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(54) Title: USE OF MANGANESE FOR ENHANCING THE GROWTH OF L. CASEI IN MIXED CULTURES

(57) Abrégé/Abstract:
The invention relates to a method for selectively enhancing the growth of bacteria from the Lactobacillus casei group in a mixed culture, by adding manganese to the culture medium. Said method is suitable in particular in the case of mixed cultures associating Lactobacillus casei with yogurt bacteria.

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(54) Title: USE OF MANGANESE FOR ENHANCING THE GROWTH OF L. CASEI IN MIXED CULTURES

(57) Abstract: The invention relates to a method for selectively enhancing the growth of bacteria from the Lactobacillus casei group in a mixed culture, by adding manganese to the culture medium. Said method is suitable in particular in the case of mixed cultures associating Lactobacillus casei with yogurt bacteria.

USE OF MANGANESE FOR ENHANCING THE GROWTH OF *L. CASEI* IN MIXED CULTURES

The invention relates to a method for enhancing the growth of bacteria from the *Lactobacillus casei* group, in mixed cultures.

The *Lactobacillus casei* group encompasses several species of lactic acid bacteria of great interest for the food industry. In particular, strains of the *Lactobacillus casei* group, belonging to the species *L. casei* subsp. *casei, L. casei* subsp. *paracasei,* and *L. casei* subsp. *rhamnosus,* have been reported to have health-promoting properties, and are used as probiotics in different food products, including in particular dairy products.

Unless otherwise specified, the terms *Lactobacillus casei* (or *L. casei)* is used hereinafter to designate any subspecies of the *Lactobacillus casei* group, and in particular any of the subspecies *casei, paracasei,* or *rhamnosus* mentioned above.

A drawback of the use of bacteria of the *Lactobacillus casei* group in the production of fermented dairy products is their slow growth in milk.

When used in pure culture, the fermentation time of *L. casei* is very long, which is a limitation in its use in industrial production.

In the manufacture of probiotic dairy products, *Lactobacillus casei* bacteria are often associated with other species of lactic acid bacteria, probiotic or not. In many cases they are combined with yogurt bacteria *(Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* or ssp. *lattis),* which give specific organoleptic properties (such as texture and flavour) to the fermented product. Yogurt bacteria grow faster in milk than *Lactobacillus casei,* thus allowing to speed up the global fermentation process. However, the fast acidification of the medium by these yogurt bacteria limits the growth of *L. casei* in the mixed culture and therefore its population in the final product.

The inventors have now found that when manganese was added to a co-culture of *Lactobacillus casei* with other lactic acid bacteria, in particular yogurt bacteria, the growth of *L. casei* was enhanced, white no effect was observed on the other lactic acid bacteria.

An object of the invention is therefore the use of manganese for selectively enhancing the growth of bacteria from the *Lactobacillus casei* group in a mixed culture containing lactic acid bacteria not belonging to the *Lactobacillus casei* group, wherein the

lactic bacteria not belonging to the *Lactobacillus casei* group are selected among *Lactobacillus delbrueckii* ssp *bulgaricus*, *Lactobacillus delbrueckii* ssp *lactis* and *Streptocccus thermophilus*.

More specifically, the invention provides a method for selectively enhancing the growth of bacteria from the *Lactobacillus casei* group in a mixed culture containing lactic acid bacteria not belonging to the *Lactobacillus casei* group, wherein said method comprises:

a) providing a fermentation medium containing manganese;

b) inoculating said fermentation medium with the bacteria;

c) fermenting the inoculated medium until it reaches a desired target pH;

d) stopping the fermentation and recovering the fermented product.

The bacteria of the Lactobacillus *casei* group are selected preferably among the species *L. casei* subsp. *casei*, *L. casei* subsp. *paracasei*, *L. casei* subsp. *rhamnosus*. The lactic bacteria not belonging to the Lactobacillus casei group are selected preferably among *Lactobacillus delbrueckii* ssp *bulgaricus*, *Lactobacillus delbrueckii* ssp *lactis* and *Streptocccus thermophilus*.

Another object of the invention is to provide a method for selectively enhancing the growth of bacteria from the *Lactobacillus casei* group in a mixed culture containing lactic acid bacteria not belonging to the *Lactobacillus casei* group, wherein the lactic bacteria not belonging to the *Lactobacillus casei* group are selected among *Lactobacillus delbrueckii* ssp *bulgaricus*, *Lactobacillus delbrueckii* ssp *lactis* and *Streptocccus thermophilus*,

wherein said method comprises:

a) providing a fermentation medium containing manganese

b) inoculating said fermentation medium with the bacteria from the *Lactobacillus casei* group and the lactic acid bacteria not belonging to the *Lactobacillus casei* group; and

c) fermenting the inoculated medium until it reaches a target pH.

Another object of the invention is to provide a method for the production of a fermented product comprising bacteria from the *Lactobacillus casei* group, wherein the lactic acid bacteria not belonging to the *Lactobacillus casei* group are selected among *Lactobacillus*

delbrueckii ssp *bulgaricus*, *Lactobacillus delbrueckii* ssp *lactis* and *Streptococcus thermophilus,*

the method comprising:

a) providing a fermentation medium containing manganese

b) inoculating said fermentation medium with the bacteria from the *Lactobacillus casei* group and with lactic acid bacteria not belonging to the *Lactobacillus casei* group;

c) fermenting the inoculated medium until it reaches a target pH; and

d) stopping the fermentation and recovering the fermented product.

The fermentation medium is preferably a dairy fermentation medium, i.e. a milk-based liquid medium. Any animal or vegetal milk source could be considered. Cow milk is however preferred. It may be for instance whole, partially or fully skimmed milk, optionally reconstituted from powdered milk. It may also consist of a milk fraction, for instance whey, or mixtures of two or more of milk fractions. Preferably, said medium will be used without supplementation other than manganese. However, it may optionally be supplemented with ingredients such as sugars, starch, thickeners, etc. provided that these ingredients do not interfere with the growth of one or more of the co-cultured strains, and provided that they are suitable for human or animal consumption.

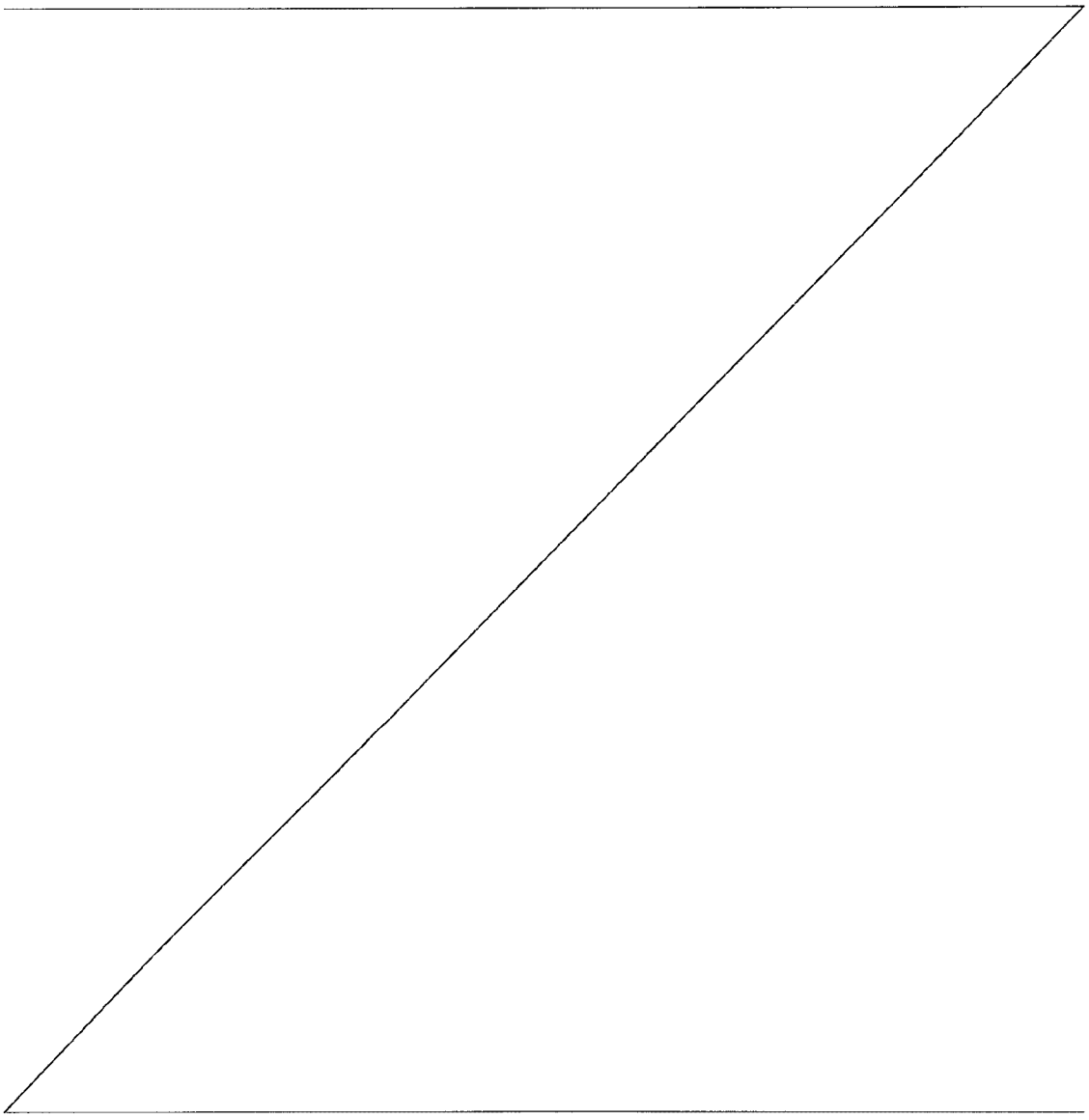
Said medium is classically sterilized before inoculation with the Lactobacillus strains. Sterilization is performed by methods known in themselves, such as heat treatment.

The pH of the fermentation medium prior to inoculation with the bacteria is preferably from 5.5 to 7, preferably of about 6.4.

According to a preferred embodiment of the invention, the fermentation medium contains at least 0.1mg/l, preferably at least 0.5mg/l, and up to 100 mg/l of manganese. Generally, it will contain from about 5 to about 60 mg/l of manganese. Manganese is added in the form of a food-grade manganese salt in appropriate amount to obtain the above-mentioned final concentrations of manganese anion in the fermentation medium. Examples of food-grade manganese salts which can be used in the method of the invention include manganese chloride, manganese oxide, manganese sulfate, manganese citrate, manganese glycerophosphate, manganese gluconate, and the like. In the case of a dairy fermentation medium, the amount of manganese added will be of at least 0.05 mg, preferably

of at least 0.45 mg, in order to take in account the amount of manganese naturally present in milk (which is of about 0,05 + /- 0,02 mg/1).

The manganese salt can be added to the fermentation medium before inoculation with the bacteria, or simultaneously with said inoculation. Advantageously, it can also be added to the *L. casei* inoculum during its preparation, for instance it can be added after concentration of the propagated bacteria, and before freezing the concentrate. In this case the amount of manganese in the inoculum will be generally of from 0,5 mg/ml to 4,5.mg/ml.



Generally, the bacteria of the *Lactobacillus casei* group are inoculated in the medium in a quantity of at least 10^4 CFU/ml, preferably of from 5 x 10^4 to 1 x 10^{10} CFU/ml, more preferably of from 10^6 to 10^8 CFU/ml, and still more preferably of about 2.10^7 CFU/ml.

The lactic acid bacteria not belonging to the *Lactobacillus casei* group are inoculated in the medium in a quantity of from 10^3 CFU/ml to 10^9 CFU/ml, preferably of from 10^5 to 10^8 CFU/ml, more preferably of from 10^6 to 10^7 CFU/ml, and still more preferably of about 5.10^6 CFU/ml.

More specifically, in the case of co-culture of *L. casei* with yogurt bacteria, *Streptococcus thermophilus* are inoculated in the medium in a quantity of from 10^5 CFU/ml to 10^8 CFU/ml, preferably of from 10^6 to 10^7 CFU/ml, more preferably of about 5. 10^6 CFU/ml, and *Lactobacillus delbrueckii* ssp. *bulgaricus* or *Lactobacillus delbrueckii* ssp *lactis* ssp. *lactis* are inoculated in the medium in a quantity of from 10^4 CFU/ml to 10^8 CFU/ml, preferably of from 10^5 to 10^7 CFU/ml, more preferably of about 10^6 CFU/ml.

After inoculation of the dairy medium, fermentation is conducted under the usual conditions suitable for growth of the inoculated bacteria.

The fermentation temperature will be generally of from 15°C to 45°C, preferably of from 25°C to 43°C, most preferably of about 36°C.

The fermentation is stopped when the fermentation medium reaches the desired target pH and/or when the *Lactobacillus casei* population reaches the desired target value.

Generally, the target pH will be of from 3.7 to 4.9, preferably of from 4 to 4.8, most preferably of about 4.6.

The target value for the *Lactobacillus casei* final population is generally of at least 1.1 times, preferably of at least 1.3 times and usually up to 1.5 times the final population obtained when the same *Lactobacillus casei* bacteria are cultured under the same conditions without Mn.

Generally, the fermentation is stopped when the *L. casei* population is of at least 10^7 CFU/ml, preferably of at least 10^7 to 10^{10} CFU/ml.

The invention also encompasses a fermented dairy product obtainable by the process of the invention. This product usually contains, in addition to the bacteria used for the fermentation, the fermented dairy medium, containing the added manganese salt. This fermented dairy product can be used as such, in particular as a food product, or a nutritional supplement.

It can also be added to a food product, in particular a dairy product such as a yogurt, or to a nutritional, pharmaceutical, or cosmetic composition. The food composition, as well as the nutritional, pharmaceutical, or cosmetic compositions comprising said fermented dairy product are also part of the invention.

The present invention is further illustrated by the additional description which follows, which refers to non-limiting examples of the implementation of the process of the invention.

EXAMPLE 1: SELECTIVE ENHANCEMENT BY MANGANESE OF GROWTH OF *L. CASEI* SSP *PARACASEI* CO-CULTURED WITH YOGOURT BACTERIA.

A dairy fermentation medium was prepared by mixing skimmed milk powder (30 g) with distilled water (920 g), sucrose (50g), with (medium A) or without (medium B) addition of monohydrate manganese sulfate at a final concentration of 61,44 mg/l, corresponding to 20mg/l of manganese cation. After 1 h rehydratation, the mix was sterilized by autoclaving for 15 mn at 115°C.

Medium A and medium B are inoculated with *Lactobacillus casei* subsp. *paracasei* DN-114 121 (3.80×10^7 CFU/ml), and a yogurt ferment (DN 542 142) consisting of: *Streptococcus thermophilus* DN-001 640 (10^6 CFU/ml), *Streptococcus thermophilus* DN-001 336 ($2. 10^6$ CFU/ml), *Streptococcus thermophilus* DN-001 236 ($7. 10^5$ CFU/ml) and *Lactobacillus bulgaricus* DN-100 290 (10^5 CFU/ml).

Fermentation was conducted at 36°C, and monitored by measuring the decrease of pH in the culture medium. Fermentation was stopped by transferring the preparation at 4°C when a target pH of 4,6 was reached.

The target pH was reached after 6,1 hours fermentation in the case of medium A, and 6,6 hours fermentation in the case of medium B.

The population of *Lactobacillus casei* subsp. *paracasei,* was evaluated at J+3 by cell count analysis. Cell count analysis was performed by serial dilutions of the fermented medium with tryptone salt solution, and plating on Petri dishes with appropriate agar medium: MRS Agar containing 1.5 g/l of BactoOXGALL (DIFCO) for *Lactobacillus casei.*

Petri dishes were incubated at 37°C during 96 H in anaerobic conditions (5% CO_2) .

The *Lactobacillus casei* population in the fermented product obtained from medium A was of 1.2×10^8 CFU/ml.

The *Lactobacillus casei* population in the fermented product obtained from medium B was of 8.8×10^7 CFU/ml.

The populations of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were not evaluated by cell counts, but by the evaluation of the acidification kinetics. It is well known that *Streptococcus thermophilus* and *Lactobacillus bulgaricus* grow faster in milk than *L. casei* and their lactic acid productions are directly correlated to their growth. Also, in the present invention, the monitoring of acidification corresponds to the monitoring of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* growth.

The experimentations were performed again with *Lactobacillus casei* subsp. *paracasei* DN-114 121, on the same dairy fermentation medium as above, with or without monohydrate manganese sulfate at a final concentration of 61,44mg/L, corresponding to 20mg/l of manganese cation.

Two yogurt ferments were tested in combination with *Lactobacillus casei*: DN 542 142 described above, and DN 543 043, consisting of a strain of *Streptococcus thermophilus* (DN-.001 171) and a strain of *Lactobacillus bulgaricus* (DN-100 182).

Lactobacillus casei subsp. *paracasei* DN-114 121 was inoculated at 3.8 10^7 CFU/ml.

DN 542 142 was inoculated at 10^6 CFU/ml for *Streptococcus thermophilus* DN-001 640 ; 2.10^6 CFU/ml for *Streptococcus thermophilus* DN-001 336; 7.10^5 CFU/ml for *Streptococcus thermophilus* DN-001 236, and 10^5 CFU/ml for *Lactobacillus bulgaricus* DN-100 290.

DN 543 043 was inoculated at 2.10^6 CFU/ml for *Streptococcus thermophilus* DN-001 171; and 7.10^4 CFU/ml for *Lactobacillus bulgaricus* DN-100 182.

Fermentation was conducted at 36°C, and monitored by measuring the decrease of pH in the culture medium. Fermentation was stopped by transferring the preparation at 4°C when a target pH of about 4.6 (in the case of the co-culture with the yogurt ferment DN 542 142), or of about 4.45 (in the case of the co-culture with the yogurt ferment DN 543 043) was reached.

The population of *Lactobacillus casei* subsp. *paracasei* was evaluated after 1 day (J+1) and 3 days (J+3) incubation at 10°C.

The results are illustrated by Figures 1 and 2 and Table I and II below.

Table I

Tests	End of culture		pH J+1 (10°C)
	pH (36°C)	Time (hours)	
543 043 + 114 121 - Mn 20mg/L	4,65	08h12	4,70
543 043 + 114 121	4,61	08h22	4,67
542 142 + 114 121 - Mn 20mg/L	4,43	06h58	4,44
542 142 + 114 121	4,47	07h08	4,46

Table II

Tests	Count ufc/ml at +10°C			
	J+1	Count. J+3	ratio. count.Mn/count. J+1	ratio. count.Mn/count. J+3
543 043 + 114 121 - Mn 20mg/L	2,70E+08	3,00E+08	1,9	2,1
543 043 + 114 121	1,46E+08	1,41E+08		
542 142 + 114 121 - Mn 20mg/L	1,15E+08	1,61E+08	1,9	1,7
542 142 + 114 121	6,15E+07	9,60E+07		

These results show that the addition of 20 mg/l of manganese has no effect on the acidification kinetics of the co-cultures. The time to reach the target pH is the same

with or without manganese. On the other hand, the addition of 20 mg/l of manganese selectively increases the population of *Lactobacillus casei.*

In some cases the population of *L. casei* was higher at J+3 than at J+1. This can be explained by the fact that the cooling of the culture at 4°C to stop the fermentation induces some stress, resulting in a reduced ability to grow when plated on petri dishes. After a few days the bacteria recover from the stress and regain their ability to restart growth.

EXAMPLE 2 : EFFECT OF MANGANESE ON GROWTH OF *L. CASEI* SSP *PARACASEI* OR *L.CASEI* SSP *RHAMNOSUS* CO-CULTURED WITH DIFFERENT YOGOURT FERMENTS.

The same experimentations as in Example 1 were performed with co-cultures of *Lactobacillus casei* subsp. *paracasei* DN-114 121 or *Lactobacillus casei* subsp. *rhamnosus* DN-116 010 with the yogurt ferment DN 543 043 described above, or the yogurt ferment DN 522 044, consisting of a strain of *Streptococcus thermophilus* (DN-001 143), *Streptococcus thermophilus* (DN-001 257) and a strain of *Lactobacillus lactis* (DN-111 224).

The experimentations were performed on the same dairy fermentation medium as in Example 1, with or without monohydrate manganese sulfate at final concentrations of 1,54 mg/L, 61,44 mg/L, 184,33 mg/L, corresponding to 0.5 mg/l, 20mg/l, and 60mg/l of manganese cation.

Lactobacillus casei subsp. *paracasei* DN-114 121 was inoculated at 3.8 10^7 CFU/ml.

Lactobacillus casei subsp. *rhamnosus* DN-116 010 was inoculated at 5.8 10^7. CFU/ml.

DN 543 043 was inoculated at 2 10^6 CFU/ml for *Streptococcus thermophilus* DN-001 171 ; and 7 10^4 CFU/ml for *Lactobacillus bulgaricus* DN- 100 182.

DN 522 044 was inoculated at 2 10^6 CFU/ml for *Streptococcus thermophilus* DN-001 143 ; 10^6 CFU/ml for *Streptococcus thermophilus* DN-001 257, 4 10^6 CFU/ml for *Streptococcus thermophilus* DN-001 623 and 2,5 10^5 CFU/ml for *Lactobacillus lactis* DN- 111 224.

Fermentation was conducted at 36°C, and monitored by measuring the decrease of pH in the culture medium. Fermentation was stopped by transfering the preparation at 4°C when a target pH of about 4.6 was reached.

The population of *Lactobacillus casei* subsp. *paracasei* was evaluated after 1 day (J+1) incubation at 10°C.

The results are illustrated by Figures 3, 4 and 5 and Tables III and IV below.

Table III

Tests	End of culture	
	pH	Time (hours)
1 - 543 043 + 114 121 0mg/L Mn	4,60	09h17
2 - 543 043 + 114 121 60mg/L Mn	4,59	09h14
3 - 543 043 + 114 121 20mg/L Mn	4,58	09h02
4 - 543 043 + 114 121 0,5mg/L Mn	4,60	09h18
5 - 543 043 + 116 010 0mg/L Mn	4,59	09h50
6 - 543 043 + 116 010 20mg/L Mn	4,60	09h06
7 - 543 043 + 116 010 60mg/L Mn	4,60	09h04
8 - 522 044 + 114 121 0mg/L Mn	4,60	09h22
9 - 522 044 + 114 121 20mg/L Mn	4,60	09h20

Table IV

Tests	Count ufc/ml at +10°C	paracasei ou rhamnosus
	Count. J+1 paracasei ou rhamnosus	count. num Mn/count. J+1
1 - 543 043 + 114 121 0mg/L Mn	1,35E+08	/
2 - 543 043 + 114 121 60mg/L Mn	3,25E+08	2,4
3 - 543 043 + 114 121 20mg/L Mn	3,13E+08	2,3
4 - 543 043 + 114 121 0,5mg/L Mn	2,99E+08	2,2
5 - 543 043 + 116 010 0mg/L Mn	2,52E+08	/
6 - 543 043 + 116 010 20mg/L Mn	3,20E+08	1,3
7 - 543 043 + 116 010 60mg/L Mn	3,30E+08	1,3
8 - 522 044 + 114 121 0mg/L Mn	1,42E+08	/
9 - 522 044 + 114 121 20mg/L Mn	3,25E+08	2,3

These results confirm that the addition of manganese has no effect on the acidification kinetics of the co-cultures. The time to reach the target pH is the same with or without manganese. On the other hand, it selectively increases the population of the bacteria of the *L. casei* group (*Lactobacillus casei* ssp *paracasei or Lactobacillus casei* ssp *rhamnosus).*

CLAIMS

1) The use of manganese for selectively enhancing the growth of bacteria from the *Lactobacillus casei* group in a mixed culture containing lactic acid bacteria not belonging to the *Lactobacillus casei* group, wherein the lactic bacteria not belonging to the *Lactobacillus casei* group are selected among *Lactobacillus delbrueckii* ssp *bulgaricus*, *Lactobacillus delbrueckii* ssp *lactis* and *Streptocccus thermophilus*.

2) The use of claim 1, wherein the bacteria of the *Lactobacillus casei* group are selected among the species *L. casei* subsp. *casei*, *L. casei* subsp. *paracasei*, and *L. casei* subsp. *rhamnosus*.

3) The use of any one of claims 1 to 2, wherein the bacteria from the *Lactobacillus casei* group and the lactic acid bacteria not belonging to the *Lactobacillus casei* group are cultivated in a dairy fermentation medium.

4) A method for selectively enhancing the growth of bacteria from the *Lactobacillus casei* group in a mixed culture containing lactic acid bacteria not belonging to the *Lactobacillus casei* group, wherein the lactic bacteria not belonging to the *Lactobacillus casei* group are selected among *Lactobacillus delbrueckii* ssp *bulgaricus*, *Lactobacillus delbrueckii* ssp *lactis* and *Streptocccus thermophilus*,

wherein said method comprises:

a) providing a fermentation medium containing manganese

b) inoculating said fermentation medium with the bacteria from the *Lactobacillus casei* group and the lactic acid bacteria not belonging to the *Lactobacillus casei* group; and

c) fermenting the inoculated medium until it reaches a target pH.

5) The method of claim 4, wherein the fermentation medium contains at least 0.1 mg/l of manganese.

6) The method of any one of claims 4 or 5, wherein the fermentation medium is a dairy fermentation medium, added with at least 0.05 mg/l of manganese.

7) The method of any one of claims 4 to 6, wherein the bacteria of the *Lactobacillus casei* group are selected among the species *L. casei* subsp. *casei, L. casei* subsp. *paracasei,* and *L. casei* subsp. *rhamnosus*.

8) The method of any one of claims 4 to 7, wherein the bacteria of the *Lactobacillus casei* group are inoculated in the medium in a quantity of at least 10^4 CFU/ml, and the lactic acid bacteria not belonging to the *Lactobacillus casei* group are inoculated in the medium in a quantity of from 10^3 CFU/ml to 10^9 CFU/ml.

9) The method of any one of claims 4 to 8, wherein the fermentation is conducted at a temperature of from 15°C to 45°C.

10) The method of any one of claims 4 to 9, wherein the fermentation is stopped when a target pH of from 3.7 to 4.9 is reached.

11) The method of any one of claims 4 to 10, wherein the final population of bacteria of the *Lactobacillus casei* group in the inoculated medium is of at least 1.1 times the final population obtained when the same *Lactobacillus casei* bacteria are cultured under the same conditions without Mn, when the inoculated medium reaches the target pH.

12) The method of any one of claims 4 to 10, wherein the final population of bacteria of the *Lactobacillus casei* group in the inoculated medium is of at least 1.3 times the final population obtained when the same *Lactobacillus casei* bacteria are cultured under the same conditions without Mn, when the inoculated medium reaches the target pH.

13) A method for the production of a fermented product comprising bacteria from the *Lactobacillus casei* group, wherein the lactic acid bacteria not belonging to the *Lactobacillus casei* group are selected among *Lactobacillus delbrueckii* ssp *bulgaricus*, *Lactobacillus delbrueckii* ssp *lactis* and *Streptococcus thermophilus*.

the method comprising:

a) providing a fermentation medium containing manganese

b) inoculating said fermentation medium with the bacteria from the *Lactobacillus casei* group and with lactic acid bacteria not belonging to the *Lactobacillus casei* group;

c) fermenting the inoculated medium until it reaches a target pH; and

d) stopping the fermentation and recovering the fermented product.

14) The method of claim 13, wherein the fermentation medium contains at least 0.1 mg/l of manganese.

15) The method of any one of claims 13 or 14, wherein the fermentation medium is a dairy fermentation medium, added with at least 0.05 mg/l of manganese.

16) The method of any one of claims 13 to 15, wherein the bacteria of the *Lactobacillus casei* group are selected among the species *L. casei* subsp. *casei, L. casei* subsp. *paracasei,* and *L. casei* subsp. *rhamnosus.*

17) The method of any one of claims 13 to 16, wherein the bacteria of the *Lactobacillus casei* group are inoculated in the medium in a quantity of at least 10^4 CFU/ml, and the lactic acid bacteria not belonging to the *Lactobacillus casei* group are inoculated in the medium in a quantity of from 10^3 CFU/ml to 10^9 CFU/ml.

18) The method of any one of claims 13 to 17, wherein the fermentation is conducted at a temperature of from 15°C to 45°C.

19) The method of any one of claims 13 to 18, wherein the fermentation is stopped when a target pH of from 3.7 to 4.9 is reached.

20) The method of any one of claims 13 to 19, wherein the final population of bacteria of the *Lactobacillus casei* group in the fermented product is of at least 1.1 times the final population obtained when the same *Lactobacillus casei* bacteria are cultured under the same conditions without Mn.

21) The method of any one of claims 13 to 19, wherein the final population of bacteria of the *Lactobacillus casei* group in the fermented product is of at least 1.3 times the final population obtained when the same *Lactobacillus casei* bacteria are cultured under the same conditions without Mn.

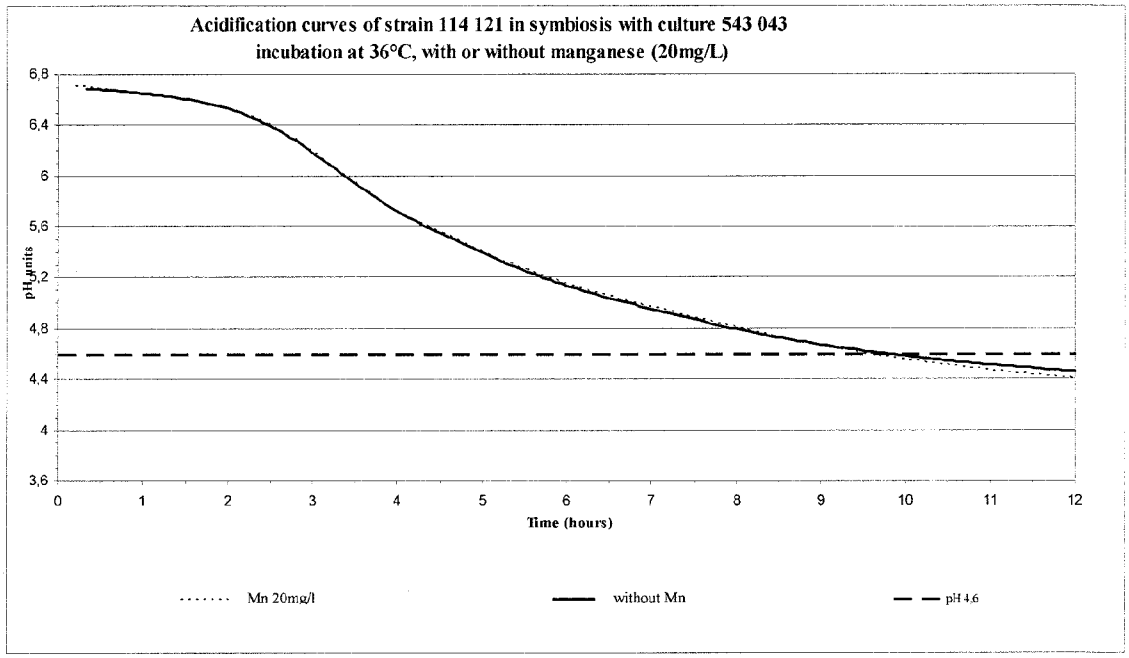


Figure 1

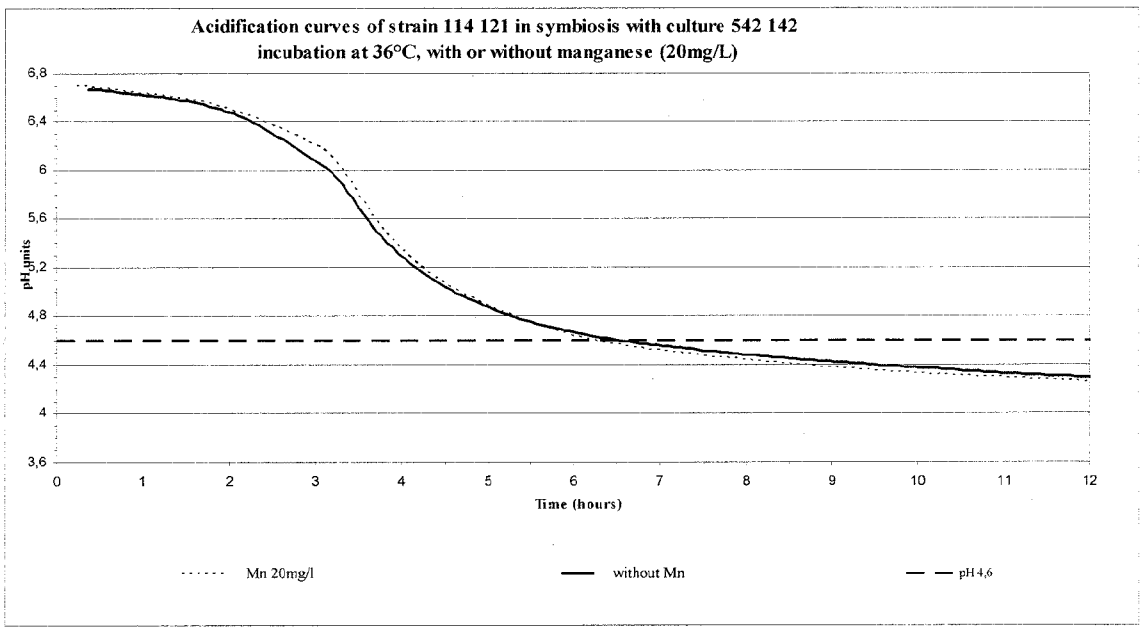


Figure 2

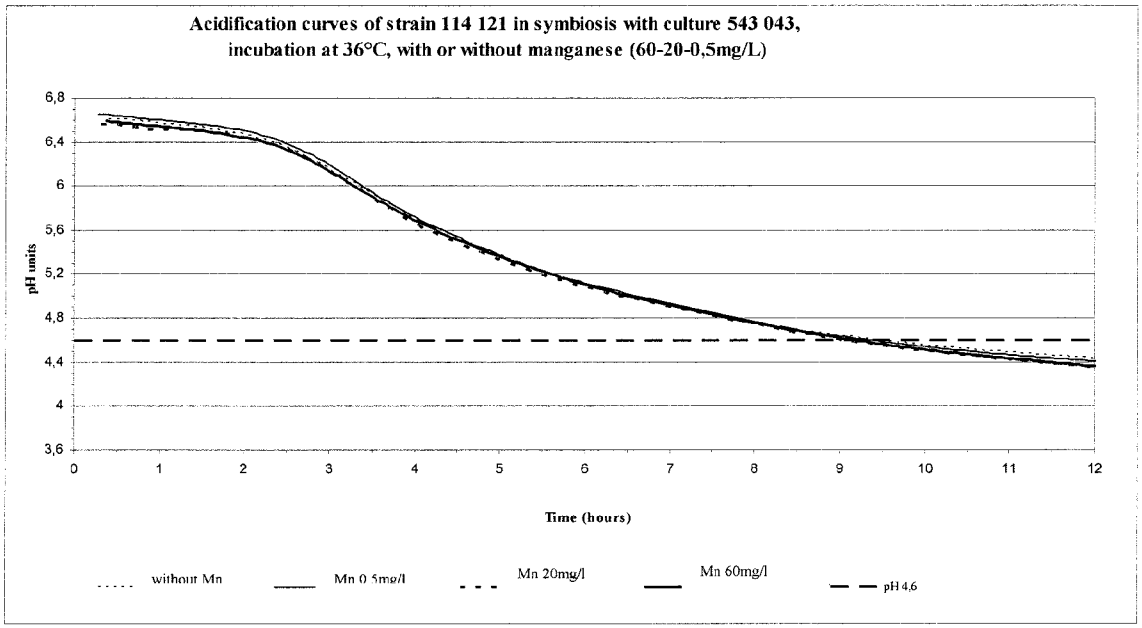


Figure 3

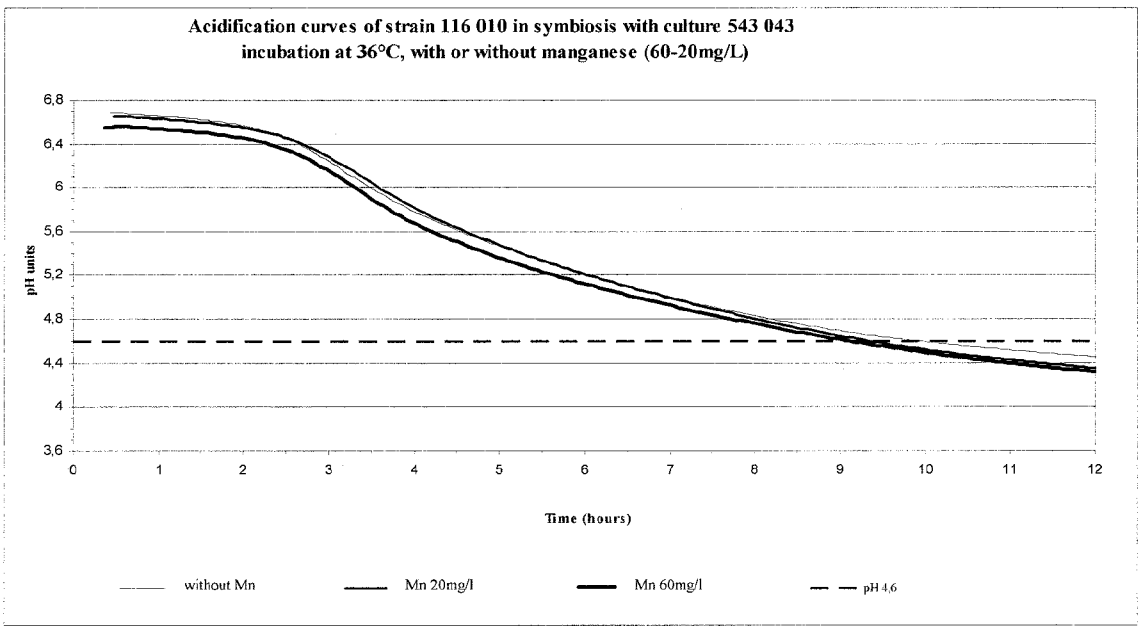


Figure 4

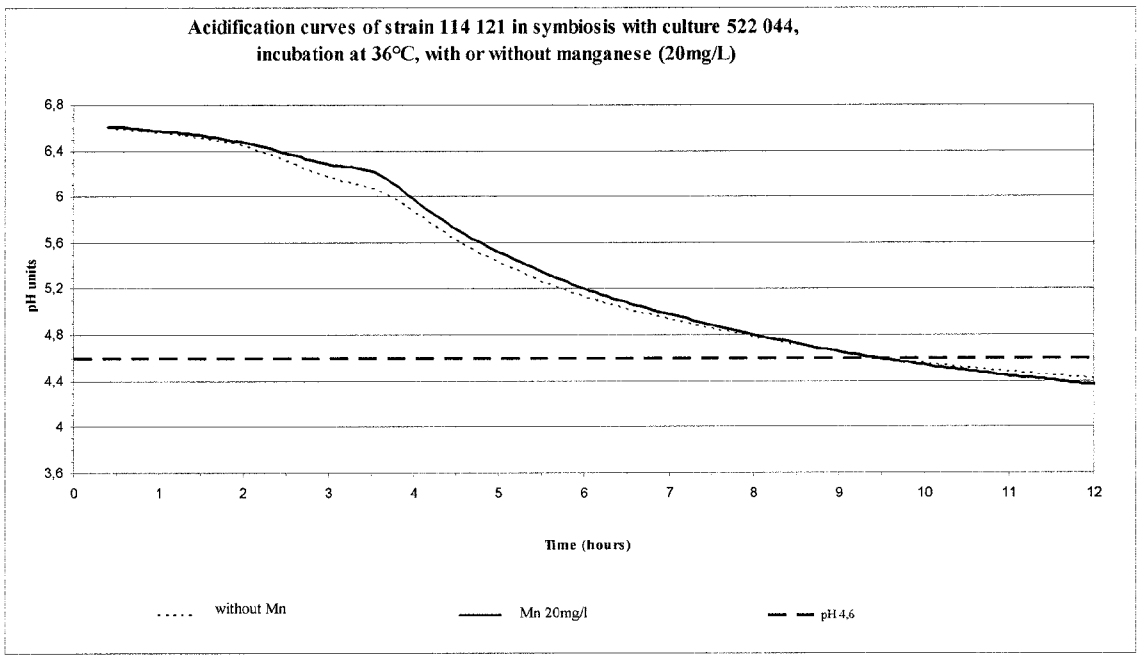

Acidification curves of strain 114 121 in symbiosis with culture 522 044,
incubation at 36°C, with or without manganese (20mg/L)
pH units
6,8
6,4
6
5,6
5,2
4,8
4,4
4
3,6
0
1
2
3
4
5
6
7
8
9
10
11
12
Time (hours)
without Mn
Mn 20mg/l
pH 4,6

Figure 5