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Full Length Research Paper

Growth and lactic acid production by *Bifidobacterium longum* and *Lactobacillus acidophilus* in goat's milk

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The fitness of a particular strains of Bifidobacteria, Lactobacilli and Streptococci for commercial utilization depends on its rapid growth and acidification of milk as well as its acid and oxygen tolerence. From 20 samples of French commercial yoghurt, one species of bifidobacteria was identified as *Bifidobacterium longum.* Also, *Lactobacillus acidophilus* was isolated and identified from fermented milk. The rate of growth was 0.37 h^{-1} on TPY medium and 0.18 h^{-1} in milk with *Bifidobactrium longum.* The production of lactic acid was compared with pure and mixed cultures and the values were 90 and 64 mM at 37°C; 82 and 140 mM at 45°C. The maximum rate of lactic acid production was obtained with mixed culture at 45°C.

Key words: *Bifidobacterium longum, Lactobacillus acidophilus,* fermented milk, growth, lactic acid.

INTRODUCTION

In recent years, there has been an increasing interest in the incorporation of the intestinal bacterial species, *Lactobacillus acidophilus* and *Bifidobacterium longum*, into fermented milk products. Studies in man have shown that these bacteria administered orally or in fermented milks have a variety of beneficial health effects in human and animal intestinal tract (Gilliland, 1990; Gibson and Wang, 1994; Link-Amster et al., 1994; Baricault et al., 1995; Lee et al., 2003).

Nutritional and health aspects of functional foods incorporating probiotic bacteria such as bifidobacteria and lactobacilli have received considerable attention. Production of high-quality fermented milk products containing these bacteria is a major challenge (Yeung et al., 2002). These organisms are often used together or in combination with conventional starters' species, such as *Streptococcus thermophilus*. Focus has generally been in incorporation of selected strains of *Bifidobacterium* spp. into milk and fermented milk products. Although a certain amount of attention has been directed towards the organoleptic characteristics of the product, most publications concerning probiotic bacteria have focused on the human health aspect. To succeed in promoting the consumption of functional probiotic products, the food industry has to satisfy the demands of the consumer (Hilde et al., 2003). The selection of strains for fermented milk production criteria does not include only the properties of intestinal effects but also the growth in milk and survival in acidic milk. Due to the repeated therapeutic effects of these organisms, Japan and several European countries have been actively involved in the development and application of bifidobacteria (Gomes and Malcata, 1999). The cultivation of bifidobacteria in milk is a difficult task compared with that a conventiolal starter because milk is an artificial medium for growth of this nutritionaly fastidious microorganisms. The aim of the present work was to study the growth and lactic acid production by pure and mixed cultures of bifidobacteria and *L. acidophilus* in milk.

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MATERIALS AND METHODS

Strains and cultivation

A total of 20 samples of French commercial yoghurt (Bifidus Actif) were

analysed for essential industrial microflora *Bifidobacterium longum, Lactobacillus acidophilus* and *Streptococcus thermophilus.* Samples were aseptically removed from containers and diluted by mixing 1 g in 9 ml of 0.1% peptone salt. Further dilutions were made as required. Isolation and enumeration of *L. acidophilus* was verified on MRS agar (Deman et al., 1960). TPY agar with lithium chloride (LiCI) 3 mg/ml and dichloxacillin 2 mg/ml was utilised for bifidobacteria (Tamime et al., 1995*).* The plates were incubated anaerobically for 24 h at 37°C, using Oxoid gas jars and anaerobic gas paks (Payne et al., 1999).

Identification of strains

Colonies and cells morphology characteristics on TPY and MRS agar were examinated. All strains were initially submatted to Gram staining, the catalase test and spore formation. The isolates considering belong to the genus *Bifidobacterium* were identified by the detection of fructose-6- phosphate phosphoketolase (F6PPK) (Scardovi, 1986). The strains were identified to species level based on their sugar fermentation pattern, as discribed by Hadadji et al. (2005) than the identity of the cultures was based on the characteristics of Bifidobacteria and Lactobacilli as discribed in Bergey's manual of determinative bacteriology (1986).

Preparation of inocula

Inocula were prepared in 10 ml of skim milk and incubated for 16 h at 37°C. They were used to inoculate the culture at 5% of each strains of *B. longum* and *L. acidophilus.*

Experimental cultures

Each strain was grown in pure culture (5% of culture starter in 200 ml skim milk, after shaking with a vortex, the culture was divided in 10 ml samples). Mixed cultures were also grown in the same conditions. The fermentation conditions were tested in duplicate in pure and mixed culture. Lactic acid production and growth were at two temperatures, 37 and 45°C.

Determination of maximum growth rate

During exponential growth, the logarithm of bacteria population varies linearly with time. Thus, the maximum growth rate (µmax) is the slope of the line. It was determined by the linear regression applied to the exponential points of the line (Bouquien et al., 1988).

Determination of maximum rate acidification

It was observed that the lactate production of culture increase linearly with time during exponential growth. The corresponding regression lines were calculated. The absolute value of their slopes is a reflection of the maximum acidification rate (Bouquien et al., 1988).

Determination of titrable acidity

Ten gram samples were taken from fermented milk, mixed and titrated with 0.11 N NaOH to a pH end point of 8.6 with constant stirring. Titrable acidity was reported as the mM of lactic acid (Bouquien et al., 1988).

Samples preparation

Fermented products were made by Bifidobacteria (5 ml of starter culture in 95 ml of reconstituted skim milk containing 0.5% cysteine hydrochloride) (Klaver et al., 1993). 10 ml of culture was then transferred into 30 ml volume test tube using strictly anaerobic conditions. The tubes were then sealed with rubber stoppers and fitted with standard plastic slip-on caps. Samples were incubated for 24 h at 37°C in the anaerobic jar.

Biochemical tests, enzymatic activity profiles and carbohydrate fermentation experiments were determined according to Tamime et al. (1995). Mixed cultures of both strains were performed. The inoculum was 5% concentration of each bacterial species.

RESULTS AND DISCUSSION

Isolation and identification studies

All pure cultures of *Bifidobacterium* obtained on TPY medium, from French yoghurt (Bifidus actif) were Gram positive, catalase negative nonsporulating and positive for F6PPK test. Growth of *Lactobacilli* and *Streptococci is* suppressed on TPY by using lithium chloride and dicloxacillin. In TPY agar, *B. longum* show mostly very elongated and relatively thin cellular elements with slightly irregular contours and rare branching. *B. longum was* distinguished from the other species of *Bifidobacterium* by the utilisation of arabinose, xylose, ribose, lactose, melesitol, trehalose and it cannot utilise gluconate, maltose and salicine. All these characteristics correspond to *B. longum* as described by Sakata et al. (2002) and Ventura and Zink (2002). Morphological features, physiological and biochemical tests were used to identify *L. acidophilus* and *S. thermophilus* (Lee et al*.,* 2003).

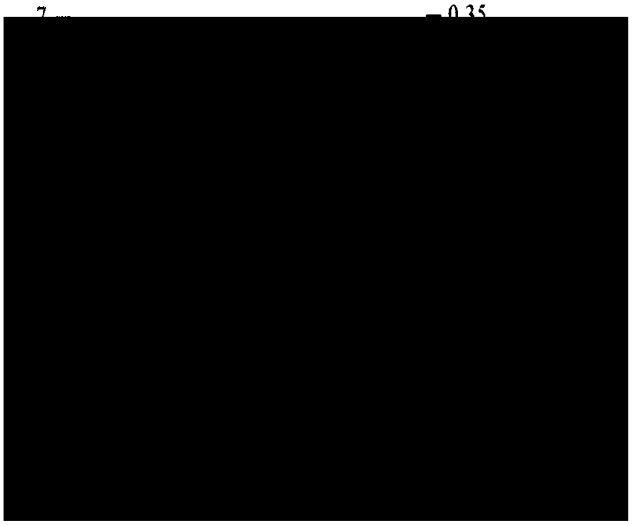

Figure 1. Kinetic growth (■) and pH change (□) of *Bifidobacterium longum* in TPY medium at 37°C.

Growth study

The kinetics growth of *B. longum* and pH evolution on TPY broth is shown in Figure 1. *B. longum* grew well in lactose TPY and the rate of growth was 0.37 h^{-1}, which is slightly higher than the value obtained by Wang and Gibson (1993). The pH of culture decreases gradually from 6.7 to 4.1 after 23 h of incubation at 37°C in lactose TPY broth; however, growth of *B. longum* remained relatively unaffected. Mitsuka et al. (1987) had shown that oligo-fructose gave a high proportion count of bifidobacteria in intestinal. These results confirm the bifidogenic effects of oligo-fructose and inulin.

Figure 2. Kinetic of lactic acid production by *Bifidobacterium longum* grown in skim milk at 37°C and standard error of deviation (± 5 %).

Kinetics of acidification

Lactic acid production in culture was proportional to the concentration of the initial inoculum starter (results not shown). In pure culture the maximum population of *B. longum* was 6.6 x 10^8 ufc/ml and the maximum growth rate was 0.18 h^{-1}. In the case of *L. acidophilus* the maximum population was 8.46 x 10^9 ufc/ml alter 24 h of incubation and the maximum growth rate was 0.26 h^{-1}. Studies of several workers (Hughes and Hoover, 1995; Baricault et al., 1995; Walker et al., 2005) showed that growth of *B. longum* and *L. acidophilus* in milk was quite variable, and after approximately 14 h growth in skim milk reached the stationary phase. In stirred yoghurt, yoghurt beverages and low milk solid yoghurts, production of polysaccharides can improve viscosity, texture, increase resistance to mechanical handling and decrease susceptibility to syneresis. The use of ropy strains of *B. longum* is particularly important to stabilise yoghurt. Zourari et al. (1992) and Gätji and Gottschalk (1991) showed that inhibition of growth and lactic acid production was caused by undissociated form of lactic acid for *lactobacillus* species and continuous cultures. Desjardins et al. (1990) remarked the same inhibition phenomenon for bifidobacteria due to the lactate and acetate accumulation, while Rogers et al. (1978) observed that growth inhibition of *Lactococcus lactis* subsp. *cremoris* by lactic acid. Lactic acid production and standard error of deviation in pure culture of *B. longum* in milk is shown in Figure 2.

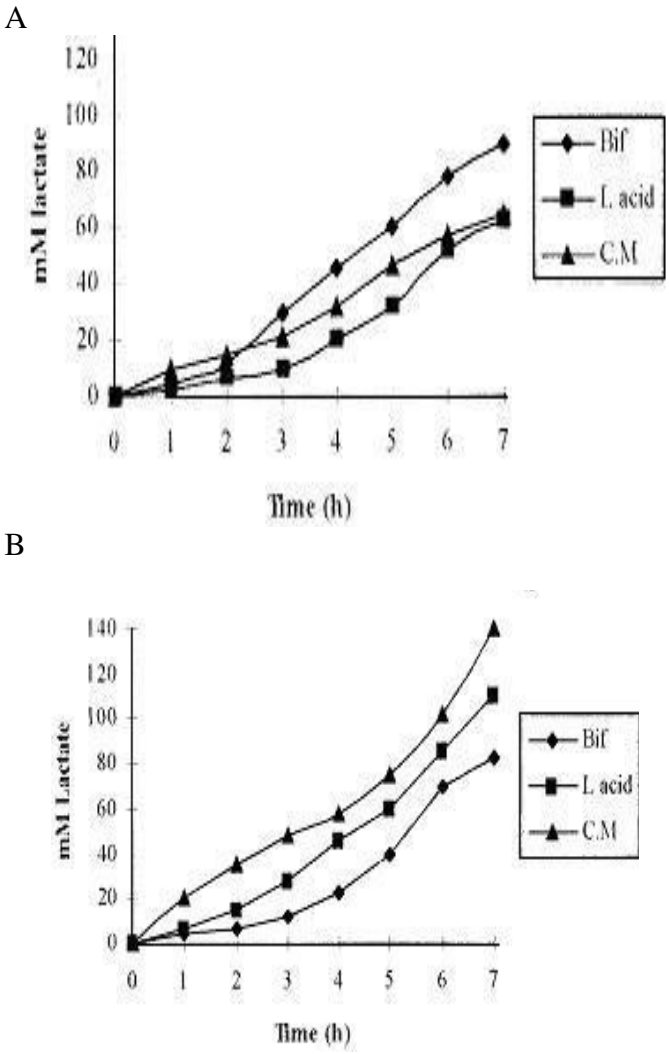

Figure 3. Kinetics of lactic acid production (mM) of pure culture of *Bifidobacterium longum* (♦),*Lactobacillus acidophiles* (■)and mixed cultures (▲) grown in skim milk at 37°C (A) and 45°C (B).

Effects of temperature

Fermentation processes were carried out for 7 h at different temperatures, and lactic acid accumulation increased with elevation temperature from 37 to 45°C in mixed culture (Figures 3A and 3B). Relative good levels

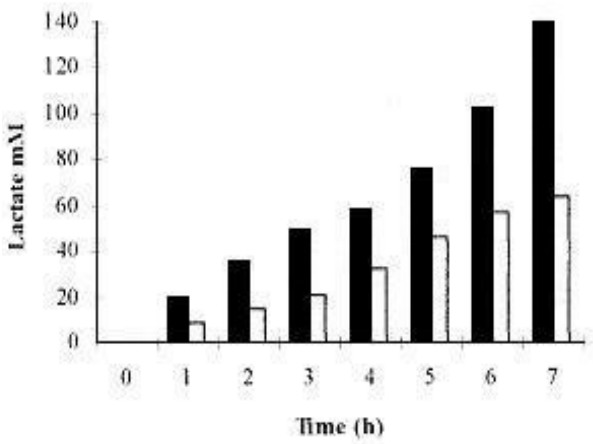

Figure 4. Comparaison of lactic acid production between mixed cultures of *Bifidobacterium longum* and *Lactobacillus acidophiles* at 37 and 45°C in skim milk.

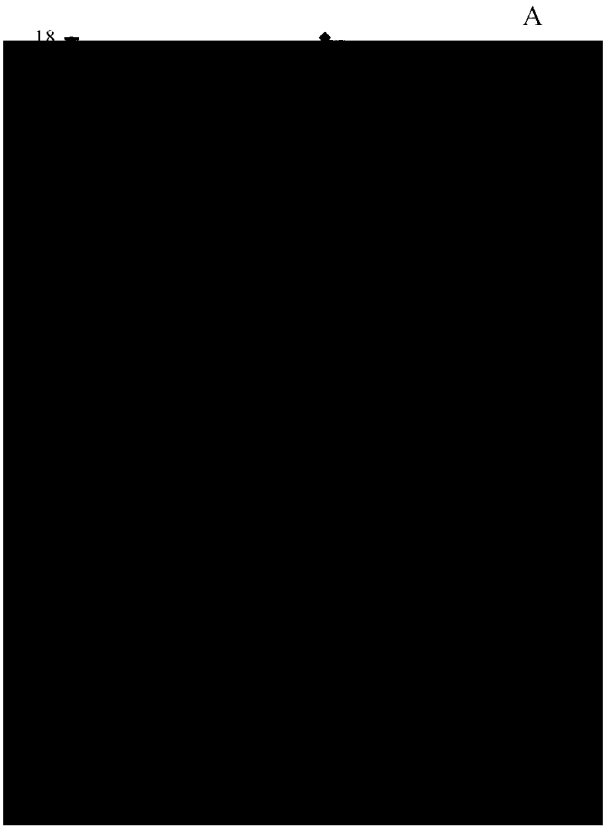

Figure 5. Kinetics of lactic acid production rate (mM/h) of pure culture of *Bifidobacterium longum* (♦), *Lactobacillus acidophilus* (■) and mixed cultures (▲) grown in skim milk at 37°C (A) and 45°C (B).

of lactic acid were produced at 37°C in the case of pure culture of *B. longum* and the maximum lactic acid production rate was 18 mM/h alter 4 h of incubation time.

In other hand, *L. acidophilus* produced a high level of lactic acid at 45°C and the maximum lactic acid production rate was 27 mM/h alter 5 h of incubation time.

The mixed culture of *B. longum* and *L. acidophilus* in skim milk led to reduction of the fermentation time compared with the pure cultures. Acidity development by mixed cultures at 45°C was higher than that developed by pure cultures. The comparison of lactic acid production at two different temperatures is shown in Figure 4. The effect of temperature on the production of lactic acid with *B. longum* is not significant, which is due to the manipulation conditions. However, the production of lactic acid with *L. acidophilus* was enhanced by 44% at 45°C.

With mixed cultures of *B. longum* and *L. acidophilus*, the production of lactic acid increased by 56% at 45°C. The positive effect of mixed cultures show probably the bifidogenic effect and the stimulant factors on the production of lactic acid. Figures 5A and 5B show that the rate of lactic acid production which was higher at 45°C. On the other hand, the maximum rate was obtained slowly with mixed culture 40 mM/h at 45°C. Wang and Gibson (1993) and Gibson et al. (1995) had shown the effects of oligo-fructose on the growth of Bifidobacteria which leads to the restoration of the intestinal microflora after antibiotherapy.

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