%.

[0029]

As used herein, "an increase in acidity is suppressed" means that an increase in the acidity from the end of fermentation to that after refrigerated storage for a certain period is 0.25% or less. In one embodiment of the present invention, a change (increase) in acidity after one week (7 days) of refrigerated storage (10°C) from the end of fermentation is, for example, 0.15% or less, preferably 0.13% or less, more preferably 0.11% or less, furthermore preferably 0.10% or less, and particularly preferably 0.09% or less. In this case, the lower limit value of a change (increase) in acidity is not particularly limited, and it is for example 0.02%.

[0030]

In one embodiment of the present invention, a change (increase) in acidity after two weeks (14 days) of refrigerated storage (10°C) from the end of fermentation is, for example, 0.25% or less, preferably 0.23% or less, more preferably 0.21% or less, furthermore preferably 0.20% or less, and particularly preferably 0.19% or less. In this case, the lower limit value of a change (increase) in acidity is not particularly limited, and it is for example 0.05%.

[0031]

In one embodiment of the present invention, the acidity at one week (7 days) of refrigerated storage (10°C) from the end of fermentation is, for example, 0.6-0.95 %, preferably 0.65-0.9%, more preferably 0.7-0.85%, furthermore preferably 0.75-0.85%, and particularly preferably 0.8-0.85%. In one embodiment of the present invention, the acidity at two weeks (14 days) of refrigerated storage (10°C) from the end of fermentation is, for example, 0.7-1.05%, preferably 0.75-1.0%, more preferably 0.8-0.95%, furthermore preferably 0.85-0.95%, and particularly

preferably 0.9-0.95%.

[0032]

As used herein, "refrigerated storage" refers to storage at a low temperature of 10°C or less, and typically it refers to storage at 5-10°C. In general, when the fermented milk to be shipped as a final product is yogurt, pH of its fresh product (at the end of fermentation) is approximately 4.0-5.0. In this case, from the viewpoint of texture and flavor (in particular, sourness) of the fermented milk, pH of the fermented milk should be, for example, 4.0 or more, preferably 4.1 or more, and more preferably 4.2 or more. In the present invention, since an increase in acidity during refrigerated storage of fermented milk is suppressed, pH of fresh products of fermented milk is, for example, 4.0-5.0, preferably 4.1-4.7, and more preferably 4.2-4.6; after refrigerated storage for about 1-2 weeks, pH of the refrigerated products of fermented milk is, for example 4.0-5.0, preferably 4.1-4.7, and more preferably 4.2-4.6.

[0033]

In one embodiment of the present invention, a change (decrease) in pH after one week (7 days) of refrigerated storage (10°C) from the end of fermentation is, for example, 0.35 or less, preferably 0.32 or less, more preferably 0.3 or less, furthermore preferably 0.27 or less, and particularly preferably 0.25 or less. The lower limit value of the change (decrease) in acidity is not particularly limited, and it is for example 0.02.

[0034]

In one embodiment of the present invention, a change (decrease) in pH after two weeks (14 days) of refrigerated storage (10°C) from the end of fermentation is, for example, 0.5 or less, preferably 0.47 or less, more preferably 0.45 or less, furthermore

preferably 0.42 or less, and particularly preferably 0.4 or less. In this case, the lower limit value of the change (decrease) in pH is not particularly limited, and it is for example 0.05.

[0035]

In one embodiment of the present invention, pH of the fermented milk after one week (7 days) of refrigerated storage (10°C) from the end of fermentation is, for example, 4.0–5.0, preferably 4.1–4.8, more preferably 4.2–4.6, furthermore preferably 4.3–4.6, and particularly preferably 4.4–4.6. In one embodiment of the present invention, pH of the fermented milk after two weeks (14 days) of refrigerated storage (10°C) from the end of fermentation is, for example, 4.0–5.0, preferably 4.1–4.8, more preferably 4.2–4.6, furthermore preferably 4.3–4.6, and particularly preferably 4.4–4.6.

[0036]

In one embodiment of the present invention, when changes in pH and changes in acidity over time are within the above ranges, quality of the fermented milk can be controlled (maintained) at predetermined quality (in particular, certain flavors) for a long period of time, and the expiration date of the fermented milk can be sufficiently prolonged.

[0037]

As used herein, "lactic acid-producing bacteria" refer to microorganisms that produce lactic acid, and include lactic acid bacteria belonging to *Lactobacillus*, *Streptococcus*, *Lactococcus*, *Leuconostoc* and *Pediococcus*, as well as *Bifidobacterium*. Here, preferable lactic acid-producing bacteria are *Lactobacillus bulgaricus* (*Lactobacillus delbrueckii* subsp. *bulgaricus*, herein also referred to as "Bulgaria bacteria" and "*L. bulgaricus*"), and *Streptococcus thermophilus* (*Streptococcus salivarius* subsp.

thermophilus, herein also referred to as "thermophilus bacteria" and "*S. thermophilus*"), which are essential for the production of yogurt, and particularly preferable bacteria are Bulgaria bacteria (*L. bulgaricus*) that produce a large amount of lactic acid in the production of yogurt.

[0038]

Regarding the Bulgaria bacteria that suppress an increase in acidity which can be used in the present invention, the present inventors have already investigated them in JP A No. 2013-116332, and found that an increase in acidity of fermented milk during refrigerated storage can be suppressed by selectively using "*Lactobacillus delbrueckii* subsp. *bulgaricus* OLL1171 having the following mycological properties: when a skim milk medium wherein its pH is adjusted to 4.2-4.3 by adding lactic acid is inoculated with said bacteria strain and the medium is maintained at 35-47°C, 15 h or longer time is required until the pH value is decreased by 0.2." Bulgaria bacteria having such properties include *Lactobacillus delbrueckii* subsp. *bulgaricus* OLL1171 (NITE BP-01569) (hereinafter also referred to as OLL1171 strain) and the like.

The *Lactobacillus delbrueckii* subsp. *bulgaricus* OLL1171 strain of the present invention has been internationally deposited at the Patent Microorganisms Depositary, National Institute of Technology and Evaluation (Room. 122, 2-5-8, Kazusa Kamatari, Kisarazu City, Chiba, 292-0818 Japan) on March 13, 2013, with the accession number: NITE BP-01569.

[0039]

Here, *L. bulgaricus* OLL1171 has the following scientific properties (morphological properties, characteristics on a medium, physiological properties, etc.).

(a) Morphological properties

Colony characteristics on a medium (BL(+) Agar, Nissui): basically circular (somewhat polymorphism), white to gray color, smooth type (partly rough type), flat

(b) Physiological properties

Bacteria form: bacilli, Gram stain: positive, Lactic acid fermentation type: homo-lactic acid fermentation, Aerobic growth: +

[0040]

(c) Other properties characterizing the microorganisms

Subspecies below subsp. cannot be distinguished by 16sRNA sequences; therefore, groEL sequence analysis is utilized for the identification of 4 subspecies of *delbrueckii* (*delbrueckii*, *bulgaricus*, *lactis*, *indicus*), and those of the following sequence can be utilized in the identification of OLL1171 strain.

groEL sequence (OLL1171 strain)

[S1]

```
AAAGGCCACCAAAGCAGCCGTTGACCAATTGCACAAGAACAGCCACAAAGTTTCCAGCCGG
GACCAAATTGCCCAAGTTGCTTCAATCTCAAGTGCTTCAAAGGAAATCGGCGACTTGATCG
CTGAAGCCATGGAAAAGGTCGGCAAGGACGGTGTTATCACCATTGAAGACTCCCGCGGGAT
CGAAACTGAACTGAGCGTGGTTGAAGGGATGCAATTCGACCGCGGCTACCTGTCCCAATAC
ATGGTAACGGACAACGACAAGATGGAAGCTGACTTGGA AAAACCCATACATCTTGATCACTG
ACAAGAAGATTTCCAACATCCAGGACATCTTGCCAATGTTGCAGGAAATCGTGCAAC
```

(SEQ ID: 1)

[0041]

In addition, *L. bulgaricus* OLL1171 has a property of suppressing acidity increase in fermented milk.

Thermophilus bacteria having no aggregability for skim milk medium, which can be used in the present invention, is a *Streptococcus thermophilus* strain "that has the following

mycological properties: when a skim milk medium (that may be mixed with yeast extract) is inoculated with said bacteria strain, cultured at 37-45°C for 12-24 h and then cooled at 1-10°C (preferably cooled for 5-15 h), no aggregate is generated," and more preferably "that has the following mycological properties: when a skim milk medium (that may be mixed with yeast extract) is inoculated with said bacteria strain, cultured at 43°C for 18 h and then cooled at 4°C for 8 h, no aggregate is generated." Examples of *Thermophilus* bacteria having such properties include *Streptococcus thermophilus* OLS3615 (NITE BP-01696) (hereinafter also referred to as OLS3615 strain), *Streptococcus thermophilus* OLS3078 (NITE BP-01697) (hereinafter also referred to as OLS3078 strain) or *Streptococcus thermophilus* OLS3290 (FERM BP-19638) (hereinafter also referred to as OLS3290 strain), and the like.

[0042]

Streptococcus thermophilus OLS3615 (NITE BP-01696) has been internationally deposited at the Patent Microorganisms Depository, National Institute of Technology and Evaluation (Room. 122, 2-5-8, Kazusa Kamatari, Kisarazu City, Chiba, 292-0818 Japan) on August 23, 2013, with the accession number: NITE BP-01696.

[0043]

Here, *S. thermophilus* OLS3615 has the following scientific properties (morphological properties, characteristics on a medium, physiological properties, etc.).

(a) Morphological properties

Colony characteristics on a medium (M17 Agar, BD): circular, white color (translucent), smooth type, hemispherical

(b) Physiological properties

Bacteria form: aureus, Gram stain: positive, Lactic acid

fermentation type: homo lactic acid fermentation, Aerobic growth:
+

[0044]

(c) Other properties characterizing the microorganisms

16s RNA gene base sequence information

[S2]

TGGCGGCGTGCCTAATACATGCAAGTAGAACGCTGAAGAGAGGAGCTTGCTCTTCTTGGAT
GAGTTGCGAACGGGTGAGTAACGCGTAGGTAACCTGCCTTGTAGCGGGGGATAACTATTGG
AAACGATAGCTAATACCGCATAACAATGGATGACACATGTCATTTATTTGAAAGGAGCAAT
TGCTCCACTACAAGATGGACCTGCGTTGTATTAGCTAGGTGAGGTAATGGCTCACCTA
GGCGACGATACATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCC
AGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGGGGCAACCCTGACCGAGCA
ACGCCGCGTGAGTGAAGAAGGTTTTTCGGATCGTAAAGCTCTGTTGTAAGTCAAGAACGGGT
GTGAGAGTGGAAAGTTCACACTGTGACGGTAGCTTACCAGAAAGGGACGGCTAACTACGT

(SEQ ID: 2)

[0045]

Streptococcus thermophilus OLS3078 (NITE BP-01697) has been internationally deposited at the Patent Microorganisms Depository, National Institute of Technology and Evaluation (Room. 122, 2-5-8, Kazusa Kamatari, Kisarazu City, Chiba, 292-0818 Japan) on August 23, 2013, with the accession number: NITE BP-01697.

[0046]

Here, *S. thermophilus* OLS3078 has the following scientific properties (morphological properties, characteristics on a medium, physiological properties, etc.).

(a) Morphological properties

Colony characteristics on a medium (M17 Agar, BD): circular, white color (translucent), smooth type, hemispherical

(b) Physiological properties

Bacteria form: aureus, Gram stain: positive, Lactic acid fermentation type: homo lactic acid fermentation, Aerobic growth: +

[0047]

(c) Other properties characterizing the microorganisms

16s RNA gene base sequence information

[S3]

TGGCGGCGTGCCTAATACATGCAAGTAGAACGCTGAAGAGAGGAGCTTGCTCTTCTTGGAT
GAGTTGCGAACGGGTGAGTAACGCGTAGGTAACCTGCCTTGTAGCGGGGGATAACTATTGG
AAACGATAGCTAATACCGCATAACAATGGATGACACATGTCATTTATTTGAAAGGAGCAAT
TGCTCCACTACAAGATGGACCTGCGTTGTATTAGCTAGTAGGTGAGGTAATGGCTCACCTA
GGCGACGATACATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCC
AGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGGGGCAACCCTGACCGAGCA
ACGCCGCGTGAGTGAAGAAGGTTTTTCGGATCGTAAAGCTCTGTTGTAAGTCAAGAACGGGT
GTGAGAGTGGAAAGTTCACACTGTGACGGTAGCTTACCAGAAAGGGACGGCTAACTACGT
(SEQ ID: 3)

[0048]

Streptococcus thermophilus OLS3290 (FERM BP-19638) has been deposited at the Patent Microorganisms Depository, National Institute of Technology and Evaluation (Room. 120, 2-5-8, Kazusa Kamatari, Kisarazu City, Chiba, 292-0818 Japan) on January 19, 2004 (domestic deposition date), then transferred to international deposition on September 6, 2013 with the accession number: FERM BP-19638.

[0049]

Here, *S. thermophilus* OLS3290 has the following scientific properties (morphological properties, characteristics on a medium, physiological properties, etc.).

(a) Morphological properties

Colony characteristics on a medium (M17 Agar, BD): circular, white

color (translucent), smooth type, hemispherical

(b) Physiological properties

Bacteria form: aureus, Gram stain: positive, Lactic acid fermentation type: homo lactic acid fermentation, Aerobic growth: +

[0050]

(c) Other properties characterizing the microorganisms

16s RNA gene base sequence information

[S4]

TGGCGGCGTGCCTAATACATGCAAGTAGAACGCTGAAGAGAGGAGCTTGCTCTTCTTGGAT
GAGTTGCGAACGGGTGAGTAACGCGTAGGTAACCTGCCTTGTAGCGGGGATAACTATTGG
AAACGATAGCTAATACCGCATAACAATGGATGACACATGTCATTTATTTGAAAGGAGCAAT
TGCTCCACTACAAGATGGACCTGCGTTGTATTAGCTAGTAGGTGAGGTAATGGCTCACCTA
GGCGACGATACATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCC
AGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGGGGCAACCCTGACCGAGCA
ACGCCGCGTGAGTGAAGAAGGTTTTTCGGATCGTAAAGCTCTGTTGTAAGTCAAGAACGGGT
GTGAGAGTGGAAAGTTCACACTGTGACGGTAGCTTACCAGAAAGGGACGGCTAACTACGT
(SEQ ID: 4)

[0051]

In the method for producing fermented milk of the present invention, conventional production steps of fermented milk can be adopted, and their preferred embodiments are described below.

[0052]

The method for producing fermented milk of the present invention comprises a blending step of raw material mix, in which raw materials are mixed (blended). In the blending step of raw material mix, conditions generally used in the production of fermented milk may be employed as appropriate. In addition, in the production method of fermented milk of the present invention, the following steps are comprised similar to conventional

methods: a step of (heat) sterilization of raw material mix, a step of cooling raw material mix, a step of adding a starter, a fermentation step, and a step of cooling fermented milk; and it is desirable to include these steps in this order. In these steps, conditions generally used in the production of fermented milk may be employed as appropriate.

[0053]

The method for producing fermented milk of the present invention may comprise a homogenization step of raw material mix. The method for producing fermented milk of the present invention may comprise the homogenization step of raw material mix, simultaneously with or subsequent to the blending step of raw material mix; prior to or subsequent to the sterilization step of raw material mix; simultaneously with, prior to or subsequent to the cooling step after the sterilization step of raw material mix; subsequent to the fermentation step; simultaneously with, prior to or subsequent to the cooling step after the fermentation step, in this order. The method for producing fermented milk of the present invention may comprise the homogenization step of raw material mix once or two or more times. In the homogenization step of raw material mix, when a homogenizer, etc. is used, the pressure of the homogenization is, for example 1-100 MPa, preferably 5-50 MPa, more preferably 8-30 MPa, and furthermore preferably 10-20 MPa in the treatment.

[0054]

The method for producing fermented milk of the present invention may comprise a deoxygenation step to deoxygenize raw material mix. In the deoxygenation step, oxygen present in the raw material mix (dissolved oxygen concentration) is reduced or removed. As a method of reducing dissolved oxygen concentration

(DO) in the raw material mix (deoxygenation method), for example, a gas replacement treatment with inert gas such as nitrogen gas, helium, neon, argon and xenon, a membrane separation treatment using oxygen permeable membrane, a degassing treatment at low pressure or in vacuum may be utilized. In the deoxygenation step, dissolved oxygen concentration of the raw material mix is reduced or removed to, for example, 5 ppm or less, preferably 3 ppm or less, more preferably 2 ppm or less, and furthermore preferably 1 ppm or less.

[0055]

The method for producing fermented milk of the present invention may comprise a deoxygenation step, simultaneously with or subsequent to the blending step of raw material mix; subsequent to the homogenization step of raw material mix; prior to or subsequent to the sterilization step of raw material mix; simultaneously with, prior to or subsequent to the cooling step of raw material mix; simultaneously with, prior to or subsequent to the adding step of starter; simultaneously with, prior to or subsequent to the fermentation step; simultaneously with, prior to or subsequent to the cooling step of fermented milk, in this order. The method for producing fermented milk of the present invention may comprise the deoxygenation step once or two or more times.

[0056]

In the deoxygenation step, fermentation time can be reduced by reducing the dissolved oxygen concentration of raw material mix at the start of the fermentation step. Furthermore, the effect of suppressing an acidity increase during refrigerated storage of fermented milk can be enhanced thereby. Thus, it is important that the dissolved oxygen concentration in the raw

material mix is maintained at a reduced level at the start of the fermentation step, and therefore, the deoxygenation step is desirably carried out simultaneously with, immediately prior to or subsequent to the adding step of starter, or simultaneously with or immediately prior to the fermentation step.

[0057]

The method for producing fermented milk of the present invention may comprise, as described above, a step of adding a starter. Starter may be a single starter wherein a single type of acid-sensitive lactic acid-producing bacteria are cultured, or a mixed starter wherein said bacteria are mixed with other microorganisms and cultured. In the present invention, microorganisms other than acid-sensitive lactic acid-producing bacteria can be used as further starter bacteria.

[0058]

As components for culturing lactic acid-producing bacteria, the use of components contained in the raw material mix is preferable, and the use of only the components contained in the raw material mix is more preferable. In specific terms, the use of raw milk, whole milk, skim milk, whole milk powder, skim milk powder, concentrated whole milk, concentrated skim milk, whey, whey powder, buttermilk, butter, cream, cheese, whey protein concentrate (WPC), whey protein isolate (WPI), α -lactalbumin (α -La) and β -lactoglobulin (β -Lg), etc. is preferable, and the use of raw milk, pasteurized milk, skim milk, skim milk powder, whole milk powder, concentrated whole milk, and/or concentrated skim milk is more preferable.

[0059]

As a growth-promoting agent for culturing lactic acid-producing bacteria, extracts such as yeast extract and meat

extract may be added to the medium for culturing starter bacteria. However, from the viewpoint of flavor of fermented milk, it is not desirable that such components are added to the raw material mix in a high concentration upon production of fermented milk. Therefore, the amount of a growth-promoting agent contained in the starter is, for example, less than 0.1 wt%, preferably less than 0.05 wt%, more preferably less than 0.01 wt%, furthermore preferably less than 0.005 wt%, and particularly preferably less than 0.001 wt%; and most preferably, no growth-promoting agent is contained in the starter.

[0060]

The number of lactic acid-producing bacteria in the starter is, for example, 10^5 - 10^{13} cfu/mL, preferably 10^6 - 10^{12} cfu/mL, more preferably 10^7 - 10^{11} cfu/mL, and furthermore preferably 10^8 - 10^{10} cfu/mL.

[0061]

In the raw material mix, the amount of a starter added is set to be, for example, 1-10 wt%, preferably 1-8 wt%, more preferably 2-6 wt%, and furthermore preferably 2-4%. In the present invention, a starter can be added to the raw material mix in accordance with ordinary methods.

[0062]

The method for producing fermented milk of the present invention comprises a fermentation step, as described above. In the present invention, from the viewpoint of efficiently obtaining fermented milk with good flavor and texture, the fermentation temperature is set to be, for example 30-50°C, preferably 35-47°C, more preferably 37-45°C, and furthermore preferably 40-43°C. In contrast, when the method comprises a deoxygenation step of raw material mix, from the viewpoint of

efficiently obtaining fermented milk with good flavor and texture, it is desirable to carry out the fermentation at relatively low temperature; therefore, the fermentation temperature is set to be, for example 28-47°C, preferably 30-45°C, more preferably 32-43°C, and furthermore preferably 35-40°C.

[0063]

In the present invention, from the viewpoint of efficiently obtaining fermented milk with good flavor and texture, the fermentation time is set to be, for example 1-48 h, preferably 2-24 h, more preferably 3-10 h, furthermore preferably 3-6 h, and particularly preferably 3-5 h. In contrast, when the method comprises a deoxygenation step of raw material mix, from the viewpoint of efficiently obtaining fermented milk with good flavor and texture, it is desirable to carry out the fermentation in relatively short time; therefore, the fermentation time is set to be, for example 1-36 h, preferably 1-12 h, more preferably 2-8 h, furthermore preferably 2-5 h, and particularly preferably 2-4 h.

[0064]

In a fermentation step, from the viewpoint of obtaining fermented milk with good flavor and texture, fermentation is terminated when the acidity of the fermented milk reaches, for example 0.6-1.0%, preferably 0.65-0.95%, more preferably 0.7-0.9%, and furthermore preferably 0.75-0.85%. In a fermentation step, from the viewpoint of obtaining fermented milk with good flavor and texture, fermentation is terminated when pH of the fermented milk reaches, for example 4.15-4.75, preferably 4.2-4.7, more preferably 4.25-4.65, and furthermore preferably 4.3-4.6.

[0065]

The method for producing fermented milk of the present invention may comprise a cooling step of fermented milk, as described above. In the cooling step of fermented milk, temperature of the fermented milk is decreased from fermentation temperature (e.g., 43°C) to a predetermined low temperature (e.g., 10°C). Then, in the cooling step of fermented milk, the temperature is decreased to 10°C or less with a cooling rate of fermented milk of, for example within 1-60 min, preferably 1-30 min, more preferably 1-10 min, and furthermore preferably 1-5 min. In this case, in order to maximally reduce acid generation in the cooling step of fermented milk, a faster cooling rate is desirable.

[0066]

The method for producing fermented milk of the present invention may comprise a crushing step of the curd of fermented milk and/or a homogenization step of fermented milk. In the crushing step of the curd of fermented milk, for example, casein particles contained in the fermented milk (solid component of the curd) are finely dispersed in the whey by applying stirring force to the fermented milk, so that the structure of the fermented milk is microparticulated. In the homogenization step of fermented milk, for example, casein particles contained in the fermented milk (solid component of the curd) are finely dispersed in the whey by extruding the fermented milk through a narrow flow passage while applying pressure, so that the structure of the fermented milk is microparticulated.

[0067]

The method for producing fermented milk of the present invention may comprise a crushing step of the curd of fermented milk and/or a homogenization step of fermented milk, subsequent

to the fermentation step; simultaneously with, prior to or subsequent to the cooling step of fermented milk, in this order. In the crushing step of the curd of fermented milk, a tank with stirring blades or a static mixer can be used. In the homogenization step of fermented milk, when a homogenizer, etc. is used, the pressure of homogenization is, for example 1-100 MPa, preferably 5-50 MPa, more preferably 8-30 MPa, and furthermore preferably 10-20 MPa in the treatment. Here, the crushing step of the curd of fermented milk is used in soft type yogurt and drinking type yogurt, which are pre-fermentation-type fermented milk, and the homogenization step of fermented milk is used in drinking type yogurt, which is pre-fermentation-type fermented milk.

[0068]

The method for producing fermented milk of the present invention may comprise a step of adding other components, wherein flavor substances, acidulants, nutrient enhancing substances, flavorings and stabilizers etc. are mixed with raw material mix and/or with fermented milk (mainly, fresh product). In the step of adding other components, fermented milk is mixed with a flowable thickened/liquid solution containing, for example, flavor substances (sugar, high-intensity sweeteners, sweeteners such as liquid sugar, pulp and juice of fruits and vegetables, etc., jams, sauces, preparations), acidulants (citric acid, lactic acid, etc.), nutrient enhancing substances (vitamins, minerals, insoluble salts (calcium phosphate, calcium carbonate, whey calcium, etc.)), flavorings (flavors), stabilizers (pectin, carboxymethylcellulose (CMC), soya polysaccharides, etc.).

[0069]

In the case of pre-fermentation-type fermented milk (soft

type yogurt and drinking type yogurt), the method for producing fermented milk of the present invention may comprise a step of adding other components, subsequent to the fermentation step; simultaneously with, prior to or subsequent to the cooling step of fermented milk; prior to or subsequent to the homogenization step, in this order. Furthermore, in the case of after-fermentation-type fermented milk (hard type yogurt and set type yogurt), the method for producing fermented milk of the present invention may comprise a step of adding other components, simultaneously with or subsequent to the blending step of raw material mix; prior to or subsequent to the sterilization step of raw material mix; simultaneously with, prior to or subsequent to the cooling step after the sterilization step of raw material mix, in this order. However, in the production steps of fermented milk, from the viewpoint of suppressing changes in temperature of other components, it is desirable that the adding step of other components is employed for pre-fermentation-type fermented milk. [0070]

In a preferred embodiment of the present invention, the additive amount of a stabilizer (pectin, carboxymethylcellulose (CMC), soya polysaccharides, etc.) in the raw material mix is, for example, 0.05-1.0 wt%, preferably 0.05-0.8 wt%, more preferably 0.1-0.5 wt%, furthermore preferably 0.1-0.4 wt%, particularly preferably 0.1-0.3 wt%; and most preferably, the raw material mix does not contain a stabilizer. In addition, in a preferred embodiment of the present invention, the additive amount of a stabilizer in the fermented milk is, for example, 0.05-1.0 wt%, preferably 0.05-0.8 wt%, more preferably 0.1-0.5 wt%, furthermore preferably 0.1-0.4 wt%, particularly preferably 0.1-0.3 wt%; and most preferably, the fermented milk does not

contain a stabilizer.

[0071]

As used herein, the term "acid-sensitive lactic acid-producing bacteria" refers to lactic acid-producing bacteria having a low activity under acidic conditions (especially at pH 4.5 or less). Specifically, acid-sensitive lactic acid-producing bacteria are lactic acid-producing bacteria that have low ability to produce lactic acid in fermented milk at pH value of 4.5 or less.

[0072]

Changes in pH can be measured in accordance with ordinary methods. Specifically, it is preferable to measure changes over time using a pH meter (commercial product). Changes in pH are measured over time, for example for 3 h or more, preferably 5 h or more, more preferably 10 h or more, and furthermore preferably 24 h or more.

[0073]

Streptococcus thermophilus of the present invention is useful for producing fermented milk wherein an increase in acidity is suppressed; in particular, when it is used in combination with Bulgaria bacteria for the production of fermented milk wherein an increase in acidity is suppressed, the present *Streptococcus thermophilus* does not inhibit the acidity-increase inhibitory action of the Bulgaria bacteria, and it is possible to produce fermented milk with good texture and fairly high viscosity.

The *Streptococcus thermophilus* of the present invention has the mycological properties of no generation of aggregates, when it is inoculated into 100 wt% of a skim milk medium (skim milk powder: 10 wt%, water: 90 wt%) (to which 0.1 wt% of yeast extract may be mixed) at an amount of 2 wt%, and typically cultured at

37-45°C for 12-24 h, then cooled at a non-frozen temperature of (approximately 1°C to) 10°C (for preferably 5-15 h), and more preferably, cultured at 43°C for 18 h then cooled at 4°C for 8 h.

[0074]

The *Streptococcus thermophilus* of the present invention can be typically screened (selected) by the following steps (a)-(d):

- (a) a step of inoculating a candidate *Streptococcus thermophilus* strain at 1-3 wt% into 100 wt% of a skim milk medium (skim milk powder: 8-12 wt%, water: 92-88 wt%) (to which 0.05-0.15 wt% of yeast extract may be mixed),
- (b) a step of culturing the mixed solution to which the candidate *Streptococcus thermophilus* strain was inoculated in the above (a) at typically 37-45°C for 12-24 h, preferably at 40-45°C for 12-24 h, then cooling at a non-frozen temperature of (approximately 1°C) to 10°C, more preferably at 1-7°C (for a cooling time of, but not particularly limited to, for example, 5-15 h, more preferably around 8 h),
- (c) a step of (visually) confirming the generation of aggregates (aggregability) in the mixture solution cooled in the above (b),
- (d) a step of determining the candidate *Streptococcus thermophilus* strain with which no aggregates are confirmed to be generated as a *Streptococcus thermophilus* strain to be used for the production of a fermented milk wherein an acidity increase is suppressed.

[0075]

In this case, in the composition of the skim milk medium in the above step (a), the content of the skim milk powder is preferably 9-11 wt%, and particularly preferably 10 wt%; and the content of water is preferably 91-89 wt%, and particularly

preferably 90 wt%. In the composition of the mixture solution in which yeast extract is mixed in the skim milk medium, the content of the yeast extract is, relative to 100 wt% of the skim milk medium, preferably 0.07-0.13 wt%, and particularly preferably 0.1 wt%.
[0076]

In addition, in the above step (b), regarding the culture conditions of the mixture solution inoculated with the candidate *Streptococcus thermophilus*, it is preferable to maintain the mixture solution at 41-44°C for 15-20 h, and particularly preferably at 43°C for 18 h. Then, regarding the cooling conditions of the mixture solution inoculated with the candidate *Streptococcus thermophilus*, it is preferable to maintain the mixture solution at 2-6°C for 6-10 h, and particularly preferably at 4°C for 8 h.

[0077]

Hereinafter, the present invention will be further described in detail based on examples; however, the present invention is not limited to these examples, and various modifications are possible without departing from the technical idea of the present invention. In this specification, unless explicitly indicated, % means wt%. When a numerical range is indicated, unless explicitly indicated otherwise, it indicates that it is possible to select any values within the range including the upper and lower limits; for example, when "1-100" is represented, it shows that it is possible to select any values from 1 to 100.

[Examples]

[0078]

[Example 1] Selection of thermophilus bacteria (1)

(a) Evaluation of physical properties in single strain of

thermophilus bacteria

Using a skim milk medium (skim milk powder: 10 wt%, water: 90 wt%) supplemented with 0.1 wt% yeast extract, thermophilus bacteria were activated twice. Then, the thermophilus bacteria were inoculated at 2 wt% into the skim milk medium excluding the yeast extract, and cultured at 43°C for 18 h. In addition, this cultured medium (culture solution) was cooled to 4°C overnight (approximately 8 h), then evaluated in terms of presence/absence of "viscosity" and "smoothness" (aggreagability: generation of aggregates). Regarding "viscosity," the culture was stirred by a medicine spoon, and the resistance of the culture was evaluated with four levels (◎; strong viscosity, O; fairly strong viscosity, Δ; viscosity is present, X; no viscosity). Regarding "smoothness," a slight amount of the culture was placed on a medicine spoon, and the presence or absence of generation of aggregates (lump) in the culture was visually inspected and evaluated with four levels (◎; smooth, O; almost no grain aggregates observed, Δ; grain aggregates observed, X; grainy: refer to Fig. 1).

Here, for the thermophilus bacteria strain used for Meiji Bulgaria Yogurt (fruit-filled soft type yogurt) (Meiji Co., Ltd. Japan: manufactured in April 2013) (hereinafter, referred to as strain Y), evaluation of both "viscosity" and "smoothness" was "O," and this was used as the index of evaluation.

[0079]

(b) Evaluation of physical properties in the combination of thermophilus bacteria and Bulgaria bacteria

Using a skim milk medium (skim milk powder: 10 wt%, water: 90 wt%) supplemented with 0.1 wt% yeast extract, each of thermophilus bacteria and Bulgaria bacteria was activated twice.

Then, the thermophilus bacteria at 1.8 wt% and Bulgaria bacteria at 0.2 wt% were inoculated into the skim milk medium excluding the yeast extract, and cultured at 43°C for 18 h. In addition, this cultured medium (culture solution) was cooled to 4°C overnight (approximately 8 h), then evaluated in terms of presence/absence of "viscosity" and "smoothness" (aggregability: generation of aggregates). Regarding "viscosity" and "smoothness," evaluation methods similar to those in the above (a) "Evaluation of physical properties in single strain of thermophilus bacteria" were adopted.

[0080]

Strains used in the above (a) and (b) are as follows.

*Thermophilus bacteria

31 strains were used from the strains of thermophilus bacteria (*Streptococcus thermophilus*) held by our company.

*Bulgaria bacteria

Lactobacillus delbrueckii subsp. *bulgaricus* OLL1171 strain was used. This strain has been deposited at the Patent Microorganisms Depositary, National Institute of Technology and Evaluation (Room. 122, 2-5-8, Kazusa Kamatari, Kisarazu City, Chiba, 292-0818 Japan) on March 13, 2013, with the accession number: NITE BP-01569 (JP A 2013-116332).

[0081]

<Results>

Of the 31 strains of thermophilus bacteria, 20 strains were evaluated to be "X" for both "viscosity" and "smoothness" in the evaluation of physical properties of single strains of thermophilus bacteria. In these 20 strains, improvement in physical properties was not observed even in combination with a Bulgaria strain. From this result, we can assume that, by

evaluating the physical properties in a single strain of thermophilus bacteria, it is possible to estimate approximate physical properties of fermented milk prepared by the combination with a strain of Bulgaria bacteria. That is, we assumed as follows: a single strain of thermophilus bacteria having good physical properties is likely to show good physical properties in combination with a strain of Bulgaria bacteria (i.e., in the fermented milk prepared using a combination with a strain of Bulgaria bacteria), and a single strain of thermophilus bacteria having poor physical properties is likely to show no improvement in physical properties even in combination with a strain of Bulgaria bacteria.

[0082]

[Example 2] Selection of thermophilus bacteria (2) (Primary screening)

258 strains were used from the strains of thermophilus bacteria held by our company, and their physical properties were evaluated in the same manner as in Example 1 (a). Table 1 shows the results.

24 strains that were evaluated to be "Δ" or better in both "viscosity" and "smoothness" were designated to be the starter candidate strains.

[0083]

[Table 1]

Table 1. Evaluation of physical properties of single strain of thermophilus bacteria (258 strains)

Viscosity	Smoothness	thermophilus bacteria
◎	○	3 strains
○	○	13 strains
Δ	◎	2 strains
Δ	○	6 strains
X	○	53 strains

X	Δ	50 strains
X	X	131 strains

[0084]

[Example 3] Evaluation of physical properties of combination of starter candidate strain (thermophilus bacteria) and Bulgaria bacteria (Secondary screening)

Each of the 24 strains of starter candidate strains (*Streptococcus thermophilus*) selected in Example 2 was combined with Bulgaria bacteria, and their physical property evaluation (viscosity and smoothness), fermentation time and storage stability were investigated.

With respect to "viscosity" and "smoothness," evaluation was performed similarly to Example 1 (a) "Evaluation of physical properties in single strain of thermophilus bacteria."

With respect to fermentation time, the time from the start of fermentation until the acidity of the culture becomes 0.7 or more to 0.8 or less was measured.

With respect to storage stability, a sample was stored at 10°C after the end of fermentation, and the acidity was measured immediately after the end of fermentation, after 1 day, after 7 days, and after 14 days; and a degree of suppression of acidity increase was used as an index. Acidity is measured by a conventional method. pH value after 14 days was also measured.

[0085]

The following bacteria strains were used.

**Thermophilus* bacteria

24 strains of starter candidate strains from the strains of thermophilus bacteria (*Streptococcus thermophilus*) held by our company were used.

*Bulgaria bacteria

Lactobacillus delbrueckii subsp. *bulgaricus* OLL1171 strain (accession number: NITE BP-01569).

2 strains with which an acidity increase of fermentation products during cold storage is suppressed (hereinafter, strain L1 and strain L2, respectively), similar to the OLL1171 strain,.

Strain Y that is the thermophilus bacteria strain used for Meiji Bulgaria Yogurt (fruit-filled soft type yogurt) (Meiji Co., Ltd. Japan: manufactured in April 2013).

[0086]

As mentioned above, the 24 strains of starter candidate strains (thermophilus bacteria) were combined with Bulgaria OLL1171 strain and their physical properties, fermentation time and storage stability were evaluated. Table 2 shows the results. As a control, a starter for Meiji Bulgaria Yogurt (fruit-filled soft type yogurt) (Meiji Co., Ltd. Japan: manufactured in April 2013) (combination of Bulgaria bacteria: strain B and thermophilus bacteria: strain Y) was evaluated in terms of its physical properties, fermentation time and storage stability. Table 3 shows the results. Here, regarding the strains in which both of "viscosity" and "smoothness" are "X," storage stability was not measured.

[0087]

Of the 24 starter candidate strains, in any combinations with OLL1171 strain and strain L1 and strain L2, strains in which both of "viscosity" and "smoothness" are not "X" include 9 strains, i.e., starter candidate strains 6, 7, 8, 9, 12, 17, 18, 19 and 22. Of these 9 strains, starter candidate strain 17 combined with any of OLL1171 strain and strain L1 and strain L2 showed longer fermentation time compared to the starter for Meiji Bulgaria Yogurt (fruit-filled soft type yogurt), and therefore, this

strain was predicted to be poor in terms of practical application.

[Table 2]

Table 2. Combination of candidate strains and OLL1171 strain

OLL1171	Fermentation time	Physical properties		End of fermentation	D1	D7	D14	
		Viscosity	Smoothness				Acidity (%)	pH
Candidate Strain 1 (CS 1)	3 h 27 min	X	X	0.75	0.74	–	–	–
CS 2	4 h 1 min	X	X	0.73	0.75	–	–	–
CS 3	4 h 54 min	X	X	0.76	0.79	–	–	–
CS 4	3 h 59 min	O	O	0.75	0.74	0.83	0.88	4.19
CS 5	3 h 26 min	O	Δ	0.76	0.76	0.93	0.95	4.13
CS 6	4 h 13 min	O	O	0.76	0.75	0.81	0.82	4.25
CS 7	3 h 16 min	O	Δ	0.75	0.75	0.81	0.95	4.15
CS 8	3 h 37 min	⊙	O	0.75	0.76	0.94	1.06	3.99
CS 9	3 h 20 min	O	Δ	0.73	0.73	0.77	0.81	4.28
CS 10	4 h 33 min	X	X	0.71	0.7	–	–	–
CS 11	4 h 39 min	X	X	0.76	0.74	–	–	–
CS 12	4 h 20 min	O	O	0.72	0.72	0.81	0.89	4.25
CS 13	6 h 3 min	X	Δ	0.72	0.72	0.77	0.8	4.53
CS 14	5 h 5 min	X	X	0.76	0.72	–	–	–
CS 15	4 h 34 min	X	Δ	0.73	0.72	0.78	0.8	4.33
CS 16	4 h 28 min	O	X	0.73	0.74	0.88	0.9	4.18
CS 17	6 h 39 min	O	X	0.74	0.71	0.81	0.87	4.17
CS 18	4 h 9 min	O	O	0.75	0.72	0.79	0.79	4.36
CS 19	3 h 41 min	⊙	O	0.72	0.72	0.84	0.91	4.18
CS 20	3 h 55 min	O	Δ	0.74	0.74	0.92	1.01	4.06
CS 21	3 h 26 min	O	X	0.74	0.74	0.76	0.82	4.33
CS 22	3 h 28 min	⊙	O	0.75	0.74	0.86	0.96	4.14
CS 23	3 h 35 min	O	Δ	0.73	0.73	0.9	0.96	4.1
CS 24	3 h 35 min	O	Δ	0.75	0.76	0.86	0.96	4.11

[0088]

[Table 3]

Table 3. Starter for Meiji Bulgaria Yogurt (fruit-filled soft type yogurt)

Strain B	Fermentation time	Physical properties		End of fermentation	D1	D7	D14	
		Viscosity	Smoothness				Acidity (%)	pH
Strain Y	3 h 20 min	O	O	0.75	0.77	0.87	0.97	4.11

[0089]

[Example 4] Evaluation in large-scale culture

One kind from the 8 starter candidate strains (candidate

strains 6, 7, 8, 9, 12, 18, 19 and 22) is combined with one kind from OLL1171 strain, strain L1 and strain L2, and their usefulness in large-scale culture was evaluated. Specifically, bulk starters were prepared with an inoculation ratio of Bulgaria bacteria : thermophilus bacteria = 1 : 9, and using these bulk starters, fermented milk was prepared. Then, physical properties and fermentation time of the fermented milk obtained were evaluated. Table 4 shows the results.

[0090]]

[Table 4]

Table 4. Evaluation of each of candidate starters in large-scale culture

	OLL1171 strain		
	Fermentation time	Physical properties	
		Viscosity	Smoothness
Candidate Strain 6 (CS 6)	3 h 30 min	O	O
CS 7	3 h 30 min	O	O
CS 8	3 h 30 min	O	O
CS 9	3 h 30 min	$\Delta \sim O$	$\Delta \sim O$
CS 12	5 h	Δ	Δ
CS 18	3 h 30 min	Δ	Δ
CS 19	3 h 30 min	O	O
CS 22	3 h 30 min	$X \sim \Delta$	$X \sim \Delta$

[0091]

From the results shown in Table 4, the following five combinations: (1) OLL1171 strain and candidate strain 6, (2) OLL1171 strain and candidate strain 7, (3) OLL1171 strain and candidate strain 8, (4) OLL1171 strain and candidate strain 9, (5) OLL1171 strain and candidate strain 19, were selected.

Then, regarding the fermented milk prepared using the combinations of these five starter candidates, as well as (6) fermented milk prepared using the starter for Meiji Bulgaria Yogurt (fruit-filled soft type yogurt) (strain B + strain Y), their storage stability (10°C, 14 days) and sensory evaluation (texture

and acidity) were investigated. The results indicated that the following starters were excellent: (1) *Lactobacillus delbrueckii* subsp. *bulgaricus* OLL1171 and candidate strain 6 (*Streptococcus thermophilus* OLS3290), (2) *Lactobacillus delbrueckii* subsp. *bulgaricus* OLL1171 and candidate strain 7 (*Streptococcus thermophilus* OLS3078), and (5) *Lactobacillus delbrueckii* subsp. *bulgaricus* OLL1171 and candidate strain 19 (*Streptococcus thermophilus* OLS3615).

[0092]

Therefore, in the following, for the production (preparation) of fermented milk, characteristics of candidate strains 6, 7 and 19 as thermophilus bacteria suitable to combine with Bulgaria bacteria (such as OLL1171 strain) were investigated in detail.

[0093]

[Experimental Example 1] Comparison of acidity increase during refrigerated (low temperature) storage of fermented milk produced using various starters

Each of the thermophilus bacteria candidate strains 6, 7 and 19 newly discovered by the above-described screening and the thermophilus bacteria for Meiji Bulgaria Yogurt (fruit-filled soft type yogurt) (strain Y), was combined with the 3 types of Bulgaria bacteria: OLL1171 strain, strain L1, and strain L2, to prepare mixed starters, and reduced skim milk (10% aqueous solution of skim milk powder) was fermented by the mixed starters to produce (prepare) fermented milk (yogurt).

Fermentation time necessary to achieve an acidity of about 0.75%, a degree of acidity increase after refrigerated storage (10°C, 14 days) (PA: Post Acidification), and physical properties were compared for each of the fermented milk. Table 5 shows the

results.

[0094]

[Table 5]

Table 5. Comparison of acidity increase during refrigerated storage in terms of various starters

Thermophilus bacteria	Bulgaria bacteria	Fermentation time	Acidity (%) at the end of fermentation [acidity1]	Acidity (%) after refrigerated storage [acidity2]	Degree of acidity increase (%) [acidity2 - acidity1]	Physical properties	
						Viscosity	Smoothness
Candidate Strain 6	OL1171 strain	3 h 20 min	0.76	0.82	0.06	O	O
	Strain L1	4 h 45 min	0.74	0.84	0.10	O	O
	Strain L2	3 h 42 min	0.77	0.85	0.08	X	X
Candidate Strain 7	OL1171 strain	3 h 20 min	0.73	0.81	0.08	O	Δ
	Strain L1	3 h 45 min	0.74	0.82	0.08	O	Δ
	Strain L2	3 h 25 min	0.77	0.87	0.10	O	O
Candidate Strain 19	OL1171 strain	3 h 41 min	0.72	0.91	0.19	⊙	O
	Strain L1	4 h 39 min	0.74	0.88	0.14	O	Δ
	Strain L2	4 h 38 min	0.75	0.92	0.17	Δ	O
Strain Y	OL1171 strain	3 h 16 min	0.75	0.85	0.10	O	Δ
	Strain L1	4 h 38 min	0.76	0.87	0.11	O	X
	Strain L2	3 h 49 min	0.77	0.98	0.21	O	O

[0095]

Compared to the cases wherein strain Y was combined with the 3 types of Bulgaria bacteria to prepare mixed starters, in the cases wherein the candidate strains of thermophilus bacteria were combined with the predetermined Bulgaria bacteria to prepare mixed starters, the fermentation time was comparable or shortened, and the degree of acidity increase (PA) after refrigerated (low temperature) storage was comparable or reduced.

At this time, compared to the cases wherein strain Y was combined with the 3 types of Bulgaria bacteria to prepare mixed starters, in the cases wherein the candidate strains of thermophilus bacteria were combined with the predetermined Bulgaria bacteria to prepare mixed starters, viscosity and smoothness of fermented milk were better.

[0096]

[Experimental Example 2] Comparison of flavor of fermented milk prepared using various starters after refrigerated (low temperature) storage (1)

In the cases wherein candidate strains 6, 7 and 19 of thermophilus bacteria were combined with the Bulgaria bacteria OLL1171 strain to prepare mixed starters, as well as in the case wherein thermophilus bacteria strain Y was combined with Bulgaria bacteria strain B to prepare a mixed starter, reduced skim milk (10% aqueous solution of skim milk powder) was fermented to produce fermented milk (yogurt).

Then, results of their sensory test after refrigerated storage (5°C, 14 days) were compared. Figure 2 shows the results.

Here, this sensory test was conducted by five expert panelists, and the case wherein thermophilus bacteria strain Y was combined with Bulgaria bacteria strain B to prepare a mixed starter was set as the standard of 0.00 (zero).

Compared to the case wherein thermophilus bacteria strain Y was combined with Bulgaria bacteria strain B to prepare a mixed starter, in the cases wherein candidate strains 6, 7 and 19 of thermophilus bacteria were combined with the Bulgaria bacteria OLL1171 strain to prepare mixed starters, the degree of acidity of the fermented milk was significantly decreased.

[0097]

In addition, in the cases wherein candidate strains 6, 7 and 19 of thermophilus bacteria were combined with the Bulgaria bacteria OLL1171 strain to prepare mixed starters, as well as in the case wherein thermophilus bacteria strain Y was combined with Bulgaria bacteria strain B to prepare a mixed starter, reduced skim milk (10% aqueous solution of skim milk powder) was fermented to produce fermented milk (yogurt).

Then, results of their sensory test immediately after production (day 1), and after refrigerated storage (5°C, 18 days) were respectively compared. Figures 3-6 show the results.

Here, this sensory test was conducted by five expert panelists, and the case wherein thermophilus bacteria strain Y was combined with Bulgaria bacteria strain B to prepare a mixed starter was set as the standard of 0.00 (zero).

Compared to the case wherein thermophilus bacteria strain Y was combined with Bulgaria bacteria strain B to prepare a mixed starter, in the cases wherein candidate strains 6, 7 and 19 of thermophilus bacteria were combined with the Bulgaria bacteria OLL1171 strain to prepare mixed starters, the degree of acidity of the fermented milk was significantly decreased.

[0098]

Furthermore, in the case wherein thermophilus bacteria candidate strain 19 was combined with the Bulgaria bacteria OLL1171 strain to prepare a mixed starter (frozen bacteria), as well as in the case wherein thermophilus bacteria strain Y was combined with Bulgaria bacteria strain B to prepare a mixed starter (frozen bacteria), skim milk was fermented, into which an aloe sauce was blended (mixed) to prepare a fermented milk (yogurt).

Then, results of their sensory test immediately after

production (day 1), and after refrigerated storage (5°C, 14 days) were respectively compared. Figures 7 and 8 show the results.

Here, this sensory test was conducted by five expert panelists, and the case wherein thermophilus bacteria strain Y was combined with Bulgaria bacteria strain B to prepare a mixed starter was set as the standard of 0.00 (zero).

Compared to the case wherein thermophilus bacteria strain Y was combined with Bulgaria bacteria strain B to prepare a mixed starter, in the case wherein thermophilus bacteria candidate strain 19 was combined with the Bulgaria bacteria OLL1171 strain to prepare a mixed starter, the degree of acidity of the fermented milk was significantly decreased.

[0099]

[Experimental Example 3] Comparison of flavor of fermented milk prepared using various starters after refrigerated (low temperature) storage (2)

In the cases wherein candidate strains 6, 7 and 19 of thermophilus bacteria were combined with the Bulgaria bacteria OLL1171 strain to prepare mixed starters, as well as in the case wherein thermophilus bacteria strain Y was combined with Bulgaria bacteria strain B to prepare a mixed starter, reduced skim milk (10% aqueous solution of skim milk powder) was fermented to produce fermented milk (yogurt).

Then, results of the analysis of aroma components immediately after production (day 1) were compared. Table 6 shows the results.

Here, aroma components contained in the headspace of a container of fermented milk were analyzed by GC-MS (n = 2), and the case wherein thermophilus bacteria strain Y was combined with Bulgaria bacteria strain B to prepare a mixed starter was set as

the standard of 0.00 (zero).

[0100]

[Table 6]

Table 6. Comparison of aroma components of fermented milk prepared using various starters

Thermophilus bacteria	Bulgaria bacteria	Acet oin	2,3-p entan ediol	Diac etyl	Ethano l	2-bu tane	Aceton e	Aceta ldehy de
Candidate Strain 6	OLL1171 strain	1.1	1.0	0.9	1.3	0.9	0.6	0.8
Candidate Strain 7	OLL1171 strain	1.1	1.0	1.3	1.3	0.9	0.6	0.8
Candidate Strain 19	OLL1171 strain	0.9	1.4	1.9	0.8	1.0	0.7	0.7
Strain Y	Strain B	1.0	1.0	1.0	1.0	1.0	1.0	1.0

[0101]

Compared to the case wherein thermophilus bacteria strain Y was combined with Bulgaria bacteria strain B to prepare a mixed starter, in the cases wherein candidate strains 6, 7 and 19 of thermophilus bacteria were combined with the Bulgaria bacteria OLL1171 strain to prepare mixed starters, in terms of comparison of structural ratio of aroma components, the amount of acetaldehyde produced was smaller and the amount of diacetyl produced was larger, showing a tendency of enhanced milk fat feel and richness taste (*kokumi*). In addition, compared to the case wherein thermophilus bacteria strain Y was combined with Bulgaria bacteria strain B to prepare a mixed starter, in the cases wherein candidate strains 6, 7 and 19 of thermophilus bacteria were combined with the Bulgaria bacteria OLL1171 strain to prepare mixed starters, the amount of diacetyl produced was larger, showing a tendency of enhanced milk fat feel and richness taste (*kokumi*).

[0102]

[Experimental example 4] Production of yogurt drinks

Thermophilus bacteria candidate strain 19 was combined with

the Bulgaria bacteria OLL1171 strain to prepare a mixed starter, and reduced skim milk (SNF 14%) was fermented for 3 h to produce yogurt drink. As a comparative example, reduced skim milk (SNF 14%) was fermented for 3 h using the starter for Meiji Bulgaria Yogurt (plain drink-type yogurt) to produce yogurt drink.

[0103]

Table 7 shows changes in acidity and pH during the fermentation time. In the present invention, an acidity increase required to produce yogurt drink could be obtained in the fermentation step.

[Table 7]

Table 7. Fermentability

	OLL1171 + candidate strain 19		Comparative example	
	SNF 14%		SNF 14%	
(min)	Acidity	pH	Acidity	pH
0	0.32	6.51	0.23	6.52
90	0.64	5.46	0.62	5.50
210	1.03	4.75	1.00	4.73
300	1.16	4.39	1.19	4.38
360	1.28	4.27	1.28	4.27

[0104]

Table 8 shows changes in acidity and pH on 1 day, 8 days, 16 days and 25 days of low temperature storage (10°C) after the end of fermentation. In the yogurt drink of the present invention, an increase in acidity was suppressed.

[Table 8]

Table 8. Storage stability (storage at 10°C)

		D+1	D+8	D+16	D+25
OLL1171 + candidate strain 19	Acidity	0.73	0.77	0.84	0.86
	pH	4.28	4.22	4.17	4.06
Comparative example	Acidity	0.75	0.89	0.97	0.99
	pH	4.23	4.02	3.94	3.89

[0105]

Sensory test was performed on 10 days of low temperature storage (10°C) after the end of fermentation. Evaluation items include degree of sweetness, degree of sourness, degree of aroma,

thick feel, refreshing feel and comprehensive evaluation; evaluation scores relative to the comparative example of 0 were expressed as 3, 2, 1, 0, -1, -2, and -3 from the better or stronger evaluation to poorer or weaker evaluation. The number of samples was 19; Table 9 shows the results.

[Table 9]

	Sweetness	Sourness	Aroma	Thick feel	Refreshing feel	Comprehensive evaluation
OLL1171 + candidate strain 19	1.7	-1.8	-0.2	0.5	-0.4	0.2
Comparative example	0	0	0	0	0	0

[0106]

The yogurt drink of the present invention showed significant differences in sweetness and sourness on 10 days of low temperature storage, with a level of significance of 1% relative to the comparative example. In addition, relative to the comparative example, the yogurt drink of the present invention showed a tendency of increased thick feel.

[0107]

In the above experiments, candidate strain 6, candidate strain 7 and candidate strain 19 with which particular utility is observed have been deposited as useful thermophilus bacteria at the depositary institution based on the Budapest Treaty, and they have been assigned with the accession numbers as follows.

[0108]

Candidate strain 6 is *Streptococcus thermophilus* OLS3290 (which has been deposited at the Patent Microorganisms Depositary, National Institute of Technology and Evaluation (Room. 120, 2-5-8, Kazusa Kamatari, Kisarazu City, Chiba, 292-0818 Japan) on January 19, 2004 (domestic deposition date), then transferred to international deposition on September 6, 2013 with the accession

number: FERM BP-19638).

[0109]

Candidate strain 7 is *Streptococcus thermophilus* OLS3078 (which has been internationally deposited at the Patent Microorganisms Depositary, National Institute of Technology and Evaluation (Room. 122, 2-5-8, Kazusa Kamatari, Kisarazu City, Chiba, 292-0818 Japan) on August 23, 2013, with the accession number: NITE BP-01697), and this is a novel strain.

[0110]

Candidate strain 19 is *Streptococcus thermophilus* OLS3615 (which has been internationally deposited at the Patent Microorganisms Depositary, National Institute of Technology and Evaluation (Room. 122, 2-5-8, Kazusa Kamatari, Kisarazu City, Chiba, 292-0818 Japan) on August 23, 2013, with the accession number: NITE BP-01696), and this is a novel strain.

[Claims]

1. A lactic acid bacteria starter comprising:
 - (a) a *Lactobacillus delbrueckii* subsp. *bulgaricus* strain having the following mycological properties: when a skim milk medium wherein its pH is adjusted to 4.2-4.3 by adding lactic acid is inoculated with said bacteria strain and the medium is maintained at 35-47°C, 15 h or longer time is required until the pH value is decreased by 0.2, and
 - (b) a *Streptococcus thermophilus* strain having the following mycological properties: when a skim milk medium is inoculated with said bacteria strain, cultured at 37-45°C for 12-24 h and then cooled at 1-10°C, aggregates are not generated.
2. The lactic acid bacteria starter according to Claim 1, wherein the *Lactobacillus delbrueckii* subsp. *bulgaricus* strain is *Lactobacillus delbrueckii* subsp. *bulgaricus* OLL1171 (NITE BP-01569).
3. The lactic acid bacteria starter according to Claim 1 or 2, wherein the *Streptococcus thermophilus* strain is one or more kinds selected from the group consisting of *Streptococcus thermophilus* OLS3615 (NITE BP-01696), *Streptococcus thermophilus* OLS3078 (NITE BP-01697), and *Streptococcus thermophilus* OLS3290 (FERM BP-19638).
4. The lactic acid bacteria starter according to any one of Claims 1 to 3, which is for the production of fermented milk wherein an increase in acidity is suppressed.

5. *Streptococcus thermophilus* OLS3615 (NITE BP-01696).
6. *Streptococcus thermophilus* OLS3078 (NITE BP-01697).
7. A fermented milk comprising *Streptococcus thermophilus* OLS3615 (NITE BP-01696) or *Streptococcus thermophilus* OLS3078 (NITE BP-01697).
8. A fermented milk comprising:
 - (a) *Lactobacillus delbrueckii* subsp. *bulgaricus* OLL1171 (NITE BP-01569), and
 - (b) one or more kinds selected from the group consisting of *Streptococcus thermophilus* OLS3615 (NITE BP-01696), *Streptococcus thermophilus* OLS3078 (NITE BP-01697) and *Streptococcus thermophilus* OLS3290 (FERM BP-19638).
9. A method for producing fermented milk, comprising a step of adding the lactic acid bacteria starter according to any one of Claims 1 to 4 to a raw material mix.
10. The production method according to Claim 9, comprising a step of fermentation at 35-45°C for any of 3-5 h.
11. The production method according to Claim 9 or 10, wherein the fermentation step is terminated when the acidity becomes 0.6 or more and 1.4 or less.
12. The production method according to any one of Claims 9 to 11, comprising a step of deoxygenation of the raw material mix.

13. A fermented milk produced by the production method according to any one of Claims 9 to 12.

14. The fermented milk according to any one of Claims 7, 8 or 13, wherein pH is 4.1-4.7.

15. The fermented milk according to any one of Claims 7, 8, 13 or 14, which is subjected to refrigerated storage.

16. The fermented milk according to Claim 15, wherein an increase in acidity is suppressed.

17. The fermented milk according to any one of Claims 7, 8, or 13 to 16, wherein the acidity is 0.6-1.4.

18. A method for screening a *Streptococcus thermophilus* strain for the production of a fermented milk wherein an acidity increase is suppressed by using the *Streptococcus thermophilus* strain selected by the screening method alone or by using the strain in combination with *Lactobacillus delbrueckii* subsp. *bulgaricus*, comprising:

(a) a step of inoculating a candidate *Streptococcus thermophilus* strain into a skim milk medium,

(b) a step of culturing and then cooling the skim milk medium inoculated with the candidate *Streptococcus thermophilus* strain in the above (a),

(c) a step of confirming the generation of aggregates in the cooled skim milk medium in the above (b), and

(d) a step of determining the candidate *Streptococcus thermophilus* strain with which no aggregates are confirmed to be

generated as a *Streptococcus thermophilus* strain for the production of a fermented milk wherein an acidity increase is suppressed.

19. The screening method according to Claim 18, wherein the amount of skim milk powder is any of 8-12 wt% and the amount of water is any of 92-88 wt%, relative to 100 wt% of the skim milk medium.

20. The screening method according to Claim 19, wherein yeast extract is mixed in the skim milk medium at any of 0.05-0.15 wt% relative to 100 wt% of the skim milk medium.

21. The screening method according to Claim 20, wherein the candidate *Streptococcus thermophilus* strain is inoculated at any of 1-3 wt% relative to 100 wt% of the skim milk medium.

22. The screening method according to any one of Claims 18 to 21, wherein the culturing in (b) is performed at 37-45°C for any of 12-24 h.

23. The screening method according to any one of Claims 18 to 22, wherein the cooling in (b) is performed at 1-10°C.