NEW BACTERIA

FIELD OF INVENTION

The present invention relates to a bacterial cell with texturizing property, starter cultures comprising the cell, and dairy products manufactured using the bacterial cell.

BACKGROUND OF INVENTION

The food industry uses numerous bacteria, in particular lactic acid bacteria (LAB), in order to improve the taste and the texture of foods but also in order to extend the shelf life of these foods. In the case of the dairy industry, LAB are used extensively in order to bring about the acidification of milk (by fermentation) but also in order to texturize the product into which they are incorporated.

Among the LAB used in the food industry, there can be mentioned the genera *Streptococcus, Lactococcus, Lactobacillus, Leuconostoc, Pediococcus* and *Bifidobacterium*.

The LAB are used extensively alone or in combination with other bacteria for the production of food products, in particular fermented products. They are used in particular in the formulation of the starter cultures used for the production of fermented milks, for example yogurts. Certain of them play a dominant role in the development of the texture of the fermented product. This characteristic is closely linked to the production of polysaccharides. Among the strains of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* it is possible to distinguish texturizing and non-texturizing strains.

In order to meet the requirements of the industry, it has become necessary to propose novel texturizing strains of LAB, in particular of *Streptococcus thermophilus* and *Lactobacillus* species. One specific focus could be to reduce for example the amount of proteins or pectin (both improving texture) in dairy products, as these are both costly.

Thus the problem that the invention proposes to resolve is to provide a strain of lactic acid bacterium having good properties for texturizing food products, especially products where the texturizing strain of the lactic acid bacterium (such as *Streptococcus thermophilus*) is used together with a strain of *a Lactobacillus* species.

SUMMARY

The present inventors have surprisingly found that the concentration of metal ions in lactic acid bacteria cells, or in starter cultures containing lactic acid bacteria cells, influence the formation of texture when such bacteria cells, or starter cultures, are used for the production of fermented milk.

In accordance with this surprising finding, the present disclosure relates to novel LAB *strains* with improved texturizing properties, and a method for producing such strains, and to fermented milk products made using such strains.

In one aspect of the present invention, there is provided a method of producing a fermented milk product comprising fermenting a milk substrate with a lactic acid bacterium (LAB) mutant strain, wherein the LAB mutant increases the viscosity of the fermented milk product, and wherein the LAB mutant is selected from the group consisting of

a) a LAB which contains less than 10 ppm (parts per million, mg/Kg dry weight) of iron ions (e.g. Fe2+), such as less than 9, less than 8 ppm, less than 6 or less than 3 ppm;

b) a LAB which contains less than 6 ppm (parts per million, mg/Kg dry weight) of Manganese ions (e.g. Mn2+), such as less than 5.5, less than 5.2 ppm, less than 5 ppm, less than 4 or less than 2 ppm;

c) a LAB which contains less than 16 ppm (parts per million, mg/Kg dry weight) in total of Fe2+ and Mn2+, such as less than 15, less than 14 ppm, less than 13 ppm, less than 10 or less than 7 ppm;

d) a LAB carrying a mutation in a gene related to the uptake of a divalent metal ion;

e) a LAB which has a changed expression of the *fur* gene caused by a partly or full inactivation of the gene, deletion of the gene or parts thereof and/or insertion of additional DNA into the gene;

f) a LAB which has a reduced expression of an *mnth* gene caused by a partly or full inactivation of the gene, deletion of the gene or parts thereof and/or insertion of additional DNA into the gene;

g) a LAB which has a reduced expression of the fatc gene or any other gene related to the uptake of iron caused by a partly or full inactivation of the gene, deletion of the gene or parts thereof and/or insertion of additional DNA into the gene; and

h) a LAB, which is resistant to tellurite, wherein a tellurite resistant mutant means a mutant which is able to grow (form a colony) on M17 agar plates containing 0.1 mM K2TeO3.

In another aspect of the present disclosure, there is provided a *Streptococcus thermophilus* strain selected from the group consisting of the *Streptococcus thermophilus* strain CHCC15712 (DSM25955) and a mutant of this strain with the same or improved properties with respect to EPS production, and wherein less than 0.1 % of the nucleotides in the bacterial genome of the mutant have been changed.

In another aspect of the present disclosure, there is provided a composition comprising a LAB (lactic acid bacterium) disclosed herein.

In a further aspect of the present disclosure, there is provided a composition comprising a LAB (lactic acid bacterium), said composition further comprises a metal ion chelator.

In another aspect of the present disclosure, there is provided a method for producing a fermented milk product comprising fermenting a milk substrate with a strain disclosed herein, or a composition disclosed herein.

In a further aspect of the present disclosure, there is provided a method for producing a fermented milk product comprising fermenting a milk substrate having a Fe^{2+} concentration below 0.25 µg/g with a LAB (lactic acid bacterium).

In another aspect of the present disclosure, there is provided a method for improving the EPS production of a LAB (lactic acid bacterium) strain, which is able to produce EPS, said method comprising growing the LAB strain in a medium, wherein Fe^{2+} ions has been removed from the medium before, during or after inoculation with the strain, wherein the resulting medium has a concentration of Fe^{2+} ions below 0.25 µg/g.

In a further aspect of the present disclosure, there is provided a method for improving the EPS production of a LAB (lactic acid bacterium) strain, which is able to produce EPS, said method comprising growing the LAB strain in a medium, wherein Mn^{2+} ions has been removed from the medium before, during or after inoculation with the strain, wherein the resulting medium has a concentration of Mn^{2+} ions below 0.025 µg/g.

The present disclosure also relates to a composition of lactic acid bacteria that are able to texturize a milk substrate, said composition comprises lactic acid bacteria having a lowered intracellular concentration of metal ions, and/or said composition comprises a lowered amount of metal ions.

It is contemplated that such cells can be obtained in different ways, such as:

- Alternative A): Genetically modification of the cell, so the cell will not take up metal ions, or only take up metal ions at a low rate, or so the the cell will not be able to synthesize fully functional ion binding proteins,
- Alternative B): Growth of the cells in a growth medium without, or having a low concentration of metal ions, e.g. by addition of a metal ion chelating agent to the medium, or
- Alternative C): Isolation of mutant cells, e.g. obtained by mutagenization treatment (including treatment with a chemical mutagen, or UV light) of a mother cell.

Relating to alternative A), the present inventors describe the finding that mutants of texturizing LAB which are perturbed (such as decreased, suppressed) in their ion metabolism will give superior texture in a fermented milk product.

The present disclosure further relates to a method for increasing the viscosity of a dairy product fermented with LAB, said method comprises fermenting a milk substrate with reduced iron (esp Fe2+) and/or manganese (esp Mn2+) content, or fermenting a milk substrate where at least one of said metal ions are made partly or fully inaccessible for the LAB.

CLAIMS

1. A method of producing a fermented milk product comprising fermenting a milk substrate with a lactic acid bacterium (LAB) mutant strain, wherein the LAB mutant increases the viscosity of the fermented milk product, and wherein the LAB mutant is selected from the group consisting of

a) a LAB which contains less than 10 ppm (parts per million, mg/Kg dry weight) of iron ions (e.g. Fe2+), such as less than 9, less than 8 ppm, less than 6 or less than 3 ppm;

b) a LAB which contains less than 6 ppm (parts per million, mg/Kg dry weight) of Manganese ions (e.g. Mn2+), such as less than 5.5, less than 5.2 ppm, less than 5 ppm, less than 4 or less than 2 ppm;

c) a LAB which contains less than 16 ppm (parts per million, mg/Kg dry weight) in total of Fe2+ and Mn2+, such as less than 15, less than 14 ppm, less than 13 ppm, less than 10 or less than 7 ppm;

d) a LAB carrying a mutation in a gene related to the uptake of a divalent metal ion;

e) a LAB which has a changed expression of the *fur* gene caused by a partly or full inactivation of the gene, deletion of the gene or parts thereof and/or insertion of additional DNA into the gene;

f) a LAB which has a reduced expression of an *mnth* gene caused by a partly or full inactivation of the gene, deletion of the gene or parts thereof and/or insertion of additional DNA into the gene;

g) a LAB which has a reduced expression of the fatc gene or any other gene related to the uptake of iron caused by a partly or full inactivation of the gene, deletion of the gene or parts thereof and/or insertion of additional DNA into the gene; and

h) a LAB, which is resistant to tellurite, wherein a tellurite resistant mutant means a mutant which is able to grow (form a colony) on M17 agar plates containing 0.1 mM K2TeO3.

2. A method according to claim 1, wherein the LAB mutant is selected from the group consisting of

a) a LAB which contains less than 10 ppm (parts per million, mg/Kg dry weight) of iron ions (e.g. Fe2+), such as less than 9, less than 8 ppm, less than 6 or less than 3 ppm;

b) a LAB which contains less than 6 ppm (parts per million, mg/Kg dry weight) of Manganese ions (e.g. Mn2+), such as less than 5.5, less than 5.2 ppm, less than 5 ppm, less than 4 or less than 2 ppm; and

c) a LAB which contains less than 16 ppm (parts per million, mg/Kg dry weight) in total of Fe2+ and Mn2+, such as less than 15, less than 14 ppm, less than 13 ppm, less than 10 or less than 7 ppm.

3. A method according to claim 1, wherein the LAB mutant is selected from the group consisting of

d) a LAB carrying a mutation in a gene related to the uptake of a divalent metal ion;

e) a LAB which has a changed expression of the *fur* gene caused by a partly or full inactivation of the gene, deletion of the gene or parts thereof and/or insertion of additional DNA into the gene;

f) a LAB which has a reduced expression of an *mnth* gene caused by a partly or full inactivation of the gene, deletion of the gene or parts thereof and/or insertion of additional DNA into the gene;

g) a LAB which has a reduced expression of the fatc gene or any other gene related to the uptake of iron caused by a partly or full inactivation of the gene, deletion of the gene or parts thereof and/or insertion of additional DNA into the gene; and

h) a LAB, which is resistant to tellurite, wherein a tellurite resistant mutant means a mutant which is able to grow (form a colony) on M17 agar plates containing 0.1 mM K2TeO3.

4. A method according to any of the preceding claims, wherein the LAB has a perturbed divalent metal ion metabolism (DMIM).

5. A method according to any of the preceding claims, wherein the divalent metal ion is selected from the group consisting of Fe2+, Mg2+, and Mn2+.

6. A method according to any of the preceding claims, wherein the LAB has been obtained by mutagenesis, and/or by genetic engineering.

7. A method according to any of the preceding claims, wherein the LAB has been obtained by growth in a medium having a concentration of divalent metal ions selected from the group consisting of Fe2+ and Mn2+, of below 0.25 microgram/gram, such as below 0.2 µg/g.

8. A method according to any of the preceding claims, wherein the LAB belongs to the species *Lactobacillus bulgaricus*.

9. A method according to any of the preceding claims, wherein the LAB belongs to the species *Streptococcus thermophilus*.

10. A method according to any of the preceding claims, wherein the LAB generates a viscosity greater than about 50 Pa.s in milk, measured as shear stress after 16 hours of growth of the bacterium in 9.5% reconstituted skim milk at 37 degrees C, when the milk is inoculated with 10E8 CFU per ml.

11. A *Streptococcus thermophilus* strain selected from the group consisting of the *Streptococcus thermophilus* strain CHCC15712 (DSM25955) and a mutant of this strain with the same or improved properties with respect to EPS production, and wherein less than 0.1 % of the nucleotides in the bacterial genome of the mutant have been changed.

12. A composition comprising a LAB (lactic acid bacterium) of claim 11.

13. A composition comprising a LAB (lactic acid bacterium), said composition further comprises a metal ion chelator.

14. A method for producing a fermented milk product comprising fermenting a milk substrate with a strain of claim 11, or a composition of claim 12.

15. A method for producing a fermented milk product comprising fermenting a milk substrate having a Fe^{2+} concentration below 0.25 µg/g with a LAB (lactic acid bacterium).

16. A method for improving the EPS production of a LAB (lactic acid bacterium) strain, which is able to produce EPS, said method comprising growing the LAB strain in a medium, wherein Fe^{2+} ions has been removed from the medium before, during or after inoculation with the strain, wherein the resulting medium has a concentration of Fe^{2+} ions below 0.25 µg/g.

17. A method for improving the EPS production of a LAB (lactic acid bacterium) strain, which is able to produce EPS, said method comprising growing the LAB strain in a medium, wherein Mn^{2+} ions has been removed from the medium before, during or after inoculation with the strain, wherein the resulting medium has a concentration of Mn^{2+} ions below 0.025 µg/g.