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Growth and Activity of Selected Probiotic Strains in UHT Milk

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ABSTRACT: The growth rate of two probiotic strains with documented health effects was studied in ultra-high temperature treated milk separately and beside each other. The probiotic strains were *Lactobacillus acidophilus* (La-5) and *Bifidobacterium lactis* (Bb-12). Fermentation was followed for 12 h at 37 ˚C and the samples were analyzed for pH, titratable acidity and viable counts of probiotic bacteria before incubation and at 2h intervals throughout the fermentation period. The obtained results showed that *Lb. acidophilus* had higher activity and growth rate than *B. lactis* when they were purely cultured in milk. Investigation on mixed culture inoculating the milk revealed that *Lb. acidophilus* had a stimulating effect on the growth rate of *B. lactis*, however, its growth wasn't significantly affected by the presence of the latter bacteria.

Keywords: *Bifidobacterium lactis, Growth Rate, Lactobacillus acidophilus, Probiotic.*

Introduction

Probiotic bacteria are microorganisms that have a beneficial effect on the intestinal function, and they can promote good health (Sanders, 1999). Some of their established health benefits include alleviation of symptoms of lactose intolerance, enhancement of the immune system, reduction of the duration of rotavirus diarrhea, a decrease in fecal bacterial enzyme activity and mutagenicity, prevention of recurrence of superficial bladder cancer, and prevention of atopic diseases (Salaminen *et al*., 1998; Naidu *et al*., 1999; Kalliomaki *et al*., 2001). Several aspects have to be taken into consideration in the selection process of probiotic organisms. Safety aspects of probiotic bacteria include specifications as to human origin, non-pathogenicity and antibiotic resistance characteristics, which have been reviewed lately (Salminen *et al*., 1998; Saarea *et al*. 2000). In recent years, there has been a growing interest in using probiotic micro-organisms as dietary adjuncts in the dairy industry (Akin *et al*., 2007). Lactic acid bacteria (LAB), in particular lactobacilli and bifidobacteria, constitute a significant proportion of the probiotic cultures used in developed countries (Oloveria *et al*., 2001; Richardson, 1996). In order to have any beneficial effect on humans, the viable cell count should be above 6 log colony forming units per milliliter (cfu ml^{-1}) to supply a sufficient "daily dose" of 10^6- 10^7 viable bacteria (Lee & Salminen, 1995; Vinderola *et al*., 2000). It is worth to mention that a standard, requiring a minimum of 10^6–10^7 cfu g^{-1} of *Lb. acidophilus* and/or Bifidobacteria in fermented milk products, has been introduced by several food organizations world-wide (IDF, 1992). Factors related to the technological and sensory aspects of the probiotic food products are important since only by consumer's satisfaction, the food industry can be successful in promotion of functional products in the future (Mattila–Sanholm *et al*., 2002). Understanding the behavior of these bacteria in milk enables

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the better uses of them in industrial application. Therefore, the objectives of this work were the monitoring of the activities and growth rates of two most commonly used probiotic strains, *Lb. acidophilus* and *B. lactis*, separately, and in the presence of each other in milk to improve their application in dairy industry.

Materials and Methods

Probiotic strains

Commercial single strain lyophilized cultures of *Lb. acidophilus* and *B. lactis* known as FD-DVS La-5 and Bb-12, were supplied by Chr. Hansen (Horsholm, Denmark).

Sample preparation

UHT milk was inoculated at the fermentation temperature (37 ˚C) by 0.01% (W/V) of cultures, separately, for the preparation of samples A and B, respectively. The same inoculation rates of two mentioned starter cultures (50:50) were applied for the preparation of AB sample.

As lower incubation temperatures (37-40˚C) were optimum for growing probiotic species (Tamime & Robinson, 1999), inoculated milks (A, B and AB), after aseptically distributing in 100-ml sterilized bottles, were incubated at 37 ˚C for 12 h. Samples were taken before incubation at 2 h intervals throughout the 12 h of fermentation time for microbiological and chemical analysis.

Microbiological analysis

Serial dilutions of samples were made in quarter strength Ringer's solution (Merck, Germany) and spread plated in duplicate on their special media. *Lb. acidophilus* was counted on MRS (De Man, Rogosa and Sharpe) agar incubated aerobically at 37 ˚C for 3 days. Viable cell numbers of *B. lactis* in the samples B and AB were counted on MRS agar and MRS-LP (Lithium Chloride and Sodium Propionate) agar, respectively, (Vinderola & Reinheimer, 1999) incubated under anaerobic conditions using GasPak system (Merck, Darmstadt, Germany) after 3 days at 37 ˚C. The latter medium allows the selective colony count of *B. lactis* in the presence of *Lb. acidophilus* (Vinderola *et al*., 2000).

Chemical analysis

Titratable acidity of the samples was measured by titrating 10 ml of the sample with 0.1 N NaOH using phenolphetalein as an indicator (Akin *et al*., 2007). All pH measurements were made using a digital pH meter with combined glass electrode and temperature probe. The pH meter was calibrated using standard buffer solutions at pH 4.0 and 7.0 (Ostile *et al*., 2005).

Statistical analysis

All measurements were performed in three replicates. Results were submitted to analysis of variance using MINITAB Software. Mean values were compared using Tukey test at $P < 0.05$.

Results and Discussion

Viable count of probiotic strains

The results for the variations of probiotic strains in the samples A, B and AB throughout 12 h incubation at 37 ˚C are shown in table 1.

This was explained by the distribution of water within the mixed system (Table 1).

The initial point of significant changes ($P< 0.05$) in the viable count of *Lb. acidophilus* in the sample A was observed after 6 h incubation. The increase in the viable bacterial numbers was continued to the end of incubation period at 37 ˚C. The viable cells of *Lb. acidophilus* reached 8.50 log cfu ml^{-1} after 12 h incubation, which showed near to 1.0 log unit increase from their initial point. Mentioned finding agrees with this fact that "milk is not, on the whole, a good growth medium for probiotic bacteria" (Shah *et al*., 1995; Schilinger,

1999; Ostile *et al*., 2005). In this respect Ostile *et al*. (2002) reported that *Lb. acidophilus* showed the most rapid increase and the highest viable cell numbers in milk incubated at 37 ˚C, attaining viable cell numbers of 8.65-9.21 log cfu ml^{-1} after 12-24 h incubation.

The viable count of *Lb. acidophilus* showed a significant increase in the sample AB after 6 h incubation. This increasing trend of *Lb. acidophilus* was similarly observed in the sample A, but it was higher in the sample AB, therefore one log unit increase was observed after 8 h incubation, and it attained 1.4 log unit at the end of incubation period. As it is shown in fig 1, the increases in the viable count of *Lb. acidophilus* in the samples A and AB were not significantly different (P>0.05) from each other except in the beginning of incubation that directly attributed to the inoculation level of *Lb. acidophilus* in the two mentioned samples.

As it is clear in table 1, the viable cell counts of *B. lactis* showed no significant changes (P>0.05) throughout 12 h incubation at 37 ˚C. Ostile *et al*. (2002) reported that *B. lactis* (Bb-12) grew slowly at 30˚C, but attained the highest increase (1.5 log unit) after 48 h incubation at this temperature. They also mentioned that the stability of the numbers of viable cell was the best at 30 and 37 ˚C. In other study Ostile *et al*. (2005) showed that *B. lactic* (Bb-12), among *Lb. acidophilus* La5, *Lb. acidophilus* 1748, *Lb. jonsonii* LA1, *Lb. rhamnosus* GG and *Lb. reuterih* SD 2112 had the lowest increase rate of viable cell numbers in milk incubated at 37 ˚C, attaining the viable cell numbers of 8.65-9.21 log cfu ml^{-1} after 12- 24 h incubation. Baron *et al*. (2000) studied bifidobacteria and found that the fermented milk produced at 30˚C by different species of bifidobacteria, showed no or only a slight increase of viable cells for all of the tested species of bifidobacteria. On the other hand, during the fermented milk production at 35˚C, the number of viable cells was increased by approximately one log unit for *B. breve, B. longum and B. infantis* strains (Baron *et al*. 2000). Our findings besides all of the above mentioned reports, showed this fact that, as milk did not contain sufficient free amino acids and peptides, it couldn't be considered as a proper media for the growth of bifidobacteria (Zourari et al., 1992; Abu-Tarboush, 1996). Therefore, Dave and Shah (1998) have shown that milk supplemented with peptides and amino acids, such as cysteine, improved the survival of bifidobacteria.

A considerable change was observed in the growth rate of *B. lactis* in the presence of *Lb. acidophilus* in the sample AB (fig 2).The viable counts of *B. lactis* were significantly (P< 0.05) increased after 6 h incubation and showed a maximum value by attaining the highest increase (approximately 1.5 log units) after 10 h incubation at 37 ˚C. Significant differences between the growth rate of *B. lactis* of the two samples (B and AB) can be attributed to the presence of *Lb. acidophilus* in the sample AB. The stimulating effects of *Lb. acidophilus* on the growth rate of *B. lactis* can be discussed from two points. 1) Although probiotic bacteria possess no complete proteolytic systems comparing them to those in yoghurt bacteria (Booth *et al*., 1990; Wohlrab & Bockelmann, 1993; Bockelmann et al., 1996; Law & Haadrikman 1997), but the proteolytic activity of *Lb. acidophilus* is higher than that of bifidobacteria (Shihata & Shah 2000; Elli et al., 1999). Therefore, *Lb. acidophilus* by mediating a kind of proteolysis provides *B. lactis* with some none protein nitrogens. 2) The consumption of dissolved oxygen, to some extent, by *Lb. acidophilus* improves conditions for the better growth rate of *B. lactis*. As oxygen definitely affects the

growth of anaerobic bifidobacteria (Kailasapathy & Sultana, 2003), so reduction of dissolved oxygen enhances the growth of *Bifidobacterium spp.* (Dave & Shah, 1996).

Table 1. Viable cell counts (log cfu ml^{-1})* of probiotics (*Lactobacillus acidophilus and Bifidobacterium lactis*) in the samples A, B and AB throughout 12 h incubation at 37 ˚C

Viable cell counts (log cfu ml^{-1})	**Time of incubation (h)**						
	0 **	**2**	**4**	**6**	**8**	**10**	**12**
***Lb. acidophilus* in Sample A**	7.68[a]	7.62[a]	7.70a	8.08[b]	8.32[b]	8.44[b]	8.50[b]
***B. lactis* in sample B**	7.98[a]	8.07[a]	8.20[a]	8.25[a]	8.23[a]	8.19[a]	7.98[a]
***Lb. acidophilus* in sample AB**	7.26[a]	7.55[a]	7.65[a]	8.13[b]	8.20[b]	8.55[b]	8.66[b]
***B. lactis* in sample AB**	7.34[a]	7.63[a]	7.78[a]	8.19[b]	8.34[b]	8.77[c]	8.72[c]

* The means shown with different letters in a row are significantly different ($P < 0.05$).
** 0 h = immediately after inoculation.

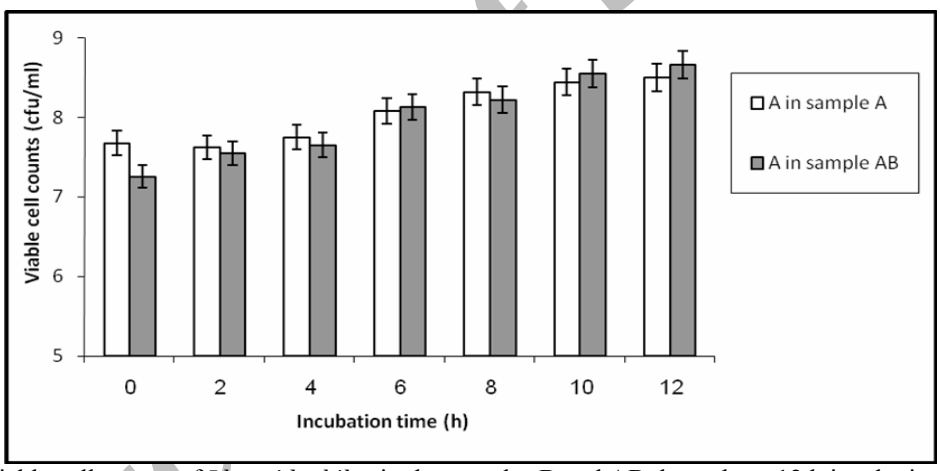

Fig. 1. Viable cell counts of *Lb. acidophilus* in the samples B and AB throughout 12 h incubation at 37 ˚C

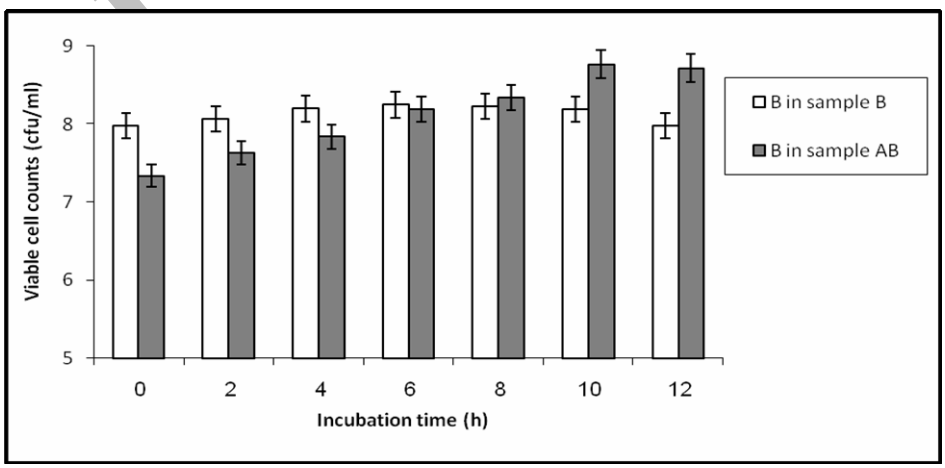

Fig. 2. Viable cell counts of *B. lactis* in the samples B and AB throughout 12 h incubation at 37 ˚C

Table 2. Mean values of three replicate measurements of pH and titratable acidity (°D) of the samples A, B and AB throughout 12 h incubation at 37 ˚C

Samples	pH / Titratable acidity (˚D) Time of incubation (h)						
	0 **	2	4	6	8	10	12
A	6.60 / 13.30	6.44 / 14.83	6.20 / 16.70	5.96 / 20.00	5.80 / 25.16	5.51 / 33.30	5.20 / 41.83
B	6.60 / 13.30	6.44 / 15. 00	6.25 / 17.30	6.13 / 19.80	6.04 / 22.10	5.92 / 23.60	5.84 / 25.60
AB	6.60 / 13.30	6.43 / 15.50	6.34 / 17.75	6.02 / 24.25	5.71 / 31.50	4.98 / 71.00	4.55 / 77.25

**0 h = immediately after inoculation.

pH and titratable acidity

The changes of pH and titratable acidity of the three samples (A, B and AB) are shown in table 2.

The pH value in the samples A, B and AB respectively was decreased from 6.6 to 5.2, 5.84 and 4.55, and the titratable acidities were increased from 13.30 to 41. 83, 25.60 and 77.25 after 12 h incubation at 37 ˚C.

The sample AB showed the most reduction of pH, and also the most increase of titratalbe acidity throughout 12 h incubation which was due to the higher activity and growth of *Lb. acidophilus* and *B. lactis* beside each other discused previously. In the sample B the changes of pH and titratable acidity were slower than those in the sample A. Mentioned results confirmed the higher activity of *Lb. acidophilus* in comparison with the poor growth of bifidobacterium spp. in milk (Ostile *et al*., 2002).

In our study the lowest viable counts of *B. lactis* in the sample B were observed in the pH of 5.84 after 12 h incubation at 37˚C, because bifidobacteria were sensitive to the level of pH variations and their growth was restricted at pH < 5.0 (Shah, 1997; Gomes & Malcata, 1999).

The optimum growth pH of *Lb. acidophilus* was at the range of 5.5- 6.0 (Gomes & Malcata, 1999) and we observed the highest viable counts of *Lb. acidophilus* at the pH value of 5.2 after incubation.

Conclusion

Findings in this work confirmed the low activity of probiotic strains in milk. *B. lactis* had no significant growth throughout 12 h incubation at 37˚C, but its growth was stimulated considerably in the mixed culture in the presence of *Lb. acidophilus* at the same fermentation condition. *Lb. acidophilus* had a higher activity in comparison with *B. lactis* in milk, while no significant changes were observed in its growth rate in the presence of *B. lactis*. All of the above results suggest that milk could be a good carrier media for *B. lactis,* but it is not suitable for producing fermented milk unless we use it in a mixed starter culture in the presence of other LABS.

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