Viability of *Bifidobacterium longum* Grown Alone or in Association with Some Strains of Lactic Acid Bacteria Under Refrigeration

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ABSTRACT

This work was undertaken to characterize the interaction between *Bifidobacterium longum* DSM 20097 and 6 strains of lactic acid bacteria belonging to *Streptococcus thermophilus, Lactococcus lactis* subsp. *lactis*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus casei* subsp. *paracasei*, *Lactobacillus acidophilus* and *Lactobacillus plantarum*. *Bifidobacterium longum* was grown in sterilized skim milk supplemented with 2% glucose, 1% yeast extract and 0.05% L-cysteine hydrochloride either separately or in co-culture with one of the above listed strains of lactic acid bacteria. Following 18 hr of incubation at 37°C, milk cultures were stored at 5±1°C for 28 days. Changes in titratable acidity, pH, organic acids, soluble tyrosine, viable counts and antibacterial activity of various milk cultures were monitored at regular intervals during refrigeration storage. Results revealed that *B. longum* survived better in the presence of *Lb. plantarum*, while its viability was drastically declined in the presence of *Lb. bulgaricus*. Among tested lactic acid organisms, *Lb. plantarum* proved to be the highest proteolytic organism, determined in terms of soluble tyrosine, and the least acid producer. These activities might be responsible for the improvement of viability of *B. longum* during refrigeration storage. Also, combining *B. longum* with *Lb. plantarum* improved the antimicrobial activity of the former against *Staphylococcus aureus* and *Bacillus subtilis* as assessed by the well-diffusion agar method.

Key words: Bifidobacteria, probiotics, lactic acid bacteria, interaction, viability.

INTRODUCTION

Bifidobacteria have attracted considerable academic and public attention because of their health benefits. These organisms are one of the predominant groups of bacteria in the normal flora of the human intestinal tract, they constitute up to 25% of the total population in the intestinal tract in adults and 95% in newborns (Yildirim & Johnson, 1998). Several studies have shown the therapeutic and nutraceutical benefits for dietary containing bifidobacteria (Zentek *et al*., 2003, Abd El-Gawad *et al*., 2004). These organisms are believed to reduce the level of cholesterol in the blood and deconjugate bile acids into free acids which are easily extracted (Jiang *et al*., 1996). They can also increase immunity of the host (Hoover, 1993, Vinderola *et al*., 2004) and are useful for improvement in lactose utilization in lactose malabsorbers (Shah & Jelen, 1990). In addition, they are able to provide unfavorable condition for growth of potential pathogenic Gram-negative bacteria in the gastrointestinal tract (Tannock, 1998). However, to achieve the desired therapeutic and health benefits, a certain level of viable count of bifidobacteria is required, for example, a fermented dairy product should contain between 10^7 and 10^8 live bifidobacteria/g when displayed in retail outlets, and consumption of 100g of this product would deliver sufficient bifidobacteria in the intestine (Blanchette *et al*., 1996).

Indeed, bifidobacteria grow poorly in milk unless it is supplemented with various nutrients. They lack the ability to generate the levels of lactic acid associated with normal yoghurt when the bifidobacteria present in a single culture, exhibit an extremely low rate of acid production, such that even overnight incubation (14-16 hours) may not provide sufficient time for the milk to reach a pH 4.3-4.4 consequently, more research has been focused on the use of mixed cultures containing bifidobacteria alongside one or more of lactic acid bacteria (LAB) (Samona & Robinson, 1994). Several species of LAB are generally used for the manufacture of fermented dairy products. Bifidobacteria are usually incorporated in yoghurt along with yoghurt culture organisms (Lankaputhra & Shah, 1995, Shah *et al*., 1995) are also being incorporated into cheeses cultures (Blanchette *et al*., 1996, Daigle *et al*., 1999). Thus, the interaction between bifidobacteria and LAB involved in milk fermentation processes needs to be studied.

Kheadr (2001) evaluated the impact of aflatoxin M1 on the viability and physiological activities of 4 bifidobacterial cultures (*B. bifidum* DSM 20456,

B. bifidum DSM BB12, *B. longum* DSM 20097 and *B. infantis* DSM 20090). These strains were grown in reconstituted skim milk with added aflatoxin M1 at concentrations of 5 or 10 µg/L and stored at 5℃ for 15 days. Among tested strains, *B. longum* DSM 20097 appeared to have a potential acidogenous capacity and higher ability to survive refrigeration conditions. In addition, *B. longum* showed the highest capability of detoxifying aflatoxin M1 and could eliminate 26-48% of the total quantity of added aflatoxin, depending on initial concentration, after 15 days of storage at 5℃. Theses characteristics make *B. longum* DSM 20097 a potential candidate for use as an adjunct in fermented milk prepared for probiotic purposes. Prior to its application in the manufacture of probiotic dairy products, it would be necessary to characterize the interaction between this strain and some lactic acid starters.

Thus, the aim of the present study was to characterize the interaction between *B. longum* DSM 20097 and some LAB when grown in co-culture in sterilized skim milk. Changes in titratable acidity, pH, organic acids, soluble tyrosine and the viable counts of *B. longum* and LAB were monitored during 28 days at refrigerated storage (5±1℃.)

MATERIALS AND METHODS

Bacterial strains and growth conditions

Bifidobacterium longum DSM 20097 (*B. longum*) was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (GmbH, Germany). *Streptococcus thermophilus* R0083 (*Str. thermophilus*), *Lactococcus lactis* subsp. *lactis* R0058 (*Lac. lactis*), *Lactobacillus acidophilus* R0052 (*Lb. acidophilus*) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (*Lb. bulgaricus*) mibl-2 were obtained from Rosell Institute Inc. (Montreal, PQ, Canada). Lactobacillus casei paracasei subsp. paracasei P/N 601385 (*Lb. paracasei*) and Lactobacillus plantarum P/N 601387 (*Lb. plantarum*) were obtained from Chr. Hansen Ltd. (Barrie, ON, Canada). All strains were reactivated from frozen stock in 20% glycerol at -80°C. *B. longum* was reactivated in de Man, Rogosa and Sharpe (MRS) broth (de Man *et al*., 1960) containing 0.05% (w/v) of filter-sterilized (0.2 µm, Nalge Co., Rochester, NY) L-cysteine hydrochloride (Hull and Roberts, 1984) and incubated anaerobically in jars using an atmosphere generation system (Oxoid AnaeroGenTM, Oxoid Ltd., Basingstoke, Hampshire, England) at 37℃ for 24 hr. *Str. thermophilus* and *Lac. lactis* were cultivated in M17 broth medium at pH 7.1 (Quelab, Montreal, PQ, Canada) supplemented with 0.5% (w/v) glucose (GM17) and incubated overnight at 37 and 30°C, respectively (Terzaghi & Sandine, 1975). All lactobacilli were grown in MRS broth and incubated anaerobically at 37℃ for 24 hr. Prior to beginning the experiment in milk, cultures were activated by three successive transfers in sterilized skim milk 1% (v/v) at 18 hr intervals.

Staphylococcus aureus ATCC 6538 (*Staph. aureus*) and *Bacillus subtilis* ATCC 6633 (*Ba. subtilis*), obtained from the American Type Culture Collection (ATCC) (Rockville, MD, USA), were used as indicator organisms for the assessment of antibacterial activity of different bacterial cultures. Both organisms were activated into nutrient broth using 1% of inocula and incubated for 18 hr at 37°C.

Media preparation

Bacteriological peptone (Oxoid) at a concentration of 0.15% (w/v) was prepared in distilled water, adjusted to pH 7.0±0.2 and autoclaved at 121℃ for 15 min. The peptone water was used as diluents throughout microbiological analyses.

The *Streptococcus thermophilus* (ST) agar medium was prepared according to the method described by Dave & Shah (1996). Inoculated plates were incubated aerobically at 37°C for 24 hr. *Lac. lactis* was enumerated on GM17 agar and incubated aerobically at 30°C for 24 hr (Terzaghi & Sandine, 1975).

The MRS agar containing nalidixic acid (15 mg/l), neomycine sulfate (100 mg/l), lithium chloride (3 g/l) and paromomycine sulfate (200 mg/l) (MRS-NNLP) was prepared according to the method described by Laroia & Martin (1991). After autoclaving the agar medium at 121℃ for 15 min, filter-sterilized NNLP and L-cysteine-HCl (0.05%, w/v) were added just before pouring into plates. *B. longum* was enumerated anaerobically at 37℃ for 72 hr.

The MRS-maltose agar was used to enumerate *Lb. acidophilus* at 43℃ for 72 hr under anaerobic condition (Tharmaraj & Shah, 2003). The MRS-sugar free medium containing 13 g/L of bacteriological agar was prepared and autoclaved at 121℃ for 15 min. Ten milliliters of filtered-sterilized 20% solutions of DL-maltose were added to 90 ml of basal agar (2% final concentration) just before pouring the agar medium.

Acidified MRS agar (pH 4.58) was used to enumerate *Lb. bulgaricus* at 45℃ for 72 hr under

anaerobic condition (Tharmaraj & Shah, 2003). The pH of MRS agar medium was adjusted to 4.58 using 1.0 M HCl and autoclaved at 121°C for 15 min.

The MRS-vancomycin agar medium was prepared as described by Tharmaraj & Shah (2003) and tested for its ability to selectively enumerate *Lb. paracasei* at 37°C for 72 hr under anaerobic condition. The MRS agar medium was autoclaved at 121°C for 15 min, cooled down to 50°C and filter-sterilized vancomycin (Sigma Chemical Co., St. Louis, MO, USA) was added just before pouring into plates to obtain 1 mg/L final concentration.

Evaluation of media selectivity

To determine the selectivity of the above media, one gram of an 18 hr milk culture of each organism was 10-fold serially diluted (10^3 to 10^7) in peptone water (0.15%, w/v). Dilutions were further plated onto different agar media and inoculated plates were incubated under conditions recommended for each medium as previously mentioned.

Preparation of reconstituted milk

Reconstituted non-fat dry milk (10%) supplemented with 2% glucose and 1% yeast extract was prepared. Two-hundred ml screw-capped glass bottles were filled with 150 ml milk. Bottles were sterilized at 121°C/15 min and cooled down to room temperature. Filter-sterilized L-cysteine-HCl was added to a final concentration of 0.05% (w/v). Bottles were then inoculated with actively growing cultures of *B. longum* or LAB to a final concentration of 1% (v/v). For mixed cultures, the final concentration of each organism was also 1% (v/v). Bottles were incubated anaerobically at 37°C for 18 hr, then transferred to a refrigerator (5±1°C) and stored for 28 days.

Sampling

Changes in titratable acidity (TA), pH , organic acids and viable bacterial counts were determined at 0, 7, 14, 21 and 28 days of storage. Tyrosine contents were determined at zero time, 14 and 28 days. Antibacterial activities were measured at zero time and after 28 days of storage. Observations at zero time mean analysis was carried out after overnight refrigeration of fermented cultures at 5±1°C.

Determination of titratable acidity and pH

One-gram of milk culture was mixed with 9 ml of distilled water (40°C) and titrated with 0.1 N NaOH using 0.5% phenolphthalein as an indicator (Dave & Shah, 1997a). The pH was measured using pH meter (Cole-Parmer Co., Chicago, IL, USA).

Determination of organic acids

Lactic and acetic acids were quantified using a Waters Associates HPLC (Milford, MA, USA) with a 300 mm x 7.8 mm i.d. interaction cation-exchange ION-300 polymer resin column (Mandel Scientific, Rockwood, ON, Canada) along with a guard column (IONGUARD GC/80 #29042) according to the method described by Doyon *et al*. (1991). Briefly, 4 g of fermented milk sample was diluted to 25 ml with 0.1N H_2SO_4, centrifuged at 3000 xg for 10 min at 4°C and filtered through 0.45 µm. A volume of 100 µl of filtered sample was injected and analysis was performed within 30 min at a flow rate of 0.4 ml/min over a temperature range of 25°C to 60°C. The mobile phase, 0.005M reagent grade sulfuric acid in HPLC grade water was vacuum-filtered on 0.45 µm HA membrane (Millipore, Billerica, MA, USA). Acetic and lactic acid (DL isomers) standards (Sigma) were prepared in HPLC grade water (lactic acid concentrations: 0.09, 0.47, 1.18, 1.89, and 2.36 mg/ml, acetic acid concentrations: 0.03, 0.16, 0.39, 0.62 and 0.77 mg/ml).

Determination of soluble tyrosine

Soluble tyrosine produced by such bacterial culture was spectrophotometrically determined according to the method described by Vakalaris & Price (1959).

Bacterial count

One gram of such milk culture was diluted with 9 ml of 0.15% (w/v) peptone water and mixed uniformly with a vortex for 1 min (Dave & Shah, 1997a). The sample was further 10-fold serially diluted in peptone water. Enumeration was carried out using pour plate technique and bacterial counts were recorded as colony forming units (cfu) per gram of fermented milk.

Assay for antimicrobial activity

The interaction between *B. longum* and LAB was investigated using well-diffusion agar technique described by Tagg *et al*. (1976). Ten ml of an overnight milk culture of such organism were centrifuged at 3000 xg for 20 min. The pH of the aqueous phase was adjusted to 7.0±0.1 with 1N NaOH, and then filter-sterilized using 0.22 µm filters. Briefly, 75 ml of molten MRS-NNLP, ST, GM17 or MRS agar tempered at 45°C were seeded

with 1% (v/v) of an overnight culture of *B. longum*, *Str. thermophilus*, *Lac. lactis*, or lactobacilli, respectively, and poured into Petri dishes (150 mm in diameter). Wells of 7 mm were cut in solidified agar and loaded with 80 µl of filter-sterilized supernatants of different strains. The plates were left at 5°C for 2 hr, to allow migration and diffusion of the tested supernatant, and incubated anaerobically at 37°C for 72 hr, except for *Str. thermophilus*, and *Lac. lactis* that were incubated aerobically at 37 and 32°C for 24 hr, respectively.

The antimicrobial activity of both single and mixed cultures were determined also by well-diffusion agar technique using *Staph. aureus* or Bacillus. subtilis as indicator organisms. Ten ml of such milk culture were prepared as described above. Molten nutrient agar at 45°C was seeded with 1% (v/v) of an overnight active culture of Staph aureus or *Ba. subtilis*. Following agar solidification, wells were cut and loaded with 80 µl of filter-sterilized supernatant. Plates were left at 5°C for 2 hr prior to incubating aerobically at 37°C for 18 hr. At the end of incubation period, plates were observed for the presence or absence of zone of inhibition and diameter of inhibition zone was recorded.

Statistical analyses

All statistical analyses were performed with Stat View SE + Graphics (Abacus Concepts, Inc., Berkeley, Calif.). Analyses of a variance (ANOVA) were performed on mean values. Estimated means were separated by Fisher's PLSD test and a difference with a $P<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Interaction between *B. longum* and LAB

The interaction between *B. longum* and 6 LAB strains belonging to *Str. thermophilus*, *Lac. lactis*, *Lb. bulgaricus*, *Lb. paracasei*, *Lb. acidophilus* and *Lb. plantarum* was tested using the well-diffusion agar method. None of tested supernatants of LAB strains appeared to inhibit the growth of *B. longum* and also *B. longum* supernatant did not have an inhibitory effect against any of the tested LAB strains.

Media selectivity

Prior to beginning the experiments, it was necessary to determine and verify the selectivity of different culture media. Consequently, the viable counts of tested strain in an overnight milk culture were enumerated on various agar media as presented in Table (1). It was obvious that *B. longum* appeared to be the only organism that could be grown on MRS-NNLP agar media under anaerobic incubation at 37°C for 72 hr. These conditions have been previously reported to be selective to bifidobacteria (Laroia & Martin, 1991). From data in Table (1), it appears that ST and GM17 agar media were particularly suitable for growing both *Str. thermophilus* and *Lac. lactis*, respectively as previously reported by Terzaghi & Sandine (1975) and Dave & Shah (1996). In accordance to Thamaraj & Shah (2003), acidified MRS (45°C/72 hr) and MRS-maltose (43°C/72 hr) agar media were suitable for selective enumeration of *Lb. bulgaricus* and *Lb. acidophilus*, respectively.

The MRS-vancomycin and acidified MRS agar media were found suitable for the enumeration of *Lb. paracasei*. However, *B. longum* could successfully grow on MRS-vancomycin agar at 37°C for 72 hr under anaerobic condition, which make this medium unsuitable for differential enumeration between *B. longum* and *Lb. paracasei*. This finding is contradictory to results of Thamaraj & Shah (2003) who reported that MRS-vancomycin agar under anaerobic incubation at 37°C for 72 hr was selective for *Lb. paracasei* which could not grow in acidified MRS at 45°C for 72 hr. Consequently, in this study, acidified MRS (45°C/72 hr under anaerobic condition) was used for selective enumeration of *Lb. paracasei*, since this medium did not appear to be suitable for the growth of *B. longum*. Indeed, acid and antibiotic tolerances are strain-dependent rather than species-related characteristics. It seems that the concentration of vancomycin (1 mg/l) added to MRS was insufficient to inhibit the growth of *B. longum*. Resistance of bifidobacteria to vancomycin has been increased and is frequently reported (Charteris *et al*., 2000, Kheadr *et al*., 2004,). Furthermore, Kheadr *et al*. (2004) evaluated the susceptibility of 18 bifidobacterial strains to vancomycin and found that none of tested strains appeared to be inhibited at vancomycin concentration of 0.5 mg/ml.

Among tested media, *Lb. plantarum* could grow on MRS-vancomycin agar aerobically which did not inhibit the growth of *B. longum* (Table 1). Selective enumeration of *Lb. plantarum*, MRS agar (37°C/72 hr) under aerobic condition was also test-

Table 1: Viable counts (log_{10}cfu/g fermented milk) of different lactic acid bacteria on different culture media and at different incubation temperatures

Bacterial Cultures	Culture Media*						
	ST agar 37°C/24 hr	GM17 32°C/48hr	MRS 37°C/72hr	Acidified-MRS (pH 4.58) 45°C/72hr.	MRS-vancomycin 37°C/72hr.	MRS-maltose 43°C/72hr.	MRS-NNLP 37°C/72hr.
B. longum	<3.00	<3.00	<3.00	<3.00	8.47	<3.00	8.30
Str. thermophilus	8.11	6.69	ND	ND	ND	ND	<3.00
Lac. lactis	6.69	8.95	ND	ND	ND	ND	<3.00
Lb. paracasei	ND	ND	6.73	9.60	9.81	6.00	<3.00
Lb. bulgaricus	ND	ND	8.08	8.17	<3.00	6.40	<3.00
Lb. acidophilus	*ND*	ND	<3.00	<3.00	7.90	8.20	<3.00
Lb. plantarum	ND	ND	8.68	<3.00	8.94	<3.00	<3.00

*ST, GM17 and MRS agar media were incubated aerobically, while acidified-MRS, MRS-vancomycin, MRS-maltose and MRS-NNLP agar media were incubated anaerobically.

ND: not detected.

ed. The aerobic incubation was used since it has been known to be inhibitory to *B. longum*.. Thus, MRS (37°C/72 hr) under aerobic incubation was used for the enumeration of *Lb. plantarum* grown either alone or in co-culture with *B. longum*.

Titratable acidity(TA) and pH

The changes in TA during refrigerated storage at 5ºC of different milk cultures are presented in Table (2). The initial TA of milk (0.15%) increased to 0.62-1.35% for single cultures and to 0.92-1.10 for mixed cultures after overnight cooling at 5ºC. Among tested strains, *Lb. bulgaricus* was the most active acid-producer, while *Lb. plantarum* showed the least capacity to acidify milk. At zero time storage, the TA values for all mixed cultures were higher as compared with values determined for *B. longum* single culture (0.88%). During storage, there were gradual increases in TA values for all cultures. Similar observation was reported by Dave & Shah, (1997b) for yoghurts stored at 5°C and attributed to the residual fermentation changes. During 28 days of storage, the increase in TA was minimal for *B. longum*/*Lb. acidophilus* culture (0.32%) as compared with *Lb. plantarum* (0.50%) and *B. longum*/*Lb. plantarum* (0.63%) cultures. In addition to residual fermentation activity, proteolytic activity can also be responsible for the increased TA values throughout storage period (Sallami *et al*., 2004).

The initial pH of milk was (6.5) decreased to 3.72-5.28 for single cultures and to 4.25-4.63 for mixed cultures after overnight cooling at 5°C (Table 3). At zero time storage, pH values for cultures where *B. longum* was mixed with *Lb. paracasei*, *Lb. acidophilus* or *Lb. plantarum* were significantly lower as compared with pH values for single cultures made with these organisms. However, *B. longum*/*Lb. bulgaricus* culture had significantly higher pH as compared with culture made with *Lb. bulgaricus* alone but lower as compared with culture made with *B. longum* alone. For all cultures, gradual decreases ($P \leq 0.05$) in pH were observed throughout the storage period. After 28 days storage, the pH dropped to 3.68-5.35 and to 3.85-4.50 for single and mixed cultures, respectively. Among mixed cultures, *B. longum*/*Lb. bulgaricus* combination produced the highest relative drop in pH values (approximately 0.4 units) during storage. This drop in pH might be responsible for the reduced viability of *B. longum*. Shah *et al*. (1995) reported that the viability of bifidobacteria was drastically reduced at low pH values (≤ 4.0). On the contrary, combining *B. longum* with either *Lb. paracasei* or *Lb. plantarum* produced the least changes in pH during storage. However, the relative decrease in pH of 0.13 for *B. longum*/*Lb. plantarum* culture did not correspond to the increase in TA by 0.63% over the storage period. The proteolytic activity of *B. longum*/*Lb. plantarum* culture, as shown by soluble tyrosine determination, may be responsible for the higher TA values (Sallami *et al*., 2004, Dabour *et al*., 2005). The authors reported that small peptides and free amino acids resulted from the hydrolysis of casein and large peptides, by the proteolytic enzymes of the bacteria, could interfere with the titration process.

Production of lactate and acetate

Data concerning changes in lactate and acetate concentration during refrigerated storage of both single and mixed cultures are presented in Table (4). Like other activities, lactate production appeared to be strain-dependent characteristic and varied significantly ($P \leq 0.05$) among strains. The amounts of lactate determined at zero time storage ranged from 3.77 to 5.15 and from 4.35 to 5.04 mg/g for single and mixed cultures, respectively. In accordance with pH, *Lb. bulgaricus* and *Lb. plantarum* showed, respectively, the highest and the least lactate production. During storage, there were significant ($P \leq 0.05$) increases in lactate content in all cultures. The maximal increases in lactate of 0.3 mg/g were observed for cultures containing *Lb. bulgaricus*, while *Lb. plantarum* containing cultures showed the minimal increases of 0.18 mg/g. After 28 days storage, the amounts of lactate ranged from 3.95 to 5.45 and from 4.50 to 5.30 mg/g for single and mixed cultures, respectively.

The production of acetate through glucose fermentation is known to be characteristically associated to the genus Bifidobacterium. Consequently, acetate production was determined for *B. longum*-containing cultures. At zero time, the concentration of acetate in *B. longum* single culture (160 µg/g) was significantly higher as compared with those (80-132 µg/g) detected in mixed cultures. During storage, significant increase ($P \leq 0.05$) in acetate concentration was observed for all cultures containing *B. longum*. After 28 days, the concentrations of acetate increased to 201 for *B. longum* and to 85-155 µg/g for mixed cultures. Among mixed cultures, acetate concentration in *B. longum*/*Lb. plantarum* culture was the highest ($P \leq 0.05$), while

Table 2: Changes in titratable acidity of single and mixed culture of *B. longum* and some lactic acid bacteria during storage at 5±1°C

Bacterial Cultures	Storage period (days)				
	0*[1]	7	14	21	28
Single cultures					
B. longum	0.88±0.03^{d,e}	0.94±0.02^{e,f}	1.02±0.04^{f,g}	1.11±0.07^{g,h,i}	1.19±0.07^{f,g}
Str. thermophilus	0.95±0.02^{c}	1.12±0.03^{c}	1.20±0.05^{c}	1.36±0.06^{b,c}	1.45±0.06^{c}
Lac. lactis	0.92±0.02^{c,d}	1.02±0.01^{d}	1.11±0.03^{d,e}	1.24±0.04^{d,e,f}	1.35±0.07^{d}
Lb. paracasei	0.79±0.04^{f}	0.85±0.02^{g}	0.95±0.05^{g,h}	1.03±0.03^{h,i}	1.14±0.03^{g}
Lb. bulgaricus	1.35±0.05^{a}	1.48±0.07^{a}	1.55±0.06^{a}	1.65±0.06^{a}	1.72±0.10^{a}
Lb. acidophilus	0.83±0.05^{e,f}	0.90±0.01^{f,g}	1.00±0.05^{f,g}	1.12±0.04^{g,h}	1.20±0.01^{f,g}
Lb. plantarum	0.62±0.02^{g}	0.75±0.02^{h}	0.92±0.03^{h}	1.02±0.02^{i}	1.12±0.06^{g}
Mixed cultures					
B. longum/Str. thermophilus	0.94±0.03^{c}	1.12±0.04^{c}	1.15±0.08^{c,d}	1.29±0.02^{c,d}	1.35±0.03^{d}
B. longum/Lac. lactis	0.92±0.04^{c,d}	1.09±0.04^{c}	1.12±0.02^{d,e}	1.25±0.03^{d,e}	1.31±0.09^{d,e}
B. longum/Lb. paracasei	0.92±0.03^{c,d}	1.00±0.03^{d}	1.06±0.04^{e,f}	1.18±0.06^{e,f,g}	1.26±0.06^{e,f}
B. longum/Lb. bulgaricus	1.10±0.04^{b}	1.20±0.05^{b}	1.35±0.07^{b}	1.44±0.05^{b}	1.59±0.07^{b}
B. longum/Lb. acidophilus	0.93±0.02^{c,d}	0.98±0.03^{d,e}	1.05±0.06^{e,f}	1.15±0.06^{f,g}	1.25±0.05^{e,f}
B. longum/Lb. plantarum	0.92±0.01^{c,d}	1.01±0.02^{d}	1.20±0.04^{c}	1.32±0.05^{c,d}	1.55±0.08^{b}

*[1]Analysis carried out after overnight storage at 5±1ºC.
Values with the same superscript letter in the same column are insignificantly different ($P \leq 0.05$).

Table 3: Changes in pH of single and mixed culture of *B. longum* and some lactic acid bacteria during storage at 5±1˚C

Bacterial Cultures	Storage period (days)				
	0*[1]	7	14	21	28
Single cultures					
B. longum	4.67±0.03f	4.69±0.05^{b,c,d}	4.58±0.04^{g,h}	4.49±0.07^{g}	4.45±0.02^{g}
Str. thermophilus	4.33±0.05^{c}	4.25±0.04^{b}	4.26±0.05^{d}	4.25±0.04^{d}	4.25±0.04^{d,e}
Lac. lactis	4.53±0.02^{e}	4.48±0.02^{b,c,d}	4.35±0.02^{e}	4.28±0.02^{d}	4.28±0..06^{e}
Lb. paracasei	4.99±0.04^{h}	4.78±0.03^{d,e,f}	4.61±0.03^{h}	4.45±0.05^{f}	4.43±0.04^{g}
Lb. bulgaricus	3.72±0.06^{a}	3.67±0.07^{a}	3.65±0.07^{a}	3.66±0.06^{a}	3.68±0.09^{a}
Lb. acidophilus	4.92±0.02^{g}	4.75±0.06^{c,d,e}	4.61±0.05^{h}	4.61±0.04^{h}	4.57±0.05^{i}
Lb. plantarum	5.28±0.01^{i}	5.37±0.02^{f}	5.40±0.02^{i}	5.40±0.01^{i}	5.35±0.04^{j}
Mixed cultures					
B. longum/Str. thermophilus	4.36±0.04^{d}	4.30±0.03^{b}	4.25±0.03^{d}	4.20±0.05^{c}	4.20±0.02^{d}
B. longum/Lac. lactis	4.32±0.03^{c,d}	4.32±0.04^{b,c}	4.16±0.05^{c}	4.04±0.02^{b}	4.00±0.04^{c}
B. longum/Lb. paracasei	4.53±0.02^{i}	4.50±0.05^{b,c,d}	4.41±0.06^{f}	4.42±0.03^{f}	4.40±0.05^{g}
B. longum/Lb. bulgaricus	4.25±0.05^{b}	4.12±0.03^{a,b}	4.05±0.05^{b}	3.9±0.07^{b}	3.85±0.09^{b}
B. longum/Lb. acidophilus	4.52±0.03^{e}	4.49±0.02^{b,c,d}	4.40±0.02^{f}	4.35±0.04^{e}	4.35±0.04^{f}
B. longum/Lb. plantarum	4.63±0.01^{f}	4.60±0.03^{e,f}	4.60±0.02^{g,h}	4.52±0.03^{g}	4.50±0.02^{h}

*[1]Analysis carried out after overnight storage at 5±1ºC.
Values with the same superscript letter in the same column are insignificantly different ($P \leq 0.05$).

Table 4: Changes in lactate (mg/g) and acetate (µg/g) concentration*[1] of single and mixed culture of *B. longum* and some lactic acid bacteria during storage at 5±1°C

Bacterial Cultures	Storage period (days)							
	0*[2]		7		14		25	
	Lactate	Acetate	Lactate	Acetate	Lactate	Acetate	Lactate	Acetate
Single cultures								
B. longum	4.58±0.02^{d}	160±3.0^{a}	4.65±0.05^{d}	187±4.3^{a}	4.70±0.02^{d}	195±5.6^{a}	4.70±0.09^{e}	201±5.5^{a}
Str. thermophilus	4.89±0.5^{c}	ND	5.00±0.04^{b,c}	ND	5.00±0.07^{c}	ND	5.04±0.05^{c}	ND
Lac. lactis	4.65±0.08^{d}	ND	4.70±0.03^{d}	ND	4.79±0.08^{d}	ND	4.85±0.06^{d}	ND
Lb. paracasei	4.09±0.07^{f}	ND	4.22±0.02^{e}	ND	4.31±0.09^{e,f}	ND	4.35±0.02^{g}	ND
Lb. bulgaricus	5.15±0.10^{a}	ND	5.38±0.01^{a}	ND	5.43±0.01^{a}	ND	5.45±0.13^{a}	ND
Lb. acidophilus	4.21±0.12^{e,f}	ND	4.25±0.05^{e}	ND	4.25±0.01^{f}	ND	4.30±0.10^{g}	ND
Lb. plantarum	3.77±0.01^{g}	ND	3.85±0.05^{f}	ND	3.92±0.02^{g}	ND	3.95±0.04^{h}	ND
Mixed cultures								
B. longum/Str. thermophilus	4.90±0.11^{b,c}	89±2.5^{e}	4.95±0.04^{c}	96±2.1^{e}	5.01±0.11^{c}	96±3.6^{e}	5.07±0.13^{c}	102±3.0^{e}
B. longum/Lac. lactis	4.95±0.03^{b,c}	120±4.8^{d}	4.99±0.03^{b,c}	129±1.1^{d}	5.04±0.13^{b,c}	130±2.3^{d}	5.10±0.20^{c}	130±1.4^{d}
B. longum/Lb. paracasei	4.60±0.06^{d}	130±1.5^{b}	4.63±0.01^{d}	142±2.3^{b,c}	4.72±0.04^{d}	142±1.1^{c}	4.75±0.12^{d,e}	145±2.4^{c}
B. longum/Lb. bulgaricus	5.04±0.09^{b}	80±2.1^{f}	5.16±0.10^{b}	85±3.1^{f}	5.22±0.16^{b}	84±2.6^{f}	5.30±0.07^{b}	85±2.6^{f}
B. longum/Lb. acidophilus	4.65±0.15^{d}	125±2.5^{c}	4.69±0.09^{d}	141±3.5^{c}	4.75±0.03^{d}	139±1.6^{c}	4.75±0.09^{d,e}	145±1.1^{c}
B. longum/Lb. plantarum	4.35±0.07^{e}	132±3.2^{b}	4.40±0.08^{e}	146±2.9^{b}	4.46±0.10^{e}	157±2.5^{b}	4.50±0.08^{f}	155±2.4^{b}

*[1]Calculated after subtractions of initial lactate and acetate concentrations (45 and 9 µg/g, respectively) found in milk prior to inoculation process.

*[2]Analysis carried out after overnight storage at 5±1°C.

ND: not detected.

Values with the same superscript letter in the same column are insignificantly different ($P \leq 0.05$).

B. longum/*Lb. bulgaricus* combination contained the least acetate concentration. The low production of acetate reported in the present study for *B. longum* is in accordance with results of Dave & Shah (1997a) who reported that the concentration of acetic acid in yoghurt-containing bifidobacteria did not exceed 190 µg/g after 35 days storage at 5°C, while lactic acid increased up to 6.0 mg/g. Indeed, some researchers have proposed a theoretical molar ratio of acetate/lactate of 1.5 (Sgorbati *et al*., 1995), while some others have proven that this ratio is not always obtained (Perrin *et al*., 2001, Van der Meulen *et al*., 2004, Ruas-Madiedo *et al*., 2005).

Production of soluble tyrosine

The changes in soluble tyrosine content, liberated by different bacterial culture, during refrigerated storage are shown in Table (5). At zero day, the quantities of tyrosine liberated by single cultures ranged from 100 to 370 µg/g and were in the order *Lb. plantarum* > *Lb. bulgaricus* > *Str. thermophilus* > *Lb. paracasei* > *B. longum* > *Lb. acidophilus* > *Lac. lactis*. This order remained till the end of storage period. In most cases, mixed cultures contained significantly higher quantities of tyrosine as compared with single cultures of the same organisms. For example, *B. longum*/ *Lb. plantarum* cultures contained, at zero time storage, 440 µg/g soluble tyrosine, while *B. longum* and *Lb. plantarum* single cultures contained, 170 and 370 µg/g, respectively. This may indicate a synergistic effect between the two organisms for the production of tyrosine and explain the increased viability of *B. longum* when grown in mixed culture with *Lb. plantarum*. Proteolytic lactobacilli have previously been reported to have a stimulatory growth interaction with several bifidobacterial strains (Klaver *et al*., 1993). Among proteolytic lactobacilli, *Lb. plantarum* have been shown to have potential proteolytic activities (El-Soda *et al*., 1986). Moreovar, Xanthopoulos *et al*. (2000) evaluated the proteolytic activity of 32 *Lb. plantarum* strains isolated from Feta cheese using o-phthaldialdehyde reagent and found that they produce 100-300 µg glycine/ml after 3 days of growth in skim milk at 30°C. Indeed, bifidobacteria are fastidious organisms that grow slowly in milk because of lack of proteolytic activity (Klaver *et al*., 1993). Furthermore, Dave & Shah (1998) reported that small peptides and free amino acids improved the viability of bifidobacteria in yoghurt. In fact, several amino acids have been reported to be either stimulatory or essential for growth of bifidobacteria, including arginine, glutamic, tyrosine, tryptophane, cysteine and valine (Poch & Bezkorovainy, 1988, 1991).

During storage, there was significant ($P \leq 0.05$) accumulation in soluble tyrosine in all cultures. After 28 days storage, the quantities of soluble tyrosine determined in single and mixed cultures ranged from 155-485 and from 206-540 µg/g, respectively (Table5).

Counts of *B. longum*

The changes in the counts of *B. longum* in single or mixed cultures during storage at 5°C for 28 days are presented in Table (6). The initial counts of *B. longum*, determined in milk immediately after inoculation, ranged from 6.80 to 6.94 log10 cfu/g. At zero time, the viable counts of *B. longum* were the highest ($P \leq 0.05$) in *B. longum*/*Lb. plantarum* culture. Also slight increase ($P > 0.05$) in the viable counts of *B. longum* was observed for *B. longum*/*Lb. paracasei* culture as compared with counts determined for single culture. Co-culturing with other LAB resulted in significant reductions in the viability of *B. longum*. The most remarkable reduction in viability was detected for *B. longum*/*Lb. bulgaricus* culture where counts of *B. longum* were 1 log10 cfu/g lower compared with those determined for single culture. The highest acidification capacity of *Lb. bulgaricus*, compared with other tested LAB, might be responsible for this remarkable decline in the viable counts of *B. longum*.

During storage, there were significant decreases in viability of *B. longum* in all cultures. By day 28, the counts of *B. longum* in the single culture were approximately 1.5 log10 cfu/g lower as compared with those determined at zero time storage. This decline is probably due to acid accumulation and/or temperature storage. The viability of bifidobacteria is significantly retarded in acidic conditions (pH below 5.0) and during refrigeration storage at 5°C (Lankaputhra & Shah, 1995). For mixed cultures, the viable counts of *B. longum* declined by 1.6, 1.4, 1.2, 2.5, 1.1 and 0.85 log10 cfu/g when grown in the presence of *Str. thermophilus*, *Lac. lactis*, *Lb. paracasei*, *Lb. bulgaricus*, *Lb. acidophilus* and *Lb. plantarum*, respectively. Among tested organisms, *B. longum* exhibited better viability when grown in mixed culture with *Lb. plantarum* and to some extent with *Lb. paracasei* and *Lb. acidophilus*. When grown in the presence of *Lb. plantarum*, *Lb. paracasei* or *Lb. acidophilus*, the viable counts of *B. longum* were 0.80, 0.30 and 0.15 log10 cfu/g higher than those deter-

Table 5: Changes in concentration of soluble tyrosine (µg/g) of single and mixed culture of *B. longum* and some lactic acid bacteria during storage at 5±1°C

Bacterial Cultures	Storage period (days)		
	0*[1]	14	28
Single cultures			
B. longum	170±12f,[g]	200±25[e,f]	220±13[f]
Str. thermophilus	203±19[d]	225±14[d,e]	237±21[e]
Lac. lactis	100±9[h]	130±19[h]	155±10[h]
Lb. paracasei	175±14[e,f]	205±24[e,f]	215±5[f]
Lb. bulgaricus	240±22[c]	290±27[c]	330±24[c]
Lb. acidophilus	150±7[g]	180±10[g]	190±23[g]
Lb. plantarum	370±26[b]	440±30[b]	485±28[b]
Mixed cultures			
B. longum/Str. thermophilus	260±16[c]	290±22[c]	310±11[c]
B. longum/Lac. lactis	164±19[f,g]	189±21[f]	206±8[f]
B. longum/Lb. paracasei	200±23[d]	240±13[d]	255±19[d]
B. longum/Lb. bulgaricus	250±7[c]	300±24[c]	325±25[c]
B. longum/Lb. acidophilus	195±9[d,e]	222±12[d,e]	248±22[d,e]
B. longum/Lb. plantarum	440±19[a]	500±25[a]	540±18[a]

*[1]Analysis carried out after overnight storage at 5±1ºC.
Values with the same superscript letter in the same column are insignificantly different ($P \leq 0.05$).

Table 6: Viable counts (log_{10} cfu/g) of *Bifidobacterium longum* grown either alone or in mixed culture with some lactic acid bacteria during storage at 5±1°C

Bacterial Cultures	Storage period (days)					
	0*[1]	0*[2]	7	14	21	28
Single cultures						
B. longum	6.85±0.09b,c	8.44±0.12b	8.15±0.11b	8.00±0.21b	7.69±0.02b	7.00±0.12c
Mixed cultures						
Str. thermophilus	6.94±0.12a,b	8.14±0.20c	7.97±0.15c	7.63±0.023c	7.09±0.014c	6.50±0.10e
Lac. lactis	6.80±0.05c	8.25±0.23c	8.15±0.12b	8.00±0.14b	7.25±0.20c	6.83±0.15d
Lb. paracasei	6.94±0.02a,b	8.50±0.30a,b	8.25±0.09b	8.15±0.23b	7.72±0.15b	7.30±0.08b
Lb. bulgaricus	6.98±0.06a	7.48±0.15d	7.00±0.12	6.68±0.24d	5.70±0.22d	5.00±0.20f
Lb. acidophilus	6.90±0.04a,b,c	8.23±0.17c	8.12±0.13b	7.97±0.13b	7.68±0.019b	7.15±0.16b
Lb. plantarum	6.80±0.05c	8.65±0.16a	8.60±0.08a	8.35±0.08a	8.00±0.21a	7.80±0.14a

*[1] Counts determined immediately after inoculation process.
*[2] Analysis carried out after overnight storage at 5±1ºC.
Values with the same superscript letter in the same column are insignificantly different ($P \leq 0.05$).

mined in single culture after 28 days storage. Low acidogenous capacity of the three organisms might be, in part, responsible for the remarkable enhancement of the viability of *B. longum*.

Counts of LAB

The changes in the viable counts of different LAB strains during refrigerated storage of their single and mixed cultures are presented in Table (7). As shown, the initial counts of each strain, determined in both single and mixed cultures immediately after inoculation, did not significantly differ. At zero time storage, the viable counts of each tested strain did not significantly differ for single and mixed cultures, except for *Str. thermophilus*. The viable counts of this organism grown in mixed culture with *B. longum* were 0.08 log10 cfu/g lower as compared with those determined in single culture.

For single culture, the decay in viable counts of tested strains throughout storage was in the order *Lb. paracasei* > *Str. thermophilus* > *Lac. lactis* > *Lb. bulgaricus* > *Lb. acidophilus* > *Lb. plantarum*. After 28 days, the viable counts of *Str. thermophilus*, *Lac. lactis*, *Lb. paracasei*, *Lb. bulgaricus*, *Lb. acidophilus* and *Lb. plantarum* were respectively 1.15, 1.07, 1.85, 0.80, 0.70 and 0.18 log10 cfu/g lower compared with counts determined for each organism at zero day storage. At 28 day storage, the viable counts of *Str. thermophilus*, *Lac. lactis* and L. acidophilus grown in mixed cultures with *B. longum* were significantly lower as compared with those determined in single cultures. However, viable counts of *Lb. paracasei*, *Lb. bulgaricus* and *Lb. plantarum* grown in mixed culture with *B. longum* did not significantly differ from those determined for single culture of such organism. At the end of storage, the reductions in viable counts of *Str. thermophilus*, *Lac. lactis*, *Lb. paracasei*, *Lb. bulgaricus*, *Lb. acidophilus* and *Lb. plantarum*, grown in co-culture with *B. longum*, were respectively1.22, 1.35, 1.58, 0.54, 1.48 and 0.45 log10 cfu/g.

Antimicrobial activity

The results given in Table (8) show the inhibitory effect of different cultures against *Staphylococcus aureus* and *Bacillus subtilis* as determined at zero time storage by the well-diffusion agar method. Among single cultures, the neutralized supernatant of *B. longum* milk culture was the only to produce inhibitory effects against both indicator organisms. This finding is in accordance with that reported previously by Kheadr (2001) who noted a remarkable antibacterial activity of *B. longum* DSM 20097 against both *Staphylococcus aureus* and *Bacillus subtilis*. When grown as co-cultures with *Lb. paracasei*, *Lb. acidophilus* or *Lb. plantarum*, *B. longum* appeared to be more active at inhibiting such indicator organism. Supernatant from *B. longum*/*Lb. bulgaricus* culture was the least active at inhibiting *Staphylococcus aureus* and did not appear to be inhibitory to *Bacillus subtilis*. The inhibitory effect observed at zero time storage appeared to be stable since similar diameters of inhibition were obtained after 28 days of storage (Figure 1).

The inhibitory effect of *B. longum* reported in the present study could be attributed to its acid-producing capacity and/or the ability to produce proteinaceous antimicrobial agents. Although, the antimicrobial activity of *B. longum* was assessed by well-diffusion agar method using neutralized supernatants, Freese *et al*. (1973) demonstrated that neutralized acids could have an antibacterial effect,and also that the undissociated forms of neutralized acetate and lactate can penetrate the cell plasma membrane. Inside the bacterial cell, such acids will dissociate, and the protons formed might inhibit many cellular metabolism machineries. In addition to acid production, many studies have attributed the antimicrobial activity of bifidobacteria to their ability to produce proteinaceous antimicrobial agents (Anand *et al*., 1984, Meghrous *et al*., 1990, Touré *et al*., 2003). To date, bifidocin B, bacteriocin produced by *B. bifidum* NCFB 1454, is the only bacteriocin that has been purified and characterized from bifidobacteria (Yildirim *et al*., 1999). Indeed, further study is needed to characterize the antimicrobial activity of *B. longum* DSM 20097 reported in the present study.

CONCLUSION

The results of the present study showed that *Lb. plantarum*, among LAB tested strains, could significantly improve viability of *B. longum*. Low acid production and high proteolytic activity of *Lb. plantarum* might be responsible for the enhancement of *B. longum* viability. Indeed, more strains and serotypes of *Lb. plantarum* and bifidobacteria need to be evaluated for their positive growth association in respect of acid production, flavour compound production, viable counts and probiotic activity.

Table 7: Viable counts (log_{10}cfu/g) of lactic acid bacteria, grown either alone or in mixed culture with *Bifidobacterium longum*, during storage at 5±1°C

Bacterial Cultures*[1]		Storage period (days)					
		0*[2]	0*[3]	7	14	21	28
Str. thermophilus	S	6.77±0.16^{f,g}	8.6±0.20^{a}	8.27±0.15^{b}	8.23±0.1^{b}	7.93±0.15^{c}	7.45±0.02^{e}
	M	6.69± 0.10^{g}	7.87±0.26^{c,d}	7.65±0.12^{d,e}	7.60±0.22^{e}	7.00±0.13^{f}	6.65±0.10^{g}
Lac. lactis	S	6.89±0.09^{f}	8.23±0.25^{a}	8.11±0.21^{a}	7.74±0.16^{b,c}	7.54±0.09^{c}	7.16±0.11^{d,e}
	M	6.81±0.13^{f}	8.15±0.21^{a}	7.84±0.23^{b}	7.65±0.19^{b,c}	7.25±0.15^{d}	6.80±0.17^{f}
Lb. paracasei	S	7.00±0.15^{h}	9.25±0.29^{a}	9.21±0.27^{a}	8.26±0.26^{c,d}	7.80±0.19^{e}	7.40±0.15^{f,g}
	M	7.09±0.20^{h}	9.27±0.32^{a}	8.77±0.21^{b}	8.38±0.23^{c}	7.99±0.32^{d,e}	7.69±0.19^{e,f}
Lb. bulgaricus	S	7.34±0.10^{f}	9.07±0.24^{a}	9.00±0.30^{a,b}	8.77±0.25^{a,b,c,d}	8.75±0.17^{b,c,d}	8.27±0.20^{e}
	M	7.53±0.19^{f}	9.00±0.26^{a,b}	9.07±0.23^{a}	8.84±0.20^{a,b,c}	8.60±0.26^{c,d}	8.46±0.19^{d,e}
Lb. acidophilus	S	5.60±0.15^{h}	7.39±0.19^{a}	7.14±0.04^{b,c}	7.17±0.11^{a,b,c}	7.00±0.09^{c,d}	6.69±0.12^{e}
	M	5.55±0.20^{h}	7.32±0.21^{a,b}	6.77±0.12^{d,e}	6.23±0.07^{f}	6.00±0.05^{f,g}	5.84±0.09^{g}
Lb. plantarum	S	6.55±0.10^{c}	8.11±0.16^{a,b}	8.07±0.20^{b}	8.07±0.19^{b}	8.00±0.21^{b}	7.93±0.15^{b,c}
	M	6.60±0.07^{c}	8.31±0.24^{a}	8.39±0.26^{a}	7.95±0.12^{b,c}	8.00±0.17^{b}	7.86±0.13^{c}

*[1] S and M: Single and mixed cultures.
*[2] Counts determined immediately after inoculation process.
*[3] Analysis carried out after overnight storage at 5±1°C.
Values with the same superscript letter in the same column are insignificantly different ($P \leq 0.05$).

Table 8: Antibacterial activity of single and mixed cultures of *Bifidobacterium longum* and some lactic acid bacteria

Bacterial Cultures	Inhibition zone (mm)*	
	Staphylococcus aureus	Bacillus subtilis
Single		
B. longum	6[c]	3[c]
Str. thermophilus	-	-
Lac. Lactis	-	-
Lb. paracasei	-	-
Lb. bulgsricus	-	-
Lb. acidophilus	-	-
Lb. plantarum	-	-
Mixed cultures		
B. longum/Str. thermophilus	6[c]	3[c]
B. longum/Lac. Lactis	5[c]	4[c]
B. longum/Lb. paracasei	8[b]	6[b]
B. longum/Lb. bulgsricus	3[d]	-
B. longum/Lb. acidophilus	8[b]	6[b]
B. longum/Lb. plantarum	10[a]	8[a]

*Measured at zero time storage.
Values with the same superscript letter in the same column are insignificantly different ($P \leq 0.05$).

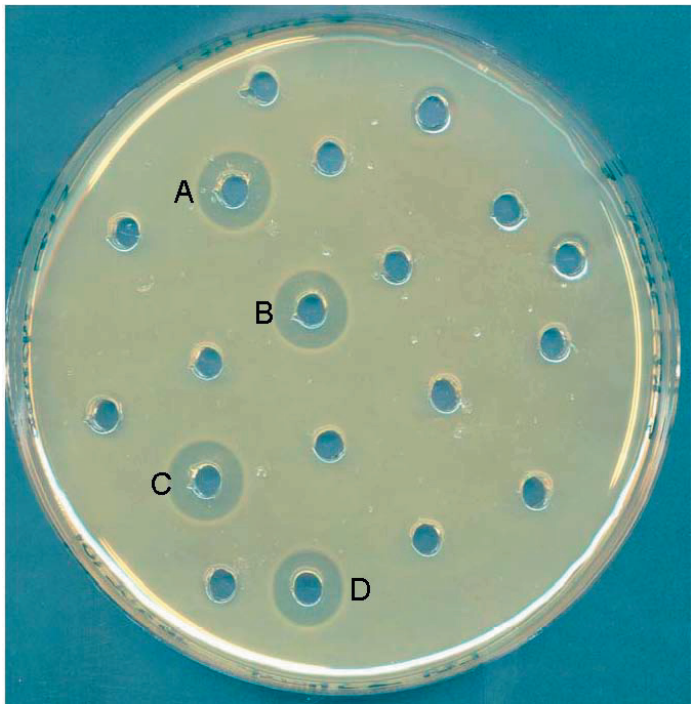

Fig. 1: Agar-well diffusion showing inhibition of *Staphylococcus aureus* by *Bifidobacterium longum* grown in skim milk either alone or in combination with lactic acid bacteria. Culture supernatants were prepared from 28-day-old milk cultures stored at 5±1°C. A, B and C represent supernatants from milk cultures where *Bifidobacterium longum* was mixed with *Lactobacillus paracasei*, *Lactobacillus acidophilus* and *Lactobacillus plantarum*, respectively, while D represents supernatant of *Bifidobacterium longum* single culture.

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دراسة حيوية بكتيريا *Bifidobacterium longum* التى نميت بمفردها أو بمشاركة بكتيريا حمض اللاكتيك تحت التبريد

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كان الهدف من إجراء هذا البحث هو دراسة العلاقة بين السلالة البكتيرية *Bifidobacterium longum* DSM 2009 وست سلالات من بكتيريا حمض اللاكتيك تابعة للأنواع التالية: *Streptococcus thermophilus*, *Lactococcus lactis* supsp. *lactis*, *Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus casei* subsp. *paracasei, Lactobacillus acidophilus*, *Lactobacillus plantarum*.

وقد تم تنمية *B. longum* فى لبن فرز معقم ومعاد ذوبانه (١٢٪ جوامد صلبة) ومقوى بإضافة ٢٪ جلوكوز، ١٪ مستخلص خميرة، ٠,٠٥٪ سيستئين وذلك إما بمفردها أو فى وجود إحدى سلالات بكتيريا حمض اللاكتيك السابق ذكرها .

وبعد إجراء عملية تخمر اللبن لمدة ١٨ ساعة على ٣٧°م تم حفظ اللبن المتخمر على ٥°م لمدة ٢٨ يوماً. وقد تم خلال هذة الفترة دراسة التغيرات فى كل من الحموضة و الـ pH وتركيز كل من حمض اللاكتيك والخليك والتيروسين الذائب وكذلك الأعداد الحية لكل سلالة بكتيرية بالإضافة إلى الخواص المضادة لنمو البكتيريا المرضة.

وقد أشارت النتائج إلى أن بكتيريا *B. longum* تحتفظ بحيويتها بدرجة أفضل عند نموها فى مزرعة مختلطة مع بكتيريا *Lactobacillus plantarum*. فى حين أن حيويتها تنخفض بدرجة ملحوظة عند تنميتها فى مزرعة مختلطة مع *Lactobacillus bulgaricus*. وقد أعزي ذلك إلى مقدرة *Lactobacillus plantarum* على إنتاج كميات كبيرة من التيروسين الذائب علاوة على انخفاض قدرتها على إنتاج الأحماض العضوية. كذلك فقد تبين أن نمو *B. longum* فى وجود *Lactobacillus plantarum* يزيد من قدرتها على انتاج المثبطات البكتيرية المعوقة لنمو كل من *Staphylococcus aureus* و *Bacillus subtilis*.