

SPECIFICATION

Method for producing foods and drinks containing bifidobacteria

5 BACKGROUND OF THE INVENTION

This invention relates to a method for producing foods and drinks containing bifidobacteria.

Bifidobacterium predominates in intestinal bacterial flora of suckling infants, and is being watched with keen interest since it has an influence on the health of breast-fed infants.

There are many reports on the study of the physiological significance of this bacterium, clarifying (1) the inhibitory effect on putrefaction by putrefactive bacteria, (2) the inhibitory effect on production of toxic amines, (3) the digestive effect on human milk casein by the action of phosphoprotein phosphatase, and (4) the effect of suppressing the growth of pathogenic bacteria by lowering intestinal pH following production of organic acids such as lactic acid, acetic acid, and formic acid.

However, this *Bifidobacterium* favourable to infants is present in a very small amount in the intestines of bottle-fed infants, which is considered to be one of the causes for their susceptibility to intestinal diseases, greater than that of breast-fed infants.

Aimed at approximating the intestinal flora of bottle-fed infants to that of breast-fed infants, an attempt has been made to produce *Bifidobacterium*-containing powdered milk for infants and to modify powdered milk for infants in such a manner as to be similar to a mother's milk.

However, due to the problems as mentioned below, it has been difficult to practice an industrial cultivation of *Bifidobacterium* in a medium consisting of milk only.

That is, as compared with dairy lactic acid bacteria which are widely used in processing milk, *Bifidobacterium* has the following problems:

- (1) An industrial mass cultivation is difficult since *Bifidobacterium* requires strict anaerobic conditions for growth, and accordingly entails a large equipment cost and requires high level cultivation techniques;
- (2) The nutritional requirement for the cultivation is complicated and fastidious and therefore the bacteria do not substantially propagate on a pure cow's milk medium containing no growth promoting substance such as yeast extract, peptone and the like; and
- (3) Acetic acid, the main metabolic product of *Bifidobacterium*, is highly stimulative, and therefore generally impairs the taste and flavour of foods and drinks containing the same.

We have found that bifidobacteria having the above mentioned properties can be prosperously propagated under the same aerobic culture conditions as in the cultivation of dairy lactic acid bacteria in a medium containing as the main component a pasty or milky product of α -starch-transformed non-glutinous or glutinous rices, polished, whole, non-polished or powdered, and further containing sugars fermentable by *Bifidobacterium*, such as glucose, lactose, fructose, galactose and the like (the sugar may vary depending on the species of *Bifidobacterium* used), and that the cultivated product has a good flavour since the fermentation product, acetic acid, well matches with the flavour of cooked rice.

SUMMARY OF THE INVENTION

The invention provides a method for producing foods and drinks containing bifidobacteria, characterised by inoculating and cultivating bifidobacteria or a mixture of bifidobacteria and lactic acid bacteria in a medium comprising α -starch-transformed rice, obtained by heating rice in water, and bifidobacteria-fermentable sugars, and optionally processing the cultivated product for preparing a food or drink, said α -starch-transformed rice being previously subjected to amylase treatment if the rice concentration in the medium is in excess of 20%.

Conveniently the fermentable sugars are themselves given by amylase treatment of the transformed rice, as described later herein along with other preferred features of the method.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1 and 2 show graphs illustrating the state of cultivation with the passage of the cultivation time.

Figure 3 shows the results of the organoleptic evaluation of a product cultivated with bifidobacteria.

DETAILED EXPLANATION OF THE INVENTION

The above mentioned properties of bifidobacteria are illustrated by the following experiments. Viable cell count expressed by count per ml was measured in accordance with the method described "J. Food Hyg. Soc. Japan" (Vol. 18, pp. 537-546, 1977). Titratable acidity was expressed by amount in ml of 0.1 N NaOH solution required to neutralize 10 ml of sample.

Experiment 1

A culture medium for this Experiment was prepared by adding about 750 ml of water to 150

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g each of washed unpolished-, whole-, and polished- non-glutinous rices, heating each mixture at 120°C for 15 minutes, emulsifying it by a mixer, adding 30 g of lactose to it and finally topping up to 1000 ml by the addition of water. A reconstituted skim milk having a non-fat milk solid concentration of 15 % was used as a reference culture medium.

Each culture medium thus prepared was placed in a 2000 ml-conical flask having a cotton plug, and was then sterilized at 120°C for 20 minutes. After cooling, 3.0 % of each starter of bifidobacteria previously prepared was inoculated in the respective medium, and was subjected to a static culture at 37°C for 24 hours. Acidity and viable cell count were then measured, and the results are shown in Table 1.

Table 1

Media	unpolished rice + lactose		whole rice + lactose		polished rice + lactose		milk	
	acidity	viable count	acidity	viable count	acidity	viable count	acidity	viable count
<i>B. bifidum</i> YIT4005	8.3	6.8×10^8	8.1	6.6×10^8	7.2	5.6×10^8	6.3	4.2×10^8
<i>B. bifidum</i> E	7.2	5.0×10^8	7.0	5.5×10^8	6.8	4.2×10^8	2.3	5.8×10^5
<i>B. breve</i> Y	9.6	7.9×10^8	9.4	7.1×10^8	8.3	6.9×10^8	3.3	3.1×10^5
<i>B. breve</i> S	9.0	6.6×10^8	9.1	6.3×10^8	8.5	5.6×10^8	2.5	4.2×10^4
<i>B. longum</i> ATCC-15707	5.8	4.1×10^8	5.6	4.3×10^8	5.0	4.0×10^8	2.8	2.0×10^4
<i>B. adolescentis</i>	5.5	3.9×10^8	5.0	3.6×10^8	4.4	2.5×10^8	2.5	3.8×10^4
<i>B. infantis</i>	4.8	1.4×10^8	4.6	1.5×10^8	3.8	8.9×10^7	2.6	5.9×10^4

Note: Starting Viable Cell Count: $8.5 \times 10^5 \sim 1.3 \times 10^7$, Acidity 1.3~1.5

In the reference milk medium, strains other than *B. bifidum* YIT 4005 (a mutant of bifidobacteria having properties of propagating under aerobic conditions in a milk medium Deposit No. FERM-3372 at Fermentation Research Institute, Government Industrial Research, Ministry of International Trade and Industry Japan) were not totally propagated. On the other hand, in the respective milky culture medium containing rice as the starting material, all the bifidobacteria were vigorously propagated.

According to other experiments wherein the concentration of culture medium was varied, it was proved that propagation was accelerated in proportion to the increase in the concentration of rice until the concentration of rice reached 20 %. If the concentration exceeds the above value, starching occurs and accordingly amylase treatment is required. However, this treatment did not have a substantial influence on the propagation.

With regard to unpolished-, whole-, and polished-glutinous rices, substantially the same results as in the above were obtained.

Experiment 2

The same procedures as in Experiment 1 were repeated, except that a medium used for this experiment was prepared by cooking unpolished-, whole-, and polished-non-glutinous rices, emulsifying it at a concentration of 15 %, and adding to the emulsion 2.0 % of lactose and 1.0-15 % of skim milk powder.

Table 2 shows the results obtained with media containing skim milk powder at a concentration of 5 %. In the media containing milk components in addition to rice and sugar, the propagation of bifidobacteria was accelerated as compared with Experiment 1 wherein milk component was not used. The effect of the milk components on accelerating propagation was enhanced in proportion to the increase in the concentration of skim milk powder until the concentration reached 10 %. If the concentration exceeded the above value, no further

enhancement of the effect on accelerating propagation was achieved.

Substantially the same results are to be obtained by the use of whey powder.

Table 2

Bifido- bacteria	rice	unpolished rice		whole rice		polished rice	
	acidity	viability count	acidity	viability count	acidity	viability count	
B.bifidum	13.5	6.2×10^9	13.3	6.0×10^9	12.6	5.8×10^9	
YIT 4005							
B.bifidum E	9.5	7.6×10^8	9.6	7.0×10^8	9.4	6.8×10^8	
B.breve Y	11.8	5.0×10^9	10.7	3.3×10^9	10.1	3.4×10^9	
B.breve S	10.0	9.0×10^8	9.0	8.2×10^8	9.1	7.8×10^8	
B.longum	7.9	6.7×10^8	7.6	5.8×10^8	7.2	5.3×10^8	
ATCC-15707							
B.adolescentis	6.5	5.1×10^8	6.3	5.0×10^8	6.0	4.1×10^8	
B.infantis	6.6	4.9×10^8	6.0	4.1×10^8	5.6	3.9×10^8	

Note: Starting Viable Cell Count: $1.0 \sim 3.4 \times 10^7$, Acidity: 1.7 ~ 2.0

Experiment 3

Two species of bifidobacteria or a mixture of bifidobacteria and lactic acid bacteria were inoculated in a milky medium prepared in such a manner as to contain 15 % of unpolished non-glutinous rice, 5 % of skim milk powder and 2 % of lactose, and was subjected to a static culture at 37°C. The inoculum of the starter of each strain was respectively 2 %. Figs. 1 and 2 show the results of the acidity and viable cell counts measured with the passage of time during cultivation in comparison with the results obtained by single cultivation of each strain. The strains used in this Experiment are as follows:

B₁: *B. bifidum* YIT 4005

B₂: *B. breve* Y

LA: *Lactobacillus acidophilus*

As can be seen from the two Figures, a certain species of strain was vigorously propagated in a rice-containing medium when subjected to a mixed cultivation.

Experiment 4

B. bifidum YIT 4005 was inoculated in a medium containing 15 % of whole rice, 5 % of skim milk powder and 2.0 % of lactose and in a skim milk medium having a non-fat milk solid concentration of 15 %, and was cultivated at 37°C for 24 hours. With regard to the culture thus obtained, organoleptic evaluation was made by a panel consisting of 10 trained panelists. The results are shown in Fig. 3.

Both cultures had almost equal values each other with regard to acidity and ratio of acetic acid/lactic acid (see Table 3). However, culture of the rice-containing medium was evaluated to have a better flavour since the flavour of cooked rice well matches with acetic acid and the stimulative smell of acetic acid is weaker as compared with the product cultivated in the milk-containing medium.

Table 3

Medium	Culture	
	Acidity	Acetic Acid/ Lactic Acid
medium consisting of milk only	6.8	1.75
medium containing rice	7.5	1.80

The present invention was accomplished in view of the above knowledge. Thus, the present invention resides in a method for producing foods and drinks containing bifidobacteria, characterized by inoculating and cultivating bifidobacteria or bifidobacteria and lactic acid

bacteria in a medium comprising a mixture containing rice treated for α -starch transformation and sugars fermentable by bifidobacteria or further containing milk components in addition to the above ingredients, and optionally processing the cultivated product into a form suitable for foods and drinks.

5 As clearly described above, any glutinous or non-glutinous rice can be used in the method of this invention, and the rice may or may not, be polished or milled, to any degree. When these 5
rices are used in a medium for cultivating bifidobacteria, they are preferably made into a
homogenous milky state by the method used in Experiment 1 or a method of grinding and
heating in water. An appropriate concentration of rice in a medium is 10-20 %, preferably 15
10 %.

Sugars such as lactose, fructose and glucose are the most preferable bifidobacteria-fermenta-
ble sugars since they are easily available and fermentable by all of the bifidobacteria. It is not
necessary to use pure sugars, but any edible material containing these fermentable sugars, for
example, lactose-containing milk, skim milk, whey and the like can be used as a medium
15 component. Milk components are particularly preferable since they accelerate the propagation of
bifidobacteria and provide a product having a good nutrition balance. A part or the whole part of
the rice may be previously treated with amylase or with amylase-producing *Aspergillus oryzae*,
Aspergillus niger, *Bacillus subtilis* and the like to produce glucose in an amount sufficient for the
propagation of bifidobacteria, and the glucose thus produced may be used as a fermentable
20 sugar for bifidobacteria. This method has advantages in that the emulsification of rice proceeds
along with saccharification, and that a unique flavour can be obtained.

The concentration of bifidobacteria-fermentable sugars in a medium should preferably be 1-5
%, more preferably about 3 %.

Any cultivation supplements or seasonings, spices, nutrients and the like may be added to a
25 medium in addition to the above components.

Bifidobacteria to be inoculated in the above mentioned homogenous milky medium are not
specially limited, and 2 species or more of bifidobacteria may be used. In combination with
bifidobacteria, Lactic acid bacteria may be inoculated, preferable examples of which include
Lactobacillus acidophilus, *L. casei*, *L. bulgaricus*, *Streptococcus thermophilus* and the like.

30 The cultivation of bifidobacteria can be carried out under aerobic conditions in the same
manner as in the cultivation of ordinary lactic acid bacteria, and strictly anaerobic conditions are
not required. This is a great advantage of this invention, and the conventional apparatus and
techniques used in the cultivation of lactic acid bacteria can be used as they are.

Under normal cultivation conditions, viable cell count reaches maximum after 18-24 hour
35 cultivation, and pH is gradually decreased because acid is formed along with the passage of the
cultivation time. The production of acetic acid by bifidobacteria starts at the initiation of
cultivation. If the cultivation is further continued, viable cell count starts to be decreased after
24 hours and the production of acid is continued for some time but the production of acid stops
to some extent. Thus, taking the use of the cultivated product into consideration, cultivation is
40 stopped when the pH of the medium reaches 4.2 to 5.6. In most cases using bifidobacteria per
se, the viable cell count of bifidobacteria should preferably be more than 10^7 per ml. According
to the culture method of this invention, it is quite easy to obtain a viable cell count of 10^{8-9} /ml.

The product of this invention contains acetic acid 80-120 mM/l and lactic acid 50-70
mM/l as the main metabolites in addition to the viable bifidobacteria. The contents of these
45 acids are not specially different from those in conventional bifidobacteria culture, but the acetic
acid well matches with emulsified rice and therefore the cultivated product of this invention
deserves much higher organoleptic evaluation than the conventional cultivated products. Thus,
the cultivated product of this invention can be served, as it is, or as a food containing viable
bifidobacteria without flavouring. The product of this invention may be served as a drink by
50 optionally adding sweetening materials, fruit juice, water, spices or the like to modulate the
concentration and flavour. The product can also be served as powdery or tablet-like foods or
medicinal preparations containing viable bifidobacteria by drying. As long as the bifidobacteria
do not perish, any conventional processing techniques and any processed forms can be used.

The present invention is further illustrated by the following Examples.

55 Example 1

To 15 Kg of fully washed polished non-glutinous rice, was added 70 liters of water, and the
resultant mixture was heated at 121°C for 15 minutes. The mixture was then emulsified in a
mixer, and the mixture was topped up to 100 liters by the addition of water. To this mixture,
60 was added 2 Kg of skim milk powder, and the mixture was sterilized at 121°C for 40 minutes.
The mixture was then cooled to 37°C while stirring.

Into the milky rice medium thus prepared, 3 % by volume of the starter of *B. bifidum*
YIT-4005 was inoculated, and was cultivated at 37°C for 24 hours.

After cultivation, the culture was homogenized in a homogenizer (150 Kg/cm²), and 40 liters
65 of syrup containing 4.8 Kg of sucrose was mixed therewith, thus producing a drink having an

acidity of 5.4 and containing bifidobacteria in a count of 4.3×10^8 /ml. The product thus obtained had a less stimulative smell and provided a satisfactory taste and flavour.

Example 2

- 5 To 15 Kg of thoroughly washed whole glutinous rice, was added 70 liters of water, and the mixture was heated at 121°C for 15 minutes. In order to facilitate emulsification, 50 g of liquifying amylase (70,000 units/g) was added to the mixture, and the mixture was emulsified in a mixer. To this mixture, 1 Kg of lactose and 10 liters of cow's milk were added, and the mixture was topped up to 100 liters by the addition of water. The mixture was then sterilized at 10 121°C for 40 minutes, and was cooled to 37°C while stirring. Into the medium thus prepared, 5 % of the starter of *B. adolescentis* was inoculated, and was cultivated at 37°C for 40 hours. After cultivation, the culture was treated in the same manner as in Example 1 to obtain a drink having an acidity of 6.1 and containing viable bifidobacteria in an amount of 8.0×10^7 /ml.

Example 3

- 15 To 2 Kg of thoroughly washed polished non-glutinous rice, was added 7 liters of water, and the mixture was heated at 121°C for 20 minutes. After cooling, 200 g of seed starter of *Aspergillus oryzae* was added to the mixture, and the mixture was shaken at 37°C for 40 hours to saccharify a part of the rice, thus forming glucose. Thereafter, the mixture was emulsified in a 20 mixer, and was topped up to 10 liters. To the emulsion thus prepared, was added 5 liters of syrup containing 600 g of sucrose and 30 g of gelatin, and the resultant mixture was sterilized. Into the medium thus prepared, 2 % each of the starter of *B. breve* Y and *B. longum* (ATCC-15707) was inoculated for culture at 37°C for 12 hours to obtain a sweetwine flavoured 25 yoghurt-like food. The product thus obtained had an acidity of 5.2, and the viable cell count was 2.2×10^8 /ml for *B. breve* Y and 7.8×10^7 /ml for *B. longum*.

Example 4

- 30 One Kg of polished nonglutinous rice was thoroughly washed and dried. The rice was then powdered by a grinder, and 5 liters of water was added to the powdered rice. The mixture was then heated at 121°C for 15 minutes. After cooling the mixture to 55°C, 2 g of saccharifying amylase (50,000 units/g) was added to the mixture while stirring thoroughly, and the resultant mixture was treated at 55°C for 1 hour. After the amylase-treatment, the mixture was topped up to 10 liters by the addition of water, and was sterilized. After cooling to 37°C, the mixture was 35 inoculated with the starter of *Saccharomyces sake*, and was cultivated at 30°C for 20 hours. Thereafter, the culture medium thus prepared was sterilized again, and 2 % each of the starter of *B. bifidum* E and *Lactobacillus acidophilus* was inoculated into the medium. The cultivation was carried out at 37°C for 30 hours. After cultivation, the culture was homogenized in a homogenizer, and 2 liters of syrup 40 containing 300 g of sucrose was added to the culture. The product thus obtained contained a small amount of alcohol, and had a titratable acidity of 9.6 and the viable cell count of 1.5×10^8 /ml for bifidobacteria and 6.6×10^7 /ml for lactobacilli.

Example 5

- 45 To the culture prepared using the same materials in the same manner as in Example 1, were added 5 Kg of skim milk powder, 5 Kg of sugar and 1 Kg of Vitamin C, and the mixture was dried by a spray drier, thus producing 26.5 Kg of powdery food containing 7.1×10^8 /g of bifidobacteria.

50 CLAIMS

1. A method for producing foods and drinks containing bifidobacteria, characterised by inoculating and cultivating bifidobacteria or a mixture of bifidobacteria and lactic acid bacteria in a medium comprising α -starch-transformed rice, obtained by heating rice in water, and bifidobacteria-fermentable sugars, and optionally processing the cultivated product for preparing 55 a food or drink, said α -starch-transformed rice being previously subjected to amylase treatment if the rice concentration in the medium is in excess of 20%.
2. A method according to claim 1, wherein said bifidobacteria-fermentable sugars are obtained by saccharifying a part or the whole of said α -starch-transformed rice by treating with amylase.
3. A method according to claim 2 wherein said amylase is produced in situ by amylase- 60 producing micro organisms selected from *Aspergillus oryzae*, *Aspergillus niger* and *Bacillus subtilis*.
4. A method according to claim 1, 2 or 3, wherein said cultivation is carried out by inoculating two or more species or strains of bifidobacteria.
5. A method according to any preceding claim, wherein said medium further comprises milk 65



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- After cultivation, the culture was treated in the same manner as in Example 1 to obtain a drink having an acidity of 6.1 and containing viable bifidobacteria in an amount of 8.0×10^7 /ml. 15

Example 3

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Example 4

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- After cultivation, the culture was homogenized in a homogenizer, and 2 liters of syrup 40 containing 300 g of sucrose was added to the culture. The product thus obtained contained a small amount of alcohol, and had a titratable acidity of 9.6 and the viable cell count of 1.5×10^8 /ml for bifidobacteria and 6.6×10^7 /ml for lactobacilli. 40

Example 5

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2. A method according to claim 1, wherein said bifidobacteria-fermentable sugars are obtained by saccharifying a part or the whole of said α -starch-transformed rice by treating with amylase. 60
3. A method according to claim 2 wherein said amylase is produced in situ by amylase-producing micro organisms selected from *Aspergillus oryzae*, *Aspergillus niger* and *Bacillus subtilis*. 60
4. A method according to claim 1, 2 or 3, wherein said cultivation is carried out by inoculating two or more species or strains of bifidobacteria. 65
5. A method according to any preceding claim, wherein said medium further comprises milk 65

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components.

6. A method according to any preceding claim, wherein the concentration of bifidobacteria-fermentable sugars in the medium is 1 to 5 percent by weight.

7. A method according to any preceding claim, wherein the cultivated product contains
5 more than 10^7 viable bifidobacteria cells/ml.

8. A method according to any preceding claim, in which cultivation is stopped when the pH of the medium is 4.2 to 5.6.

9. A method according to claim 1, wherein said cultivation is carried out substantially as herein described in any one of the Examples.

10 10. A food or drink when prepared by the method of any one of claims 1 to 9.

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