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SURVIVAL AND THERAPEUTIC POTENTIAL OF PROBIOTIC MICROORGANISMS IN FERMENTED MILK

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The survival and therapeutic potential of probiotic microorganisms in fermented milk is estimated on the basis of the number present. The level of probiotic microorganisms which has a positive prophylactic effect and therefore therapeutic potential is $>10^6$cfu/ml. After the fermentation process and during the storage period the number of probiotic microorganisms in fermented milk should remain 10^6cfu/ml. One mono and two mixed cultures of lactic acid bacteria were investigated. The mono culture consisted of Lactobacillus acidophilus. The first mixed culture contained L.acidophilus, Bifidobacterium spp. and Streptococcus salivarius subs. thermophilus. The second mixed culture, besides the above mentioned bacteria also contained Bifidobacterium longum BB 536. Milk fermentation was carried out in laboratory conditions using sterile 9% reconstituited skimmed milk, incubated at 37 ^{0}C till the optimal pH value for fermented milk was obtained.

The number of lactic acid bacteria and change of pH value were determined after l, 4 and 8 days storage at 8±l ^{0}C .

It was found that Lactobacillus acidophilus number in the milk fermented with the monoculture remained above the therapeutical minimum during 8 days storage. Moreover, the number of Lactobacillus acidophilus and Bifidobacterium spp. in the milk fermented with mixed culture I remained above the therapeutical minimum during the storage period of 8 days. The number of Bifidobacterium longum BB 536 in fermented milk obtained with mixed culture II also remained above the therapeutical minimum during the storage period of 8 days.

Key words: fermented milk, probiotic microorganisms, therapeutic potential

INTRODUCTION

The most recent generation of fermented milk includes probiotic microorganisms in its composition. The term "probiotic" was defined by Lilly and Sillwell (l965) as the products of some microorganisms which positively influence

the growth of other microorganisms, mostly the intestinal microflora. According to new demands, the term probiotic has changed its meaning, and now it refers not only to microbial products but to the microorganism itself. Even foods that contain probiotic microorganisms, mostly fermented milk products, are usually called probiotics. According to Fuller (1989), probiotics are defined as food supplements in the form of living microorganisms, which have a positive effect on the hosts health by maintaining a favourable balance of the intestinal microflora. The mechanism by which the probiotical effect of some strains of *Lactobacillus* and *Bifidobacterium spp.* is explained, includes a decrease of cholesterol level in blood (Biss *et al.*, 1971, Rašić and Kurman 1983, Kim 1989), deconjugation of bile acids to free acids which can then be easily excreted and an increase of calcium absorption (Kuebler, 1983), although Modler and co-workers (1990) did not find that bifidobacteria decreased the cholesterol level in blood. *Bifidobacterium spp.* also have a favourable effect on the host immune system (Yamazaki *et al.* 1982, Moineau and Goulet 1991, Kanbe 1992, de Simone *et al.*, 1989, de Vrese and Barth 1991). They decrease the intestinal microflora bacterial enzymes - beta glucuronidase and beta glucosidase (Goldin and Gorbach 1977, Rowland and Grasso 1975) which are responsible for catalysing carcinogenic amine conversion and in that way decrease the risk of intestinal cancer. Nowadays one criterion for *Bifidobacterium spp.* strain selection demands the chosen strains to show beta-galactosidase activity, which may improve the intraintestinal digestion of lactose, and thus increase lactose tolerance in lactose-intolerant persons (Kallweit *et al.*, 1988). Out of a great number of strains that have been examined as possible probiotics, Bifidobacterium longum BB 536 emerged with favourable probiotic characteristics which are completely documented (Morinaga Milk Industry 1998).

Beside the cited positive characteristics, probiotic microorganisms must also show technological acceptability. By the term "technologically acceptable" de Vrese and Schresenmeir (1998) understand that, after addition to milk the microorganisms should grow before and/or after fermentation and/or at least survive the fermentation process. The taste and structure of fermented probiotic products obtained from milk, must not be worse than products obtained in the traditional way. A prophylactic effect of these microorganisms can be achieved if the number of lactobacilli and bifidobacteria in the fermented milk is 10^6 cfu/g or ml. It is well known that *Lactobacillus acidophilus* and *Bifidobacterium spp.* are poor producers of acid which is responsible for the regular fermentation course and achievment of the optimal pH. Therefore, *Streptococcus salivarius subsp. thermophilus* and *Lactobacillus delbrueckii subsp. lactis* are introduced in mixed cultures to shorten the fermentation period. The aim of this work was to determine whether the numbers of *Lactobacillus acidophilus, Bifidobacterium spp.* and *Bifidobacterium longum* BB 536 are maintained at a therapeutical level in fermented milk during the declared allowed storage period.

MATERIALS AND METHODS

One mono and two mixed cultures of lactic acid bacteria were examined. The mono culture consisted of Lactobacillus acidophilus. The first mixed culture contained strains of *L.acidophilus, Bifidobacterium spp.* and *Streptococcus*

salivarius subs. thermophilus. The other mixed culture contained the same bacteria and also Bifidobacterium longum BB 536.

Fermentation was carried out in laboratory conditions in sterile 9% reconstituited skimmed milk, incubated at 37^{0}C till the optimal pH value, for fermented milk was obtained. After finishing the production process the fermented milk was cooled and bacterial numbers determined 1, 4 and 8 days during storage at 8 1^{0}C.

The number of lactic acid bacteria was determined using suitable nutrient media for colony growth. The results were calculated as log I0 cfu/ml.

The number of lactobacilli were obtained on AC-agar (Wiesby GmbH Application), bifidobacteria on CPA- 0.5% agar (Wiesby GmbH Application), streptococci on M I7 agar (Merck). The participation of *Bifidobacterium longum* BB 536 in the total number of bifidobacteria during the storage period was determined according to the morphological characteristics of colonies grown on CPA 0.5%.

RESULTS AND DISCUSSION

The change of *L.acidophilus* number and pH value in mono culture during fermentation at 37 ^{0}C for 7h is presented in Table 1.

Table 1. Change of *L. acidophilus* number and pH value during fermentation for 7h at 37^{0}C and storage at 8 ± 1 °C.

Time	log_{10}cfu/ml	pH
Beginning of fermentation	6.10	6.30
End of fermentation	7.26	4.45
1 day storage	7.20	4.47
4 days storage	7.14	4.36
8 days storage	6.70	4.32

Milk fermented with *L. acidophilus*, had a microorganism count of 7.26 log I0 cfu/ml and pH 4.45. During 8 days storage at 8±1^{0}C, the number of lactobacilli decreased from 7.20 to 6,70 log_{10}cfu/ml. The change in *Lactobacillus acidophilus* numbers was accompanied by a change of pH value. The first day after fermentation the pH remained at 4.47, but after 8 days storage at 8 ±1^{0}C it was 4.32. The small change of bacterial number and pH value during the storing period showed that there was no significant post fermentation acidity, and that the number of living *L.acidophilus* remained above the therapeutical minimum of 10^6 cfu/ml.

Changes in bacterial number and pH value for the first mixed culture are presented in Table 2.

It can be noticed that the number of lactobacilli and bifidobacteria at the beginning of fermentation were already above the therapeutical minimum (>6 log_{10}/ml). During fermentation at 37^{0}C for 7 h the streptococci multiplied intensively and increased from 5.74 log_{10} cfu/ml to 8.11 log_{10} cfu/ml. During the

Table 2. Changes in the number of lactic acid bacteria and pH during the fermentation for 7h at 37 °C with the first mixed culture and storage at 8 ± 1 °C.

Time	Number of lactic acid bacteria (log_{10}cfu/ml)			pH
	L. acidophilus	*Bifidobacterium spp.*	*St. salivarius subsp. thermophilus*	
Beginning of fermentation	6.17	6.49	5.74	6.30
End of fermentation	7.50	7.16	8.11	4.45
1 day storage	7.14	6.50	7.70	4.37
4 days storage	7.11	6.40	7.70	4.55
8 days storage	7.11	6.20	7.70	4.55

same interval the number of lactobacilli increased by 1.33 log_{10} cfu/ml, and the number of bifidobacteria by 0.67 log_{10} cfu/ml.

During the storage period the number of lactobacilli decreased less than in the monoculture during the same period (Tables 1 and 2). After cooling the number of bifidobacteria in the mixed culture decreased rapidly from 7.16 to 6.50 log_{10} cfu/ml, but after 8 days storage, the number of bifidobacteria had further declined slightly to 6.20 log_{10} cfu/ml. The number of streptococci also decreased significantly immediately after cooling, but did not change during the storage period. In the same period the pH of the fermented product had an optimal value.

The number of microorganisms from the second mixed culture during 8 days storage at 8±1 ^{0}C is presented in Table 3.

Table 3. The number of lactic acid bacteria from mixed culture II in fermented milk during 8 days storage at 8 ± 1 °C.

Storage for	pH	Lactic acid bacteria number (log_{10}cfu/ml)		
		Bifidobacterium spp.	*Lactobacillus acidophilus*	*St. salivarius subsp. thermophilus*
1 day	4.32	7.25	7.15	9.18
4 days	4.31	7.49	7.13	9.18
8 days	4.25	7.19	7.14	9.14

The results show that during storage of milk fermented with mixed culture II, the number of bifidobacteria increased from 2.5 to 4.9x 10^7 cfu/ml on the fourth day, which confirms its technological acceptability according to de Vres and Schrezenmeir (1998).

The participation of *Bifidobacterium longum* BB 536 in the total bifidobacteria number in fermented milk with mixed culture II during 8 days storage at 8±1 ^{0}C is presented in Table 4.

Table 4. Participation of *Bifidobacterium longum* BB 536 in the total bifidobacteria number in fermented milk with mixed culture II during 8 days storage at 8 ± 1 °C.

Storage conditions 8 ± 1°C	pH	Lactic acid bacteria number (log_{10}cfu/ml)	
		Bifidobacterium spp.	*Bifidobacterium longum BB536*
1 days	4.32	7.25	6.97
4 days	4.31	7.49	6.20
8 days	4.25	7.19	6.23

The number of *Bifidobacterium longum* BB 536 in fermented milk after the first day of storage was 6.97 log_{10}cfu/ml. On the fourth and eighth day of storage at 8±1^{0}C this had decreased to 6.20 and 6.23 log_{10}cfu/ml, respectively. However, the fermented milk obtained with *Bifidobacterium longum* BB536 fulfilled the therapeutic requirements throughout the storage period of 8 days (Hansen 1987, Reuter 1990), because the number of *Bifidobacterium longum* BB536 was > 10^6/ml.

CONCLUSIONS

On the basis of the results obtained, following conclusions can be drawn:

Lactobacillus acidophilus number in fermented milk obtained with a monoculture was maintained above the therapeutical minimum during 8 days storage at 8±1^{0}C.

The number of Lactobacillus acidophilus and *Bifidobacterium spp.* in fermented milk obtained with mixed culture I remained above the therapeutical minimum during storage for 8 days at 8±1^{0}C .

The number of *Bifidobacterium longum* BB 536 in fermented milk obtained with mixed culture II remained above the therapeutical minimum during storage for 8 days at 8 1^{0}C.

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PREŽIVLJAVANJE I TERAPEUTSKI POTENCIJAL PROBIOTSKIH MIKROORGANIZAMA U FERMENTISANOM MLEKU

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SADRŽAJ

Pre'ivljavanje i terapeutski potencijal probiotskih mikroorganizama se procenjuju na osnovu broja ovih mikroorganizama u fermentisanom mleku. Terapeutski potencijal je onaj nivo probiotskih mikroorganizama koji ima pozitivno profilaktičko delovanje a ono se ostvaruje ako je njihov broj u fermentisanom mleku 10^6cfu/ml. Posle fermentacije i za vreme perioda čuvanja fermentisanog mleka broj probiotskih mikroorganizama treba da iznosi 10^6cfu/ml.

Materijal u ovim ispitivanjima su predstavljale jedna mono kultura i dve mešovite kulture bakterija mlečne kiseline. Mono kultura se sastojala od *Lactobacillus acidophilus* a prva mešovita kultura od tri vrste mikroorganizama: *Lactobacillus acidophilus, Bifidobacterium spp.* i *Streptococcus salivarius subs.*

thermophilus. Druga mešovita kultura je pored bakterija koje ulaze u sastav prve mešovite kulture sadr'avala i *Bifidobacterium longum* BB536.

Fermentacija mleka je izvođena u laboratorijskim uslovima u sterilnom 9% obranom mleku a inkubacija je sprovedena pri 37 °C do postizanja optimalnog pH za pojedina fermentisana mleka. Broj bakterija mlečne kiseline određivan je na podlogama odgovarajućeg sastava i iskazan kao $\log_{10}$cfu/ml.

Laktobacili su dokazivani na AC-agaru (Wiesby GmbH Application), bifidobakterije na CPA-0,5% agaru (Wiesby GmbH Application) a streptokoke na M17 agaru (Merck). Broj bakterija mlečne kiseline i promena pH određivani su posle 1., 4. i 8.-og dana čuvanja na 8±1°C.

Na osnovu rezultata dobijenih u eksperimentu izvedeni su sledeći zaključci: 1. broj *Lactobacillus acidophilus* u fermentisanom mleku dobijenom sa mono kulturom tokom perioda čuvanja od 8 dana na 8+1°C održava se iznad terapeutskog minimuma. 2. broj *Lactobacillus acidophilus* i *Bifidobacterium spp.* u fermentisanom mleku dobijenom sa mešovitom kulturom I tokom perioda čuvanja od 8 dana na 8±1 °C održava se iznad terapeutskog minimuma. 3. broj *Bifidobacterium longum* BB 536 u fermentisanom mleku dobijenom sa mešovitom kulturom II tokom perioda čuvanja od 8 dana na 8±1 °C održava se iznad terapeutskog minimuma.