

METHOD FOR CULTURING LACTIC ACID BACTERIA AT HIGH CONCENTRATION

[BACKGROUND OF THE INVENTION]

[0001] Field of Invention

The present invention relates to a method for culturing lactic acid bacterium and more specifically relates to a culturing method that can increase the concentration of lactic acid bacterium.

[0002] Background Art

With culturing lactic acid bacterium, lactose is metabolized to lactic acid. The lactic acid generally lowers pH of the medium (culture solution), and, consequently, the growth of lactic acid bacterium is suppressed. In order to avoid the suppression of lactic acid bacterium growth and to grow lactic acid bacterium at a high concentration, for example, a method has been employed in which a mixture is cultured while maintaining and controlling pH of the medium (culture solution) in a predetermined range by adding an alkali (for example, potassium carbonate or sodium hydroxide) to the medium (culture solution) during culturing of lactic acid bacterium, while measuring pH of the medium (a culture solution).

[0003] For example, some methods that can increase the concentration of lactic acid bacterium have been proposed, for instance, a method for fermenting a microorganism while preventing a lowering in pH by adding magnesium carbonate (JP 2004-057020A (PTL 1)), a method for fermentation using a substance that promotes the growth of lactic acid bacterium (JP 2006-230259A (PTL 2), JP H07(1995)-099968A (PTL 3), and JP H07(1995)-099967A (PTL 4)), a method for fermentation in which lactic acid is removed during culturing of lactic acid bacterium (JP 2001-211878A (PTL 5)), a process for preparing a medium that can easily recover lactic acid bacterium from a culture solution (JP 2007-089511A (PTL 6)), and a method for co-culturing of lactic acid bacterium and filamentous fungi (JP 2006-238743A (PTL 7)) have been proposed.

[0004] The genus *Lactococcus* that metabolizes arginine is known in the art (NPLs 1 and 2) and in its metabolic pathway of arginine metabolism, a reaction in which arginine deiminase and ornithine carbamyltransferase are involved is also known. As far as the present

inventors know, however, there is no report that refers to a change in pH during arginine metabolization or a reaction thereof.

[0005] Further, JP H07(1995)-184687A (PTL 8) describes a method for simply examining lactic acid bacterium constituting a lactic acid bacterium starter in which a lactic acid bacterium starter is cultured in a lactic acid growing medium containing arginine. However, the amount of arginine added is very small, and here an increase in pH is not contemplated. Further, JP 2009-112205A (PTL 9) describes a process for producing an L-ornithine-containing material, which comprises the step of fermenting an L-arginine-containing protein using lactic acid bacterium. This document, however, does not describe that L-arginine is solely added.

[Citation List]

[Patent Literature]

[0006]

[PTL 1] JP 2004-057020A

[PTL 2] JP 2006-230259A

[PTL 3] JP H07 (1995)-099968A

[PTL 4] JP H07 (1995)-099967A

[PTL 5] JP 2001-211878A

[PTL 6] JP 2007-089511A

[PTL 7] JP 2006-238743A

[PTL 8] JP H07 (1995)-184687A

[PTL 9] JP 2009-112205A

[Non Patent Literature]

[0007]

[NPL 1] Crow V. L., T. D. Thomas. 1982. Arginine metabolism in lactic streptococci. J. Bacteriol. 150:1024-1032.

[NPL 2] Poolman B., A. J. M. Driessen, W. N. Konings. 1987. Regulation of arginine-ornithine exchange and arginine deiminase pathway in *Streptococcus lactis*. Bacteriol., 169 5597-5604

[SUMMARY OF THE INVENTION]

[Technical Problem]

[0008] The present inventors have now found that lactic acid bacterium can be cultured at a high concentration by culturing lactic acid

bacterium having a citrulline producing ability in a medium to which arginine is added for the purpose of suppressing a lowering in pH of the medium. More specifically, the present inventors have found that lactic acid bacterium can be cultured at a high concentration by basically controlling and regulating a culture temperature only.

[0009] Accordingly, an object of the present invention is to provide a culturing method that can simply increase the concentration of lactic acid bacterium.

[Solution to Problem]

[0010] Thus, the present invention relates to the following embodiments (1) to (7).

(1) A method for culturing lactic acid bacterium, comprising culturing lactic acid bacterium having a metabolic ability of converting arginine into citrulline in a medium to which arginine is added in order to suppress a lowering of pH of the medium.

(2) The method for culturing lactic acid bacterium according to (1), wherein the number of microbial cells per mL of a culture solution of lactic acid bacterium is twice or more the number of microbial cells when cultured in an arginine-free medium.

(3) The method for culturing lactic acid bacterium according to (1) or (2), wherein lactic acid bacterium has a metabolic ability of converting citrulline into ornithine after the conversion of arginine into citrulline.

(4) The method for culturing lactic acid bacterium according to any one of (1) to (3), wherein arginine is added in an amount of 0.5 to 15% by mass to the medium.

(5) The method for culturing lactic acid bacterium according to any one of (1) to (4), wherein the lactic acid bacterium is one or more members selected from the group consisting of *Lactococcus lactis*, *Lactobacillus fermentum*, *Lactobacillus buchneri*, *Enterococcus faecalis*, *Oenococcus oeni*, *Pediococcus pentosaceus*, *Lactobacillus amylovorus*, *Lactobacillus brevis*, *Lactobacillus reuteri*, *Lactobacillus acidophilus*, *Lactobacillus crispatus*, and *Lactobacillus delbrueckii*.

(6) The method for culturing lactic acid bacterium according to (5), wherein the lactic acid bacterium is one or more members selected from the group consisting of *Lactococcus lactis* spp. *lactis*, *Lactococcus lactis* spp. *cremoris*, *Lactic acid bacterium reuteri*, and *Lactic acid bacterium brevis*.

(7) The method for culturing lactic acid bacterium according to any one of (1) to (6), wherein the culture solution is not stirred.

[Advantageous effect]

[0011] In the method for culturing lactic acid bacterium according to the present invention, lactic acid bacterium can be cultured at a high concentration by culturing lactic acid bacterium having a citrulline producing ability in a medium with arginine added thereto while basically controlling and regulating a culture temperature only. Accordingly, in the method for culturing lactic acid bacterium according to the present invention, lactic acid bacterium can be cultured at a high temperature without the need to provide an apparatus/equipment that measures pH of a culture solution while adding an alkali and an apparatus/equipment that adds an alkali to a culture solution with a change (a lowering) in pH of the culture solution. Specifically, basically, lactic acid bacterium can be cultured at a high concentration only using a culturing tank without a special apparatus/equipment, and, thus, troubles are less likely to occur in the culturing step. Accordingly, the method for culturing lactic acid bacterium according to the present invention is more advantageous than conventional methods in that lactic acid bacterium can simply be cultured at a high concentration. Thus, the method for culturing lactic acid bacterium according to the present invention is highly useful.

[BRIEF DESCRIPTION OF THE DRAWINGS]

[0012]

[Fig. 1] is a graph illustrating a change in pH in culturing *Lactococcus lactis* spp. *lactis* JCM5805T strain, IFO12007 strain, MEP1706301 strain, MEP1706302 strain, and MEP1706303 strain in a MRS medium to which arginine is added.

[Fig. 2] is a graph illustrating a change in pH in culturing *Lactococcus lactis* spp. *lactis* JCM16167T strain, MEP1706304 strain, and MEP1706305 strain in a MRS medium to which arginine is added.

[Fig. 3] is a graph illustrating a change in pH in culturing a *Lactobacillus reuteri* JCM1112T strain and *Lactobacillus brevis* JCM1061 strain in a MRS medium to which arginine is added.

[Fig. 4] is a graph illustrating a change in pH in culturing *Lactococcus*

lactis spp. *lactis* JCM5805T strain, MEP1706302 strain, and MEP1706303 strain in a reconstituted skim milk to which arginine is added.

[Fig. 5] is a graph illustrating a change in pH in culturing *Lactococcus lactis* spp. *lactis* MEP1706302 strain in a reconstituted skim milk and a reconstituted whey medium to which arginine is added.

[DESCRIPTION OF EMBODIMENTS]

[0013] Culturing at high concentration

In the present invention, lactic acid bacterium at least having a metabolic ability of converting arginine into citrulline is cultured in a medium to which arginine is added. As a result, lactic acid bacterium can be cultured at a high concentration. Specifically, in the present invention, the number of microbial cells (viable cell count) per mL of a culture solution of lactic acid bacterium is preferably twice or more, more preferably thrice or more, still more preferably four times or more, particularly preferably five times or more, that in an arginine-free medium.

[0014] Lactic acid bacterium

In the present invention, while lactic acid bacterium has at least a metabolic ability of converting arginine into citrulline, in one embodiment of the present invention, preferably, lactic acid bacterium has a metabolic ability of, after converting arginine into citrulline, converting citrulline to ornithine.

[0015] In the present invention, a mechanism (reason) through which lactic acid bacterium can be cultured at a high concentration by adding arginine to the medium is considered to be as follows. Specifically, in lactic acid bacterium having a metabolic ability of converting at least arginine into citrulline, further in lactic acid bacterium having a metabolic ability of, after converting arginine into citrulline, converting citrulline to ornithine, arginine deiminase converts arginine into citrulline, and carbamoyltransferase converts citrulline to ornithine. In these reactions, deamination of arginine and citrulline occurs, leading to evolution of ammonia. It is considered that the ammonia suppresses a lowering in pH of the medium involved in the production of lactic acid and lactic acid bacterium can be cultured at a high concentration.

[0016] In the present invention, examples of preferred lactic acid bacterium include lactic acid bacterium belonging to *Lactococcus lactis*, *Lactobacillus fermentum*, *Lactobacillus buchneri*, *Enterococcus faecalis*, *Oenococcus oeni*, *Pediococcus pentosaceus*, *Lactobacillus amylovorus*, *Lactobacillus brevis*, *Lactobacillus reuteri*, *Lactobacillus acidophilus*, *Lactobacillus crispatus*, and *Lactobacillus delbrueckii*.

[0017] In the present invention, examples of more preferred lactic acid bacterium include *Lactococcus lactis* ssp *lactis*, *Lactococcus lactis* ssp *cremoris*, *Lactobacillus reuteri*, and *Lactobacillus brevis*, specifically *Lactococcus lactis* ssp *lactis* JCM5805T, *Lactococcus lactis* ssp *lactis* IFO12007, *Lactococcus lactis* ssp *lactis* MEP1706301, *Lactococcus lactis* ssp *lactis* MEP1706302, *Lactococcus lactis* ssp *lactis* MEP1706303, *Lactococcus lactis* ssp *cremoris* JCM16167T, *Lactococcus lactis* ssp *cremoris* MEP1706304, *Lactococcus lactis* ssp *cremoris* MEP1706305, *Lactobacillus reuteri* JCM1112T, and *Lactobacillus brevis* JCM1061.

[0018] Medium

In the present invention, the medium can be properly selected, and examples of preferred media include media containing defatted milks, defatted concentrated milks, powdered skim milks (reconstituted skim milks) and proteolyzates of these skim milk ingredients, wheys, whey concentrates, whey powders (reconstituted wheys), and proteolyzates of these whey ingredients, and raw milks, sterilized milks (whole milks), whole milk concentrated milks, and powdered whole milks (reconstituted whole milks) and proteolyzates of these whole milk ingredients. Media containing skim milks, powdered skim milks, wheys, and whey powders are more preferred.

[0019] Addition of arginine

In the present invention, lactic acid bacterium is cultured in a medium to which arginine is added. The amount of arginine added to the medium can be properly determined by taking into consideration, for example, the amount of microbial cells (microbial cell count), the amount of production of lactic acid, and the amount of production of ammonia considered to suppress a lowering in pH. For example, the amount of arginine is preferably 0.5 to 15% by mass, and the lower limit of the amount is more preferably 1% by mass, still more preferably 2%

by mass, while the upper limit of the addition amount is preferably 12% by mass. The amount can make a proper production of ammonia which is considered to suppress a lowering in pH.

[0020] In the present invention, while arginine can be added to the medium before the start of culturing of lactic acid bacterium, and additional arginine to the culture solution during culturing of lactic acid bacterium is not indispensable, an embodiment (a concept) in which arginine is additionally added to the culture solution during culturing of lactic acid bacterium with considering pH of the culture solution is not excluded. Further, the present invention includes an embodiment in which arginine is added to a culture solution only during culturing of lactic acid bacterium without the addition of arginine to the medium before the start of culturing of lactic acid bacterium. The addition of arginine during culturing of lactic acid bacterium may be carried out intermittently or continuously or in an embodiment in which the addition is carried out in a combination of an intermittent matter with a continuous manner. Even in an embodiment in which arginine is added to the culture solution during culturing of lactic acid bacterium, the amount of arginine added in terms of total amount is preferably as described above.

[0021] In the present invention, the expression "medium to which arginine is added" means a medium to which arginine has been added artificially or actively with a view to at least suppressing a lowering in pH of the medium or the culture solution. Accordingly, in culturing of lactic acid bacterium, for example, an embodiment where arginine is incidentally contained in the medium or the culture solution is not embraced within the scope of the present invention. However, an embodiment where arginine is contained in the medium or the culture solution (for example, in a very small amount) under a natural law or under such conditions that persons cannot be involved is not excluded.

[0022] Culturing conditions

In the present invention, culturing conditions are not particularly limited, and the culturing temperature can be a temperature optimal to lactic acid bacterium that is actually cultured. For example, the culturing temperature is preferably 28 to 40°C, more preferably 29 to 38°C, still more preferably 30 to 36°C. The culturing temperature is preferably controlled and regulated in a desired range during culturing of

lactic acid bacterium. While the culturing time can be properly determined according to the type of lactic acid bacterium that is actually cultured, for example, the culturing time is preferably 4 to 48 hr, more preferably 8 to 36 hr, still more preferably 12 to 24 hr. In the present invention, the culture solution may not be stirred during culturing of lactic acid bacterium, and lactic acid bacterium can be cultured at a high concentration under such conditions. Therefore, preferably, the culture solution is not stirred from the viewpoint of reducing the amount of energy and electric expense involved in stirring of the culture solution during culturing of lactic acid bacterium. In the present invention, while stirring the culture solution during culturing of lactic acid bacterium is not indispensable, an embodiment (a concept) where the culture solution is stirred during culturing of lactic acid bacterium is not excluded. That is, lactic acid bacterium can be cultured at a higher concentration by properly stirring the culture solution during culturing of lactic acid bacterium.

[0023] In the present invention, a lowering in pH of the culture solution is suppressed as compared with the case where arginine is not added to the medium. As demonstrated in Examples that will be described later, in the present invention, pH of the medium is often once lowered with the elapse of the culturing time, but thereafter, the lowering of pH is reversed. In the present invention, as compared with the case where arginine is not added, pH of the medium is sometimes gradually lowered with the elapse of time even still in a high pH state. In the present invention, as compared with the case where arginine is not added, the profile of a change in pH of the medium is not particularly limited as long as the lowering in pH of the medium is suppressed and a desired high microbial cell concentration can be realized.

[0024] In the present invention, preferably, lactic acid bacterium is subjected to activation culturing before the culturing. Conditions for activation culturing may be properly determined according to the type of lactic acid bacterium.

[0025] Lactic acid bacterium obtained at a high concentration thus obtained can be used either as such or after centrifugation of microbial cells or the like. Specifically, it can be used as a starter for the production of fermented foods. With Lactic acid bacterium at a high

concentration, useful ingredients that affect flavor, texture, physical properties, and quality and functional ingredients that metabolize lactic acid bacterium can be obtained in a large amount.

[EXAMPLES]

[0026] The present invention is further illustrated by the following Examples that are not intended as a limitation of the scope of the present invention.

[0027] In the following Examples, the percentage is by mass unless otherwise specified. Further, the microbial cell concentration was measured by a measuring method for the number of lactic acid bacterium cells specified in "the Ministerial Ordinance concerning Compositional Standards, Etc. for Milk and Milk Products (Ministerial Ordinance concerning Milk, Japan). The culturing temperature of a BCP medium was 30°C. The concentration of ammonia was measured with a biosensor (BF-7, manufactured by Oji Scientific Instruments). The concentration of arginine, the concentration of citrulline, the concentration of ornithine, and the concentration of lactic acid were measured by a HPLC method.

[0028] Example 1

The following five strains of *Lactococcus lactis* ssp *lactis* having a metabolic ability of converting arginine into ornithine were provided.

- (1) *Lactococcus lactis* ssp *lactis* JCM5805T
- (2) *Lactococcus lactis* ssp *lactis* IFO12007
- (3) *Lactococcus lactis* ssp *lactis* MEP1706301
- (4) *Lactococcus lactis* ssp *lactis* MEP1706302
- (5) *Lactococcus lactis* ssp *lactis* MEP1706303

The above strains (3) to (5) are strains separated from raw milks originated from Tokachi district by the applicant of the present invention.

[0029] In an ordinary MRS medium, the above strains were activated several times before use. At the outset, a MRS medium containing 1% of glucose and 2% of arginine added thereto or a MRS medium containing 1% of glucose added thereto was inoculated with 1%, based on the amount of the medium, of a culture solution of the activated

strain. Glucose was mixed into a MRS medium before sterilization (121°C, 15 min) of the MRS medium, and arginine was mixed into a MRS medium after a procedure consisting of sterilizing the MRS medium, neutralizing the MRS medium with citric acid (in an amount by a factor of 0.4) and performing mechanical sterilization. The MRS medium originally contains 2% of glucose, and, thus, at an initial point in this experiment, the MRS medium contains 3% of glucose. Culturing was carried out under conditions of 30°C and 16 hr, and stirring was not carried out during culturing.

[0030] A change in pH during culturing with the elapse of time was illustrated in Fig. 1. For culturing of all the strains, in the medium to which arginine was not added, pH of the culture solution was lowered to around 4.5. On the other hand, in the medium to which arginine is added, pH was lowered to around 5.5 and was then turned into an increase in pH, resulting in suppression of the lowering in pH.

[0031] After the culturing, the microbial cell concentration and OD (600 nm) were measured. The results are shown in Table 1. In the medium to which arginine is added, as compared with the medium to which arginine was not added, the microbial cell concentration was increased by a factor of 2.2 to 8.5 times, and OD of the culture solution was increased by a factor of 2.2 to 4.1.

[0032] For culture solutions after culturing, the concentration of arginine (Arg), the concentration of citrulline (Cit), and the concentration of ornithine (Orn) were measured. The results are shown in Table 2. A major part of arginine initially added to the medium was converted to ornithine. From the results, it was considered that, in the process of converting arginine to ornithine, ammonia was released to increase pH of the culture solution and the increase in pH of the culture solution promoted the growth of microbial cells, leading to an increased microbial cell concentration (cell count) after the culturing.

[0033]

Table 1: Influence of *Lactococcus lactis* ssp *lactis* on microbial cell growth in MRS medium to which arginine is added

Strain NO	Vial cell count [cfu/mL]		Vial cell count ratio	OD (600 nm)		OD ratio
	Arg ⁻	Arg ⁺		Arg ⁻	Arg ⁺	
JCM5805T	7.2×10 ⁸	1.6×10 ⁹	2.2	2.0	4.5	2.2
IFO12007	1.0×10 ⁹	4.2×10 ⁹	4.2	2.4	5.6	2.3
MEP1706301	1.9×10 ⁹	1.6×10 ¹⁰	8.5	2.2	9.1	4.1
MEP1706302	1.6×10 ⁹	8.3×10 ⁹	5.2			
MEP1706303	1.6×10 ⁹	8.5×10 ⁹	5.2	2.6	10.1	3.8

Arg⁻: Results of culturing in MRS medium containing 1% of glucose added thereto.

Arg⁺: Results of culturing in MRS medium containing 1% of glucose and 2% of arginine added thereto.

[0034]

Table 2: Concentration of Arg, Cit, and Orn after culturing of *Lactococcus lactis* ssp *lactis* in MRS medium to which arginine is added

Strain NO	Arg ⁻			Arg ⁺		
	Arg [mM]	Cit [mM]	Orn [mM]	Arg [mM]	Cit [mM]	Orn [mM]
JCM5805T	0.0	0.3	1.0	19.6	10.2	83.0
IFO12007	0.0	0.0	1.5	0.0	7.5	120.2
MEP1706301	0.0	0.3	1.3	0.0	10.0	105.3
MEP1706302	0.0	0.0	1.5	0.0	6.0	111.6
MEP1706303	0.0	0.3	1.1	0.0	6.0	106.3

Arginine concentration at 0 hr in culturing was 121 mM.

Arg⁻: Results of culturing in MRS medium containing 1% of glucose added thereto.

Arg⁺: Results of culturing in MRS medium containing 1% of glucose and 2% of arginine added thereto.

[0035] Example 2

The following three strains of *Lactococcus lactis* ssp *cremoris* having a metabolic ability of converting arginine to ornithine was provided. These strains were cultured under the same conditions as in Example 1.

(6) *Lactococcus lactis* ssp *cremoris* JCM16167T

(7) *Lactococcus lactis* ssp *cremoris* MEP1706304

(8) *Lactococcus lactis* ssp *cremoris* MEP1706305

The above strains (7) and (8) are strains separated from

raw milks originated from Tokachi district by the applicant of the present invention.

[0036] A change in pH during culturing with the elapse of time is shown in Fig. 2. For culturing of all the strains, a lowering in pH was suppressed in media to which arginine is added.

[0037] The microbial cell concentration and OD (600 nm) of the culture solutions after culturing are shown in Table 3. In the medium to which arginine is added, as compared with the medium to which arginine was not added, the microbial cell concentration was increased by a factor of 2.7 to 6.2 times, and OD of the culture solution was increased by a factor of 1.4 to 4.5. Also from the results, it was considered that, in the process of converting arginine to ornithine, ammonia was released to increase pH of the culture solution and the increase in pH of the culture solution promoted the growth of microbial cells.

[0038]

Table 3: Influence of *Lactococcus lactis* ssp *cremoris* on microbial cell growth in MRS medium to which arginine is added

Strain NO	Vial cell count [cfu/mL]		Vial cell count ratio	OD (600 nm)		OD ratio
	Arg ⁻	Arg ⁺		Arg ⁻	Arg ⁺	
JCM16167T	2.4×10 ⁸	6.6×10 ⁸	2.7	1.6	2.2	1.4
MEP1706304	1.2×10 ⁹	3.3×10 ⁹	2.9	2.4	9.4	3.9
MEP1706305	7.1×10 ⁹	4.4×10 ⁹	6.2	2.2	10.1	4.5

[0039] Example 3

The following two strains of *Lactococcus lactis* ssp *cremoris* having a metabolic ability of converting arginine to ornithine were provided. These strains were cultured under the same conditions as in Example 1, except that the culturing temperature was 37°C.

(9) *Lactobacillus reuteri* JCM1112T

(10) *Lactobacillus brevis* JCM1061

[0040] A change in pH during culturing with the elapse of time is shown in Fig. 3. For culturing of all the strains, a lowering in pH was suppressed in media to which arginine is added.

[0041] OD (600 nm) of the culture solution after the culturing is shown in Table 4. In the medium to which arginine is added, as compared with the medium to which arginine was not added, OD of the

culture solution was increased by a factor of 1.1 to 1.4. Also from the results, it was considered that, lactic acid bacterium converted arginine to ornithine, in the process ammonia was released to increase pH of the culture solution and the increase in pH of the culture solution promoted the growth of microbial cells.

[0042]

Table 4: Influence of lactic acid bacterium belonging to genus *Lactobacillus* in MRS medium with arginine added thereto

Strain NO	OD (600 nm)		OD ratio
	Arg ⁻	Arg ⁺	Arg ⁺ / Arg ⁻
JCM112T	8.87	9.68	1.1
JCM1061	2.37	3.29	1.4

[0043] Example 4

The following three strains used in Example 1 were cultured under the same conditions as in Example 1, except that a medium composed mainly of a milk medium was used instead of the medium composed mainly of the MRS medium.

- (1) *Lactococcus lactis* ssp *lactis* JCM5805T
- (4) *Lactococcus lactis* ssp *lactis* MEP1706302
- (5) *Lactococcus lactis* ssp *lactis* MEP1706303

[0044] Before use, the strains were activated several times in an ordinary MRS medium. A medium prepared by adding 0.5% of a yeast extract (P2G: ASAHI FOOD & HEALTHCARE, LTD.), 2% of arginine, and 0.8% of citric acid to a reconstituted skim milk (a 10% aqueous solution of a powdered skim milk (low heat) manufactured by Meiji Co., Ltd.) and sterilizing the mixture (95°C, 5 min) (hereinafter referred to also as "a reconstituted skim milk medium") was used as the medium. The reconstituted skim milk medium was inoculated with the activated strain culture solution at a proportion of 1% to the medium followed by culturing. The culturing was carried out under conditions of 30°C and 16 hr, and stirring was not carried out during the culturing.

[0045] A change in pH during culturing with the elapse of time is shown in Fig. 5. For culturing of all the strains, a lowering in pH was suppressed in media to which arginine is added.

[0046] The microbial cell concentration of the culture solutions after culturing are shown in Table 5. In the medium to which arginine is

added, as compared with the medium to which arginine was not added, the microbial cell concentration was increased by a factor of 1.2 to 2.2. It was confirmed that, when the medium was composed mainly of the milk medium, as with the medium composed mainly of the MRS medium, the addition of arginine suppressed a lowering in pH of the culture solution and, at the same time, promoted the growth of microbial cells, making it possible to culture lactic acid bacterium at a high concentration.

[0047]

Table 5: Influence of *Lactococcus lactis* ssp *lactis* on microbial cell growth in reconstituted skim milk medium to which arginine is added

Strain NO	Vial cell count [cfu/mL]		Vial cell count ratio
	Arg ⁻	Arg ⁺	Arg ⁺ / Arg ⁻
JCM5805T	2.0×10^9	2.8×10^9	1.4
MEP1706302	3.8×10^9	8.4×10^9	2.2
MEP1706303	9.3×10^9	1.1×10^{10}	1.2

[0048] Example 5

The following strain was cultured under the same conditions as in Example 4, except that the addition amount of arginine was varied to vary the composition of the medium.

(4) *Lactococcus lactis* ssp *lactis* MEP1706302

[0049] A medium prepared by adding 0.1% of a yeast extract (P2G: ASAHI FOOD & HEALTHCARE, LTD.), 0, 2, 3, or 4% of arginine, and citric acid (in an amount by a factor of 0.4 of the arginine concentration) to a reconstituted skim milk medium (a 10% aqueous solution of a powdered skim milk (low heat), manufactured by Meiji Co., Ltd.) and sterilizing the mixture (95°C, 5 min), and a medium prepared by adding 0.1% of a yeast extract, 3% of arginine, and 1.2% of citric acid to a reconstituted whey medium (a 10% aqueous solution of a whey powder, manufactured by Meiji Co., Ltd.) and sterilizing the mixture (hereinafter referred to also as "a reconstituted whey medium") were used as the medium. The reconstituted skim milk medium was inoculated with the activated strain culture solution at a proportion of 1% to the medium followed by culturing. The culturing was carried out under conditions of 30°C and 16 hr, and stirring was not carried out during the culturing.

[0050] A change in pH during culturing with the elapse of time is

shown in Fig. 5. In the medium to which arginine is added, pH was increased 8 to 10 hr after the start of culturing, and pH was then lowered. The larger the addition amount of arginine, the later the timing and the larger the variation width of the pH increase. There was no clear difference in a change in pH with the elapse of time between the reconstituted skim milk medium and the reconstituted whey medium.

[0051] The concentration of arginine, the concentration of ornithine, the concentration of ammonia, the concentration of lactic acid, and the concentration of microbial cells were measured. The results are shown in Table 6. It was confirmed that arginine was converted to ornithine between 8 hr and 16 hr after the start of culturing and that, simultaneously with the start of converting from arginine into ornithine, the production of ammonia was started and the concentration of ammonia was increased to about twice the concentration of ornithine. The reason why the pH value of the culture solution was not lowered despite the fact that the concentration of lactic acid was increased with the elapse of culturing time, was believed to reside in that pH was neutralized with ammonia produced.

[0052] A comparison of the concentration of microbial cells in the reconstituted skim milk 16 hr after the start of culturing has revealed that the microbial cell concentration was 3.0×10^9 when the arginine concentration was 0%; was 1.0×10^{10} when the arginine concentration was 2%; was 1.3×10^{10} when the arginine concentration was 3%; and was 1.6×10^{10} when the arginine concentration was 4%, demonstrating that the microbial cell concentration increased with an increase in arginine addition concentration. There was no clear difference in microbial cell concentration between the reconstituted skim milk medium and the reconstituted whey medium. That is, it was confirmed that, even in the reconstituted whey medium, as with the reconstituted skim milk medium, the addition of arginine can suppress a lowering in pH of the culture solution and, at the same time, promotes the growth of microbial cells, making it possible to culture lactic acid bacterium at a high concentration.

[0053]

Table 6: Concentration of arginine (Arg), ornithine (Orn), ammonia, lactic acid, and microbial cells after culturing of *Lactococcus lactis* ssp *lactis* MEP1706302 in reconstituted skim milk medium and reconstituted whey medium to which arginine is added

Medium	Culturing time [hr]	pH [-]	Arg [mM]	Orn [mM]	Ammonia [mM]	Lactic acid [mM]	Vial cell count [cfu/mL]
Reconstituted skim milk medium Arg: 0%	2	6.36	0	0	2	8	2.3×10^8
	4	5.48	0	0	5	36	1.5×10^9
	8	4.46	0	0	5	76	2.8×10^9
	16	4.24	0	0	6	102	3.0×10^9
Reconstituted skim milk medium Arg: 2%	2	7.02	122	0	2	4	1.4×10^8
	4	6.70	115	0	4	11	6.4×10^8
	8	5.11	102	11	61	78	3.5×10^9
	16	5.39	0	106	247	269	1.0×10^{10}
Reconstituted skim milk medium Arg: 3%	2	7.11	167	0	2	7	1.5×10^8
	4	6.90	196	0	6	14	5.5×10^8
	8	5.58	193	0	37	80	3.3×10^9
	16	5.91	0	163	337	317	1.3×10^{10}
Reconstituted skim milk medium Arg: 4%	2	7.05	228	0	2	1	1.2×10^8
	4	6.85	253	0	5	7	4.9×10^8
	8	5.69	246	0	29	68	3.3×10^9
	16	5.99	18	203	470	349	1.6×10^{10}
Reconstituted whey medium Arg: 3%	2	7.05	219	0	2	7	2.3×10^8
	4	6.83	187	0	5	12	5.3×10^8
	8	5.46	190	0	40	77	3.5×10^9
	16	5.87	0	192	336	379	1.2×10^{10}

[0054] Example 6

The following strain was cultured in the same manner as in Example 5, except that the addition amount of arginine was varied and the culturing time was not more than 22 hr.

(4) *Lactococcus lactis* ssp *lactis* MEP1706302

The concentration of arginine, the concentration of citrulline, the concentration of ornithine, the concentration of lactic acid, and the concentration of microbial cells were measured 16 hr and 22 hr after the start of culturing. The results were as shown in Table 7.

[0055]

Table 7: Concentration of arginine (Arg), ornithine (Orn), lactic acid, and microbial cells after culturing of *Lactococcus lactis* ssp *lactis* MEP1706302 in reconstituted skim milk medium to which arginine is added

Medium	Culturing time [hr]	Arg [mM]	Cit [mM]	Orn [mM]	Lactic acid [mM]	Vial cell count [cfu/mL]
Arg: 0%	16	0	0	0	76	1.8×10^9
	22	0	0	5	83	2.4×10^9
Arg: 4%	16	26	20	163	275	1.4×10^{10}
	22	0	38	194	400	1.7×10^{10}
Arg: 7%	16	263	16	97	185	1.1×10^{10}
	22	77	61	230	332	1.3×10^{10}
Arg: 10%	16	517	11	30	119	7.4×10^9
	22	412	42	131	227	9.6×10^9

[CLAIMS]

1. A method for culturing lactic acid bacterium, comprising the step of culturing lactic acid bacterium having a metabolic ability of converting arginine into citrulline in a medium to which arginine is added in order to suppress a lowering of pH of the medium.
2. The method for culturing lactic acid bacterium according to claim 1, wherein the number of microbial cells per mL of a culture solution of lactic acid bacterium is twice or more the number of microbial cells when cultured in an arginine-free medium.
3. The method for culturing lactic acid bacterium according to claim 1 or 2, wherein lactic acid bacterium has a metabolic ability of converting citrulline into ornithine after the conversion of arginine into citrulline.
4. The method for culturing lactic acid bacterium according to any one of claims 1 to 3, wherein arginine is added in an amount of 0.5 to 15% by mass to the medium.
5. The method for culturing lactic acid bacterium according to any one of claims 1 to 4, wherein the lactic acid bacterium is one or more members selected from the group consisting of *Lactococcus lactis*, *Lactobacillus fermentum*, *Lactobacillus buchneri*, *Enterococcus faecalis*, *Oenococcus oeni*, *Pediococcus pentosaceus*, *Lactobacillus amylovorus*, *Lactobacillus brevis*, *Lactobacillus reuteri*, *Lactobacillus acidophilus*, *Lactobacillus crispatus*, and *Lactobacillus delbrueckii*.
6. The method for culturing lactic acid bacterium according to claim 5, wherein the lactic acid bacterium is one or more members selected from the group consisting of *Lactococcus lactis* spp. *lactis*, *Lactococcus lactis* spp. *cremoris*, *Lactobacillus reuteri*, and *Lactobacillus brevis*.
7. The method for culturing lactic acid bacterium according to any one of claims 1 to 6, wherein the culture solution is not stirred.