

DESCRIPTION

TITLE OF INVENTION: HEAT-RESISTANT ACIDOPHILIC BACTERIA
PROLIFERATION INHIBITOR AND METHOD FOR INHIBITING HEAT-
5 RESISTANT ACIDOPHILIC BACTERIA PROLIFERATION

TECHNICAL FIELD

[0001]

The present invention relates to a heat-resistant
10 acidophilic bacteria growth inhibitor. The present
invention also relates to, for example, a method of
inhibiting heat-resistant acidophilic bacteria growth.

BACKGROUND ART

15 [0002]

It is important to prevent contamination of
microorganisms such as bacteria in commercial beverages to
increase the preservability. For example, heat-resistant
acidophilic bacteria such as *Alicyclobacillus*
20 *acidoterrestris* have an action that converts a fruit juice
component into guaiacol, which is an odor causing
substance. Thus, the growth of the bacteria is known to
cause quality deterioration of food or beverages.

[0003]

25 3-Hydroxybutyric acid (3HB) is a type of short-chain
fatty acid and is produced as an energy source when glucose
in the body is depleted during carbohydrate restriction or
intense exercise, for example. It has been reported that
3-hydroxybutyric acid has various physiological functions
30 in addition to the action as an energy source. For
example, Patent Literature 1 discloses a collagenase MMP1
and MMP3 production inhibitor containing R-3-hydroxybutyric
acid as an active ingredient.

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CITATION LIST

- Patent Literature

[0004]

Patent Literature 1: JP 2018-158897 A

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SUMMARY OF INVENTION

- Technical Problem

[0005]

Heat-resistant acidophilic bacteria are resistant to heat and can grow under acidic conditions. Thus, a component having antibacterial action against heat-resistant acidophilic bacteria and usable in food or beverages is useful in the field of food or beverages and other like fields.

[0006]

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The present invention aims to provide a heat-resistant acidophilic bacteria growth inhibitor. The present invention also aims to provide a method of inhibiting heat-resistant acidophilic bacteria growth in a beverage.

20

- Solution to Problem

[0007]

As a result of extensive studies to solve the above problem, the present inventors found that 3-hydroxybutyric acid or a salt thereof has growth inhibitory action against heat-resistant acidophilic bacteria. The antibacterial action of 3-hydroxybutyric acid or a salt thereof is yet to be reported.

[0008]

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Specifically, the present invention relates, but is not limited, to a heat-resistant acidophilic bacteria growth inhibitor, a method of inhibiting heat-resistant acidophilic bacteria growth in a beverage, and the like, which are defined below.

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(1) A heat-resistant acidophilic bacteria growth

inhibitor, containing 3-hydroxybutyric acid or a salt thereof as an active ingredient.

(2) The heat-resistant acidophilic bacteria growth inhibitor according to (1) above, which is for beverages.

5 (3) The heat-resistant acidophilic bacteria growth inhibitor according to (1) or (2) above, wherein the heat-resistant acidophilic bacteria are *Alicyclobacillus acidoterrestris*.

10 (4) A method of inhibiting heat-resistant acidophilic bacteria growth in a beverage, including adding 3-hydroxybutyric acid or a salt thereof to a beverage to a 3-hydroxybutyric acid equivalent concentration of 0.2 wt% or more.

15 (5) The method according to (4) above, wherein a pH of the beverage is adjusted to 6.5 or less.

(6) The method according to (4) or (5) above, wherein a pH of the beverage is adjusted to 2.5 to 4.6.

(7) The method according to any one of (4) to (6) above, wherein the heat-resistant acidophilic bacteria are
20 *Alicyclobacillus acidoterrestris*.

(8) The method according to any one of (4) to (7) above, wherein the beverage is a tea-based beverage, a coffee beverage, a non-alcoholic beer-taste beverage, a carbonated beverage, a functional beverage, a
25 fruit/vegetable-based beverage, a lactic beverage, a soymilk beverage, or a flavored water.

(9) The method according to any one of (4) to (8) above, wherein the beverage is a non-alcoholic beer-taste beverage, a functional beverage, a carbonated beverage, or
30 a fruit/vegetable-based beverage.

(10) Use of 3-hydroxybutyric acid or a salt thereof for inhibiting heat-resistant acidophilic bacteria growth.

(11) A beverage containing the heat-resistant acidophilic bacteria growth inhibitor according to any one
35 of (1) to (3) above.

(12) The beverage according to (11) above, wherein a 3-hydroxybutyric acid equivalent concentration of 3-hydroxybutyric acid or a salt thereof is 0.2 wt% or more.

5 - Advantageous Effects of Invention
[0009]

The present invention can provide a heat-resistant acidophilic bacteria growth inhibitor. The present invention can also provide a method of inhibiting heat-resistant acidophilic bacteria growth in a beverage.

DESCRIPTION OF EMBODIMENTS

[0010]

The heat-resistant acidophilic bacteria growth inhibitor of the present invention contains 3-hydroxybutyric acid or a salt thereof. The heat-resistant acidophilic bacteria growth inhibitor of the present invention contains 3-hydroxybutyric acid or a salt thereof as an active ingredient. 3-Hydroxybutyric acid or a salt thereof has antibacterial action against heat-resistant acidophilic bacteria and thus can be used to inhibit heat-resistant acidophilic bacteria growth.

[0011]

3-Hydroxybutyric acid is a type of short-chain fatty acid and is also referred to as " β -hydroxybutyric acid". In the present invention, 3-hydroxybutyric acid may be in the R-form (R-3-hydroxybutyric acid), the S-form (S-3-hydroxybutyric acid), or a mixture of the R-form and the S-form. In one embodiment, preferably, 3-hydroxybutyric acid is a mixture of R-3-hydroxybutyric acid and S-3-hydroxybutyric acid.

[0012]

The salt of 3-hydroxybutyric acid is not limited as long as it is a pharmacologically acceptable salt or a dietary acceptable salt. Examples include metal salts such

as sodium salt, potassium salt, magnesium salt, and calcium salt, and basic amino acid salts such as arginine salt.

[0013]

The 3-hydroxybutyric acid or a salt thereof may be derived from any source and produced by any method. It may be a chemically synthesized product or may be a product produced by a fermentation method or an enzymatic method. A commercial 3-hydroxybutyric acid or a salt thereof is available, and a commercial product can also be used.

10 [0014]

The amount of 3-hydroxybutyric acid or a salt thereof in the heat-resistant acidophilic bacteria growth inhibitor of the present invention can be set as appropriate. For example, a 3-hydroxybutyric acid equivalent amount of 3-hydroxybutyric acid or a salt thereof in the heat-resistant acidophilic bacteria growth inhibitor of the present invention is preferably 0.2 wt% or more, more preferably 0.5 wt% or more. The 3-hydroxybutyric acid equivalent amount of 3-hydroxybutyric acid or a salt thereof in the heat-resistant acidophilic bacteria growth inhibitor of the present invention may be 40 wt% or less or 20 wt% or less, for example. The heat-resistant acidophilic bacteria growth inhibitor of the present invention may contain a solvent, an excipient, and the like.

25 [0015]

The heat-resistant acidophilic bacteria may be bacteria belonging to the genus *Alicyclobacillus*. Examples include *Alicyclobacillus acidoterrestris* and *Alicyclobacillus acidifilus*. In particular, *Alicyclobacillus acidoterrestris* are more preferred as the heat-resistant acidophilic bacteria.

3-Hydroxybutyric acid and salts thereof show antibacterial action against the heat-resistant acidophilic bacteria.

35 *Alicyclobacillus acidoterrestris* are bacteria

belonging to the genus *Alicyclobacillus* consisting of Gram-positive spore-forming bacteria.

In one embodiment, the heat-resistant acidophilic bacteria growth inhibitor of the present invention can be suitably used to inhibit *Alicyclobacillus acidoterrestris* growth.

[0016]

The heat-resistant acidophilic bacteria growth inhibitor of the present invention is used to inhibit heat-resistant acidophilic bacteria growth in a food or beverage and the like. For example, adding the heat-resistant acidophilic bacteria growth inhibitor of the present invention to a food or beverage can inhibit heat-resistant acidophilic bacteria growth in the food or beverage. The food or beverage is not an exclusive target for which the heat-resistant acidophilic bacteria growth inhibitor of the present invention is used. The heat-resistant acidophilic bacteria growth inhibitor of the present invention can also be added, for example, to a product such as a pharmaceutical product or a quasi-pharmaceutical product to inhibit heat-resistant acidophilic bacteria growth in the pharmaceutical product or the quasi-pharmaceutical product. For example, a food or beverage, a pharmaceutical product, a quasi-pharmaceutical product, or the like in which heat-resistant acidophilic bacteria growth is inhibited can be obtained by using 3-hydroxybutyric acid or a salt thereof. The target is preferably a liquid. The food or beverage is preferably a beverage (liquid beverage).

[0017]

In one embodiment, the heat-resistant acidophilic bacteria growth inhibitor of the present invention is suitably used to inhibit heat-resistant acidophilic bacteria growth in a beverage. In one embodiment, preferably, the heat-resistant acidophilic bacteria growth inhibitor of the present invention is a heat-resistant

acidophilic bacteria growth inhibitor for beverages. A preferred beverage is one with a pH of 6.5 or less, because heat-resistant acidophilic bacteria usually prefer acidic conditions. Adding 3-hydroxybutyric acid or a salt thereof
5 to a beverage with a pH of 6.5 or less can effectively inhibit heat-resistant acidophilic bacteria growth in the beverage. The pH of the beverage is preferably 6.4 or less, more preferably 2.0 to 4.6, particularly preferably 2.5 to 4.0. Use of the heat-resistant acidophilic bacteria
10 growth inhibitor of the present invention can effectively inhibit heat-resistant acidophilic bacteria growth in a beverage with a pH in the above range. Use of the heat-resistant acidophilic bacteria growth inhibitor of the present invention can inhibit heat-resistant acidophilic
15 bacteria growth in, for example, a beverage with a pH in the above range, compared to no use of 3-hydroxybutyric acid or a salt thereof.

Herein, the pH is measured at 25°C. The pH can be measured with a commercial pH meter.

20 [0018]

The heat-resistant acidophilic bacteria growth inhibitor of the present invention may be used in any amount that can achieve the effect of inhibiting heat-resistant acidophilic bacteria growth in a food or beverage
25 or the like. For example, when the heat-resistant acidophilic bacteria growth inhibitor of the present invention is used in a beverage, preferably, it is used in an amount such that the 3-hydroxybutyric acid equivalent concentration of 3-hydroxybutyric acid or a salt thereof in
30 the beverage is preferably 0.2 wt% or more, more preferably 0.3 wt% or more, still more preferably 0.4 wt% or more. A larger amount of 3-hydroxybutyric acid or a salt thereof is expected to produce a higher effect of inhibiting heat-resistant acidophilic bacteria growth. At the same time,
35 preferably, the heat-resistant acidophilic bacteria growth

inhibitor is used in an amount such that the 3-hydroxybutyric acid equivalent concentration of 3-hydroxybutyric acid or a salt thereof in a beverage is preferably 5 wt% or less, more preferably 4 wt% or less, still more preferably 3 wt% or less, in terms of aroma and taste of the beverage. In one embodiment, preferably, the heat-resistant acidophilic bacteria growth inhibitor of the present invention is used in a beverage in an amount such that the 3-hydroxybutyric acid equivalent concentration of 3-hydroxybutyric acid or a salt thereof in the beverage is preferably 0.2 to 5 wt%, more preferably 0.3 to 4 wt%, still more preferably 0.4 to 3 wt%. In one embodiment, in the case of inhibiting heat-resistant acidophilic bacteria growth in a liquid such as a beverage, the heat-resistant acidophilic bacteria growth inhibitor is preferably used such that the concentration of 3-hydroxybutyric acid or a salt thereof in the liquid is in the above concentration range.

Regarding 3-hydroxybutyric acid or a salt thereof, in the case of 3-hydroxybutyric acid, the expression "the 3-hydroxybutyric acid equivalent amount (or concentration)" means the amount (or concentration) of 3-hydroxybutyric acid. In the case of a salt of 3-hydroxybutyric acid, the expression means the amount (or concentration) of 3-hydroxybutyric acid having the same number of moles as that of the salt.

[0019]

The concentration of 3-hydroxybutyric acid or a salt thereof can be measured by high performance liquid chromatography (HPLC) (e.g., LC-10 Series available from Shimadzu Corporation). The following measurement conditions and the like can be employed.

Column: SHIM-PACK SCR-102H (8 mm I.D. × 300 mm L)
(available from Shimadzu Corporation)

Mobile phase: mixture of water, p-toluenesulfonic acid,

EDTA, and Bis-Tris

Flow rate: 0.8 ml/min

Column temperature: 40°C

Detection: electrical conductivity (with polarity set to
5 positive)

Analysis time: 30 min

[0020]

The present invention also encompasses a method of
inhibiting heat-resistant acidophilic bacteria growth in a
10 beverage. The method of inhibiting heat-resistant
acidophilic bacteria growth in a beverage of the present
invention is a method of inhibiting heat-resistant
acidophilic bacteria growth in a beverage, including adding
3-hydroxybutyric acid or a salt thereof to a beverage to a
15 3-hydroxybutyric acid equivalent concentration of 0.2 wt%
or more.

Preferred embodiments of 3-hydroxybutyric acid or a
salt thereof are as described above for the heat-resistant
acidophilic bacteria growth inhibitor of the present
20 invention.

[0021]

In the method of the present invention, 3-
hydroxybutyric acid or a salt thereof is added to a
beverage to a 3-hydroxybutyric acid equivalent
25 concentration of 0.2 wt% or more. When the 3-
hydroxybutyric acid equivalent concentration of 3-
hydroxybutyric acid or a salt thereof in a beverage is 0.2
wt% or more, the heat-resistant acidophilic bacteria growth
in the beverage can be inhibited.

30 In the method of the present invention, preferably,
3-hydroxybutyric acid or a salt thereof is added to a
beverage in an amount such that the 3-hydroxybutyric acid
equivalent concentration of 3-hydroxybutyric acid or a salt
thereof in the beverage is preferably 0.3 wt% or more, more
35 preferably 0.4 wt% or more. Adding the above amount of 3-

hydroxybutyric acid or a salt thereof to a beverage can more effectively inhibit heat-resistant acidophilic bacteria growth in the beverage. Preferably, 3-hydroxybutyric acid or a salt thereof is added to a beverage in an amount such that the 3-hydroxybutyric acid equivalent concentration of 3-hydroxybutyric acid or a salt thereof in the beverage is preferably 5 wt% or less, more preferably 4 wt% or less, still more preferably 3 wt% or less, in terms of aroma and taste of the beverage.

In one embodiment, in the method of the present invention, preferably, 3-hydroxybutyric acid or a salt thereof is added to a beverage such that the 3-hydroxybutyric acid equivalent concentration of 3-hydroxybutyric acid or a salt thereof in the beverage is preferably 0.2 to 5 wt%, more preferably 0.3 to 4 wt%, still more preferably 0.4 to 2 wt%.

In the method of the present invention, the concentration of 3-hydroxybutyric acid or a salt thereof in a beverage is not limited as long as the final concentration thereof is in the above concentration range. 3-Hydroxybutyric acid or a salt thereof may be added to a beverage during production thereof.

[0022]

The method of inhibiting heat-resistant acidophilic bacteria growth of the present invention is suitably used as a method of inhibiting heat-resistant acidophilic bacteria growth in a beverage with a pH of 6.5 or less. Use of 3-hydroxybutyric acid or a salt thereof can effectively inhibit heat-resistant acidophilic bacteria growth in a beverage with a pH of 6.5 or less. The pH of the beverage is more preferably 6.4 or less, still more preferably 2.0 to 4.6, particularly preferably 2.5 to 4.0. According to the manufacturing standards specified in the Food Sanitation Act of Japan, a product with a pH of less than 4.0 is required to be heat-sterilized at 65°C for 10

minutes or under equivalent or more severe conditions, and a product with a pH of 4.0 or more and less than 4.6 is required to be heat-sterilized at 85°C for 30 minutes or under equivalent or more severe conditions. Yet, with such
5 heat-sterilization, a risk of microbial contamination by heat-resistant acidophilic bacteria may remain. Use of the method of inhibiting heat-resistant acidophilic bacteria growth of the present invention can effectively inhibit heat-resistant acidophilic bacteria growth in a beverage
10 with a pH of preferably 6.5 or less, more preferably 6.4 or less, still more preferably 2.0 to 4.6, particularly preferably 2.5 to 4.0, compared to no use of 3-hydroxybutyric acid or a salt thereof.

[0023]

15 In the method of inhibiting heat-resistant acidophilic bacteria growth in a beverage of the present invention, the pH of the beverage may be adjusted as needed. In the method of the present invention, the pH of the beverage may be adjusted to 6.5 or less. In one
20 embodiment, the method of inhibiting heat-resistant acidophilic bacteria growth in a beverage of the present invention may include a step of adjusting the pH of the beverage to 6.5 or less. The method of inhibiting heat-resistant acidophilic bacteria growth in a beverage of the
25 present invention may include a step of adjusting the pH of the beverage to 6.4 or less, 2.0 to 4.6, or 2.5 to 4.0. In one embodiment, the pH of the beverage may be adjusted to less than 4.6 or less than 4.0.

The pH of the beverage is not limited as long as the
30 final pH is in the above range. The pH may be adjusted before adding 3-hydroxybutyric acid or a salt thereof or after adding 3-hydroxybutyric acid or a salt thereof. In one embodiment of the method of the present invention, 3-hydroxybutyric acid or a salt thereof may be added to a
35 beverage with a pH of 6.5 or less. In the method of the

present invention, the pH of the beverage to which 3-hydroxybutyric acid or a salt thereof is added may be adjusted to 6.5 or less as needed.

The pH can be adjusted by, for example, a known
5 method such as adding a pH adjuster as needed.

[0024]

The method of inhibiting heat-resistant acidophilic bacteria growth in a beverage of the present invention may include a step of heating the beverage. The beverage may
10 be heated before adding 3-hydroxybutyric acid or a salt thereof or after adding 3-hydroxybutyric acid or a salt thereof, with the latter being preferred. The heating conditions are not limited. For example, the beverage is heated at 65°C to 130°C, preferably 85°C to 120°C, for 10
15 to 40 minutes. As long as the sterilization value equivalent to that under the above conditions can be obtained, the beverage may be heated at any appropriate temperature for several seconds, for example, 5 to 30 seconds.

20 [0025]

In the method of inhibiting heat-resistant acidophilic bacteria growth in a beverage, the heat-resistant acidophilic bacteria and preferred embodiments thereof are as described above. Preferably, the heat-resistant acidophilic bacteria are *Alicyclobacillus acidoterrestris*. The method of the present invention can
25 be suitably used to inhibit the growth of the microorganisms in a beverage.

[0026]

30 The beverage for which the heat-resistant acidophilic bacteria growth inhibitor and the method of inhibiting heat-resistant acidophilic bacteria growth of the present invention are used may be a non-alcoholic beverage, an alcoholic beverage, or the like. A preferred beverage is a
35 non-alcoholic beverage. The term "non-alcoholic beverage"

as used herein refers to a beverage having an ethanol concentration of 1.2 v/v% or less, preferably 1.0 v/v% or less, more preferably 0.5 v/v% or less.

A preferred non-alcoholic beverage is, for example, a
5 tea-based beverage, a coffee beverage, a non-alcoholic beer-taste beverage, a carbonated beverage, a functional beverage, a fruit/vegetable-based beverage, a lactic beverage, a soymilk beverage, or flavored water.

A more preferred beverage for which the heat-
10 resistant acidophilic bacteria growth inhibitor and the method of inhibiting heat-resistant acidophilic bacteria growth of the present invention are used is a non-alcoholic beer-taste beverage, a functional beverage, a carbonated beverage, or a fruit/vegetable-based beverage. This is
15 because these beverages usually have a pH of 6.5 or less and the possibility of heat-resistant acidophilic bacteria growth in these beverages is high. In particular, a functional beverage or a fruit/vegetable-based beverage is more preferred.

20 [0027]

A preferred tea-based beverage is a black tea beverage or a sugarless tea beverage. Examples of the sugarless tea beverage include green tea beverages, oolong tea beverages, barley tea beverages, brown rice tea
25 beverages, adlay tea beverages, and sugarless black tea beverages.

A preferred coffee beverage is a packaged coffee beverage or a liquid coffee.

[0028]

30 The term "non-alcoholic beer-taste beverages" as used herein refers to carbonated beverages with a beer-like flavor, which are non-fermented non-alcohol beverages. Such beverages are substantially free of alcohol. Here, the non-alcoholic beer-taste beverages do not exclude
35 beverages containing a trace amount (undetectable amount)

of alcohol.

[0029]

A preferred carbonated beverage is a cola-flavored beverage, a clear carbonated beverage, ginger ale, a fruit
5 juice-based carbonated beverage, a milk-containing carbonated beverage, or a sugarless carbonated beverage.

Examples of the functional beverage include sports drinks, energy drinks, health-supporting beverages, and jelly drink pouches. A preferred functional beverage is a
10 sports drink, an energy drink, or a health-supporting beverage.

A preferred fruit/vegetable-based beverage is a 100% fruit juice, a fruit-containing beverage, a soft drink with a low fruit juice content, a pulp-containing fruit juice,
15 or a pulp-containing beverage.

[0030]

The present invention also encompasses use of 3-hydroxybutyric acid or a salt thereof for inhibiting heat-resistant acidophilic bacteria growth. The present
20 invention also encompasses use of 3-hydroxybutyric acid or a salt thereof for producing a heat-resistant acidophilic bacteria growth inhibitor. 3-Hydroxybutyric acid or a salt thereof can be used as an active ingredient for inhibiting heat-resistant acidophilic bacteria growth. In the above
25 uses, 3-hydroxybutyric acid or a salt thereof, preferred embodiments, and the like are as described above. A target and preferred embodiments thereof for which 3-hydroxybutyric acid or a salt thereof is used are the same as the target and preferred embodiments thereof for which
30 the heat-resistant acidophilic bacteria growth inhibitor described above is used. Preferably, 3-hydroxybutyric acid or a salt thereof is used to inhibit heat-resistant acidophilic bacteria growth preferably in a liquid, more preferably in a beverage. The beverage, preferred
35 embodiments thereof, and the like are as described above.

In one embodiment, the 3-hydroxybutyric acid equivalent concentration of 3-hydroxybutyric acid or a salt thereof in a target such as a beverage is preferably 0.2 wt% or more, more preferably 0.2 to 5 wt%, still more preferably 0.3 to 4 wt%, particularly preferably 0.4 to 3 wt%. In one embodiment, preferably, 3-hydroxybutyric acid or a salt thereof is added to a target such that the concentration of 3-hydroxybutyric acid or a salt thereof is in the above concentration range.

10 A beverage in which heat-resistant acidophilic bacteria growth is inhibited can be obtained by adding the heat-resistant acidophilic bacteria growth inhibitor of the present invention to the beverage. The present invention also encompasses a beverage containing the heat-resistant acidophilic bacteria growth inhibitor. The beverage, preferred embodiments thereof, and the like are as described above. The 3-hydroxybutyric acid equivalent concentration of 3-hydroxybutyric acid or a salt thereof in the beverage is preferably 0.2 wt% or more, more preferably 15 0.2 to 5 wt%, still more preferably 0.3 to 4 wt%, particularly preferably 0.4 to 3 wt%.

[0031]

Herein, in each numerical range defined by the lower limit and the upper limit, i.e., "the lower limit to the upper limit", the lower limit and the upper limit are inclusive. For example, the range defined by "1 to 2" means 1 or more and 2 or less, with 1 and 2 being inclusive. Herein, the range may be any combination of any upper limit and any lower limit.

30

EXAMPLES

[0032]

The following provides Examples that more specifically describe the present invention. The present invention is not limited to these Examples.

35

[0033]

In the following Examples, DL-sodium 3-hydroxybutyrate (Sigma-Aldrich) (a mixture of sodium R-hydroxybutyrate and sodium S-hydroxybutyrate (molar ratio
5 of R-form to S-form is 50:50), hereinafter described as "3HB-Na") was used as sodium 3-hydroxybutyrate.

Heat-resistant acidophilic bacteria "*Alicyclobacillus acidoterrestris* NBRC 108913" were used as test bacteria.

[0034]

10 <Example 1>

The minimum inhibitory concentration (MIC) of 3HB-Na against the test bacteria was measured.

(Test method)

The liquid medium dilution method was used.

15 (1) Preparation of test samples

3HB-Na and 6N HCl were mixed to prepare a 3HB-Na solution (3HB-Na concentration: 20 wt%). The 3HB-Na solution was diluted in a YSG liquid medium to prepare a sample having a 3HB-Na concentration of 2 wt%. This sample
20 was diluted in a YSG liquid medium to prepare a two-fold dilution series with 10 levels in total, whereby test samples (No. 1 to No. 10) were obtained. The 3HB-Na concentration (10 levels, 0.004 to 2 wt%) in each test sample is described below. The value in the parenthesis
25 indicates the 3-hydroxybutyric acid (3HB) equivalent concentration.

No. 1: 2 wt% (3HB equivalent: 1.6 wt%), No. 2: 1 wt% (3HB equivalent: 0.8 wt%), No. 3: 0.5 wt% (3HB equivalent: 0.4 wt%), No. 4: 0.25 wt% (3HB equivalent: 0.2 wt%), No. 5:
30 0.125 wt% (3HB equivalent: 0.1 wt%), No. 6: 0.063 wt% (3HB equivalent: 0.05 wt%), No. 7: 0.031 wt% (3HB equivalent: 0.025 wt%), No. 8: 0.016 wt% (3HB equivalent: 0.013 wt%), No. 9: 0.008 wt% (3HB equivalent: 0.007 wt%), No. 10: 0.004 wt% (3HB equivalent: 0.003 wt%)

35 A YSG liquid medium without 3HB-Na was used as

control.

[0035]

(2) pH measurement

5 The pH (25°C) of each test sample was measured with a pH meter (AS600 available from AS ONE Corporation).

[0036]

(3) Preparation of test bacterial solution

10 The test bacteria were inoculated into a YSG agar medium and cultured at 45°C for 24 hours. After culturing, the bacterial count was adjusted to 10^7 CFU/mL using saline, whereby a test bacterial solution was obtained.

[0037]

(4) Inoculation and culturing of test bacterial solution

15 The test samples (Nos. 1 to 10) prepared above were used as test media. The test bacterial solution was inoculated into each test medium and cultured at 45°C for 24 hours. The test was performed with $n = 3$ for each test sample.

(5) Determination of MIC

20 After culturing, the growth or non-growth of the test bacteria was observed with the naked eye, and the MIC was determined.

[0038]

(Results)

25 Table 1 shows the pH measurement results of each test sample. Table 2 shows the MIC measurement results of 3HB-Na against *Alicyclobacillus acidoterrestris*. In Table 1, the control is a buffer with a pH of 4.0 (Nacalai Tesque Inc.). In the MIC test, the control showed good growth of
30 the test bacteria.

[0039]

[Table 1]

	Test sample										Control
No.	1	2	3	4	5	6	7	8	9	10	
3HB-Na concentration (wt%)	2	1	0.5	0.25	0.125	0.063	0.031	0.016	0.008	0.004	0
pH	3.91	3.94	3.97	3.98	3.99	3.98	4.00	3.98	3.94	3.88	3.82

[0040]

[Table 2]

	Test sample										MIC (%)
No.	1	2	3	4	5	6	7	8	9	10	
3HB-Na concentration (wt%)	2	1	0.5	0.25	0.125	0.063	0.031	0.016	0.008	0.004	
Determination	-	-	-	-	+	+	+	+	+	+	0.25
	-	-	-	-	+	+	+	+	+	+	0.25
	-	-	-	-	+	+	+	+	+	+	0.25

5 [0041]

The "Determination" in Table 2 is the evaluation by testing each specimen (test sample) three times (n = 3). (Criteria)

"+": Growth was observed.

10 "-": No growth was observed.

[0042]

3HB-Na had an action to inhibit the growth of the test bacteria. The MIC of 3HB-Na against the test bacteria was 0.25 wt% (3-hydroxybutyric acid equivalent concentration: 0.2 wt%).

15

[0043]

The results show that 3-hydroxybutyric acid or a salt thereof has antibacterial action against heat-resistant acidophilic bacteria, i.e., a heat-resistant acidophilic bacteria growth inhibitory action.

20

INDUSTRIAL APPLICABILITY

[0044]

The present invention is useful in the field of food or beverages, for example.

25

CLAIMS

Claim 1. A heat-resistant acidophilic bacteria growth inhibitor, comprising:

5 3-hydroxybutyric acid or a salt thereof as an active ingredient.

Claim 2. The heat-resistant acidophilic bacteria growth inhibitor according to claim 1,

10 which is for beverages.

Claim 3. The heat-resistant acidophilic bacteria growth inhibitor according to claim 1 or 2,

15 wherein the heat-resistant acidophilic bacteria are *Alicyclobacillus acidoterrestris*.

Claim 4. A method of inhibiting heat-resistant acidophilic bacteria growth in a beverage, comprising:

20 adding 3-hydroxybutyric acid or a salt thereof to a beverage to a 3-hydroxybutyric acid equivalent concentration of 0.2 wt% or more.

Claim 5. The method according to claim 4,

25 wherein a pH of the beverage is adjusted to 6.5 or less.

Claim 6. The method according to claim 4 or 5,

30 wherein a pH of the beverage is adjusted to 2.5 to 4.6.

Claim 7. The method according to claim 4 or 5,

 wherein the heat-resistant acidophilic bacteria are *Alicyclobacillus acidoterrestris*.

35 Claim 8. The method according to claim 4 or 5,

wherein the beverage is a tea-based beverage, a coffee beverage, a non-alcoholic beer-taste beverage, a carbonated beverage, a functional beverage, a fruit/vegetable-based beverage, a lactic beverage, a
5 soymilk beverage, or a flavored water.

Claim 9. The method according to claim 4 or 5,
wherein the beverage is a non-alcoholic beer-taste beverage, a functional beverage, a carbonated beverage, or
10 a fruit/vegetable-based beverage.

Claim 10. Use of 3-hydroxybutyric acid or a salt thereof for inhibiting heat-resistant acidophilic bacteria growth.
15

Claim 11. A beverage comprising:
the heat-resistant acidophilic bacteria growth inhibitor according to claim 1 or 2.

20 Claim 12. The beverage according to claim 11,
wherein a 3-hydroxybutyric acid equivalent concentration of 3-hydroxybutyric acid or a salt thereof is 0.2 wt% or more.