

DESCRIPTION

LACTIC ACID BACTERIA FERMENTED SUBSTANCE AND FERMENTED MILK FOOD
PRODUCT CONTAINING THE SAME

5

TECHNICAL FIELD

[0001] The present invention relates to lactic acid bacteria
fermentation products, and more specifically to lactic acid
bacteria fermentation products, which contain viable lactic acid
10 bacteria at high concentration, and also to fermented milk
products which contain such lactic acid bacteria fermentation
products.

BACKGROUND ART

15 [0002] Culture of lactic acid bacteria is carried out in
various manners; most widely by using animal milk in the
production of lactic acid bacteria preparations, and also in
production of fermented milk, lactic acid bacteria beverages,
cheese, etc. However, generally, lactic acid bacteria have
20 different auxotrophy depending on the species, and in general
do not sufficiently grow in a medium consisting of only animal
milk. Therefore, even with a strain having relatively good
proliferability among lactic acid bacteria, culture should be
continued for as many as several days to obtain a fermentation
25 product such as fermented milk or lactic acid bacteria beverage,
of sufficient acidity upon its production.

[0003] Such long-time culture of lactic acid bacteria,
however, causes reduction in the viable cell count, and therefore,
is not necessarily considered to be a preferred culturing method
30 for the production of lactic acid bacteria beverages, fermented
milk, or the like all of which place importance on the viable
cell count in expectation of various physiological effects.

[0004] In producing various beverages or foods each of which
places importance on the flavor of a lactic acid bacteria
35 fermentation product, on the other hand, strains to be used cannot
be selected solely from the viewpoint of their proliferativeness.
Lactic acid bacteria of poor proliferativeness may be used in

some instance for the availability of fermentation products with good flavor.

[0005] In the culture of lactic acid bacteria, it is, therefore, common practice to add various growth-promoting substances beforehand to a medium for the purpose of improving the efficiency of the culture. Examples of growth-promoting substance, which are generally considered to be effective, include chlorella extract, iron salts, vitamins, proteolytes including amino acids or peptides, and yeast extract.

10 [0006] Further, as other techniques intended to promote the growth of lactic acid bacteria, there have been recently, reported a method making use of an aqueous extract of sake lees and/or an aqueous extract of sake lees which has been treated with a protease (Patent Document 1), a method making use of an
15 extract from leaves of a plant of Coffea arabica (Patent Document 2), a method making use of papaya flesh parts including their skins (Patent Document 3), a method making use of an extract from algal bodies of marine microalgae (Patent Document 4), a method making use of one or more vegetables or the like selected
20 from the group consisting of brocolli, cauliflower, kale, sheperd's purse, radish, tower mustard, celery-leaved buttercup, white celery mustard, Japanese bittercress, yellow rocket, watercress, leaf mustard, brown mustard, wasabi (green horseradish paste), herbaceous perennial, long Japanese turnip,
25 Japanese pickling turnip, turnip, oilseed rape, cabbage, spinach, komatuna (Brassica campestris var. peruviridis), celery, parsley, lettuce and apple (Patent Document 5), a method making use of one or more kinds of vegetables or the like selected from the group consisting of dishcloth gourd, cucumber, sweet melon,
30 pumpkin, yam, taro, 'KONJAK', Japanese radish, carrot, tomato, green pepper, okra, Welsh onion, Chinese cabbage, bean sprouts and tangerine orange (Patent Document 6), a method making use of a tea extract (Patent Documents 7 and 8), a method making use of a calcium salt (Patent Document 9) and a method making
35 use of an extract of ginger, tea or green onion (Patent Document 10), etc.

[0007] In order to maintain the usefulness or the efficacy

of lactic acid bacteria, however, it is necessary not only to promote the growth of the bacteria but also to reduce death of the bacteria and to improve the viability of the bacteria in the fermentation product by the lactic acid bacteria. Generally,
5 a reduction in the viability of lactic acid bacteria becomes pronounced when preparing a low-fat fermented milk food containing lactic acid bacteria fermentation product of skim milk powder or the like, or when lactic acid fermentation has proceeded too much. The reduction in the viability of lactic
10 acid bacteria, therefore, becomes more serious when preparing a low-calorie fermented milk food or a low-pH fermented milk food. Chlorella or the like is known as a material usable to prevent a viability reduction of lactic acid bacteria, and to maintain the cell count of lactic acid bacteria in a fermented
15 milk food.

[0008] In the production of a beverage or food such as a lactic acid bacteria fermentation product or a fermented milk food containing the same, however, a conventionally-known substance added for promoting the growth of lactic acid bacteria or a
20 conventionally-known substance added for improving the viability of lactic acid bacteria may affect the flavor itself of the product in many instances and may also cause a rise in product cost, when used in such an amount as bringing about sufficient effects. Furthermore, even if it is possible to
25 maintain a state that a large amount of viable lactic acid bacteria are contained, the lactic acid bacteria can not be maintained active, thereby making it difficult to expect sufficient physiological effects in some instances.

[0009]

30 [Patent Document 1] JP-A-05-015366
[Patent Document 2] JP-A-06-125771
[Patent Document 3] JP-A-07-023777
[Patent Document 4] JP-A -07-051057
[Patent Document 5] JP-A-11-266860
35 [Patent Document 6] JP-A-02-242667
[Patent Document 7] JP-B- 2667421
[Patent Document 8] JP-B-3223326

[Patent Document 9] JP-B-2673333

[Patent Document 10] JP-A-2001-190272

DISCLOSURE OF THE INVENTION

5 Problems to be solved by the Invention

[0010] An object of the present invention is, therefore, to find a novel substance, the mere addition and mixing of which to a medium makes it possible not only to easily increase the viable cell count of lactic acid bacteria but also to maintain
10 the viable cell count even after the preparation of a final product without developing problems about the flavor and taste, and to use the substance for the provision of a lactic acid bacteria fermentation product, said fermentation product containing a number of viable lactic acid bacteria, or a beverage or food
15 making use of the fermentation product.

Means for solving the Problems

[0011] To achieve the above-described object, the present inventors have conducted extensive research. As a result, it
20 has been found that without impairing the flavor and taste of a fermentation product to be obtained by lactic acid bacteria, the proliferative activities of the lactic acid bacteria can be easily improved by adding a novel extract of a specific plant to a medium and culturing the lactic acid bacteria there. Further,
25 the present inventors have also found that culture of lactic acid bacteria on a medium, which contains the above-mentioned extract and a specific fatty acid, makes it possible to obtain a lactic acid bacteria fermentation product which contains viable lactic acid bacteria at high concentration without a reduction
30 in their activities. Furthermore, the present inventors have also found that various beverages or foods, such as fermented milk foods, prepared by the above-mentioned methods are free of any problem in their flavor and taste, leading to the completion of the present invention.

35 [0012] In one aspect of the present invention, there is thus provided a lactic acid bacteria fermentation product, which has been obtained by culturing lactic acid bacteria on a medium

comprising an extract of at least one food material selected from the group consisting of rice bran, persimmon leaves, perilla, Houttuynia cordata Thunb, Eucommia ulmoides Oliv., turmeric, clove, cinnamon and Rubus suavissimus S. Lee (Rosaceae).

5 [0013] In another aspect of the present invention, there is also provided a lactic acid bacteria fermentation product, which has been obtained by culturing lactic acid bacteria on a medium comprising an extract of at least one food material selected from the group consisting of rice bran, persimmon leaves, perilla,
10 Houttuynia cordata Thunb, Eucommia ulmoides Oliv., turmeric, clove, cinnamon and Rubus suavissimus S. Lee (Rosaceae), and oleic acid or a derivative thereof.

[0014] In a further aspect of the present invention, there is also provided a fermented milk food comprising the
15 above-described fermentation product.

[0015] In a still further aspect of the present invention, there is also provided a method for producing a lactic acid bacteria fermentation product, comprising culturing lactic acid bacteria on a medium comprising an extract of at least one food
20 material selected from the group consisting of rice bran, persimmon leaves, perilla, Houttuynia cordata Thunb, Eucommia ulmoides Oliv., turmeric, clove, cinnamon and Rubus suavissimus S. Lee (Rosaceae).

[0016] In a yet further aspect of the present invention, there is also provided a method for producing a lactic acid bacteria fermentation product, comprising culturing lactic acid bacteria on a medium comprising an extract of at least one food material selected from the group consisting of rice bran, persimmon leaves, perilla, Houttuynia cordata Thunb, Eucommia ulmoides Oliv.,
25 turmeric, clove, cinnamon and Rubus suavissimus S. Lee (Rosaceae),
30 and oleic acid or a derivative thereof.

EFFECT OF THE INVENTION

[0017] The extract, which is useful in the lactic acid bacteria
35 fermentation product of the present invention and has been derived from at least one food material selected from the group consisting of rice bran, persimmon leaves, perilla, Houttuynia

cordata Thunb, Eucommia ulmoides Oliv., turmeric, clove, cinnamon and Rubus suavissimus S. Lee (Rosaceae), has excellent growth-promoting effects or viability-improving effects for the lactic acid bacteria and moreover, has practically no effect
5 on the flavor and the taste. A fermented milk food, which has been obtained by adding and mixing the extract and contains the lactic acid bacteria fermentation product, is therefore excellent for the promotion of health, and has high utility as a beverage or food which does not undergo any much deterioration
10 in the flavor and taste.

[0018] In particular, the combined use of the above-described extract with oleic acid or a derivative thereof can reduce the death of bacteria even in a low-fat fermented milk food or low-pH fermented milk food, thereby guaranteeing the viable cell count
15 in the product and their viability.

BEST MODE FOR CARRYING OUT THE INVENTION

[0019] The lactic acid bacteria fermentation product of the present invention is obtained by fermenting lactic acid bacteria
20 under conventionally-known culture conditions, except for the use of a medium which contains an extract of at least one food material selected from the group consisting of rice bran, persimmon leaves, perilla, Houttuynia cordata Thunb, Eucommia ulmoides Oliv., turmeric, clove, cinnamon and Rubus suavissimus
25 S. Lee (Rosaceae) (hereinafter, it may be simply called to as "an extract").

[0020] Among the food materials which can each be used as a raw material for the above-mentioned extract, rice bran is a mixture of perocarps, aleurone layers and germs of kernels
30 (brown rice) available from Oryza sativa without the chaff of paddy. This rice bran is known to have effects such as immunity enhancement, fatty liver prevention and the like.

[0021] Persimmon leaves include leaves of the plant of Diospyros Kaki Thunb, Diospyros lotus L., or Diopyros lotus L.
35 var. glabra Makino. In the present invention, Diospyros Kaki Thunb are particularly preferred among the plants of the genus Diospyros because the leaves are known to have effects such as

suppressing sneezing, nasal congestion, runny nose and the like.

[0022] Perilla includes Perilla frutescens (L.) Britton var. acuta Kudo, Perilla frutescens (L.) Britton var. acuta Kudo forma viridis Makino, Perilla frutescens (L.) Britton var. crispa (Thunb) Decne. In the present invention, Perilla frutescens (L.) Britton var. acuta Kudo is particularly preferred. To obtain an extract from perilla, leaves, branches and seeds can be used, with the use of leaves being particularly preferred. Perilla is known to have effects such as antiallergic effects, hypoglycemic effects and skin rejuvenation.

[0023] Houttuynia cordata Thunb. is a plant belonging to Houttuynia cordata. To obtain an extract from Houttuynia cordata Thunb., aerial grass parts and branch parts can be used, with the use of grass parts being particularly preferred. Houttuynia cordata Thunb. is known to have mucosal inflammation suppressing effects.

[0024] Eucommia ulmoides Oliv. is a plant belonging to Eucommia ulmoides. To obtain an extract from Eucommia ulmoides Oliv., leaves and branches can be used, with the use of leaves being particularly preferred. Eucommia ulmoides Oliv. is known to have effects such as blood pressure control, stress relief and prevention of lifestyle related diseases.

[0025] Turmeric is the rootstock of Curcuma longa L. or Curcuma aromatica Salisb. In the present invention, Curcuma longa L. is particularly preferred among plants belonging to Curcuma. Curcuma longa L. is known to have effects such as hepatic function improving effects, hangover preventing effects, gastric antisecretory effects and gastrointestinal dysfunction improving effects.

[0026] Clove is the bud of Syzygium aromaticum (L.) Merr. et Perry or Eugenia caryophyllata Thunb. Clove is known to have preservation effects, uterine contraction activities, dental pain reduction effects, and the like.

[0027] Cinnamon is the bark of Cinnamomum zeylanicum Nees or Cinnamomum cassia Blume. Cinnamomum zeylanicum Nees is particularly preferred among these cinnamomum plants. Cinnamon is known to have effects such as antibacterial activities,

body-warming effects, antipyretic effects, digestive system activation effects, amelioration effects for various cold symptoms, indigestion relief, diarrhea relief and nausea relief.

[0028] Rubus sauvissimus S. Lee (Rosaceae) is a plant
5 belonging to Rubus. To obtain an extract from Rubus sauvissimus
S. Lee (Rosaceae), its leaves and stem can be used, with the
use of its leaves being particularly preferred. Rubus
sauvissimus S. Lee (Rosaceae) is attracting attention in recent
years for its anti-inflammatory activities and antiallergic
10 effects.

[0029] To obtain an extract from one or more of the
above-described food materials, it is only necessary to extract
with a solvent the food material or materials either as they
are or after optionally applying processings such as washing,
15 peeling, drying and/or crushing. Such extracts may be used
either singly or in combination. A mixed extract may also be
used, which is obtained by mixing a plurality of food materials
and extracting them. Among these extracts, preferred are an
extract from persimmon leaves and an extract from Rubus
20 sauvissimus S. Lee (Rosaceae).

[0030] Solvents usable in the extraction include water and
organic solvents such as lower alcohols having 1 to 5 carbon
atoms, e.g., ethanol, ethyl acetate, glycerol and propylene
glycol. Two or more of these solvents may be used together as
25 a mixed solvent. Among these solvents, water and aqueous
solvents such as water-lower alcohols are particularly
preferred.

[0031] No particular limitation is imposed on the extraction
method of an extract from one or more of the above-mentioned
30 food materials with the above-mentioned solvent, but acid
extraction is preferred as it can efficiently extract from the
food material or materials components which enhance the
proliferative activities of lactic acid bacteria and can also
bring about excellent growth-promoting effects even when the
35 extract is added in a small amount. Acid extraction can
preferably be performed under an acidic condition of pH 4.0 or
lower, especially pH 3.0 to 4.0. No particular limitation is

imposed on acid ingredient adapted to regulate the pH of the solvent in this acid extraction, and any ingredient can be used insofar as it is acidic. Among such acid ingredients, preferred are organic acids such as citric acid, malic acid, tartaric acid, succinic acid, lactic acid and acetic acid.

[0032] Furthermore, extraction conditions for the extract with the use of the above-mentioned solvent are not particularly limited, and the extraction processing can be carried out, for example, by treatment for 30 to 60 minutes preferably at 60°C to 120°C, more preferably at 80°C to 100°C.

[0033] The extract obtained as described above may be used as a solution as obtained immediately after the extraction, or as a concentrated extract obtained by purification and concentration of the obtained extract by means of ultrafiltration, centrifugation or the like, or as a powdery extract obtained by further drying the concentrated extract by means of spray drying, freeze drying or the like.

[0034] Upon adding the above-mentioned extract to a medium where lactic acid bacteria can grow, its amount may preferably be determined after an experimental verification since the resulting growth-promoting effects may differ depending on the strains to be cultured, the composition of the medium and the application of the cultured product. In general, however, the extract may be added in an amount preferably of about 0.01 to 10% by weight (hereinafter, simply referred to as "%"), more preferably of about 0.01% to 5% as calculated in terms of an extract having 10 degrees Brix (sugar content).

[0035] These extracts may be added in an amount greater than 10% or more. However, the growth-promoting effects may not be brought about as much as proportional to the amount added. On the contrary, such an excessively large amount of the extract may affect the flavor and taste of the beverage or food containing the resulting medium. It is, therefore, not preferred to add the extract in such an excessively large amount. An amount of such an extract smaller than 0.01%, on the other hand, may not bring about the growth-promoting effects sufficiently and, therefore, is not preferred.

[0036] In the present invention, it is possible to obtain synergic growth-promoting effects and viability-improving effects for lactic acid bacteria by adding oleic acid or a derivative thereof (hereinafter, simply referred to as "the oleic acid") to the medium containing the extract. No particular limitation is imposed on the oleic acid to be added together with the extract to the medium, and illustrative are free oleic acid, inorganic salts of oleic acid, and sugar esters, glycerides, sorbitan esters and propylene glycol esters, which are used commonly as emulsifiers, and contain oleic acid as their fatty acid moiety. It is also possible to use food materials which contain a large amount of the oleic acid. It is, however, to be noted that among those containing the oleic acid in their structures, those having such a form as lysolecithin or the like may not be able to obtain the effects of maintaining the bacterial cell count and activities in the lactic acid bacteria fermentation product of the invention.

[0037] Preferred specific examples of the oleic acid include oleate salts such as sodium oleate and potassium oleate, and oleate esters such as glyceryl oleate, polyglyceryl oleate acid ester and sucrose oleate. Among the above-described oleate esters, glyceryl oleate or polyglyceryl oleate is preferred for its high effects of increasing the cell count and improving viability upon completion of the culture. From the standpoint in physical properties such as solubility in media, sucrose oleate is preferred. These oleic acids may be used singly or in combination.

[0038] The oleic acid can preferably be added to a medium in such an amount that its final concentration in the product becomes 5 to 50 ppm, preferably 5 to 25 ppm in terms of oleic acid. An amount of the oleic acid smaller than 5 ppm may not be able to sufficiently exhibit the synergic effects of activating growth and suppressing death of bacteria in the product when used in combination with the extract. An amount of the oleic acid greater than 50 ppm, on the other hand, may develop a problem in cost and may inhibit the proliferability of bacteria, and therefore, is not preferred.

[0039] In the present invention, the timing of addition of the extract and the oleic acid to a medium can preferably be, but not limited thereto, before the fermentation by lactic acid bacteria. They can also be added during the fermentation by
5 lactic acid bacteria, or after the completion of the fermentation by lactic acid bacteria. They can be added in several portions. It is particularly preferred to add the extract and the oleic acids before the fermentation by lactic acid bacteria, because the cell count and the viability of the bacteria upon completion
10 of the culture can be maintained at high levels.

[0040] Media to which the extract and the oleic acid are to be added include animal milk media composed of fresh milks such as cow milk, goat milk, horse milk and sheep milk or dairy products such as skim milk powder, whole milk powder and fresh cream,
15 and various synthetic media. These media may be those containing ingredients which are used in ordinary media for lactic acid bacteria. Such ingredients include, for examples, vitamins such as vitamin A, vitamin Bs, vitamin C and vitamin E, various peptides and amino acids, and salts such as calcium salts and
20 magnesium salts.

[0041] In the present invention, no particular limitation is imposed on the lactic acid bacteria to be used for culture insofar as it is a microorganism commonly used in the production of foods. Illustrative are bacteria of the genus *Lactobacillus*
25 such as *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus cremoris*, *Lactobacillus helveticus*, *Lactobacillus salivarius*, *Lactobacillus gasseri*, *Lactobacillus fermentum*, *Lactobacillus yoghurtii*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus delbrueckii* subsp. *delbrueckii* and *Lactobacillus johnsonii*, bacteria of the genus *Streptococcus* such as *Streptococcus thermophilus*, bacteria of
30 genus *Lactococcus* such as *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus plantarum* and *Lactococcus raffinolactis*, bacteria of the genus *Enterococcus* such as *Enterococcus faecalis* and *Enterococcus faecium*. Among
35 these lactic acid bacteria, it is preferred to use one or more species selected from the group consisting of the bacteria of

the genus *Lactobacillus*, the bacteria of the genus *Streptococcus* and the bacteria of the genus *Lactococcus*. It is to be noted that the term "lactic acid bacteria" as used herein means facultative anaerobic bacteria, and does not include the bacteria of the genus *Bifidobacteria*, which are anaerobic bacteria.

[0042] The above-mentioned lactic acid bacteria also include those which do not grow sufficiently with media composed of animal milks, and the extract for use in the present invention brings about particularly remarkable effects in the culture of such bacteria. Specifically, excellent growth-promoting effects can be obtained when the extract is added to media upon culturing lactic acid bacteria such as *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus cremoris*, *Lactobacillus helveticus*, *Lactobacillus gasseri*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus* and *Lactococcus lactis* subsp. *lactis*.

[0043] No particular limitations are imposed on the culture conditions for lactic acid bacteria in order to obtain the lactic acid bacteria fermentation product of the present invention. For example, however, culture may be conducted at about 30 to 40°C for 1 to 7 days. As further conditions for such culture, a method suited for lactic acid bacteria to be cultured may be selected from a standing, stirring, shaking, aeration or like method.

[0044] The lactic acid bacteria fermentation product obtained as described above contains viable lactic acid bacteria at high concentration without a reduction in their proliferativeness. This product can be mixed with other auxiliary materials, the addition of which to foods is generally approved, to produce fermented milk foods.

[0045] The term "fermented milk foods" includes fermented milks, dairy products, beverages such as lactic acid bacteria beverages, hard yogurt, soft yogurt, plain yogurt and further, kefir, cheese, etc., which are defined by the Ministerial Ordinance concerning Compositional Standards, etc. for Milk and Milk Products. Fermented milk foods of the present invention, therefore, include various beverages and foods making use of

various lactic acid bacteria, for example, fermented milks, lactic acid bacteria beverages, kefir, cheese and the like, which can be of the plain type, flavored type, fruit type, sweetened type, soft type, drink type, solid (hard) type or frozen type.

5 [0046] These fermented milk foods are obtained by adding, to the above-described lactic acid bacteria fermentation product, a sweetener such as starch syrup and various other food materials, for example, optional ingredients such as various carbohydrates, thickeners, emulsifiers and various vitamins, as needed.

10 Specific examples of these food materials include carbohydrates such as sucrose, glucose, fructose, paratinose, trehalose, lactose, xylose and maltose; glycoalcohols such as sorbitol, xylitol, erythritol, lactitol, palatinate, reduced starch syrup and reduced maltose syrup; sweeteners of high sweetness intensity

15 such as aspartame, thaumatin, sucralose, acesulfame K and stevia; various thickeners (stabilizers) such as agar, gelatin, carrageenan, guar gum, xanthane gum, pectin, locust bean gum, gellan gum, carboxymethylcellulose, soybean polysaccharides and propylene glycol alginate; emulsifiers such as sucrose fatty

20 acid esters, glycerine fatty acid esters, polyglycerine fatty acid esters, sorbitan fatty acid esters and lecithin; milk fats such as cream; butter and sour cream; sour seasonings such as citric acid, lactic acid, acetic acid, malic acid, tartaric acid and gluconic acid; various vitamins such as vitamin A, vitamin

25 Bs, vitamin C and vitamin E; minerals such as calcium, magnesium, zinc, iron and manganese; and flavorings such as yogurt, berry, orange, Chinese quince, perilla, citrus, apple, mint, grape, apricot, pear, custard cream, peach, melon, banana, tropical, herb, black tea and coffee.

30 [0047] The fermented milk food obtained as described-above has a high utility as a beverage or food, which has good flavor and taste, is excellent in health promotion, and does not undergo much deteriorations in flavor and taste. In addition, the lactic acid bacteria fermentation product of the present invention is

35 excellent in growth-promoting effects and viability-improving effects for lactic acid bacteria owing to the extract added to the culture, and therefore, has and maintains a sufficient lactic

acid bacteria cell count. When oleic acid or the like is incorporated in the medium in addition to the extract, synergic effects are recognized with respect to the growth-promoting effects and viability-improving effects of lactic acid bacteria.

5 [0048] Although the mechanism of action of the extract on the growth-promoting effects and viability-improving effects for lactic acid bacteria in the present invention has not been elucidated yet, it is presumed that the extract contains abundant minerals, and these minerals contribute to the growth
10 promotion and viability improvement of lactic acid bacteria. It is also presumed that, when the extract is combined with oleic acids or the like, synergic effects of the minerals and oleic acid or the like achieve the growth promotion and viability improvement of lactic acid bacteria.

15

EXMAPLES

[0049] The present invention will hereinafter be described in further detail based on Examples. It should, however, be borne in mind that the present invention is by no means limited
20 to the following examples.

[0050] Example 1

<Extract Preparation 1>

Turmeric (the rootstock of Curcuma longa L.), the aerial grass part of Houttuynia cordata Thunb., leaves of Eucommia ulmoides Oliv., persimmon leaves (leaves of Diospyros kaki Thunb.), leaves of Perilla frutescens (L.) Britton var. acuta Kudo, clove (the bud of Syzygium aromaticum (L.) Merr. et Perry) and cinnamon (the bark of Cinnamomum zeylanium Nees) were each separately subjected to processings such as peeling and crushing,
25 and then extracted for 60 minutes with hot water of 90°C (in an amount 10 times as much as the weight of the corresponding raw material) to prepare extracts of turmeric, Houttuynia cordata Thunb., Eucommia ulmoides Oliv., persimmon leaves, perilla, clove and cinnamon, respectively. The extracts were each
30 separately concentrated to 10 degrees Brix in an evaporator.

35

[0051] Example 2

<Comparison in the Proliferability of Lactic Acid Bacteria>

As a basal medium, 12% skim milk powder, was furnished. The extracts of turmeric, Houttuynia cordata Thunb., Eucommia ulmoides Oliv., persimmon leaves, perilla, clove, and cinnamon, which had been prepared and adjusted to 10 degrees Brix in Example 1, were added at 1% to aliquots of the basal medium, respectively, followed by sterilization to prepare sterilized media. To each of those media, a starter of Lactobacillus casei YIT9029 was inoculated at 1%, and the bacteria strain was then cultured at 37°C for 48 hours. Employed as a comparative medium was one prepared by adding "MEAST" (trademark for brewery beer yeast autolysate; product of Asahi Food and Healthcare Co., Ltd.) at 0.15% to the basal medium and then sterilizing the medium. The amount of "MEAST" so added is the upper limit of a range in which its adverse effects on the flavor and taste of the culture is acceptable.

[0052] The proliferabilities of the lactic acid bacteria in the respective cultures were then compared relying upon the acidities of the cultures (titration values of 0.1 N caustic soda when portions (9 g) of the respective cultures were taken and an organic acid in the respective cultured portions 0.1 N caustic soda until pH 8.5 was reached; unit: mL) as indices. The results are shown below in Table 1.

[0053]

[Table 1]

Basal medium	MEAST	Turmeric extract	<u>Houttuynia cordata Thunb.</u> extract	<u>Eucommia ulmoides Oliv.</u> extract	Persimmon leaf extract	Perilla extract	Clove extract	Cinnamon extract
Acidity								
8.2	10.1	11.1	10.9	11.0	11.3	10.7	10.9	10.7

25

[0054] As is clear from Table 1, it has been confirmed that the acidity becomes higher in a medium with an extract of turmeric, Houttuynia cordata Thunb., Eucommia ulmoides Oliv., persimmon leaves, perilla, clove or cinnamon added therein, than in a medium without the addition of any extract or a medium with "MEAST" added therein. This indicates that the growth of lactic acid

bacteria can be promoted by these extracts.

[0055] Example 3

<Verification of Effects of Acid Extraction Extract on Proliferability of Lactic Acid Bacteria>

5 Under similar conditions as in the extract preparation in Example 1 except for the use of water and aqueous solutions, the pHs of which had been adjusted to 3.0, 4.0 and 5.0, respectively, with citric acid instead of hot water, persimmon leaves were treated. To aliquots of a 15% skim milk powder medium (with 10 3% glucose contained therein), said aliquots containing the thus-obtained extracts added therein at 1%, respectively, the starter of Lactobacillus casei YIT9029 was inoculated at 1%. The bacteria strain was then cultured at 35°C for 5 days. The acidities of the resultant cultures were measured in a similar 15 manner as in Example 2. The results are shown in Table 2.

[0056]

[Table 2]

Test Strain	Hot water	pH 3.0	pH. 4.0	pH 5.0
<u>Lactobacillus casei</u> YIT9029	23.1	24.4	24.5	23.5

[0057] As shown in Table 2, it has been confirmed that the 20 proliferability for lactic acid bacteria when tends to become remarkable with an extract obtained by adjusting the pH of an extraction solvent to 5.0 or less.

[0058] Example 4

<Extract Preparation 2>

25 Turmeric (the rootstock of Curcuma longa L.), the aerial grass part of Houttuynia cordata Thunb., leaves of Eucommia ulmoides Oliv., persimmon leaves (leaves of Diospyros kaki Thunb.), leaves of Perilla frutescens (L.) Britton var. acuta Kudo, clove (the bud of Syzygium aromaticum (L.) Merr. et Perry) 30 and cinnamon (the bark of Cinnamomum zeylanium Nees), were each separately subjected to processings such as peeling and crushing, and then extracted under similar conditions as in Example 1 except for the use of water and an aqueous solution, the pH of which had been adjusted to pH 4.0 with citric acid, (in amounts 10 35 times as much as the weight of the corresponding raw material)

to prepare extracts of turmeric, Houttuynia cordata Thunb., Eucommia ulmoides Oliv., persimmon leaves, perilla, clove and cinnamon, respectively. They were each separately concentrated to 10 degrees Brix, in an evaporator.

5 [0059] Example 5

<Verification of Effects of Extract on Proliferability of Lactic Acid Bacteria >

As a basal medium, 16% skim milk powder was furnished. The extract of turmeric, Houttuynia cordata Thunb., Eucommia ulmoides Oliv., persimmon leaves, perilla, clove and cinnamon by 1%, which had been adjusted to 10 degrees Brix in Example 4, were added at 1% to aliquots of the basal medium to prepare media, respectively. Into each of those media, the starters of various lactic acid bacteria strains were inoculated at 0.1%, and those bacteria strains were cultured at 37°C for 48 hours.

[0060] In the above culture were used Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus cremoris, Lactobacillus helveticus, Lactobacillus gasseri, Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophilus and Lactococcus lactis subsp. lactis.

[0061] The acidities of the resultant cultures were measured in a similar manner as in Example 2 to compare the proliferabilities of the various lactic acid bacteria. The results are shown in Table 3.

25 [0062]

[Table 3]

Test strain	Basal medium	Turmeric extract	<u>Houttuynia cordata</u> Thunb. extract	<u>Eucommia ulmoides</u> Oliv. extract	Persimmon leaf extract	Perilla extract	Clove extract	Cinnamon extract
	Acidity							
<u>Lactobacillus casei</u> YIT9029	8.1	13.5	13.1	13.0	14.0	12.2	14.1	13.2
<u>Lactobacillus acidophilus</u> YIT0070	9.0	11.4	10.7	11.1	11.7	11.4	10.0	10.4
<u>Lactobacillus cremoris</u>	1.4	6.7	6.1	6.4	7.1	5.8	6.2	6.1

YIT2002								
<u>Lactobacillus helveticus</u> YIT0100	17.2	17.8	17.2	17.5	17.7	17.4	17.5	17.6
<u>Lactobacillus gasseri</u> YIT0192	2.8	9.1	9.5	9.8	10.0	8.1	8.1	7.2
<u>Lactobacillus delbrueckii</u> subsp. <u>bulgaricus</u> YIT0098	14.9	16.1	15.8	16.4	16.2	15.4	16.7	16.1
<u>Streptococcus thermophilus</u> YIT2001	7.6	8.9	8.5	8.9	8.7	7.9	8.4	8.1
<u>Lactococcus lactis</u> subsp. <u>lactis</u> YIT2013	6.2	7.2	6.8	6.9	7.0	6.2	6.4	6.5

[0063] As is clear from Table 3, the effects of these extracts on the proliferability of various lactic acid bacteria have been confirmed with substantially all the strains, although they vary depending on the species of the strains. Remarkable effects have been confirmed particularly with the extracts of turmeric, Houttuynia cordata Thunb., Eucommia ulmoides Oliv. and persimmon leaves. Further, the proliferative effects have been confirmed to have a tendency of giving excellent effects to strains which are poor in proliferation on the basal medium. This suggests even when lactic acid bacteria hard to grow in an animal medium is used, the use of these extracts make it possible to easily obtain a culture of a large cell count.

[0064] Example 6

15 <Preparation of Lactic Acid Bacteria Beverage>

A 15% skim milk powder medium (with 3% of glucose contained therein) was furnished as a basal medium. The various extracts prepared in Example 4 were added at 0.1% to aliquots of the basal medium to provide test media, respectively. After sterilizing those media under heat, the starter of Lactobacillus casei YIT9029 was inoculated at 0.5% to the respective media, and the bacteria strain was cultured at 35°C for 5 days to obtain respective cultures. Each culture was homogenized at 15 MPa, and to 20 parts by weight of that culture, 80 parts by weight

of a 15% sugar solution, which had been sterilized at 100°C for 5 minutes, was added, and a yogurt flavoring (product of Yakult Material Co., Ltd.) was further added at 0.1% to prepare a dairy product. A taste test was conducted by five well-experienced
5 5 assessors on each of the dairy products obtained as described above. No difference was confirmed between any of the lactic acid bacteria beverages and the control product which contained the culture obtained with the use of the basal medium.

[0065] In addition, the various extracts were assessed to
10 give no flavor and /or taste-related effects to the basal medium and to match very well. It has, therefore, been also confirmed that their use in cultures for beverages or foods such as lactic acid bacteria beverages does not lead to deteriorations in their flavors or tastes.

15 [0066] Example 7

<Effects of Added Amount of Persimmon leaf Extract on Flavor and Taste and Proliferative Effects>

(1) Preparation of Persimmon Leaf Extracts

Using water and a solution, the pH of which has been adjusted
20 to 4.0 with citric acid, in amounts of 10 times as much as persimmon leaves, persimmon leaf extracts were prepared under similar conditions as in Example 1. Those extracts were each separately concentrated to 10 degrees Brix in an evaporator.

[0067]

25 (2) Determination of an amount to be added

To aliquots of a 15% skim milk powder medium (with 3% of glucose contained therein) the persimmon leaf extracts prepared above in (1) was added at concentrations in a range of 0.01 to 10%, respectively, followed by sterilization at 100°C for 60
30 minutes to prepare media for culturing lactic acid bacteria. To those media, the starter of Lactobacillus casei YIT9029 was inoculated at 1%, and the bacteria strain was cultured at 35°C until the acidities (titration values of 0.1 N sodium hydroxide required for the neutralization of 9 g portions of the respective
35 samples) became 24. The cell count of the lactic acid bacteria in each of the cultures was determined by BCP medium. Each culture was homogenized at 15 MPa, and to 20 parts by weight

of the homogenized culture, 80 parts by weight of a 15% sugar solution, which had been sterilized at 100°C for 5 minutes, was added, and a yogurt flavoring (product of Yakult Material Co., Ltd.) was further added at 0.1% to prepare a dairy product. With respect to such dairy products a flavor and taste assessment was conducted by five trained organoleptic assessors based on the following standards. The results are shown in Table 4.

[0068] <Assessment standards>

(Ranking) (Description)

- 10 A: Very good
B: Good
C: Average
D: Poor
E: Very poor

15 [0069]

[Table 4]

	Added amount of persimmon leaf extract (%)	Culture time (hrs)	Viable cell count of lactic acid bacteria (/mL)	Flavor and taste assessment
Not added	0	192	1.9×10^9	A
Water extraction	0.01	147	2.7×10^9	A
	0.1	130	3.2×10^9	A
	1	121	5.5×10^9	B
	5	116	5.3×10^9	B
	10	116	5.2×10^9	D
Acid extraction (pH 4.0)	0.01	128	3.5×10^9	A
	0.1	120	5.4×10^9	A
	1	117	5.7×10^9	B
	5	117	5.9×10^9	B
	10	115	5.5×10^9	D

[0070] It has been confirmed from Table 4 that the addition of an extract of persimmon leaves at 0.1% or so is effective for the promotion of culture by lactic acid bacteria, and moreover, can increase the viable cell count of lactic acid bacteria. It has also been ascertained that the addition of an extract of

persimmon leaves even as much as 10% to a medium can not bring about any additional excellent effects in proportion to the amounts added, but on the contrary, the flavor and taste derived from the extract tend to affect the flavor and taste of the product.

- 5 It has also been confirmed that the growth-promoting effects of the extract are exhibited more remarkably with one obtained by acid extraction than with one obtained by water extraction.

[0071] Example 8

<Extract Preparation 3>

- 10 Ricebran (a mixture of pericarps, aleurone layers and germs of kernels (brown rice) available from Oryza sativa without the chaff of paddy), turmeric (the rootstock of Curcuma longa L.), aerial grass part of Houttuynia cordata Thunb., layers of Eucommia ulmoides Oliv., persimmon leaves (leaves of Diospyros
15 kaki Thunb.), leaves of Perilla frutescens (L.) Britton var. acuta Kudo, clove (the bud of Syzygium aromaticum (L.) Merr. et Perry) and cinnamon (the bark of Cinnamomum zeylanium Nees) were each separately subjected to processings such as peeling and crushing, and then extracted for 60 minutes with hot water of
20 80°C (in an amount 10 times as much as the weight of the corresponding raw material) to prepare extracts of turmeric, Houttuynia cordata Thunb., Eucommia ulmoides Oliv., persimmon leaves, perilla, clove and cinnamon, respectively. The extracts were each separately concentrated to 10 degrees Brix
25 in an evaporator.

[0072] Example 9

<Determination of Lactic Acid Bacteria Cell Count upon Completion of Culture (1)>

- To aliquots of a 15% skim milk powder medium (with 3% of
30 glucose contained therein) as a basal medium, the extracts of ricebran, persimmon leaves, perilla, Houttuynia cordata Thunb., Eucommia ulmoides Thunb., turmeric, clove and cinnamon, which were prepared and adjusted to 10 degrees Brix in Example 8, were added at 1%, respectively, followed by sterilization at 100°C
35 for 60 minutes to prepare sterilized media. To those sterilized media, the starter of Lactobacillus casei YIT9029 was inoculated at 1%, and the bacteria strain was cultured at 37°C until the

pHs of the respective media reached 3.7. The viable cell counts were similarly determined upon completion of the culture. In addition, a medium with sodium oleate added in place of the above-described extract to give a concentration of 25 ppm in terms of oleic acid and another medium with both of the above-described extract and sodium oleate added were also prepared. The viable cell counts were similarly determined upon completion of the culture. It is to be noted that determination of each viable cell count was performed by counting colonies formed after incubating the corresponding sample, which had been suitably diluted in a physiological solution, at 37°C for 3 days on BCP medium. The results are shown in Table 5.

[0073]

[Table 5]

	Additive	Cell count of lactic acid bacteria (cfu/mL)		Additive(s)	Cell count of lactic acid bacteria (cfu/mL)
Comparative Product 1	Not added	1.9×10^9	Comparative Product 2	Sodium oleate	2.1×10^9
Invention Product 1	Rice bran extract	4.7×10^9	Invention Product 2	Rice bran extract, sodium oleate	7.1×10^9
Invention Product 3	Persimmon leaf extract	5.2×10^9	Invention Product 4	Persimmon leaf extract, sodium oleate	7.8×10^9
Invention Product 5	Perilla extract	3.6×10^9	Invention Product 6	Perilla extract, sodium oleate	6.6×10^9
Invention Product 7	<u>Houttuynia cordata</u> Thunb. extract	4.2×10^9	Invention Product 8	<u>Houttuynia cordata</u> Thunb. extract, sodium oleate	7.0×10^9
Invention Product 9	<u>Eucommia ulmoides</u> Oliv. extract	4.3×10^9	Invention Product 10	<u>Eucommia ulmoides</u> Oliv. extract, sodium oleate	7.4×10^9
Invention	Turmeric extract	4.2×10^9	Invention	Turmeric extract,	6.8×10^9

Product 11			Product 12	sodium oleate	
Invention Product 13	Clove extract	4.5×10^9	Invention Product 14	Clove extract, sodium oleate	6.9×10^9
Invention Product 15	Cinnamon extract	4.4×10^9	Example Product 16	Cinnamon extract, sodium oleate	6.4×10^9

[0074] It has been indicated from Table 5 that the combined use of any one of extracts of rice bran, persimmon leaves, perilla, Houttuynia cordata Thunb., Eucommia ulmoides Thunb., turmeric, clove and cinnamon with sodium oleate can synergically increase the cell count of lactic acid bacteria upon completion of the culture as compared with the single use of the same extract or sodium oleate.

[0075] Example 10

<Determination of Viable Cell Count of Lactic Acid Bacteria in Dairy Product (1)>

The cultures prepared in Example 9 (Comparative Products 1 and 2 and Invention Products 3 and 4) were each separately homogenized at 15 MPa. To aliquots (20 parts by weight) of those homogenized cultures, 80 parts by weight of a 15% sugar solution which had been sterilized at 100°C for 5 minutes were added, followed by the further addition of a yogurt flavoring at 0.1% to prepare dairy products. Those dairy products were filled in containers, and the viable cell counts were determined in a similar manner as in Example 9 immediately after the production of the dairy products and after their storage at 10°C for 14 days. The results are shown in Table 6.

[0076]

[Table 6]

	Additive(s)	Cell count of lactic acid bacteria (cfu/mL)	
		Immediately after production	After storage at 10°C for 14 days
Comparative Product 3	Not added	4.2×10^8	1.1×10^8
Comparative	Sodium oleate 25 ppm	9.0×10^8	4.4×10^8

Product 4			
Invention Product 17	Persimmon leaf extract 1% by weight	1.0×10^9	3.8×10^8
Invention Product 18	Persimmon leaf extract 1% by weight Sodium oleate 25 ppm	1.8×10^9	1.1×10^9

[0077] It has been indicated from Table 6 that a dairy product obtained by using, as a raw material, a culture prepared by using an extract of persimmon leaves and sodium oleate in combination is excellent in its effect to suppress changes in the cell count of lactic acid bacteria in the product during storage as compared with a dairy product available from the use of a culture which contains none of them (not added) or contains only one of them.

[0078] Example 11

<Determination of Viable Cell Count of Lactic Acid Bacteria upon Completion of Culture (2)>

Lactobacillus casei YIT9029 was cultured under similar conditions as in Example 9 except that to aliquots of the basal medium prepared in Example 9, various oleate-based emulsifiers were added respectively, each in combination with 1% of the extract of persimmon leaves prepared in Example 8, such that the emulsifiers amounted to 25 ppm in terms of the content of oleic acid. The viable cell counts of the bacteria in the resulting cultures were determined according to the method of Example 9. The results are shown in Table 7.

[0079]

[Table 7]

	Additive(s)	Cell count of Lactic acid bacteria(cfu/mL)
Invention Product 19	Persimmon leaf extract	2.3×10^9
Invention Product 20	Persimmon leaf extract, sodium oleate	7.1×10^9
Invention Product 21	Persimmon leaf extract, glyceryl oleate	7.3×10^9
Invention Product 22	Persimmon leaf extract, pentaglyceryl trioleate	3.9×10^9
Invention Product 23	Persimmon leaf extract,	6.9×10^9

	hexaglyceryl monooleate	
Invention Product 24	Persimmon leaf extract, decaglyceryl decaoleate	4.2×10^9
Invention Product 25	Persimmon leaf extract, sucrose oleate	7.0×10^9
Invention Product 26	Persimmon leaf extract, glyceryl oleate	3.2×10^9

[0080] It has been indicated from Table 7 that the use of the oleic acid as derived from any one of the emulsifiers can provide the resulting culture with a higher cell count of lactic acid bacteria owing to the use of the extract of persimmon leaves in combination.

The use of glyceryl oleate, hexaglyceryl monooleate or sucrose oleate among these emulsifiers can bring about remarkable effects.

[0081] Example 12

<Determination of Viable Cell Count of Lactic Acid Bacteria upon Completion of Culture (3)>

Under similar conditions as in the extract preparation in Example 8 except for the use of water and aqueous solutions, the pHs of which has been adjusted to pH to 3.0, 4.0 and 5.0, respectively with citric acid instead of hot water, rice bran, persimmon leaves, Eucommia ulmoides Thunb., turmeric and clove were each treated to prepare their extracts of 10 degrees Brix. To aliquots of a 15% skim milk powder medium with the thus-prepared extracts added at 0.1%, respectively, sodium oleate was added at 25 ppm in terms of the content of oleic acid, and further, the starter of Lactobacillus casei YIT9029 was inoculated at 1%. The bacteria strain was then cultured at 37°C until the pH reached 3.7. The viable cell counts of lactic acid bacteria in the resultant cultures were determined according to the method of Example 9. The results are shown in Table 8.

[0082]

[Table 8]

	Additives	Extraction pH	Cell count of lactic acid
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			bacteria (cfu/mL)
Invention Product 27	Rice bran extract, sodium oleate	5.0	7.1×10^9
Invention Product 28		4.0	9.0×10^9
Invention Product 29		3.0	9.4×10^9
Invention Product 30	Persimmon leaf extract, sodium oleate	5.0	8.5×10^9
Invention Product 31		4.0	8.8×10^9
Invention Product 32		3.0	9.6×10^9
Invention Product 33	<u>Eucommia ulmoides</u>	5.0	7.0×10^9
Invention Product 34	<u>Oliv.</u> extract, sodium oleate	4.0	8.4×10^9
Invention Product 35		3.0	9.1×10^9
Invention Product 36	Turmeric extract, sodium oleate	5.0	7.4×10^9
Invention Product 37		4.0	8.5×10^9
Invention Product 38		3.0	8.4×10^9
Invention Product 39	Clove extract, sodium oleate	5.0	7.0×10^9
Invention Product 40		4.0	8.5×10^9
Invention Product 41		3.0	8.4×10^9

[0083] It has been indicated from Table 8 that an extract obtained by acid extraction tends to provide a higher cell count upon completion of culture as the pH of the solvent used in the extraction becomes lower. This effect is pronouncedly observed especially with various extracts obtained at pH 5.0 or lower, more preferably, pH 4.0 or lower.

[0084] Example 13

<Determination of Viable Cell Count of Lactic Acid Bacteria upon Completion of Culture (4)>

Using a citric acid solution of pH 4.0, an extract of persimmon leaves of 10 degrees Brix was prepared under similar conditions as in Example 8. To 10% skim milk powder, the extract was added at 1% and further, sodium oleate was also added at 25ppm in terms of oleic acid. The resultant mixture was sterilized to prepare a sterilized medium. To aliquots of that sterilized medium, starters of various lactic acid bacteria were inoculated at 0.1%, respectively and the bacteria strains were cultured at 37°C for 24 hours. As the lactic acid bacteria, were used Lactobacillus bulgaricus YIT0098, Lactobacillus

acidophilus YIT0071 and Lactobacillus casei YIT9029. In addition, those lactic acid bacteria were cultured in similar manner as described above in a 10% skim milk powder medium for the sake of comparison. The lactic acid bacteria cell counts
5 in the resultant cultures were determined in a similar manner as in Example 9. The results are shown in Table 9.

[0085]

[Table 9]

	Additives	Cell count of lactic acid bacteria (cfu/mL)	
		Medium with no addition	Medium with addition
<u>Lactobacillus bulgaricus</u> YIT0098	Persimmon leaf extract, sodium oleate	3.3×10^8	6.8×10^8
<u>Lactobacillus acidophilus</u> YIT0071		3.5×10^6	2.4×10^7
<u>Lactobacillus casei</u> YIT9029		7.7×10^8	5.0×10^9

10 [0086] It has been indicated from Table 9 that the effect of increasing the lactic acid bacteria cell count, which is available from the combined use of an acid-extracted persimmon leaf extract and sodium oleate, can be recognized for all lactic acid bacteria although the effect has been confirmed to somewhat
15 differ depending on the species of the lactic acid bacteria.

[0087] Example 14

<Determination of Viable Cell Count of Lactic Acid Bacteria upon Completion of Culture (5)>

20 Using a citric acid solution of pH 4.0, an extract of persimmon leaves of 10 degrees Brix was prepared under similar conditions as in Example 8. The extract and glyceryl oleate, as oleic acid, were added to aliquots of a 15% skim milk powder medium (with 3% of glucose contained therein) such that their added amounts reached those shown below in Table 10, respectively.
25 The resultant media were sterilized at 100°C for 60 minutes, to prepare sterilized media. To the respective sterilized media, the starter of Lactobacillus casei YIT9029 was inoculated at 1%, and the bacteria strain was cultured at 37°C until their pHs reached 3.7. In addition, culture was similarly carried

out as a control by adding an yeast extract (product of DIFCO), which is generally known as a culture promoter, at 0.2% to the medium. The lactic acid bacteria cell counts in the resultant cultures were determined in a similar manner as in Example 9.

5 The results are shown in Table 10.

[0088]

[Table 10]

Added amount of persimmon leaf extract (%) \ Added amount of oleic acid (ppm)	0	5	25	50
0	2.3×10^9			
0.01		5.0×10^9	5.1×10^9	5.4×10^9
0.1		7.4×10^9	8.0×10^9	7.6×10^9
5.0		8.5×10^9	9.0×10^9	9.1×10^9
10.0		9.1×10^9	9.5×10^9	9.4×10^9
Yeast extract (0.2)	2.7×10^9			

[0089] It has been confirmed from Table 10 that the effect of increasing the viable cell count be clearly recognized by adding 0.1% or more of the persimmon leaf extract and 5 ppm or more of oleic acid in combination. It has also been indicated that the resulting viable cell count is greater than that available from the addition of an yeast extract.

15 [0090] Example 15

<Determination of Viable Cell Count of Lactic Acid Bacteria in Dairy Product (2)>

Using the cultures prepared in Example 14, dairy product were produced in a similar manner as in Example 10. A flavor and taste assessment was performed by five trained organoleptic assessors on those dairy products on the basis of the following standards. The results are shown in Table 11.

[0091] <Assessment standards>

	(Ranking)	(Description)
25	A:	Very good
	B:	Good
	C:	Average
	D:	Poor
	E:	Very poor

[0092]

[Table 11]

Added amount of oleic acid (ppm) Added amount of persimmon leaf extract(%)	0	5	25	50
0	B			
0.01		B	B	B
0.1		B	B	B
5.0		B	B	B
10.0		C	C	C
Yeast extract (0.2)	D			

[0093] It has been indicated from Table 11 that when addition of the extract of persimmon leaves at 10% to the medium, i.e., at 2% per dairy product affects the flavor and taste of the dairy product irrespective of the added amount of oleic acid and therefore, that this added amount can be considered to be the acceptable addition upper limit. It is to be noted that even with the addition amount of the persimmon leaf extract in that amount, the product had a better flavor and taste than that available from the addition of the yeast extract.

[0094] Example 16

<Extract Preparation 4>

Leaves of Rubus suavissimus S. Lee (Rosaceae) were subjected to processings such as peeling, crushing and roasting, and then extracted for 60 minutes with hot water of 90°C (in an amount of 10 times as much as the weight of the leaves of Rubus suavissimus S. Lee (Rosaceae)), to prepare an extract of Rubus suavissimus S. Lee (Rosaceae). The resultant extract was concentrated to 10 degrees Brix in an evaporator.

[0095] Example 17

<Verification of the Effects for Lactic Acid Bacteria (1)>

As a basal medium, 12% skim milk powder was furnished. The extract of Rubus suavissimus S. Lee (Rosaceae), which had been prepared and adjusted to 10 degrees Brix in Example 16, was added

at 0.5% to the basal medium followed by sterilization to prepare a sterilized medium. To that sterilized medium, the starter of Lactobacillus casei YIT9029 was inoculated at 1%, and the bacteria strain was cultured at 37°C for 48 hours. Employed as a comparative example was one prepared by adding "MEAST" (trademark for brewery yeast autolysate; product of Asahi Food and Healthcare Co., Ltd.) at 0.15% to the basal medium and then sterilizing the medium. The amount of "MEAST" so added is the upper limit of a range in which its adverse effects on the flavor and taste of the culture is acceptable.

[0096] The proliferability of the lactic acid bacteria in which the culture was then compared relying upon the acidity of the culture (titration value of 0.1 N caustic soda when a portion (9 g) of culture was taken and an organic acid in the culture portion was reached; unit: mL as an index). The results are shown in Table 12.

[0097]

[Table 12]

	Acidity
Basal medium	8.0
"MEAST"	10.0
<u>Rubus suavissimus</u> S. Lee (Rosaceae) extract	11.7

[0098] As evident from Table 12, it has been confirmed that the acidity becomes higher in a medium with the Rubus suavissimus S. Lee (Rosaceae) extract added therein than in a medium with no addition or with "MEAST" added therein. This indicates that the proliferability of lactic acid bacteria can be promoted by a Rubus suavissimus S. Lee (Rosaceae) extract.

[0099] Example 18

<Verification of the Effects for Lactic Acid Bacteria (2)>

Under similar conditions as in the extract preparation method in Example 16 except for the use of aqueous solutions (90°C), the pHs of which has been adjusted to pH 3.0, 4.0 and 5.0, respectively with citric acid instead of hot water, leaves of Rubus suavissimus S. Lee (Rosaceae) were treated to prepare Rubus suavissimus S. Lee (Rosaceae) extracts of 10 degrees Brix.

To aliquots of a 15% skim milk powder medium (with 3% of glucose contained therein), said aliquots containing the thus-obtained extracts added therein at 1%, respectively the starter of Lactobacillus casei YIT9029 was inoculated at 1%. The bacteria strain was cultured at 35°C for 5 days. The acidities of the resultant cultures were measured in a similar manner as in Example 17. The results are shown in Table 13.

[0100]

[Table 13]

Test Strain	Hot water	pH 3.0	pH 4.0	pH 5.0
<u>Lactobacillus casei</u> YIT9029	23.2	24.6	24.8	23.8

[0101] As shown in Table 13, it has been confirmed that the proliferability for lactic acid bacteria tends to become remarkable with a Rubus suavissimus S. Lee (Rosaceae) extract obtained by adjusting the pH of an extraction solvent to 5.0 or lower.

[0102] Example 19

<Extract Preparation 5>

Leaves of Rubus suavissimus S. Lee (Rosaceae) were subjected to processings such as peeling, crushing and roasting, and then extracted under similar conditions as in Example 16 with an aqueous solution of citric acid adjusted to pH 4.0 (in an amount of 10 times as much as the weight of the leaves of Rubus suavissimus S. Lee (Rosaceae)) to prepare an extract of Rubus suavissimus S. Lee (Rosaceae). The thus-obtained extract was concentrated to 10 degrees Brix in an evaporator.

[0103] Example 20

<Verification of the Effects for Lactic Acid Bacteria (3)>

A 16% skim milk powder was furnished as a basal medium, and to medium, the Rubus suavissimus S. Lee (Rosaceae) extract adjusted to 10 degrees Brix in Example 19 at 1% to prepare a medium. To aliquots of that medium, starters of various lactic acid bacteria were inoculated at 0.1%, and the bacteria strains were cultured at 37°C for 48 hours.

[0104] In above culture were used Lactobacillus casei,

Lactobacillus acidophilus, Lactobacillus cremoris,
Lactobacillus helveticus, Lactobacillus gasseri, Lactobacillus
delbrueckii subsp. bulgaricus, Streptococcus thermophilus and
Lactococcus lactis subsp. lactis.

5 [0105] The acidities of the resultant cultures were measured
in a similar manner as in Example 17 to compare the proliferability
of the various lactic acid bacteria. The results are shown in
Table 14.

[0106]

10 [Table 14]

Test Strain	Basal medium	<u>Rubus suavissimus</u> S. Lee (Rosaceae) Extract
<u>Lactobacillus casei</u> YIT9029	8.7	14.5
<u>Lactobacillus acidophilus</u> YIT0070	9.2	11.4
<u>Lactobacillus cremoris</u> YIT2002	1.2	6.5
<u>Lactobacillus helveticus</u> YIT0100	17.0	17.0
<u>Lactobacillus gasseri</u> YIT0192	2.2	11.0
<u>Lactobacillus delbrueckii</u> subsp. <u>bulgaricus</u> YIT0098	14.5	16.5
<u>Streptococcus thermophilus</u> YIT2001	7.0	8.2
<u>Lactococcus lactis</u> subsp. <u>lactis</u> YIT2013	6.4	6.8

[0107] As is clear from Table 14, the effects of the Rubus
suavissimus S. Lee (Rosaceae) extract on the proliferability
of the various lactic acid bacteria have been confirmed with
15 substantially all the strains, although they vary depending on
the species of the strains. Further, these proliferative
effects have been confirmed to have tendency of giving excellent
effects to strains which are not very good in proliferation on
the basal medium. This suggests that even when lactic acid
20 bacteria hard to grow in an animal medium is used, the use of
a Rubus suavissimus S. Lee (Rosaceae) extract makes it possible
to easily obtain a fermentation product with a large number of
bacteria cell count.

[0108] Example 21

25 <Investigation on the Amount of Rubus suavissimus S. Lee

(Rosaceae) Extract to be Added>

(1) Preparation of Rubus suavisissimus S. Lee (Rosaceae) Extract

Using an aqueous solution of citric acid the pH of which had been adjusted to pH 4.0, in an amount as much as leaves of
5 Rubus suavisissimus S. Lee (Rosaceae), a Rubus suavisissimus S. Lee (Rosaceae) extract was prepared under similar conditions as in Example 16. The extract was then concentrated to 10 degrees Brix in an evaporator.

[0109]

10 (2) Determination of an amount to be added

To aliquots of a 15% skim milk powder medium (with 3% of glucose contained therein), Rubus suavisissimus S. Lee (Rosaceae) extract of 10 degrees Brix, which was prepared above in (1) was added at concentrations in a range of 0.01 to 10%, respectively,
15 followed by sterilization at 100°C for 60 minutes to prepare a medium for culturing lactic acid bacteria. To those media, the starter of Lactobacillus casei YIT9029 was inoculated at 1%, and the bacteria strain was cultured at 35°C until the acidities (titration values of 0.1 N sodium hydroxide required
20 for neutralization of 9 g portions of respective samples) became 24. The cell count of the lactic acid bacteria in each of the cultures was determined by BCP medium. The culture was homogenized at 15 MPa, and to 20 parts by weight of the homogenized culture, 80 parts by weight of a 15% sugar solution, which had
25 been sterilized at 100°C for 5 minutes at 100°C were added, and a yogurt flavoring (product of Yakult Material Co., Ltd.) was further added at 0.1% to prepare a dairy product. With respect to such dairy products, a flavor and taste assessment was performed by five trained organoleptic assessors on the base
30 of the following standards. The results are shown in Table 15.

[0110] <Assessment standards>

	(Ranking)	(Description)
	A:	Very good
	B:	Good
35	C:	Average
	D:	Poor
	E:	Very poor

[0111]

[Table 15]

	Added amount of <u>Rubus suavisissimus</u> S. Lee (Rosaceae) extract (%)	Culture time (hrs)	Viable cell count of lactic acid bacteria (cfu/mL)	Flavor and taste assessment
Not added	0	184	1.2×10^9	A
Water extraction	0.01	144	2.3×10^9	A
	0.1	123	3.0×10^9	A
	1	120	4.2×10^9	A
	5	118	4.5×10^9	B
	10	116	4.8×10^9	C
Acid extraction (pH 4.0)	0.01	132	3.0×10^9	A
	0.1	121	4.2×10^9	A
	1	118	5.1×10^9	A
	5	115	4.9×10^9	B
	10	115	5.3×10^9	C

[0112] It has been confirmed from Table 15 that the addition
 5 at 0.01% or so of an extract of Rubus suavisissimus S. Lee (Rosaceae)
 can bring about proliferative effects for lactic acid bacteria,
 and moreover, can increase the viable cell count of lactic acid
 bacteria. It has also been ascertained that the addition of
 an extract Rubus suavisissimus S. Lee (Rosaceae) even as much as
 10 more than 10% does not bring about extra effect in proportion
 to the amount so added, but on the contrary, tends to affect
 the flavor and taste of the product. It has also been confirmed
 that the effects of the extract are exhibited more remarkably
 15 by water extraction.

[0113] Example 22

<Verification of the Effects for Lactic Acid Bacteria (4)>

To aliquots of a 15% skim milk powder medium (with 3% of
 glucose contained therein) as a basal medium, the Rubus
 20 suavisissimus S. Lee (Rosaceae) extracts, which were prepared and
 adjusted to 10 degrees Brix in Example 16 and Example 19,

respectively were added at 1%, respectively, followed by sterilization at 100°C for 60 minutes to prepare sterilized media. To those media, the starter of Lactobacillus casei YIT9029 was inoculated at 1%, and the bacteria strain, which was cultured at 37°C until the pHs of the respective media reached 3.7. The viable cell counts were determined upon completion of the culture. In addition, a medium with sodium oleate added instead of the above-described extract to give a concentration of 25 ppm in terms of oleic acid and another medium both of the above-described extract and sodium oleate added were also prepared. The viable cell counts were similarly determined upon completion of the culture. It is to be noted that the determination of each viable cell count was performed by counting the colonies formed after incubating the corresponding sample, which has been suitably diluted in a physiological solution, saline solution at 37°C for 3 days on BCP medium. The results are shown in Table 16.

[0114]

[Table 16]

	Additive(s)	Cell count of lactic acid bacteria (cfu/mL)
Comparative Product 5	Not added	1.7×10^9
Invention Product 42	<u>Rubus suavissimus</u> S. Lee (Rosaceae) extract (hot water)	4.1×10^9
Invention Product 43	<u>Rubus suavissimus</u> S. Lee (Rosaceae) extract (pH 4.0)	5.4×10^9
Comparative Product 6	Sodium oleate	2.5×10^9
Invention Product 44	<u>Rubus suavissimus</u> S. Lee (Rosaceae) extract (hot water), sodium oleate	5.5×10^9
Invention Product 45	<u>Rubus suavissimus</u> S. Lee (Rosaceae) extract (pH 4.0), sodium oleate	6.5×10^9

[0115] It has been confirmed from Table 16 that the combined use of any one of the Rubus suavissimus S. Lee (Rosaceae) extracts with sodium oleate can synergically increase the cell count of

lactic acid bacteria as compared with the single use of the corresponding Rubus suavisissimus S. Lee (Rosaceae) extract.

[0116] Example 23

<Verification of the Effects for Lactic Acid Bacteria (5)>

5 The lactic acid bacteria fermentation products prepared in Example 22 (Invention Products 42, 43, 44 and 45) were each separately homogenized at 15 MPa, and to 20 parts by weight aliquots of the homogenized products, 80 parts by weight aliquots of a 15% sugar solution, which had been sterilized at 100°C for 10 5 minutes were added, and a yogurt flavoring was further added at 0.1% to prepare dairy products. Those dairy products were filled in containers, respectively and the viable cell counts of the lactic acid bacteria in the respective dairy products were determined in a similar manner as in Example 22 immediately 15 after their preparation and after their storage at 10°C for 14 days. The results are shown in Table 17.

[0117]

[Table 17]

	Additive(s)	Cell count of lactic acid bacteria (cfu/mL)	
		Immediately after preparation	After storage at 10°C for 14 days
Comparative Product 7	Not added	3.4×10^8	9.7×10^7
Invention Product 46	<u>Rubus suavisissimus</u> S. Lee (Rosaceae) extract (hot water) 1% by weight	8.2×10^8	4.4×10^8
Invention Product 47	<u>Rubus suavisissimus</u> S. Lee (Rosaceae) extract (hot water) 1% by weight Sodium oleate 25 ppm	1.3×10^9	7.4×10^8
Invention Product 48	<u>Rubus suavisissimus</u> S. Lee (Rosaceae) extract (pH 4.0) 1% by weight	1.0×10^9	5.4×10^8
Invention Product 49	<u>Rubus suavisissimus</u> S. Lee (Rosaceae) extract (pH 4.0) 1% by weight Sodium oleate 25 ppm	1.4×10^9	8.4×10^8

20 [0118] It has been indicated from Table 17 that a dairy product obtained by using, as a raw material, a lactic acid bacteria

fermentation product, which has been prepared by using a Rubus suavisissimus S. Lee (Rosaceae) extract singly, or a Rubus suavisissimus S. Lee (Rosaceae) extract and sodium oleate in combination, is excellent in the effect of suppressing changes in the cell count of lactic acid bacteria in the product during storage as compared with a dairy product obtained by using a lactic acid bacteria fermentation product prepared with none of them. Further, the use of a Rubus suavisissimus S. Lee (Rosaceae) extract in combination with sodium oleate can synergically bring about the effects as opposed to the single use of the Rubus suavisissimus S. Lee (Rosaceae) extract.

[0119] Example 24

<Verification of the Effects for Lactic Acid Bacteria (6)>

Using an aqueous citric acid solution of pH 4.0, an extract of Rubus suavisissimus S. Lee (Rosaceae) of 10 degrees Brix was prepared under similar conditions as in Example 16. To 10% skim milk powder, that extract was added at 1% and further, sodium oleate was also added at 25 ppm in term of oleic acid. The resultant mixture was sterilized to prepare a sterilized medium. To aliquots of that medium, starters of various lactic acid bacteria were inoculated at 0.1%, respectively and the bacteria strains were cultured at 37°C for 24 hours. As the lactic acid bacteria, were used Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus acidophilus and Lactobacillus casei. As comparative examples, those lactic acid bacteria were also cultured in a similar manner as described above by using 10% skim milk powder as a medium. The cell counts of lactic acid bacteria in the resultant cultures were determined in a similar manner as in Example 22. The results are shown in Table 18.

[0120]

[Table 18]

Test Strain	Cell count of lactic acid bacteria (cfu/mL)	
	Medium with no addition	Medium with addition
<u>Lactobacillus bulgaricus</u> YIT0098	3.0×10^8	6.5×10^8
<u>Lactobacillus acidophilus</u> YIT0071	3.5×10^8	6.4×10^8
<u>Lactobacillus casei</u> YIT9029	8.2×10^8	2.4×10^9

[0121] It has been indicated from Table 18 that the effects of a Rubus suavisissimus S. Lee (Rosaceae) extract and oleic acid can be recognized for all lactic acid bacteria, although the effects have been confirmed to somewhat differ depending on the species of the lactic acid bacteria.

[0122] Example 25

<Verification of the Effects for Lactic Acid Bacteria (7)>

To aliquots of a 15% skim milk powder medium (with 3% glucose contained therein) as a basal medium, various oleate-based emulsifiers were added respectively, each in combination with 1% of the Rubus suavisissimus S. Lee (Rosaceae) extract prepared and adjusted to 10 degreed Brix in Example 19, such that the emulsifiers amounted to 25 ppm in terms of the content of oleic acid. The resulting mixtures were then sterilized at 100°C for 60 minutes to prepare sterilized media, respectively. To those media, the starter of Lactobacillus casei YIT9029 was inoculated at 1%, and the bacteria strain was cultured at 37°C until the pHs of the respective media reached 3.7. The viable cell counts were measured in a similar manner as in Example 21. The results are shown in Table 19.

[0123]

[Table 19]

	Added material	Cell count of lactic acid bacteria (cfu/mL)
Invention Product 50	<u>Rubus suavisissimus</u> S. Lee (Rosaceae) extract	1.8×10^9
Invention Product 51	<u>Rubus suavisissimus</u> S. Lee (Rosaceae) extract, sodium oleate	6.0×10^9
Invention Product 52	<u>Rubus suavisissimus</u> S. Lee (Rosaceae) extract, monoglyceryl oleate	6.5×10^9
Invention Product 53	<u>Rubus suavisissimus</u> S. Lee (Rosaceae) extract, pentaglyceryl trioleate	4.2×10^9
Invention Product 54	<u>Rubus suavisissimus</u> S. Lee (Rosaceae) extract, hexaglyceryl monooleate	5.0×10^9
Invention Product 55	<u>Rubus suavisissimus</u> S. Lee (Rosaceae) extract, decaglycerine decaoleate	4.2×10^9
Invention Product 56	<u>Rubus suavisissimus</u> S. Lee (Rosaceae) extract,	6.4×10^9

	sucrose oleate	
Invention Product 57	<u>Rubus suavisissimus</u> S. Lee (Rosaceae) extract, glyceryl oleate	3.1×10^9

[0124] It has been confirmed that as shown in Table 19, the use of the oleic acid as derived from any one of the emulsifiers can bring about proliferative effects for lactic acid bacteria owing to the use of the extract of Rubus suavisissimus S. Lee (Rosaceae) in combination. The use of sodium oleate monoglyceryl oleate or sucrose oleate among these emulsifiers can bring about remarkable effects.

[0125] Example 26

10 <Verification of the Effects for Lactic Acid Bacteria (8)>

Using an aqueous citric acid solution of pH 4.0, an extract of Rubus suavisissimus S. Lee (Rosaceae) adjusted to 10 degrees Brix was prepared under similar conditions as in Example 16. The extract and glyceryl oleate, as oleic acid, were added to 15 aliquots of 15% skim milk powder medium (with 3% of glucose contained therein) such that their added amount reached those shown below in Table 20, respectively. The resultant media were sterilized at 100°C for 60 minutes to prepare sterilized media. To the respective sterilized media, the starter of Lactobacillus casei YIT9029 was inoculated at 1%, and the bacteria strain was 20 cultured at 37°C until their pHs reached 3.7. In addition, culture was similarly carried out as a control by adding a yeast extract (product of DIFCO), which is generally known as a culture promoter, at 0.2% to the medium. The lactic acid bacteria cell 25 counts in the resultant cultures were determined in a similar manner as in Example 22. The results are shown in Table 20.

[0126]

[Table 20]

Added amount of oleic acid (ppm)				
Added amount of <u>Rubus suavisissimus</u>	0	1	25	50

<u>S. Lee</u> (Rosaceae) extract (%)				
0	1.8×10^9			
0.01	2.8×10^9	3.5×10^9	4.2×10^9	4.0×10^9
0.1	4.0×10^9	4.9×10^9	5.9×10^9	6.2×10^9
1.0	4.9×10^9	6.1×10^9	8.1×10^9	7.8×10^9
5.0	5.2×10^9	6.3×10^9	8.2×10^9	8.5×10^9
10.0	5.0×10^9	6.1×10^9	8.4×10^9	8.3×10^9
Yeast extract (0.2)	2.3×10^9			

[0127] As shown in Table 20, proliferative effects for lactic acid bacteria can be recognized by the addition of oleic acid at 0.01 ppm or higher.

5 [0128] Example 27

<Verification of the Effects for Lactic Acid Bacteria (9)>

Using the lactic acid fermentation product prepared in Example 26, dairy products were produced in a similar manner as in Example 21. A flavor and test assessment was performed by five trained organoleptic assessors on those dairy products on the basis of similar assessment standards as in Example 21. The results are shown in Table 21.

[0129]

[Table 21]

Added amount of <u>Rubus suavissimus</u> <u>S. Lee</u> (Rosaceae) extract (%)	Added amount of oleic acid(ppm)	0	1	25	50
0		A			
0.01		A	A	A	A
0.1		A	A	A	A
1.0		A	A	A	A
5.0		B	B	B	B
10.0		C	C	C	C
Yeast extract (0.2)		D			

15

[0130] It has been found from Table 21 that similarly to the indication of Table 15, the addition of the Rubus suavissimus S. Lee (Rosaceae) extract at 10% to the medium, i.e., at 2% per

product affects the flavor and taste of the product irrespective of the added amount of oleic acid. It is to be noted that even with the addition of the Rubus suavissimus S. Lee (Rosaceae) extract is that amount, the product had a better flavor and taste
5 than that available from the addition of the yeast extract.

INDUSTRIAL APPLICABILITY

[0131] The lactic acid bacteria fermentation product of the present invention has a large amount of viable cell count of
10 lacticacidbacteria. The fermentation product does not undergo much deteriorations in flavor and taste as death of the lactic acid bacteria can be reduced. Accordingly, this lactic acid bacteria fermentation product can be suitably used as a raw
material for various fermented dairy foods.

15

CLAIMS

1. A lactic acid bacteria fermentation product, which has been obtained by culturing lactic acid bacteria on a medium comprising an extract obtained by acid extraction of at least one food material selected from the group consisting of rice bran, persimmon leaves, perilla, Houttuynia cordata Thunb, Eucommia ulmoides Oliv., turmeric, clove, cinnamon and Rubus suavissimus S. Lee (Rosaceae).
2. A fermentation product according to claim 1, wherein the extract has been obtained by acid extraction under an acidic condition not higher than pH 4.0.
3. A fermentation product according to any one of claims 1 to 2, wherein the extract amounts to a range of 0.01 to 10% by weight.
4. A lactic acid bacteria fermentation product, which has been obtained by culturing lactic acid bacteria on a medium comprising an extract obtained by acid extraction of at least one food material selected from the group consisting of rice bran, persimmon leaves, perilla, Houttuynia cordata Thunb, Eucommia ulmoides Oliv., turmeric, clove, cinnamon and Rubus suavissimus S. Lee (Rosaceae), and oleic acid ester selected from the group consisting of glyceryl oleate, polyglyceryl oleate ester and sucrose oleate, or a metal salt of oleic acid.
5. A fermentation product according to claim 4, wherein the oleic acid ester selected from the group consisting of glyceryl oleate, polyglyceryl oleate ester and sucrose oleate, or a metal salt of oleic acid amounts to a range of 1 to 50 ppm.
6. A fermentation product according to claim 4, wherein the extract has been obtained by acid extraction under an acidic condition not higher than pH 4.0 or less.
7. A fermentation product according to any one of claims 4 to 6, wherein the extract amounts to a range of 0.01 to 10% by weight.
8. A fermented milk food comprising a fermentation product according to any one of claims 1 to 7.
9. A method for producing a lactic acid bacteria

fermentation product, comprising culturing lactic acid bacteria on a medium comprising an extract obtained by acid extraction of at least one food material selected from the group consisting of rice bran, persimmon leaves, perilla, Houttuynia cordata Thunb,
5 Eucommia ulmoides Oliv., turmeric, clove, cinnamon and Rubus suavissimus S. Lee (Rosaceae).

10 10. A method for producing a lactic acid bacteria fermentation product, comprising culturing lactic acid bacteria on a medium comprising an extract obtained by acid extraction of at least one food material selected from the group consisting of rice bran, persimmon leaves, perilla, Houttuynia cordata Thunb,
Eucommia ulmoides Oliv., turmeric, clove, cinnamon and Rubus suavissimus S. Lee (Rosaceae), and oleic acid ester selected from the group consisting of glyceryl oleate, polyglyceryl oleate
15 ester and sucrose oleate, or a metal salt of oleic acid.