

Flavour modulation by bio-processing using cream-flavour forming bacteria strains

5 Field of invention

The present invention relates to the generation of flavour and aroma in milk-based products. The generation of flavour and aroma in milk-based products is achieved using
10 bacteria strains during fermentation of a milk source.

Background of the invention

Fermentation is a conversion of carbohydrates to organic
15 acids or other compounds using bacteria strains.

Fermented milk products are major consumer products. Fermented milk products can be, for example, cheeses, buttermilks and yoghurts. Fermented milk products are
20 manufactured by fermenting a milk source.

A milk source, for example milk, contains the carbohydrate lactose. During fermentation of the milk source the bacteria strains ferment the carbohydrate lactose to
25 produce lactic acid. The production of lactic acid results in an acidification of the milk source during the manufacture of the fermented milk product. During fermentation of the milk source, other reactions may occur between other substances present in the milk source and
30 the bacterial strains.

A fermentation of the milk source with bacteria strains is responsible for a generation of a flavour and aroma in the fermented milk products. Furthermore the fermentation of

the milk source with the bacteria strains increases a shelf-life of the fermented milk products.

The bacteria strains used to ferment the milk source can
5 be lactic acid bacteria strains. The lactic acid bacteria strains include Lactobacillus, Leuconostoc, Pediococcus, Lactococcus and Streptococcus; as well as the more peripheral Aerococcus, Carnobacterium, Enterococcus, Oenococcus, Sporolactobacillus, Teragenococcus, Vagococcus
10 and Weisella; these lactic acid bacteria strains belong to the order Lactobacillales.

An international patent application publication No. W0 2008/049581 by the Applicant Nestec SA is titled "Taste
15 and flavour modulation by biotransformation in milk products". The international patent application publication No. W0 2008/049581 discloses a method to promote a non-savoury flavour in a food product.

20 An international patent application publication No. W0 02/085131 by the Applicant New Zealand Dairy Board is titled "Method of preparing savoury-flavoured products by fermentation of proteins". The international patent application publication No. 02/085131 discloses a method
25 for the manufacture of a savoury flavoured product from a source of protein using a combination of two distinct strains of bacteria. The source of protein may be a plant soy, wheat, rice, milk or whey. A first strain of bacteria is selected from the group Macroccoccus, Micrococcus, Enterococcus, Staphylococcus, Brevibacterium, Anthrobacter
30 and Corynebacterium, preferably Macroccoccus caseolyticus. A second strain of bacteria is selected from the lactic acid bacteria - Lactococcus, Lactobacillus, Pediococcus or Leuconostoc. The savoury flavoured product may be combined
35 with other ingredients to form products such as cheese,

protein-water gels, yoghurts, creams, custards, sauces and confectionary products.

An international patent application publication No. W0
5 02/00845 by the Applicant Nizo Food Research is titled
"Enhanced flavour production in or relating to food by
cultivation of various food grade micro-organisms". The
international patent application publication No. W0
02/00845 discloses new mixed cultures of two or more
10 micro-organism strains wherein at least one of the micro-
organism strains which are comprised in said mixed culture
is individually selected on the basis of its ability to
perform part of an enzymatic pathway, and said two or more
selected micro-organism strains together form a complete
15 pathway towards a desired flavour component. The mixed
culture is a culture for the production of a fermented
product, such as yogurt or cheese or sausage. Said two or
more micro-organism strains are preferably co-cultivated.
Particular and preferred embodiments are starter cultures
20 for the manufacture of cheese. The mixed culture
comprising a combination of various *Lactococcus* strains
and a combination of a *Brevibacterium* strain and a
Staphylococcus strain, respectively.

25 An article by Monnet et al. in a Journal of
Microbiological Methods 37 (1999) pp 183-185 is titled "An
improved method for screening alpha-acetolactate producing
mutants". The article by Monnet et al. discloses that a
bacterial strain *Lactococcus Lactis* ssp. *Lactis* Biovar.
30 *Diacetylactis* is used in the dairy industry to produce
diacetyl. Diacetyl is a major flavour compound in
cultured dairy products.

An article by Boumerdassi et al. in a Journal of Dairy
35 Science Vol. 80 Issue 4 (1997) pp 634-639 is titled

“Effect of citrate on production of diacetyl and acetoin by *Lactococcus Lactis* ssp. *Lactis* CNRZ 483 cultivated in the presence of oxygen”. The article by Monnet et al. discloses the effects of trisodium citrate addition on growth and formation of diacetyl and acetoin by *Lactococcus Lactis* ssp. *Lactis* CNRZ 483 in a whey based medium.

The article “Characterisation of a malty-compound producing *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* C1 strain isolated from naturally fermented milk” by Mutukumira et al. (2009) *Milchwissenschaft* 64(1) pp. 26-29, relates to a strain that produced acceptable fermented milk to a sensory panel despite the presence of a slight malty flavour.

The article “Diacetyl production by different strains of *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuconostoc* spp.” by Hugenholtz and Starrenburg (1992) *Appl. Microbiol. Biotechnol* 38, pp. 17-22, relates to the comparison of several strains for product formation from citrate in milk cultures.

However, due to a number of the lactic acid bacteria strains and their interactions with individuals, a selection of certain lactic acid bacteria strains to produce certain flavours and aromas in the fermented milk products is not predictable.

Fermented milk products have a wide variety of flavours and aromas depending upon the milk source and the lactic acid bacteria strains used to ferment the milk source.

There is a need to provide methods and lactic acid bacteria strains that are responsible for specific flavours and aromas in the fermented milk products.

- 5 Furthermore, artificial additives are negatively perceived by the consumer. There a need to provide flavour and aromas in a natural way that avoids artificial additives.

- 10 There is also a need to provide flavour and aromas which can be used in a wide range of foods.

There is thus a need to overcome the aforementioned problems in the art.

15 **Summary of invention**

- In a first aspect the present invention relates to a lactic acid bacterium, *Lactococcus lactis* subsp. *lactis* *diacetylactis* (CNCM No. I-4404). The present invention
20 also relates to a lactic acid bacterium *Lactococcus lactis* subsp. *lactis* *diacetylactis* (CNCM No. I-4405).

- In a further aspect the present invention relates to a method for the manufacture of a fermented milk product.
25 The fermented milk product has at least a cream flavour and aroma. The method comprises providing a milk source, optionally adding citrate to the milk source to form a supplemented milk source, adding to the milk or supplemented milk source a *Lactococcus lactis* subsp. *lactis* *diacetylactis* (CNCM No. I-4404) or a *Lactococcus*
30 *lactis* subsp. *lactis* *diacetylactis* (CNCM No. I-4405) to form a mixture. The mixture is then fermented to manufacture the fermented milk product.

In a further aspect the present invention relates to a fermented milk product with at least a cream flavour and aroma obtainable by the aforementioned method.

- 5 In a further aspect the present invention relates to a product for consumption by a mammal comprising a fermented milk product with at least a cream flavour and aroma.

In a further aspect the present invention relates to a
10 food product comprising a lactic acid bacterium, *Lactococcus lactis* subsp. *lactis diacetylactis* (CNCM No. I-4404) or a *Lactococcus lactis* subsp. *lactis diacetylactis* (CNCM No. I-4405) and at least one of diacetyl, acetoin and 4-dihydroxy-3,4-dimethyl-2,5-
15 hexanedione.

In a further aspect the present invention relates to a culture comprising lactic acid bacterium, *Lactococcus lactis* subsp. *lactis diacetylactis* (CNCM No. I-4404) or a
20 *Lactococcus lactis* subsp. *lactis diacetylactis* (CNCM No. I-4405).

In a further aspect the present invention relates to a use of a lactic acid bacterium, *Lactococcus lactis* subsp. *lactis diacetylactis* (CNCM No. I-4404) or a *Lactococcus lactis* subsp. *lactis diacetylactis* (CNCM No. I-4405) to
25 impart at least a cream flavour and aroma to a milk source.

30 In a further aspect the present invention relates to a use of a lactic acid bacterium, *Lactococcus lactis* subsp. *lactis diacetylactis* (CNCM No. I-4404) or a *Lactococcus lactis* subsp. *lactis diacetylactis* (CNCM No. I-4405) for the manufacture of 3,4-dihydroxy-3,4-dimethyl-2,5-
35 hexanedione.

The present inventors were surprised to find that a lactic acid bacterium, *Lactococcus lactis* subsp. *lactis* diacetylactis (CNCM No. I-4404) or *Lactococcus lactis* subsp. *lactis* diacetylactis (CNCM No. I-4405) imparts such flavour and aroma to the fermented milk products.

Detailed description of invention

For a complete understanding of the present invention and the advantages thereof, reference is made to the following detailed description of the invention.

It should be appreciated that various aspects of the present invention are merely illustrative of the specific ways to make and use the present invention.

The various aspects of the present invention can be combined with other aspects of the present invention and do not limit the scope of the invention when taken into consideration with the claims and the following detailed description.

The present invention concerns fermented milk products. The fermented milk products are manufactured by a fermentation of a milk source with a lactic acid bacterium to provide flavour and aroma to the fermented milk products.

The lactic acid bacterium is a *Lactococcus lactis* subsp. *lactis* diacetylactis (CNCM No. I-4404) or a *Lactococcus lactis* subsp. *lactis* diacetylactis (CNCM No. I-4405). The lactic acid bacterium, *Lactococcus lactis* subsp. *lactis* diacetylactis (CNCM No. I-4404) and the *Lactococcus lactis* subsp. *lactis* diacetylactis (CNCM No. I-4405) were

deposited on 25-November-2010 with the Institut Pasteur -
Collection Nationale de Cultures de Mico-organisme (CNCM).

The milk source can be any type of milk, such as cow milk,
5 sheep milk, goat milk and buffalo milk or any mixtures
thereof. The milk source may be UHT-treated milk,
pasteurised milk or non-pasteurised milk. The milk source
may be full fat milk, a skimmed milk or semi-skimmed milk.
Furthermore the milk source may be a fresh milk,
10 recombined milk and milk containing vegetable fat and any
mixtures thereof.

A conversion of citrate into volatile flavour and aroma
compounds plays an important role in food technology. The
15 conversion of citrate into volatile flavour and aroma
compounds can be achieved by the lactic acid bacterium.
Many milk ingredients contain citrate; however to increase
formation it can be desired to supplement the milk source
with citrate.

20 The citrate is converted into volatile flavour and aroma
compounds during fermentation. The volatile flavour and
aroma compounds have at least a cream like flavour and
aroma as discussed below.

25 The citrate compound e.g. trisodium citrate is added to
the milk source in an amount of 0.01 to 5 wt%, preferably
0.01-2 wt%, more preferably in an amount of 0.03-1.0 wt%,
most preferably 0.05-0.3 wt%.

30 To the supplemented milk source is added the lactic acid
bacterium, *Lactococcus lactis* subsp. *lactis* *diacetylactis*
(CNCM No. I-4404) or the *Lactococcus lactis* subsp. *lactis*
diacetylactis (CNCM No. I-4405).

35

Lipase can also be added to the milk source or the mixture to produce an enhanced flavour and aroma in the fermented milk products. Lipase hydrolyses fats in the milk source to form for example di-glycerides, monoglycerides and free fatty acids or any mixtures thereof. The di-glycerides, monoglycerides and free fatty acids impart a cream-like flavour to the fermented milk product. Therefore the use of lipase enhances the cream like flavour in the fermented milk product.

10

Lactase can also be added to the milk source or the mixture to produce desired flavour and aroma in the fermented milk products. Lactase hydrolyses the disaccharide lactose in the milk source into galactose and glucose. Glucose and galactose are used as flavour precursors for caramel-like and sweetened condensed milk-like flavour formation.

15

If the milk source is non-pasteurised, the milk source or the supplemented milk source may be pasteurised, subjected to ultra-high temperature treatment (UHT-milk) or sterilised under conditions known in the art. The pasteurisation, ultra-high temperature treatment and sterilisation is carried out in a temperature range of 70°C to 150°C for a time of between 2s to 20min. Alternatively, the milk source may be heat-treated prior to being the supplemented milk source.

20

25

The mixture of the supplemented milk source and the lactic acid bacterium (*Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*) is then fermented to manufacture the fermented milk product with the creamy flavour and aroma. The fermentation is allowed to take place for between 6 and 24 hours at a temperature of approximately 30°C.

30

35

Optionally, fermentation improving cofactors such as alpha-ketoglutarate, manganese or magnesium salts may also be added prior to the fermentation.

5 Depending upon the milk source it is to be appreciated that the fermented milk product with a creamy flavour and aroma can be in the form of slurry (yogurt like) or a liquid. The fermented milk product can be dried or concentrated.

10

The fermented milk product with the creamy flavour and aroma can be dried, preferably by spray-drying and then converted into a powder.

15 The fermented milk product with the creamy flavour and aroma can have applications in food products and during a manufacture of food products. For example, the powder with the creamy flavour and aroma can have applications in the beverage industry to impart the malty flavour and aroma to
20 beverages. For example, the powder with the creamy flavour and aroma can have applications in the food industry to impart the creamy flavour and aroma to foodstuffs.

Examples

25

The manufactured fermented milk products were analysed by an electronic nose based on mass spectrometry and gas chromatography coupled to mass spectrometry (GC-MS).

30 Analysis with electronic nose based on mass spectrometry is a direct analysis method wherein the fermented milk product is placed directly into the ion source without the need for separation procedures and is therefore time-saving. A determination of volatiles from such a resultant
35 mass spectra contains limited information for the

identification of aroma components. Unequivocal identification of the single compounds present is not possible without prior separation and selective fragmentation i.e. GC-MS.

5

Gas chromatography coupled to mass spectrometry (GC-MS) provides the necessary separation and detection of volatiles. GC-MS is used for obtaining MS fragments belonging to a specific aroma component. The unambiguous identification of the molecules by GC-MS in combination with olfactometry analysis is mandatory for analysing volatiles with a specific odour.

10

Commonly used extraction methods for the isolation of volatiles from fermented milk products are vacuum distillation followed by solvent extraction, purge and trap (PT) and headspace techniques such as headspace solid-phase micro extraction (HS-SPME). The purge and trap (PT) and headspace techniques methods identify volatiles with different yield performances, but with comparable reproducibility. PT appeared to be a more sensitive whereas SPME is a more rapid and less expensive technique.

20

The reagents where used, were used as received without prior treatment unless otherwise stated.

25

Example 1

A - Reactivation of lactic acid bacterium

30

The *Lactococcus lactis* subsp. *lactis* *diacetylactis* (CNCM No. I-4404) in ampoules was reactivated with 1 ml reconstituted milk under sterile conditions, transferred into sterile glass tubes containing 9 ml reconstituted

milk and incubated aerobically at 30°C for 24 h in the dark.

The bacteria were then stored at 6°C for two weeks and
5 subsequently inoculated at 0.5% ((v/v) 0.05/10 ml medium)
in a culture.

The culture was M17x (M17 Terzaghi Bouillon, Merck 1.15029
and 5g/l glucose (Merck 8342)). After the growth phase (3
10 days) the flasks were stored at 6°C to form the
reactivated lactic acid bacterium.

Alternatively the culture can be skimmed milk.

15 B - Milk source supplementation with trisodium citrate

A 100 mM trisodium citrate solution in water was
manufactured. The trisodium citrate solution was filtrated
through a pore size of 0.45µm (Schleicher & Schuell,
20 Whatmann, FP 30/0.45µm, 7bar max. CA-S). 500 µl of the
trisodium citrate solution was added to 4.5 ml UHT-milk
(dilution 1:10) to obtain a supplemented milk source with
a final concentration of 10mM in 5.0ml.

25 C - Fermentation

Fermentation in UHT-milk was performed by two approaches
(I - II).

30 **I:** Index (Inside needle dynamic extraction; Hamilton)
headspace sampling of volatile compound fragments in non-
supplemented UHT-milk.

II: Tenax (accumulation adsorbens, Marin-Epagnier,
35 Switzerland) headspace sampling of volatile compounds in

supplemented milk source UHT-milk (10 mM trisodium citrate).

5 An aliquot of 50 μ l of the reactivated lactic acid bacterium was transferred in 5 ml supplemented milk source UHT milk (1% inoculation) under sterile conditions and incubated at 30°C aerobically for 16-24 hours in the dark.

10 An addition of 2.8g NaCl into the headspace vials helped to expel the volatiles from the fermented milk product into the headspace to get more intense release of the volatiles.

15 An electronic nose detected the volatile compound fragments at a range of m/z 40 - 100 for the experiment with non-supplemented UHT-milk (i.e. no trisodium citrate) and at m/z 10 - 160 for the experiment with supplemented UHT-milk (i.e. with trisodium citrate).

20 Principle component analysis (PCA) was calculated using the software program "The Unscrambler" (version 9.7). The results were calculated with logarithmised raw data and exclusion of the water and milk blanks. The calculations were done with all variables (MS fragments) included to
25 group the strains in relation to similar MS-fragment patterns and abundance of compounds.

D - Electronic nose measurements

30 Analysis of the fermented milk product by the electronic nose measurements in supplemented milk source UHT-milk was conducted. II: Tenax headspace measurement with 10mM trisodium citrate supplemented UHT-milk. GC-MS fragments $[M]^+$ were 27, 29, 43, 45, 60, 70, 86, 87, 88 and 135.

E - pH and redox potential

A pH of the fermented milk product was determined to be 2.2 with a redox potential of -10mV.

5

F - Sensory assessment of fermented milk product

After fermentation the glass vials were kept closed until sensory evaluation started. Seven persons attended the sensory assessment of the fermented milk product. The sensory assessment was based on the following attributes, scoring is noted with a X. A blank sample (incubated milk) was given as a reference. In order to test the influence of the trisodium citrate samples were also prepared without the trisodium citrate (addition of sterile water only) and presented to the panel. The results are shown below, wherein an X indicated a sensory perception of the fermented milk product to the panellist.

20	Buttery	XXXX
	Bitter/-almond	XX
	Flower-like	X
	Bread-like	X
	Creamy	XXXX
25	Caramel	-
	Strawberry	-
	Fresh	-
	Fruity	-
	Yeast	X
30	Honey	X
	Yoghurt	-
	Cheesy	-
	Milky	-
	Malty	XX
35	Almond	XX

	Nutty	X
	Paper-like	-
	Sweet	X
	Acidic	X
5	Salty	-
	Vanilla	-

The results of the sensory assessment of the fermented milk product demonstrate that the fermented milk product
10 has a creamy buttery like flavour and aroma.

Example 2

A - Reactivation of lactic acid bacterium

15

The *Lactococcus lactis* subsp. *lactis* *diacetylactis* (CNCM No. I-4405) in ampoules was reactivated with 1 ml reconstituted milk under sterile conditions, transferred into sterile glass tubes containing 9 ml reconstituted
20 milk and incubated aerobically at 30°C for 24 h in the dark.

The bacteria were then stored at 6°C for two weeks and subsequently inoculated at 0.5% ((v/v) 0.05/10 ml medium)
25 in a culture.

The culture was M17x (M17 Terzaghi Bouillon, Merck 1.15029 and 5g/l glucose (Merck 8342). After the growth phase (3 days) the flasks were stored at 6°C to form the
30 reactivated lactic acid bacterium.

Alternatively the culture can be skimmed milk.

35

B - Milk source supplementation with trisodium citrate

A 100 mM trisodium citrate solution in water was manufactured. The trisodium citrate solution was filtrated
5 through a pore size of 0.45µm (Schleicher & Schuell, Whatmann, FP 30/0.45µm, 7bar max. CA-S). 500 µl of the trisodium citrate solution was added to 4.5 ml UHT-milk (dilution 1:10) to obtain a supplemented milk source with a final concentration of 10mM in 5.0ml. To the
10 supplemented milk source was added lipase 100 mM.

C - Fermentation

Fermentation in UHT-milk was performed by two approaches
15 (I - II).

I: Index (Inside needle dynamic extraction; Hamilton) headspace sampling of volatile compound fragments in non-supplemented UHT-milk.

20 II: Tenax (accumulation adsorbens, Marin-Epagnier, Switzerland) headspace sampling of volatile compounds in supplemented UHT-milk (10 mM L-leucine, L-isoleucine, L-valine and L-phenylalanine).

25 An aliquot of 50 µl of the reactivated lactic acid bacterium was transferred in 5 ml supplemented milk source UHT milk (1% inoculation) under sterile conditions and incubated at 30°C aerobically for 22 hours in the dark.

30 An addition of 2.8g NaCl into the headspace vials helped to expel the volatiles from the fermented milk product into the headspace to get more intense release of the volatiles.

An electronic nose detected the volatile compound fragments at a range of m/z 40 – 100 for the experiment with non-supplemented UHT-milk (i.e. no trisodium citrate) and at m/z 10 – 160 for the experiment with supplemented
5 UHT-milk (i.e. with trisodium citrate).

Principle component analysis (PCA) was calculated using the software program "The Unscrambler" (version 9.7). The results were calculated with logarithmised raw data and
10 exclusion of the water and milk blanks. The calculations were done with all variables (MS fragments) included to group the strains in relation to similar MS-fragment patterns and abundance of compounds.

15 D - Electronic nose measurements

Analysis of the fermented milk product by the electronic nose measurements in supplemented milk source UHT-milk was conducted. II: Tenax headspace measurement with 10mM
20 trisodium citrate supplemented UHT-milk. GC-MS fragments $[M]^+$ were 43, 55, 71, 77, 60, 88, 89, 99, 114, 120 and 131.

F - Sensory assessment of fermented milk product

25 After bacterial fermentation the glass vials were kept close until sensory evaluation started. Ten persons attended the sensory assessment of the fermented milk product. The sensory evaluation was a taste evaluation in
30 order to gain information on the in mouth - effect and taste of the obtained fermented milk products.

In each case the samples were pasteurised (85 °C for 15 min in a water bath) and diluted to 1 % in UHT milk (at a

temperature 20–25°C). The results shown below detail the inferences of the panellists.

	Panellist	Blank Incubated UHT-milk	Fermented milk product
5			
	1	Slightly milky	Intense creamy, Butter
	2	Milky, UHT-milk Slightly Sour	Sweet/creamy, Buttery
10	3	Milky, Slight almond, Bitter	Malty, butter
	4	Milky	Cream like
	5	Milky, Cooked	Intense creamy
15	6	Milk powder, Sweet	Creamy, Buttery
	7	Milky, Fatty, Buttery, Sweet	Butterish - cream
	8	-	Malty butter
	9	-	Creamy butter
20	10	-	Buttery

The results of the sensory assessment of fermented milk product demonstrate that the fermented milk product had a predominantly creamy like flavour and aroma.

25

In order to determine the volatile responsible for the creamy buttery flavour an analysis of the GS mass spectra was made with known flavour compounds. It was found that the mass spectrum of 3,4-dihydroxy-3,4-dimethyl-2,5
 30 hexanedione was identical to the mass spectrum of the volatile responsible for the creamy buttery flavour. Furthermore it was found that the compound 3,4-dihydroxy-3,4-dimethyl-2,5 hexanedione has a butter like flavour (see for example US patent No. 4,889,736). Thus it was
 35 surprising that the lactic acid bacterium, *Lactococcus lactis* subsp. *lactis* *diacetylactis* (CNCM No. I-4404) or the *Lactococcus lactis* subsp. *lactis* *diacetylactis* (CNCM

No. I-4405) can be used to manufacture 3,4-dihydroxy-3,4-dimethyl-2,5-hexanedione.

Having thus described the present invention in detail, it
5 is to be understood that the detailed description is not intended to limit the scope of the invention thereof.

What is desired to be protected by letters patent is set forth in the following claims.

10

PCT

Print Out (Original in Electronic Form)

(This sheet is not part of and does not count as a sheet of the international application)

0-1	Form PCT/RO/134 (SAFE) Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)	
0-1-1	Prepared Using	PCT Online Filing Version 3.5.000.225 MT/FOP 20020701/0.20.5.20
0-2	International Application No.	
0-3	Applicant's or agent's file reference	11101-WO-PCT

1	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
1-1	page	5
1-2	line	19
1-3	Identification of deposit	
1-3-1	Name of depositary institution	CNCM Collection nationale de cultures de micro-organismes
1-3-2	Address of depositary institution	Institut Pasteur, 28, rue du Dr Roux, 75724 Paris Cedex 15, France
1-3-3	Date of deposit	25 November 2010 (25.11.2010)
1-3-4	Accession Number	CNCM I-4404
1-5	Designated States for Which Indications are Made	All designations
2	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
2-1	page	5
2-2	line	21
2-3	Identification of deposit	
2-3-1	Name of depositary institution	CNCM Collection nationale de cultures de micro-organismes
2-3-2	Address of depositary institution	Institut Pasteur, 28, rue du Dr Roux, 75724 Paris Cedex 15, France
2-3-3	Date of deposit	25 November 2010 (25.11.2010)
2-3-4	Accession Number	CNCM I-4405
2-5	Designated States for Which Indications are Made	All designations

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0-4	This form was received with the international application: (yes or no)	yes
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Claims

1. A lactic acid bacterium, *Lactococcus lactis* subsp.
5 *lactis diacetylactis* (CNCM No. I-4404).
2. A lactic acid bacterium, *Lactococcus lactis* subsp.
 lactis diacetylactis (CNCM No. I-4405).
- 10 3. A method for the manufacture of a fermented milk
 product with at least a cream flavour and aroma
 comprising:
 - providing a milk source,
 - optionally adding citrate to the milk source to form
15 a supplemented milk source,
 - adding to the milk or supplemented milk source a
 Lactococcus lactis subsp. *lactis diacetylactis* (CNCM
 No. I-4404) or a *Lactococcus lactis* subsp. *lactis*
 diacetylactis (CNCM No. I-4405) to form a mixture;
20 and
 - fermenting the mixture to manufacture the fermented
 milk product.
- 25 4. The method according to claims 3 further comprising
 concentrating the fermented milk product and forming
 a fermented milk product concentrate.
- 30 5. The method according to any one of claims 3 or 4
 further comprising drying the fermented milk product
 or fermented milk concentrate and forming a powder.
6. The method according to any one of claims 3 to 5,
 wherein the milk source is selected from at least one
 of full fat milk, skimmed milk, semi-skimmed milk,

fresh milk, recombined milk, cream, buttermilk, whey and milk containing vegetable fat.

- 5 7. The method according to any one of claims 3 to 6 further comprising an addition of at least one of a lipase enzyme and a lactase enzyme to the milk source.
- 10 8. The method according to any one of claims 3 to 7 further comprising adding a fermentation co-factor to the milk source.
- 15 9. The method according to any one of claims 3 to 8, wherein citrate, preferably trisodium citrate is added to the milk source in an amount of 0.01 to 5 wt%.
- 20 10. A fermented milk product with at least a cream flavour and aroma obtainable by the method of any one of claims 3 to 9.
- 25 11. A product for consumption by a mammal comprising the fermented milk product of claim 10.
- 30 12. A food product comprising a lactic acid bacterium, *Lactococcus lactis* subsp. *lactis* diacetylactis (CNCM No. I-4404) or a *Lactococcus lactis* subsp. *lactis* diacetylactis (CNCM No. I-4405) and at least one of diacetyl, acetoin and 4-dihydroxy-3,4-dimethyl-2,5-hexanedione.
- 35 13. A culture comprising lactic acid bacterium, *Lactococcus lactis* subsp. *lactis* diacetylactis (CNCM No. I-4404) or a *Lactococcus lactis* subsp. *lactis* diacetylactis (CNCM No. I-4405).

14. A use of a lactic acid bacterium, *Lactococcus lactis* subsp. *lactis diacetylactis* (CNCM No. I-4404) or a *Lactococcus lactis* subsp. *lactis diacetylactis* (CNCM No. I-4405) to impart least a cream flavour and aroma to a milk source.

15. A use of a lactic acid bacterium, *Lactococcus lactis* subsp. *lactis diacetylactis* (CNCM No. I-4404) or a *Lactococcus lactis* subsp. *lactis diacetylactis* (CNCM No. I-4405) for the manufacture of 3,4-dihydroxy-3,4-dimethyl-2,5-hexanedione.