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Growth of probiotic bacteria and bifidobacteria in a soy yogurt formulation

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Abstract

Soy beverage and cows' milk yogurts were produced with *Steptococcus thermophilus* (ATCC 4356) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (IM 025). The drop in pH during fermentation was faster in the soy beverage than in cows' milk, but the final pH values were similar. Yogurts were prepared with a yogurt starter in conjunction with either the probiotic bacteria *Lactobacillus johnsonii* NCC533 (La-1), *Lactobacillus rhamnosus* ATCC 53103 (GG) or human derived bifidobacteria. The presence of the probiotic bacteria did not affect the growth of the yogurt strains. Approximately 2 log increases in both *L. rhamnosus* GG and *L. johnsonii* La-1 were observed when each was added with the yogurt strains in both cows' milk and the soy beverage. Two of the five bifidobacteria strains grew well in the cows' milk and soy beverage during fermentation with the yogurt bacteria. High pressure liquid chromatography (HPLC) analyses showed that the probiotic bacteria and the bifidobacteria were using different sugars to support their growth, depending on whether the bacteria were growing in cows' milk or soy beverage.

Keywords: Soy beverage; Yogurt; Probiotic; Bifidobacteria

1. Introduction

Yogurt is produced by adding two starter cultures, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* to milk (Tamime and Marshall, 1997). During the fermentation, hydrolysis of the milk proteins occurs, the pH drops, the viscosity increases, and bacterial metabolites are produced that contribute to the taste and possibly to the health promoting properties of yogurt. Several health benefits have been reported for traditional yogurt (Boudraa et al., 1990; Marteau et al., 1990; Bakalinsky et al., 1996; Rachid et al., 2002), and this healthy image is enhanced by supplementation with probiotic bacteria.

Probiotic bacteria are defined as "live microorganisms that when administered in adequate amounts confer a health benefit on the host" (FAO, 2001). Fermented foods that have potential probiotic properties are produced worldwide from a variety of food substrates (Farnworth, 2005). Probiotics have been used for the treatment of various types of diarrhoea (Sarker et al., 2005; Szymanski et al., 2006), urogenital infections (Reid et al., 2003), and gastrointestinal diseases such as Crohn's disease (Bousvaros et al., 2005) and pouchitis (Kuehbacher et al., 2006), although there is still no consensus about their effectiveness (Lin, 2003; Reid and Hammond, 2005; Senok et al., 2005). Lactic acid bacteria including lactobacilli and bifidobacteria are the most common bacterial species considered as potential probiotics (Sanders, 1997).

Yogurt produced from cows' milk is consumed in both developing and industrialized countries. However, the demand for alternatives to cows' milk is growing due to problems with allergenicity, desire for vegetarian alternatives, etc., and therefore interest in a soy-based yogurt has developed. Probiotic milk-based yogurts are now being marketed, and consequently it would be desirable to know if probiotic bacteria can also be incorporated into soy-based yogurt-type fermentations.

Probiotic bacteria generally do not grow rapidly in cows' milk. Thus, in yoghurt manufacture, they do not attain as high numbers as the starter cultures (Champagne et al., 2005). However, many studies indicate that soy is a good substrate for probiotic bacteria (Angeles and Marth, 1971; Mital et al., 1974; Scalabrini et al., 1998), but not for the traditional yoghurt starter *L. delbrueckii* subsp. *bulgaricus* (Mital et al., 1974; Karleskind et al., 1991;

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Wang et al., 2002). These findings suggest that some probiotic bacteria could better compete with yoghurt cultures in a soy-based substrate. Unfortunately, little information is available on the growth of probiotic bacteria in mixed cultures with yoghurt strains in soy substrates, because most studies on the growth of probiotics in soy extracts have been carried out using pure cultures (Angeles and Marth, 1971; Murti et al., 1993b; Kamaly, 1997; Hou et al., 2000; Desai et al., 2002). Although some data are available on mixed cultures of probiotics and *S. thermophilus* (Mital et al., 1974; Wang et al., 2002, 2003, 2004), little is known of more complex mixtures involving both traditional yoghurt strains. Results of Murti et al. (1993b) suggest that *Bifidobacterium* can indeed grow more extensively in soy than in cows' milk under comparable conditions. However, very wide variations have been noted in the growth abilities of strains within a given species (Murti et al., 1993a; Scalabrini et al., 1998), and more data are needed to better characterize the potential of soy as a substrate to support good growth of bifidobacteria in combination with yoghurt strains.

Lactobacilli are also extensively used as probiotics. Soy has been examined as a substrate for the *Lactobacillus* species *L. casei* (Murti et al., 1993b; Garro et al., 1999), *L. helveticus* (Angeles and Marth, 1971; Murti et al., 1993b), *L. fermenti* (Mital et al., 1974; Chumchuere and Robinson, 1999), *L. fermentum* (Garro et al., 2001, 2004), *L. reuteri* (Tzortzis et al., 2004) and *L. acidophilus* (Mital et al., 1974; Murti et al., 1993b; Shelef et al., 1998; Wang et al., 2002, 2003), but no information is available on the growth of the species *L. rhamnosus* or *L. johnsonii* in soy-based mixed cultures. These two species are also recognized as important probiotics in dairy products (Ouwehand et al., 2003) and investigation of their growth in soy products is warranted.

The purpose of this study was to measure the ability of *L. rhamnosus*, *L. johnsonii* and various bifidobacteria to grow in mixed cultures with yogurt strains in a soy beverage and cows' milk.

2. Materials and methods

2.1. Production of yogurt

Batches of yogurt were prepared in sterilized glass bottles, containing 500 ml of either homogenized cows' milk (Bio Lait Biologique, Liberté Co., Candiac, QC, Canada) containing 3.3% protein, and 2% milk fat, or a soy beverage (So Nice Organic Original, SoyWorld Inc., Vancouver, BC, Canada) containing 2.4% protein, 1.2% fat, evaporated cane juice, and 14 vitamins and minerals. Control yogurts were prepared by inoculating cows' milk and soy beverage with about 3×10^5 cfu/ml of each of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* cultures, both of which were obtained from Abiasa Co., Saint Hyacinthe, QC, Canada. All fermentations were carried out for 12 h at 41 ± 1 °C, in a waterbath, without agitation.

2.2. Yogurt containing probiotic bacteria

The list of probiotic bacteria tested for their ability to grow during fermentation of either cows' milk or soy beverage is shown in Table 1. Before inoculation, the probiotic bacteria were replicated twice for 16 h at 37 °C under an atmosphere of (85% nitrogen, 10% oxygen, and 5% carbon dioxide in de Man Rogosa Sharp (MRS) broth (Difco, Sparks, MD, USA) supplemented with cysteine, 0.5 g/l. Cultures were centrifuged to pellet the cells which were then resuspended in cows' milk or soy beverage. Probiotic bacteria were added at numbers of 1×10^5 cfu/500 ml of milk or soy beverage at the same time that the yogurt starter bacteria were added. Each fermentation was repeated three times.

Table 1
Bacteria used in the study

Bacterium	Number Code	Source
Lactobacillus acidophilus	ATCC 4356	ATCC
Lactobacillus delbrueckii subsp. *bulgaricus*	IM 025	Abiasa
Lactobacillus johnsonii	NCC533 (La-1)	Nestle
Lactobacillus rhamnosus	ATCC 53103 (GG)	ATCC
Bifidobacterium sp.	RBL 00064	INAF/Fliss
Bifidobacterium sp.	RBL 00066	INAF/Fliss
Bifidobacterium sp.	RBL 00079	INAF/Fliss
Bifidobacterium sp.	RBL 00080	INAF/Fliss
Bifidobacterium sp.	RBL 00084	INAF/Fliss
Streptococcus thermophilus	IM 111	Abiasa

2.3. Enumeration of bacteria

Each 2 h, a 1 ml sample was taken from each yogurt. Serial ten-fold dilutions were prepared in a solution of 0.9% NaCl (w/v) and 0.1% (w/v) bacto peptone (Difco) and suitable dilutions were plated on appropriate media. *L. delbrueckii* subsp. *bulgaricus* were enumerated on lactobacilli MRS broth (Difco) supplemented with agar, 15 g/l (Difco); *S. thermophilus* were enumerated on M17 broth (Difco) supplemented with lactose, 5 g/l (Mallinckrodt, St. Louis, MO, USA) and agar, 15 g/l (Difco); *Bifidobacterium* sp. RBL 00064, RBL 00066, RBL 00079, RBL 00080 and RBL 00084 were enumerated on Columbia agar base medium (CAB; Becton Dickinson, Cockeysville, MD, USA) supplemented with cysteine HCl, 0.5 g/l; raffinose, 0.5 g/l; lithium chloride, 2 g/l; and sodium propionate, 3 g/l. *L. johnsonii* La-1, *L. rhamnosus* GG, and *L. acidophilus* were enumerated on a modified MRS agar containing proteose peptone, 10 g/l (Becton Dickinson); beef extract, 10 g/l (Becton Dickinson); yeast extract, 5 g/l (Becton Dickinson); maltose, 20 g/l; polysorbate 80, 1 g/l; ammonium citrate 2 g/l; sodium acetate, 5 g/l; magnesium sulphate, 0.1 g/l; manganese sulphate, 0.05 g/l; dipotassium phosphate, 2 g/l; and agar, 15 g/l. The medium was supplemented with maltose, 20 g/l for enumeration of *L. johnsonii* La-1, or mannitol, 20 g/l for enumeration of *L. rhamnosus* GG. Enumeration of the target organisms could be performed using these media since *L. delbrueckii* subsp. *bulgaricus* formed pin point colonies on them, and such colonies were disregarded.

2.4. High pressure liquid chromatography (HPLC) analysis

At 4, 8, and 12 h after the start of the fermentation, 1 ml samples were taken for sugar and organic acid analyses. Samples were extracted with 95% ethanol supplemented with arabinose as an

internal standard. After mixing and centrifugation, the supernatant was applied to a SepPak C18 column (Waters Corp., Milford, MA, USA) that had been washed with methanol. Sugars were eluted with 5 ml of methanol followed by 5 ml of water. A Waters 600 HPLC system fitted with a Ion 33, Interaction 300 mm × 7.8 mm column (Mandel Scientific Co., St. Laurant, QC, Canada), using 0.005N H_2SO_4 at a flow rate of 0.4 ml/min mobile phase, a Waters 410 refractive index detector (Millipore, Milford MA, USA) as well as a Waters 996 photodiode array detector was used to analyse for lactose, glucose, galactose, stachyose, raffinose, sucrose, fructose, acetic acid, and lactic acid. Identified peaks were quantified using authentic standards. Samples were obtained from three independent fermentations. Values were compared to those obtained from unfermented products.

2.5. Sugar utilization by bifidobacteria

An API 50CH sugar utilization kit (BioMérieux, St. Laurent, QC, Canada) was used to screen the bifidobacteria isolates for utilization of sugars. In addition, the ability to utilize stachyose was evaluated using an MRS medium without sugar, as previously described, with the addition of 0.5% (w/v) filter-sterilized stachyose. Growth was evaluated by comparing the turbidity of an inoculated tube that was not incubated to that of one that was incubated for 48 h under anaerobic conditions.

2.6. Data presentation and statistical analyses

Data discussed are the averages from three fermentations. Analyses of variance were carried out where indicated using SAS version 8.02 (SAS Institute Inc., Cary, NC, USA). Comparisons were considered significantly different if $p < 0.05$.

3. Results

3.1. Growth of yogurt starter cultures

Fig. 1 shows the growth pattern and pH changes that occurred when the two yogurt producing bacteria *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* were inoculated together into either cows' milk or soy beverage. Twelve hours were required to reach a pH of 4.3 in both cases (Fig. 1). The *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* strains used in this study grew well in the soy beverage and their population stabilized after 6 h and 10 h incubation, respectively. When cows' milk was inoculated with both *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, their numbers increased steadily during the 12 h fermentation. After 12 h, the numbers of *S. thermophilus* in both the cows' milk and the soy beverage were higher than those of *L. delbrueckii* subsp. *bulgaricus*.

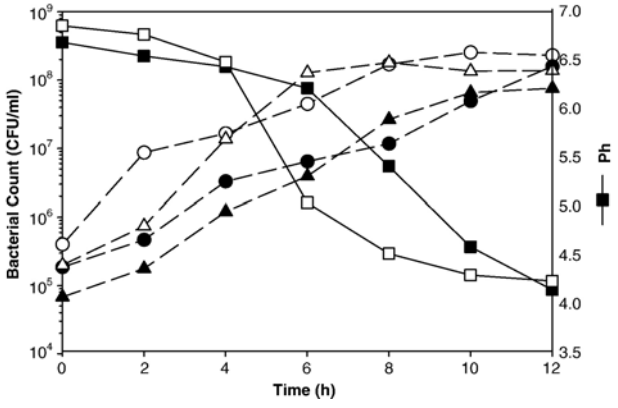

Fig. 1. Growth of *L. delbrueckii* subsp. *bulgaricus* and (●) and *S. thermophilus* (○) in cows' milk; *L. delbrueckii* subsp. *bulgaricus* (▲) and *S. thermophilus* (△) in soy beverage; and pH values of cows' milk (■) and soy beverage (□) during their fermentations. Data points are averages from three independent fermentations.

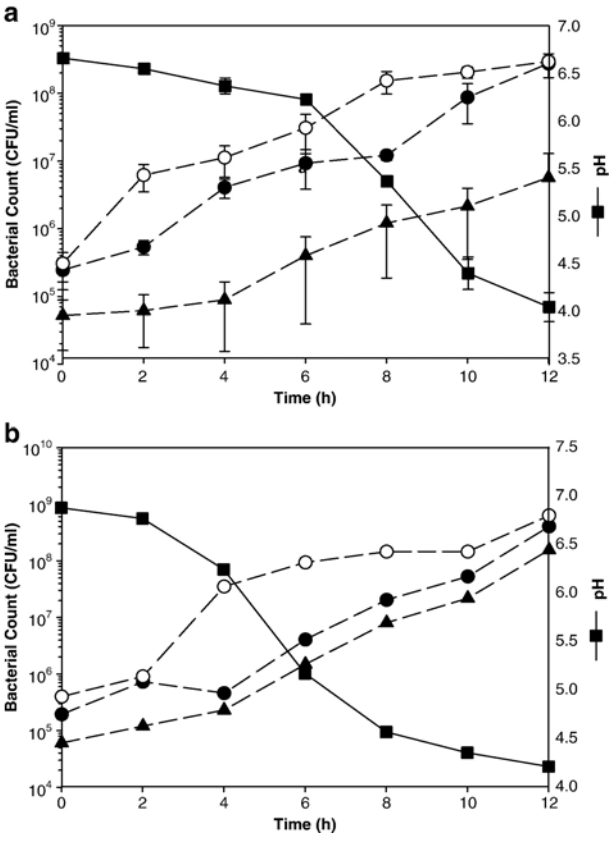

Fig. 2. Growth of *L. delbrueckii* subsp. *bulgaricus* (●), *S. thermophilus* (○), and *L. rhamnosus* (▲), and pH (■) during fermentation of (a) cows' milk or (b) soy beverage Data points are averages of three independent fermentations. Bars on data points represent standard errors.

The final pH value of both milk and soy beverage were similar (Fig. 1), but the pH declined faster in the soy beverage than in the cows' milk.

The presence of the probiotics did not affect the growth patterns of the yogurt strains, and there were many phenomena that were constant in all the treatments (Figs. 1–5). Data are presented in one Fig. 2a to illustrate typical means and standard errors of the means. In all cases, *S. thermophilus* growth was faster in soy beverage than in milk; growth of *L. delbrueckii* subsp. *bulgaricusi* was always slower in soy beverage than in milk; *S. thermophilus* growth in soy stopped after about 6 h incubation with numbers at approximately 2×10^8 cfu/ml; in both the soy beverage and cows' milk the growth of *L. delbrueckii* subsp. *bulgaricus* was slower than that of *S. thermophilus* but

continued for a longer time; and after 6 h incubation, *S. thermophilus* was dominant, but after 12 h the ratios of the numbers of *S. thermophilus* to those of *L. delbrueckii* subsp. *bulgaricus* were close to 1:1 in milk, and approximately 2:1 in soy beverage.

The probiotic strains did not have an obvious effect on acidification. In all cases (Figs. 1–5), the drop in pH was faster in soy beverage than in milk, and the pH values after 12 h incubation at 41 °C were not greatly affected by the presence of the probiotic strains.

3.2. Growth of the probiotic strains

Approximately 2 log increases in the numbers of both *L. rhamnosus* GG and *L. johnsonii* La-1 were observed when either of these probiotic strains was added together with the yogurt strains in cows' milk (Figs. 2a, 3a) or the soy beverage (Figs. 2b, 3b). The numbers of both *L. rhamnosus* GG and *L. johnsonii* La-1 were less than the numbers of the yogurt bacteria after 12 h fermentation. Although acidification was faster in the soy beverage than in milk, growth of both probiotic lactobacilli was more extensive in the soy beverage than in milk. This resulted in *L. rhamnosus* GG and *L. johnsonii* La-1 populations being respectively 3 and 5 times higher in the fermented soy beverage than in the fermented milk after 12 h

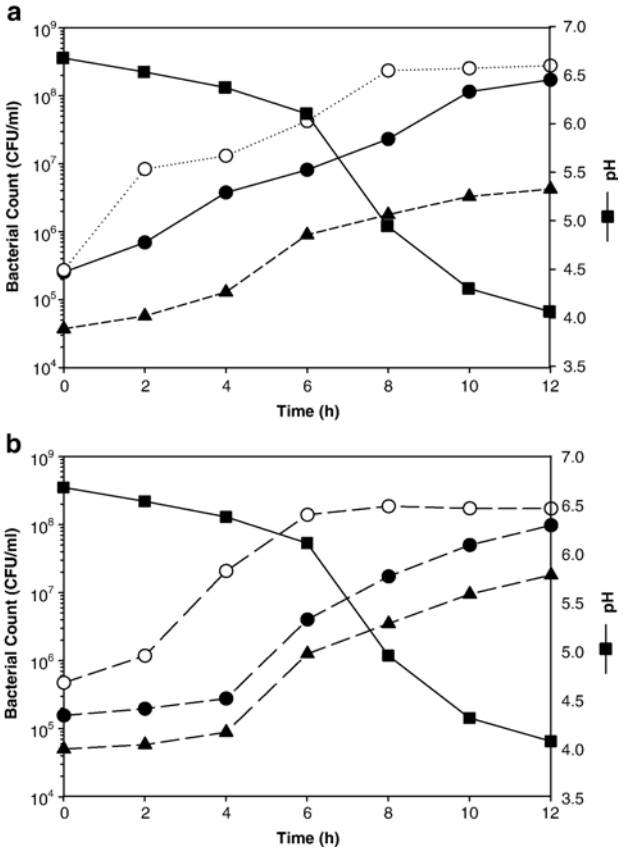

Fig. 3. Growth of *L. delbrueckii* subsp. *bulgaricus* (●), *S. thermophilus* (○), and *L. johnsonii* (▲), and pH (■) during fermentation of (a) cows' milk or (b) soy beverage. Data points are averages of three independent fermentations.

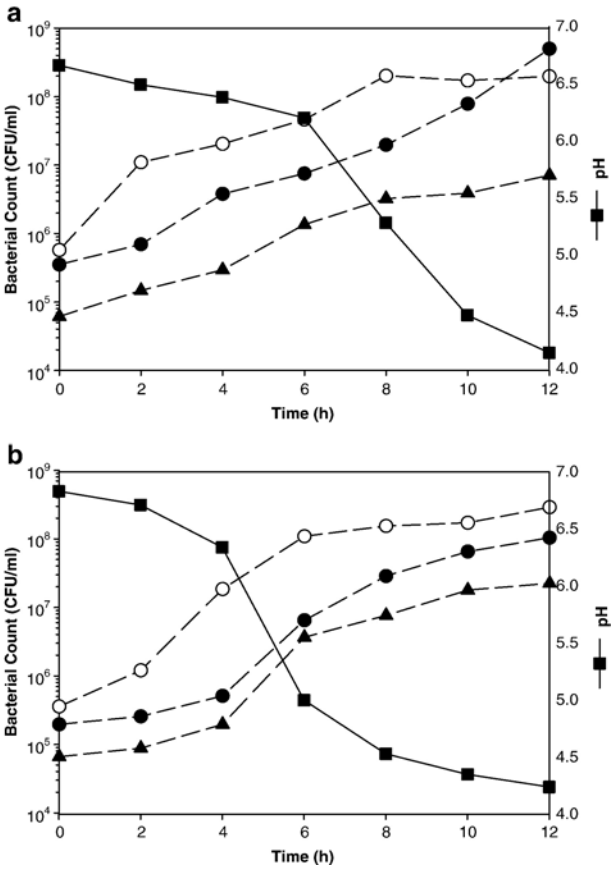

Fig. 4. Growth of *L. delbrueckii* subsp. *bulgaricus* (●), *S. thermophilus* (○), and *B.* RLB00064 (▲), and pH (■) during fermentation of (a) cows' milk or (b) soy beverage. Data points are averages of three independent fermentations.

incubation (Figs. 2 and 3). Of the two probiotic lactobacilli, *L. rhamnosus* GG showed the greatest ability to grow with the yogurt starter in the soy beverage.

The final pH values of the yogurts were between 4.0 and 4.3. The final pH value of the cow's milk yogurt plus probiotic bacteria was lower than that of the fermented soy beverage yogurt plus probiotic bacteria; but the difference was not significant ($p > 0.05$).

Two bifidobacteria strains, RBL 00079 and RBL 00064, grew well in cows' milk or soy beverage during fermentation with the yogurt bacteria. However, their growth patterns were different. Both bifidobacteria grew steadily in the cows' milk (Figs. 4a, 5a), but in the soy beverage there was little growth during the first 4 h (Figs. 4b, 5b). At the end of 12 h, the soy beverage yogurt had approximately four times more *Bifidobacterium* RBL 00079 or RBL 00064 than the cows' milk yogurt. The final pH values of the yogurts containing the bifidobacteria were similar to those of the yogurts containing probiotic bacteria.

3.3. Sugar assimilation and organic acid production

HPLC analyses showed that the probiotics and bifidobacteria were using different sugars to support their growth in cows'

milk or soy beverage. In the cows' milk the concentration of lactose declined over time, whether or not the milk contained added probiotic bacteria or bifidobacteria. As lactose values were declining, the concentrations of galactose in all the fermenting milks increased. The glucose concentration in cows' milk remained low during the 12 h fermentation.

The sugar utilization tests indicated that none of the five bifidobacteria strains used glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, *N*-acetyl-glucosamine, amygdaline, arbutine, salicine, D-celiobiose, xylitol, gentiobiose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-cetogluconate, or potassium 5-cetogluconate. All the bifidobacteria used D-galactose, D-glucose, D-fructose, and D-mannose, D-melezitose, D-raffinose and starch. *Bifidobacterium* RBL 00079 differed from the other isolates in that it was not able to use D-turanose and D-lyxose. However, *B*. RBL 00079 was the only bifidobacteria isolate able to utilize stachyose.

The differences between the initial and final concentration of sugars in the cows' milk and soy beverage containing probiotic bacteria or *Bifidobacterium* 00079 are presented in Table 2. Lactose and fructose were the primary sugars used in fermentations of cows' milk and soy beverage, respectively. For fermentations of cows' milk or soy beverage, the final concentrations of sugars were not significantly affected ($p>0.05$) by the addition of *L. rhamnosus* GG, *L. johnsonii* La-1 or *Bifidobacterium* RBL 00079.

Table 2
Changes in sugar concentrations (g/100 ml) resulting from fermentations of cows' milk or soy beverage for 12 h by *Lactobacillus johnsonii* (La-1), *Lactobacillus rhamnosus* GG (GG) or *Bifidobacterium* RBL 0079 (Bif79)

Sugar	Cows' milk			Soy beverage		
	La-1	GG	Bif79	La-1	GG	Bif79
Lactose	1.73	1.82	1.88	na[a]	na	na
Glucose	−0.06	−0.06	−0.01	0.33	0.33	0.34
Galactose	−0.01	−0.01	−0.01	na	na	na
Stachyose	na	na	na	0.25	0.21	0.18
Raffinose	na	na	na	0.01	0.01	0.02
Sucrose	na	na	na	0.38	0.48	0.28
Fructose	na	na	na	1.84	1.82	1.83

[a] na=not applicable.

After 12 h, the lactic acid concentrations in cows' milk and soy beverage fermentations were 0.63–0.82 g /100 ml and 0.38–0.39 g/100 ml, respectively. Levels of acetic acid in all fermentations were between 0.01 and 0.05 g /100 ml.

4. Discussion

The falls of the pH values were faster in the soy beverage than in milk. This suggests a greater rate of production of organic acids in the soy beverage than in milk, but the HPLC data did not show this to be the case. It has been observed that, for a given titratable acidity level, pH values are lower in soy blends than in milk (Angeles and Marth, 1971). Therefore, the higher apparent acidification rates observed in soy blends reflect the lower buffering ability of soy protein as compared to milk proteins.

In order to better examine the effects of substrate on the growth of strains and their ratios in mixed cultures, inoculation rates were kept under 10^6 cfu/ml, which is about 10 times lower than in industrial practices. This resulted in somewhat lengthy fermentations times. The growth patterns of the yogurt starters in milk were in line with data in the literature, which show that the streptococci initiate the milk fermentation and that lactobacilli contribute to acidification later in the incubation (Tamime and Robinson, 1985). This study showed that this pattern also occurs in soy beverage fermentation.

Growth of *L. delbrueckii* subsp. *bulgaricus* was initially slower in soy beverage than in cows' milk. This was expected, because most *L. delbrueckii* subsp. *bulgaricus* strains do not grow well in soy beverages (Mital et al., 1974; Karleskind et al., 1991; Murti et al., 1993b). However, after 4 h incubation, growth of the organism improved significantly. That could be due to the faster drop of the pH of soy beverage than of cows' milk, to pH values which are favourable to the growth of lactobacilli. These data suggest that some of the symbiotic elements of the relationship between the two yogurt strains, which are well documented in milk fermentation (Tamime and Robinson, 1985), might occur in the soy beverage also.

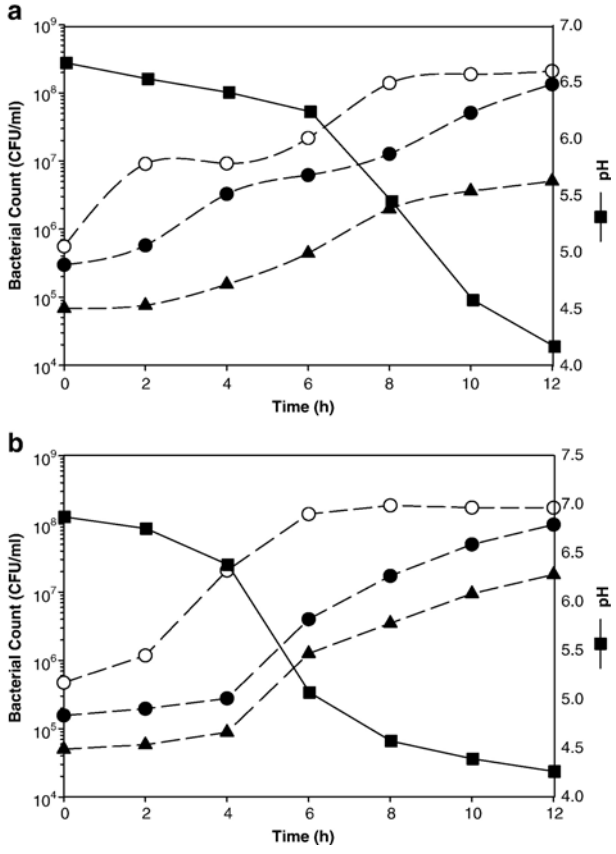

Fig. 5. Growth of *L. delbrueckii* subsp. *bulgaricus* (●), *S. thermophilus* (○), and *B.* RLB00079 (▲), and pH (■) during fermentation of (a) cows' milk or (b) soy beverage. Data points are averages of three independent fermentations.

During incubation, for the first 6 h, growth of *S. thermophilus* was faster in soy beverage than in milk. This confirms the data in the literature which show that *S. thermophilus* is well able to grow in soy beverages because of its ability to use sucrose (Angeles and Marth, 1971; Karleskind et al., 1991; Chumchuere and Robinson, 1999). Cane sugar added to the soy beverage in this study may also have contributed to this faster growth. However, growth of *S. thermophilus* stopped after 6 h during soy beverage fermentation, which was 2 h earlier than during fermentation of cows' milk. This may be due to the faster drop in pH during soy beverage fermentation.

Cows' milk often does not support extensive growth of bacteria because of the lack of free amino acids. Yogurt starter cultures therefore contain bacteria with proteolytic activity, such as *L. delbrueckii* subsp. *bulgaricus*, that break down protein to produce amino acids that support the growth of nonproteolytic bacteria. In traditional yogurt, *S. thermophilus* is generally the beneficiary of this proteolysis, but in our fermentations the probiotics could also have benefited from it.

In some yogurt production, supplements such as whey powder, whey protein concentrates or acid casein hydrolysates are added to reduce the time required for fermentation with *L. acidophilus*, because they provide amino acids and/or carbohydrates to support the growth of the organism (Dave and Shah, 1998). Growth of probiotic bacteria and bifidobacteria in both cows' milk and soy beverage may also be dependent on the liberation of amino acids by other bacteria. Except for the growth of *L. rhamnosus* GG in the soy beverage, in all the other yogurts containing *L. rhamnosus*, *L. johnsonii* La-1, *Bifidobacterium* RBL 00079 or *Bifidobacterium* RBL 00064, the growth of the added bacteria was delayed for 2 to 4 h after the start of the fermentation. Presumably this time was needed to generate enough amino acids to support growth. However, other factors could also be involved such as reduction of the free oxygen level as a result of *S. thermophilus* growth, or a drop in pH to a level more favourable for the probiotics. More data are thus needed to explain the behaviours of probiotics in mixed cultures with yogurt strains.

The utilization of sugars in the soy beverage was different than that reported by Hou et al. (2000), who showed that concentrations of sucrose, raffinose and stachyose decreased during fermentation by *Bifidobacterium infantis* and *Bifidobacterium longum* of a laboratory prepared soymilk. At the same time, the concentrations of the monosaccharides fructose, glucose and galactose increased. In our experiment, fructose was the sugar most utilized; glucose, raffinose and stachyose were used much less. This discrepancy might be due to the numbers of probiotics involved. Due to the rapid growth of *S. thermophilus*, and to a lesser extent of *L. delbrueckii* subsp. *bulgaricus*, the bifidobacteria did not reach numbers $>3\times10^7$ cfu/ml. Therefore, except for *L. rhamnosus* GG, the carbohydrate assimilation patterns observed probably reflected the metabolism of the yogurt strains, which were 10 times more numerous. In addition, the initial high concentration of fructose in the soy beverage, together with the low concentrations of raffinose and stachyose may have affected the fermentations.

It has been suggested that fermented dairy products require probiotic bacteria at 10^7 cfu/ml in order to give health effects in the gastro-intestinal tract when consumed (Ouwehand and Salminen, 1998). However, in fermented soy products containing a mixed culture, the numbers of probiotic bacteria might be insufficient to impart beneficial effects. This study showed that when the probiotic bacteria population remained below 3×10^7 cfu/ml while that of the total yogurt starter reached 5×10^8 cfu/ml, the effects on the final carbohydrate concentrations are minimal. This suggests that the effects of probiotic bacteria on sensory properties would also be limited. In some instances this could be desirable, but not always. The presence of probiotic bacteria could reduce the levels of *n*-hexanal and pentanal compounds responsible for the "bean" flavour of soy products (Desai et al., 2002) or increase the level of free isoflavones in soy products (Choi et al., 2002; Tsangalis et al., 2003).

It has been reported that soymilk itself will support the growth of bifidobacteria, but at rates less than those in MRS broth or reconstituted skim milk (Kamaly, 1997). Bifidobacteria have α-galactosidase activity (Roy et al., 1992) that allows them to utilize sugars such as raffinose and stachyose, and sufficient proteolytic activity to support growth in soymilk (Tamime et al., 1995; Kamaly, 1997). We found that all four human derived bifidobacteria could use raffinose but only *Bifidobacterium* RBL 00079 was able to metabolise stachyose. This would imply that *Bifidobacterium* RBL 00079 would be the best of these four human derived strains for inclusion in a soy-based product.

The bifidobacteria that we used were originally isolated from humans, and therefore their nutrient requirements may be very specific. Even though *Bifidobacterium* RBL 00079 can utilize sugars found in soy beverage, the slow initial growth of the bifidobacteria in the soy beverage may indicate a need for other nutrients that only become available in large quantities after the two yoghurt starter culture bacteria have established the fermentation process.

This study is the first report of the potential of *L. rhamnosus* GG and *L. johnsonii* La-1 as probiotics in fermented soy products with yogurt cultures. These lactobacilli can compete better with the yogurt strains in a soy beverage than in cows' milk. Soy-based yogurt containing *L. rhamnosus* GG and *L. johnsonii* La-1 are therefore possible.

Although probiotic bacteria grow in cows' milk and soy substrates, they still remain a minor fraction of the total population when yogurt starters are present. Approaches such as milk supplementation or changes in inoculation levels are available to manufacturers who wish to increase the probiotic bacteria content in their fermented dairy products (Champagne et al., 2005). Further studies are needed, however, to determine if these strategies can also be effective with soy-based media.

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