Growth Enhancement of *Bifidobacterium lactis* Bo and *Lactobacillus acidophilus* Ki by Milk Hydrolyzates

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ABSTRACT

The determination of the best conditions of preparation of a (tentatively) probiotic starter culture that might be suitable for cheese making composed solely of *Bifidobacterium lactis* Bo and *Lactobacillus acidophilus* Ki is critical if a consistently reliable acid production is to be achieved, especially because bifidobacteria have stringent requirements for growth. Therefore, we determined whether *B. lactis* Bo and *L. acidophilus* Ki required or benefitted from the addition of milk hydrolyzates (brought about by proteinase or neuramidase as the nitrogen source). The growth and acid production of *B. lactis* in milk were affected by the addition of proteinase-mediated hydrolyzate and, to a lesser extent, by neuramidase-mediated hydrolyzate; a higher degree of hydrolysis of either hydrolyzate resulted in greater biomass increase and greater acid production. This result suggests that the poor growth of bifidobacteria in milk is due partially to the lack of small peptides and free amino acids. The rates of growth and acidification by *B. lactis* were enhanced when cocultured with *L. acidophilus* (1:1 inoculum ratio). Conversely, the growth rates and acid production of *L. acidophilus* were not positively affected by the addition of either milk hydrolyzate. Although *L. acidophilus* grew slowly, its proteolytic system was apparently able to generate its own nitrogen source. Nevertheless, coculture with *B. lactis* (1:1 inoculum ratio) led to enhanced rates of growth and acidification when compared with that of the single strain, suggesting some degree of symbiosis between the strains.

(Key words: *Bifidobacterium lactis* Bo, *Lactobacillus acidophilus* Ki, growth, milk hydrolyzate)

Abbreviation key: DH = degree of hydrolysis of the milk hydrolyzate; MHN = milk hydrolyzate obtained from treatment of milk with neuramidase; MHP = milk hydrolyzate obtained from treatment of milk with proteinase.

INTRODUCTION

Microorganisms that are used for the production of fermented dairy products are selected for their suitable growth characteristics in milk as well as for consistently reliable acid production (16). The starters used to manufacture these products should lead to the development of good organoleptic properties and inhibit the growth of spoilage or potentially pathogenic microorganisms. The incorporation of intestinal species (e.g., *Lactobacillus acidophilus* and *Bifidobacterium* spp.) in milk products (e.g., fermented milk and yogurt, ice cream, and cheese) has been considered by a number of researchers (3, 8, 9, 18, 26) because of the potential health-promoting benefits of these bacteria. Dietary supplementation with bifidobacteria or factors with bifidogenic activity might in fact encourage resistance to intestinal infections. As a result of their bile resistance, *L. acidophilus* Ki and *Bifidobacterium lactis* Bo (14) are able to establish themselves in the intestine and are important in the microbial ecology of the intestinal environment; furthermore, these bacteria have been shown to control serum cholesterol concentrations (32) and to increase the colonization resistance against pathogenic bacteria such as *Salmonella enteritidis* and *Clostridium perfringens* (31). In addition to the beneficial role of *L. acidophilus* and *B. lactis* after consumption, high growth and acidification rates and good acid and oxygen tolerances have been considered to be important properties in the choice of such specific starter strains.

The cultivation of bifidobacteria in milk is a difficult task compared with that of conventional starters because milk is an artificial medium for growth of these nutritionally fastidious microorganisms (24). Although milk contains all of the essential nutrients for growth, these nutrients do not always exist in acceptable forms or in optimal concentrations;
in fact, several amino acids that are either stimulatory or essential for growth of bifidobacteria and lactobacilli [e.g., arginine, glutamic acid, isoleucine, leucine, tryptophan, tyrosine, cysteine, and valine (20, 21)] are generally present in insufficient amounts either unbound or as low molecular mass peptides (30). Available nitrogen therefore limits growth unless growth-promoting elements are added to the milk. Without such compounds, the growth rate of each of these species may differ considerably, and well-controlled fermentations would be difficult to achieve. Rasic and Kurmann (24) and Modler (19) have reviewed various bifidus factors and have emphasized their strain dependence. Among those factors are milk-based media supplemented with specific protein hydrolyzates (20, 22, 23), carbohydrates (7, 24), and organic and yeast extracts (16).

In attempts to establish good criteria for the preparation of a mixed starter composed solely of B. lactis and L. acidophilus, growth and acidification rates were studied using selected strains under conditions of low redox potential, focusing on the role of the nitrogen source reflected by addition of various milk hydrolyzates. Subsequent coculture experiments were done to determine the response of each strain to presence of the other coculture.

MATERIALS AND METHODS

Strains and Cultivation

Single strains of B. lactis Bo (order code number 42) and L. acidophilus Ki (order code number 41) in the form of starter concentrates were obtained from NIZO (Nederlands Instituut voor Zuivelonderzoek, Ede, The Netherlands) and were routinely stored at −40°C (12). Prior to use, the frozen starter concentrates were melted at 20°C for 30 min.

Growth Supplements

Milk was hydrolyzed with enzymes obtained from either Aspergillus sp. (Protease 2A™; Amano Pharmaceutical, Nagoya, Japan; MHP) or Bacillus subtilis (Neutrase™; Novo-Nordisk, Bagsvaerd, Denmark; MHN) that possessed high proteolytic activity. Hydrolysis was carried out using the following protocol: skim milk to which skim milk powder had been previously added to standardize the protein content to 60 g/kg was treated at 85°C for 5 min and 140°C for 5 s. The milk was brought to 45°C, and the proteolytic enzyme was added at a concentration of 20 g/kg of milk protein. Incubation time was 0.5, 2, 8, or 24 h. The hydrolyzed milk was then held at 85°C for 3 min, cooled, refrigerated, and held at 4°C.

Growth Experiments

Both B. lactis and L. acidophilus were cultivated in skim milk that was UHT-treated using the VTIS (vacuum treatment with injection of steam) method (deaeerated milk with an oxygen mass fraction <2 mg/kg was obtained via vacuum treatment after injection of steam during UHT treatment for 5 s at 140°C). Prior to inoculation, the milk was cooled to 37°C and supplemented with filter-sterilized 0.5 g/L of cysteine-HCl to lower the redox potential. Milk hydrolyzates were added at 50 ml/L. Inoculation was with 50 ml/L of frozen starter concentrate; then, milks were transferred aseptically to 50-ml flat-bottomed glass flasks that were entirely filled (to exclude oxygen completely) and stoppered. Samples were incubated without agitation at 37°C for up to 24 h. One flask was randomly removed at each of 0, 2, 4, 6, 8, 10, and 24 h and assayed for growth and acid production. Each fermentation was performed in duplicate.

Analytical Methods

Bacterial growth was estimated using both spectrophotometry and colony plate counts. For the measurement of cell density, 1-ml milk samples were clarified with 9 ml of 2 g/L of alkaline EDTA (pH 12) according to the method of Kanasaki et al. (10). Absorbance at 460 and 600 nm was measured for L. acidophilus and B. lactis, respectively.

To determine counts (colony-forming units) of B. lactis and L. acidophilus, samples were decimally diluted in sterile peptone-saline solution (1 g/L of peptone and 8.5 g/L of saline) (Oxoid, Basingstoke, United Kingdom) and pour plated on MRS agar supplemented with 0.5 g/L of L-cysteine-HCl or TGV agar (tryptone-glucose-meat extract) (6), respectively. Plates were incubated anaerobically for 48 h at 37°C using the Gas Pak culture system (BBL, Cockeysville, MD). For the cocultures, each individual strain was counted on spread plates of MRS agar with 0.5 g/L of L-cysteine-HCl to which 2 g/L of bile salts (Difco, Detroit, MI) and 50 ml/L of sheep blood were added as selective agents (13) or on spread plates of TGV agar supplemented with 20 g/L NaCl, respectively. Individual strains were confirmed by visual inspection of colony morphology and microscopic observation.

The titratable acidity, expressed as equivalent lactic acid, was determined as described elsewhere (2).
Milk coagulation was monitored over 24 h. All analyses were conducted in duplicate.

Lactic and acetic acids in the joint cultures were quantified by HPLC (Perkin-Elmer, Beaconsfield, United Kingdom) using the method described by Gomes et al. (8). For sample preparation, a 10-g sample was diluted to 50 ml with 1 M HClO4 to precipitate milk proteins; the suspension was kept overnight at 4°C and filtered (0.45-μm Millipore SLGV filters; Millipore, Bedford, MA) prior to injection. All analyses were conducted in duplicate.

The degree of hydrolysis (DH) of each milk hydrolyzate was evaluated by indirectly measuring the amount of alpha-amino nitrogen using the formaldehyde titration method (33). The total content of peptide bonds used for the calculation of DH was 8.32 eq/kg of protein (1). The protein concentrations of the different milk hydrolyzates were determined by the micro-Kjeldahl method as described by Koops et al. (15) using 6.38 as the conversion factor. All analyses were conducted in triplicate.

Profiles of the molecular mass distribution of all milk hydrolyzates were determined by FPLC® (Pharmacia LKB Biotechnology, Uppsala, Sweden) using a Superose-12 column. A previously degassed buffer (0.05 M Tris HCl, pH 7.0) was used as eluant at a flow rate of 0.4 ml/min. Sample injection volume was 100 μl, and effluent absorbance was monitored at 280 nm. Each sample was filtered through a 0.2-μm membrane filter (Millipore, Bedford, MA) prior to chromatographic analysis. Protein standards were BSA (67,000 Da), bovine β-LG (36,000 Da), bovine α-LA (14,000 Da), β-chain of insulin (5750 Da) and nicotineamide adenine dinucleotide (669 Da). The void volume was determined by the use of blue dextran. Integration of peaks in the chromatograms was performed to permit comparison of the molecular mass distribution of protein components from milk hydrolyzates at various degrees of hydrolysis; for this purpose, four ranges were considered: molecular mass <500, between 500 and 2000, between 2000 and 5000, and >5000; each was expressed as a percentage of the total area.

The amino acid composition of the 24-h hydrolyzates was assayed by the Waters Pico-Tag™ (Waters, Milford, MA) method; for sample preparation, the sample was centrifuged at 7500 × g for 10 min, and the supernatant, with Norleucine (Sigma Chemical, St. Louis, MO) added as internal standard, was filtered through an ultrafiltration membrane with a 10-kDa molecular mass cutoff (Ultrafree MC from Millipore) and derivatized according to the Pico-Tag™ protocol. All analyses were conducted in duplicate.

**Statistical Analyses**

Analysis of variance was used to determine the effect of type and DH of the hydrolyzate on the relative absorbance, titratable acidity, decrease in pH, and increase in log colony-forming units per milliliter for both B. lactis and L. acidophilus as pure cultures. The concentrations of acetic and lactic acids and the log concentrations of the mixed cultures were similarly analyzed. The manipulated variables tested were 1) inoculum ratio, hydrolyzate concentration, and time (in the first experimental phase) and 2) hydrolyzate type and time (in the latter phase once the inoculum ratio was chosen). Fisher’s protected least significant difference t test at the 5% significance level was applied to all experimental results to assess significant differences within pairs. All statistical analyses were done with the Statview™ package (Abacus Concepts, Berkeley, CA).

**RESULTS**

**Milk Hydrolyzates**

The degrees of enzymatic hydrolysis reported for milk were dependent on the enzyme used. Differences in DH of MHP and MHN may be reflected as differences in growth and acidification rates of pure cultures of B. lactis and L. acidophilus in supplemented milk (Table 1).

As expected, the molecular mass distribution of peptides contained in the milk hydrolyzates varied with the DH, as is apparent from inspection of Table 2. The higher the DH in either MHP or MHN, the greater was the contents in low molecular mass peptides (considered as the 500 to 2000-Da and <500-Da fractions grouped together). In fact, MHP was essentially composed of these small peptides by 24 h of hydrolysis; its MHN counterpart contained significant portions of larger peptides (>5000 Da; Table 2). The 30-h MHP exhibited a qualitative profile similar to the 24-h MHP, but the 48-h MHP no longer contained intermediate molecular mass peptides (2000 to 5000 Da). In contrast, the 30- and 48-h MHN showed qualitative profiles that were similar to those of the 24-h MHN but that were different with respect to the contents of intermediate and low molecular mass peptides (or peptides <5000 Da, considered as combination of the three appropriate fractions). In terms of the low molecular mass peptides in the 24-h, 30-h, and 48-h MHN, the sum of such fractions increased with DH. Furthermore, a decrease in the 500- to 2000-Da fraction was associated with an increase in the <500-Da fraction.
The total concentration of all free amino acids of the 24-h hydrolyzates (except cysteine, which could not be determined) was much higher in MHP (25 g/L) than in MHN (0.5 g/L). Phenylalanine was the most abundant free amino acid (42.3%) in MHN, followed by methionine, lysine, and leucine at approximately similar concentrations (8.3, 8.1, and 7.8%, respectively); leucine was the most abundant free amino acid (18.6%) in MHP, followed by lysine, phenylalanine, and tyrosine (12.1, 9.7, and 7.4%, respectively).

Growth and Acidification of Pure Cultures

The effect of inoculum size of starter concentrates of both B. lactis and L. acidophilus was determined in the range of 1 to 10%, and correlation between inoculum size and acid production was good. An inoculum size of 5% was chosen to carry out further experiments. Similar inoculum levels have been applied by other researchers (23) in growth studies with bifidobacteria, and inoculum level was also recommended as a selection criterion for starters in fermented milk products (16).

The growth of B. lactis on unsupplemented milk was poor. When supplemented with milk hydrolyzate, growth was improved (P < 0.05); the exponential phase of growth occurred during the first 8 to 10 h following inoculation, and milk supplemented with MHP fractions produced better growth performances than that supplemented with MHN fractions (P < 0.05) (Table 1). In addition, correlations were observed between growth and acidification and between DH of MHP (r = 0.89 and r = 0.89, respectively) or MHN (r = 0.93 and r = 0.87, respectively). Milk enriched with either MHP or MHN at higher DH exhibited higher rates of production of biomass and acid.

Unlike B. lactis, the response of L. acidophilus in terms of biomass and acid production to all DH of either MHP or MHN was of small or no statistical significance, respectively. The growth and acidifica-
TABLE 3. Effects of inoculum ratio and supplementation on the growth of Bifidobacterium lactis Bo and Lactobacillus acidophilus Ki in mixed culture by 30-h fermentation at 37°C.

<table>
<thead>
<tr>
<th>Inoculum ratio1</th>
<th>Culture medium</th>
<th>Viability (log(cfu/ml))</th>
<th>Development (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B. lactis</td>
<td>L. acidophilus</td>
</tr>
<tr>
<td>1:1</td>
<td>Milk</td>
<td>8.37a</td>
<td>8.72a</td>
</tr>
<tr>
<td>1:10</td>
<td>Milk</td>
<td>7.95b</td>
<td>8.30b</td>
</tr>
<tr>
<td>10:1</td>
<td>Milk</td>
<td>9.14a</td>
<td>8.42c</td>
</tr>
<tr>
<td>1:1</td>
<td>Milk + 24-h MHP</td>
<td>8.95c</td>
<td>8.51a</td>
</tr>
<tr>
<td>1:10</td>
<td>Milk + 24-h MHP</td>
<td>8.67a</td>
<td>8.51b</td>
</tr>
<tr>
<td>10:1</td>
<td>Milk + 24-h MHP</td>
<td>8.99c</td>
<td>8.18a</td>
</tr>
</tbody>
</table>

1MHP = milk hydrolyzate produced by Proteinase 2A™ (Amano, Nagoya, Japan); MHN = milk hydrolyzate produced by Neutrase™ (Novo-Nordisk, Bagsvaerd, Denmark).

Growth and Acidification of Mixed Cultures

To test whether the growth of B. lactis was enhanced by the addition of a bacterial strain with proteolytic activity in a coculture environment, a first set of growth experiments was performed in milk supplemented with 24-h MHP and inoculated with B. lactis to L. acidophilus in ratios of 10:1, 1:1, and 1:10. Analysis of variance showed that the inoculum ratio and fermentation time affected all growth and acidification parameters assayed (P < 0.05); the addition of hydrolyzate exerted a significant effect only on the viable numbers of B. lactis (Table 3). Maximum viable cell numbers of B. lactis were higher (P < 0.05) at the 1:1 and 10:1 inoculum ratios than those determined at the 1:10 inoculum ratio (Table 3). A similar trend was observed for viable cell numbers of L. acidophilus, although, in this case, maximum viability occurred at the 1:1 and 1:10 inoculum ratios (Table 3). The similar mean levels of fermentation by 30
milk hydrolyzate (Figure 1b). Although lactic acid products were positively affected by the addition of parameters of pure cultures of $B. \text{lactis}$ or MHN, acetic acid concentrations were positively increased by the addition of MHP and MHN, which is consistent with the coculture effect on lactic and acetic acid production in pure culture (Figure 1b). Interestingly, the coculture effect on lactic and acetic acid production was substantially larger than the addition of effects of the corresponding acid production in pure culture (Figure 1b).

The efficiency of addition of milk hydrolyzate on $B. \text{lactis}$ and coculture with $L. \text{acidophilus}$ could be related to the directly accessible pools of free amino acids or bioactive peptides in MHP as a potential source of nitrogen for cell growth and concomitant proteolytic activity of $L. \text{acidophilus}$. A further series of experiments, in which the inoculum ratio of these strains was 1:1 and either 24-h MHP or MHN was added, was conducted to check this effect using pure cultures of $B. \text{lactis}$ and $L. \text{acidophilus}$ in milk supplemented with 24-h MHP as a control to confirm the symbiotic relationship between the two strains. A clear difference ($P < 0.05$) in viable cell numbers of both strains and concentrations of lactic and acetic acids was observed between pure and mixed cultures supplemented with MHP (Figure 1), which suggests that the stimulatory effect could not be attributed solely to addition of a nitrogen source, as provided by the milk hydrolyzate. At the inoculum ratio of 1:1, the presence of a milk hydrolyzate supplement, whether MHP or MHN, had no significant effect on the growth of either $B. \text{lactis}$ or $L. \text{acidophilus}$ after 30 h of incubation (Figure 1a). Conversely, fermentation end products were positively affected by the addition of milk hydrolyzate (Figure 1b). Although lactic acid concentrations were increased by the addition of MHP or MHN, acetic acid concentrations were positively affected by only MHP, which is consistent with the results obtained for growth and acidification parameters of pure cultures of $B. \text{lactis}$. Interestingly, the coculture effect on lactic and acetic acid production was substantially larger than the addition of effects of the corresponding acid production in pure culture (Figure 1b).

**DISCUSSION**

The results of this study have shown that, under aerobic conditions, $B. \text{lactis}$ was not able to grow adequately in pure milk. The moderate growth observed could be due to the low redox potential or to the heat treatment of the milk; the latter is known to be important in reducing the amount of oxygen dissolved (16), in leading to slight proteolysis (5), and in releasing stimulatory sulphydryl compounds (21). Similar observations were made by Misra and Kuila (18) who prepared a milk containing $B. \text{bifidum}$. Several researchers (4, 27) have also reported poor growth of bifidobacteria in whole milk, which was attributed to a lack of essential vitamins (4, 25) or a lack of essential amino acids in free form or in the form of peptides (27); results reported in the present study substantiate the latter requirement for bifidobacterial growth and acidification.

The addition of MHP and MHN fractions increased the amount of free amino acids and small peptides that were present in milk, thus promoting the growth of $B. \text{lactis}$; however, both the type of enzyme and the DH affected the nature and molecular mass distribution of the peptides contained in the hydrolyzates, as previously observed by Proulx et al. (22). The MHP offered a wider array of peptides, especially those <2000 Da, and free amino acids than did MHN in all fractions tested at the same hydrolysis time, which may have accounted for the growth differences observed (22). Although low molecular mass peptides in the 500- to 2000-Da fraction were still present in a significant amount in the MHP by 24 h of hydrolysis, the lower fraction (<500 Da) was more abundant and could be assimilated more easily by $B. \text{lactis}$. Moreover, similar contents of the 500- to 2000-Da fraction in the 24-h MHN but significantly lower contents of the <500-Da fraction did not show comparable trends in the growth and acidification behavior of $B. \text{lactis}$, which further supports our reasoning. Bifidobacteria possess aminopeptidases, iminopeptidases, dipeptidases (5), and several transport systems of amino acids and peptides; it is the combined action of these enzymes and transport systems that determines which amino acids become available for growth. Furthermore, the analysis of the amino acid composition of the 24-h MHP indicates a higher absolute concentration of all free amino acids as well as higher percentages of the essential amino acids leucine, phenylalanine, and tyrosine compared with the amino acid composition of MHN. Similarly, threonine, which has been identified as a growth-promoting substance of bifidobacteria (17), is present in MHP but not in MHN. Growth studies conducted by Klaver et
al. (13) further support our observations concerning MHP with regard to amino acid effects. Despite these observations, the bifidobacterial growth in milk supplemented with MHN, which was similar to that reported (13) in milk supplemented with an extra five times concentrated mixture of free amino acids, may lead to speculation about the importance of the low molecular mass peptides that are present in MHN, as claimed by Poch and Bezkorovainy (21) and Proulx et al. (22, 23). In addition, an increase in the DH of either MHP and MHN (30 h and 48 h of enzymic incubation) with a concomitant increase in free amino acid content did not show a dose-dependent stimulatory response; hence, it appears that further degradation of milk protein was not essential for nitrogen utilization by B. lactis. However, strains could utilize growth promoters to a maximum concentration beyond which extra addition of growth promoter does not enhance growth further. Therefore, specific studies to determine whether 5% inocula of milk hydrolyzates offer optimum concentrations for nutritional requirements, in either free amino acid or low molecular mass peptide form, were required and have accordingly been carried out in our laboratory.

For end product formation, measured as the titratable acidity in milk, results were comparable with those reported by Klaver et al. (13) for casitone addition to milk, but the time required to reach such values in milk supplemented with 24-h hydrolyzates was reduced. The associated acceleration of milk coagulation provided further evidence to confirm these observations. Such a decrease in fermentation

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**Figure 1.** Bacterial counts of Bifidobacterium lactis Bo (a) and Lactobacillus acidophilus Ki (b) and fermentation end products expressed as lactic acid (c) or acetic acid (d) cocultured in milk, incubated at 37°C, and inoculated at the ratio of B. lactis to L. acidophilus of 1:1 without milk hydrolyzate addition (●), with the addition of Protease 2A™ (Amano Nagoya, Japan) milk hydrolyzate (MHP) (▲), and with the addition of Neutrase™ (Novo-Nordisk Bagsvaerd, Denmark) milk hydrolyzate (MHN) (○). Single cultures of B. lactis and L. acidophilus in milk with added MHP and incubated at 37°C are denoted as (○) and (△), respectively. The calculated values for the theoretical sum of lactic and acetic acids produced by Bifidobacterium lactis Bo (○) and Lactobacillus acidophilus Ki (△) in single cultures are denoted by a dashed line. Plotted data are the mean values of duplicate experiments.
time provides additional advantages to the use of MHP as a growth supplement in fermented milk products [such as cheese (8)] for rapidly growing and acidifying probiotic strains; however, an increase in titratable acidity was not simultaneous with an increase in growth rate of B. lactis, except for the 24-h MHP and MHN tested. This uncoupling effect (metabolic activity continues into the stationary phase of growth), which was more visible at lower DH of MHP and MHN, may reflect the small differences in order of magnitude between biomass and acid production when the effect of DH is considered.

As opposed to B. lactis, the growth and acidification activities of L. acidophilus were not significantly influenced in a positive manner by the addition of either milk hydrolyzate; although the strain grew slowly, this growth was unaffected by the milk hydrolyzate supplement. In fact, the high concentrations of free amino acids in the 24-h MHP and MHN appeared to result in inhibition of its growth and acid-producing capacity; MHP affected L. acidophilus more deleteriously. The increase in short peptide and free amino acid contents as a function of DH may have caused an off-balance in the milk medium and in the cell transfer system and consequently suppressed growth activity. Similar inhibitory effects occurred when B. infantis was grown in a synthetic medium that was supplemented with 2% of the amino acid fraction of a casein hydrolyzate (23).

During the growth of B. lactis in milk containing L. acidophilus, the cell density of B. lactis progressively increased, thus demonstrating that L. acidophilus, via its proteolytic activity, was important in supplying utilisable nitrogen. The L. acidophilus not only can use peptides and free amino acids for its own growth and acidification activity, but also can hydrolyze milk caseins using proteinases near the cell-wall surface (30) to produce more low molecular mass nitrogen (especially peptides) to continue growth and, consequently, aid in the growth of and acidification by B. lactis. Stimulatory interactions involving nitrogen requirements have previously been observed between several bifidobacterial strains and proteolytic lactobacilli (13); however, the results in this study seem to indicate that coculturing of bifidobacteria with a proteolytic strain may not be the sole reason for its growth enhancement because the MHP supplement provided an additional fortification element, especially during the initial stages of incubation.

According to Sasaki et al. (28), a ratio of 10:1 of Lactobacillus bifidus (now known as Bifidobacterium bifidum) to L. acidophilus was considered to be adequate and essential for the growth of B. bifidum when in mixed culture. Our results agree only partially with this report because maximum viable numbers and concentrations of fermentation end products (except acetic acid) were attained at an inoculum ratio for B. lactis and L. acidophilus of 1:1 in plain milk. Conversely, acetic acid concentrations were maximum at a 10:1 inoculum ratio, which is justified in view of the heterofermentative nature of bifidobacteria (29), and this observation may also explain requirements previously claimed (28).

The growth of L. acidophilus in a coculture with B. lactis at a 1:1 inoculum ratio was enhanced relative to the pure culture, and no inhibition occurred in the presence of either MHP or MHN. This enhancement was possibly due to the buffering effect of acetate (pKₐ of 4.8), essentially produced by the latter strain, which prevented sharp declines in milk pH (11), and may also suggest a certain degree of symbiosis between the two strains.

On the whole, our results suggest that the eventual selection of the strains B. lactis Bo and L. acidophilus Ki as a mixed thermophilic starter to produce a cheese product not only would have the advantage of rapid growth and production of lactic and acetic acids (8), but also would likely increase the probiotic nutritional value of the final product (31, 32).

ACKNOWLEDGMENTS

Financial support for A.M.P. Gomes, provided by Ph.D. fellowships administered by Junta National de Investigação Científica e Tecnológica (Portugal) through the programs CIENCIA (BD 1734-1F) and PRAXIS XXI (BD/3160/94), is hereby gratefully acknowledged. The Protease 2A™ was a gift from Merck-Nederland BV. The authors are deeply indebted in particular to Jan de Wouters and in general to NIZO for permission to use its microbiology facilities.

REFERENCES

PROMOTION OF THE GROWTH OF BIFIDOBACTERIA


