THE DYNAMICS OF GROWING OF *BIFIDOBACTERIUM LACTIS* IN SUBSTRATE ENRICHED WITH LACTULOSE

ILZE BEITĀNE, INGA CIPROVIČA
Latvia University of Agriculture
e-mail: inga.ciprovica@llu.lv

Abstract

Lactulose is recognized world-wide as one of the most clinically reliable bifidogenic factor. Lactulose is used in various types of food products (infant formula, baby food, confectionary, soft drink, milk products) and also pharmaceutically to improve hepatic encephalopathy and constipation (Mizota, 1996; Strohmaier, 1998). The objective of this study was to investigate the influence of concentration of lactulose on growing of *Bifidobacterium lactis* in milk.

A bifidogenic factor, lactulose was added (1, 2, 3, 4 and 5%) into 100g of milk. The milk samples were inoculated with *Bifidobacterium lactis* (BB-12, Chr. Hansen, Denmark) and incubated at 38 °C for 16 hours.

Lactulose as a prebiotic influences the growth of bifidobacteria in milk. Trends of development of acidity in the milk samples with or without lactulose were not similar. Laboratory studies have generally shown that growth of *Bifidobacterium lactis* in milk depends on the concentration of lactulose. Data on the final cell count in fermented milk indicates that increasing the lactulose concentration from 2% to 3% enhanced the growth of the *Bifidobacterium lactis*, whereas no significant difference between lactulose concentration 4–5% and control sample was observed.

Present results furthermore indicate that finding combination of prebiotic and probiotic pairs where the prebiotic would benefit the specific probiotic strain, e.g. during production and formulation into foods, is not a simple task.

Key words: lactulose, bifidogenic factors, bifidobacteria, milk.

Introduction

In the area of functional food there is currently considerable interest to increase the number of "beneficial" microorganisms in milk products that may confer health benefits to the consumer.

Food manufacturers use two strategies to achieve this goal. The direct approach is to supply the fermented dairy products such as yoghurts with live preparations of the microorganisms. In this case one of the perceived difficulties with probiotics is that after ingestion, a substantial proportion of them are killed by adverse conditions: stomach acid, bile salts, pancreatic enzymes, in upper gut before they reach the colon where the main population of bacteria resides (Saxelin et al., 1999). The other approach has been taken that non-digestible carbohydrate food supplements are given in products. They support and stimulate the growth of lactic acid bacteria in the colonic microflora. These food components have been termed prebiotics.

Prebiotics are defined as nondigestible food that may beneficially affect the host by selectively stimulating the growth and/or the activity of a limited number of bacteria in the colon (Gibson et al., 1995). Thus, to be effective, prebiotics must escape digestion in the upper gastrointestinal tract and be used by a limited number of the microorganisms comprising the colonic microflora. Prebiotics are principally oligosaccharides, e.g. fructo-oligosaccharides, galacto-oligosaccharides, lactulose, inulin, and also polysaccharides such as certain forms of resistant starch.

Several studies have indicated that these sugars are not degraded in the upper gastrointestinal tract and reach the colon in an intact form and are utilized by colonic microflora. They mainly stimulate the growth of bifidobacteria, for which reason they are referred to as bifidogenic factors (Vuyst, 2000). Inulin and fructo-oligosaccharides are now the most widely used prebiotics. Lactulose is recognized world-wide as one of the most clinically reliable bifidogenic factors. Lactulose is a disaccharide comprised of the sugars D-galactose and D-fructose. The sugars are linked by a beta-glycosidic linkage, making it resistant to hydrolysis by human digestive enzymes. Besides, during heating of milk, lactose may isomerise into lactulose. Lactulose is formed in fairly large quantities, from 300 to over 1000 mg l^{-1} in sterilized milk (Walstra, Geurts, Noomen et al., 1999). Therefore, lactulose is not only a semisyntethic disaccharide, but a native component in thermally processed milk and also important factor for growth of bifidobacteria in milk.

Also, the further development of synbiotics may improve the effectiveness of probiotic strains and appropriate health-stimulating substrates, in particular reaching an increased number of ingested bacteria reaching the colon in a viable form. As we know, synbiotics are defined as a mixture of probiotics and prebiotics that improve the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, either by stimulating the growth or by metabolically activating the health promoting bacteria (Gibson et al., 1995; Lewis et al., 1998).

However, less is known about the interaction between different combinations of probiotics and prebiotics, although this is necessary to have a rationale for selecting different probiotics and prebiotics and developing efficacious synbiotics. Moreover, it is essential that these components be developed as the active ingredients of the food products that are ultimately intended for human consumption, in particular when probiotic, prebiotic or synbiotic is considered a food ingredient or food supplement (Vuyst, 2004).

The objective of this study was to investigate the influence of concentration of lactulose on growing of bifidobacteria in milk.

Materials and methods

The research was performed in the microbiological laboratory of the Department of Food Technology of Latvia University of Agriculture.

The strain of *Bifidobacterium lactis* (freeze – dried starter culture Bb-12, Chr. Hansen, Denmark) was used. During the experiments, the culture was maintained at – 18 °C.

The lactulose syrup (Duphalac®, Netherland) was used as a bifidogenic factor for growing of bifidobacteria in milk. The composition of the syrup of lactulose was as follows (%): lactulose – 67, lactose – less than 6, galactose – less than 10.

The *Bifidobacteria lactis* was incubated in milk. Different lactulose contents (1, 2, 3, 4 and 5%) were added individually in the 100 g milk. The *Bifidobacterium lactis* were inoculated with 2 ml of milk suspension (about $1*10^6$ bifidobacteria) and cultured at 36 °C for 16 hours. During the incubation after each 2 hours, the increase of bifidobacteria was estimated by cell count techniques. The acidity (Therner degree, °Th) of fermented milk was determined by titrimetric method using 0, 1 M NaOH solution.

Fermentations were performed in triplicate, and the analyses were carried out in duplicate. The data given here are the mean values of the measurements.

Results and discussion

In contrast with lactobacilli, bifidobacteria exhibit a weak growth in milk or do not grow at all in milk. The addition of growth promoting factors, such as vitamin-enriched protein hydrolysates, or sources of carbohydrates, for example lactulose, in milk stimulates growth of bifidobacteria (Modler, 1994). The obtained results are given in Figures 1 and 2, respectively.

The rate of acid development is a critical factor in milk fermentation by bifidobacteria. Also the chemical composition of the fermentation medium for growth (for instance, the carbohydrate source, total solid content, availability of nutrients and growing parameters, dissolved oxygen content), the cultivation conditions (for instance, the level of inoculation, the incubation temperature, the fermentation time), final acidity, etc. may affect the viability of probiotic organisms in fermented products. With increased substrate level (4% and 5%) in milk, growth of acidity was poorer. Trends of development of acidity in the milk samples with or without lactulose were not similar.

The study has shown that oligosaccharides such as lactulose, when included in milk, stimulate the growth of bifidobacteria in fermentation process. Laboratory studies have generally shown that growth of *Bifidobacterium lactis* (BB-12, Chr.Hansen) depends on the concentration of lactulose in milk. The lactulose concentrations of 2% and 3% still were the best, and the difference to control sample was larger compared to the experiment with the 5% lactulose concentration. With increased concentration of lactulose (4–5%), growth of bifidobacteria was poorer. Data on the final cell count in fermented milk indicates that increasing the lactulose concentration from 2% to 3% enhanced the growth of *Bifidobacterium lactis*, whereas no significant difference between lactulose concentration 4% and 5% and control sample was observed.

Shin, Lee, Petska, and Ustunol (2000) were able to show that adding large quantities (5%) of galacto-oligosaccharides and especially fructo-oligosaccharides to skimmed milk enhanced the survival of the two *Bifidobacterium* strains. Since we studied different prebiotic (lactulose) and *Bifidobacterium lactis,* our results are not comparable to the results of Shin et al. (2000). However, the possibility that by using larger quantities of prebiotic (5% in

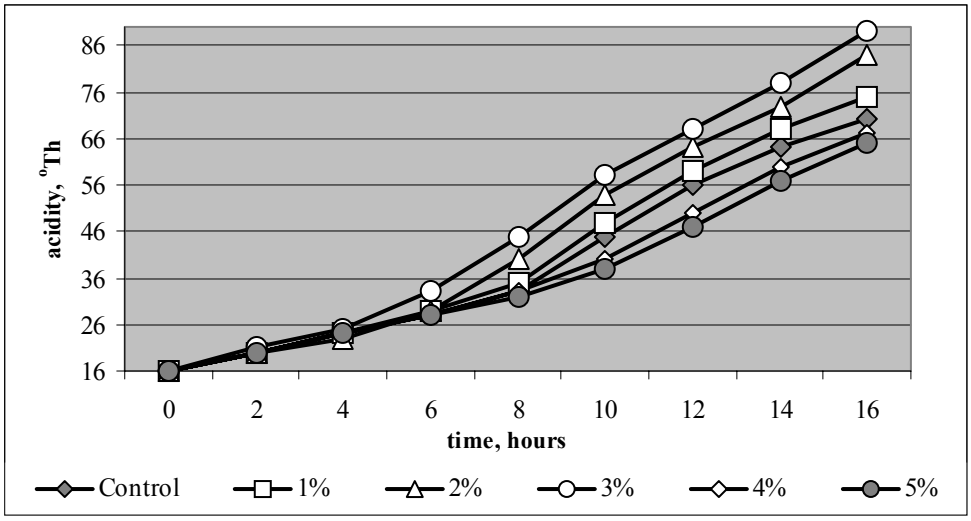

Fig. 1. The rate of acid development in milk fermentation by bifidobacteria.

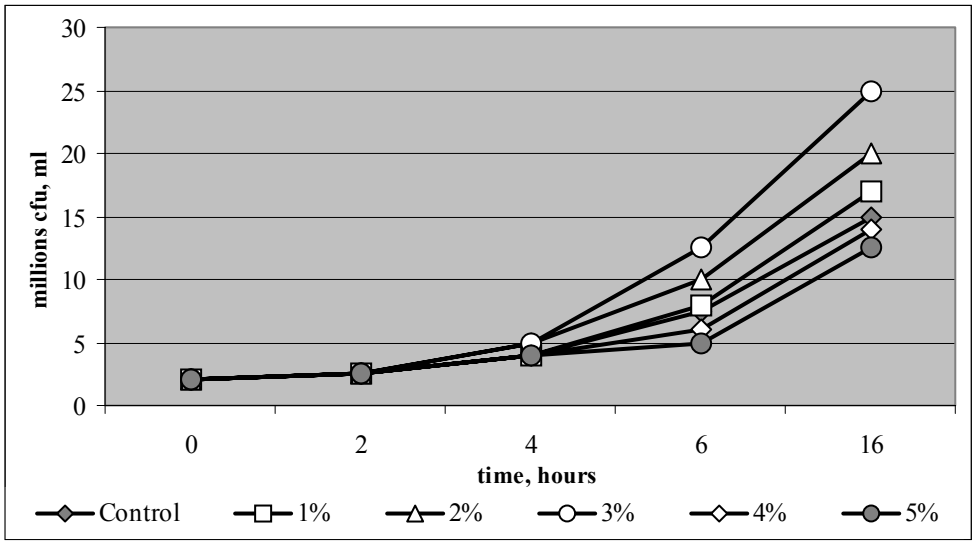

Fig. 2. The dynamic of growing of Bifidobacterium lactis in substrate enriched with lactulose.

the present study) a more pronounced effect on the properties of the tested *Bifidobacterium lactis* could have been detected cannot be ruled out.

Present results furthermore indicate that finding combination of prebiotic and probiotic pairs where the prebiotic would benefit the specific probiotic strain, e.g. during production and formulation into foods, is not a simple task.

Conclusions

1. Trends of development of acidity in the milk samples with or without lactulose were not similar.

2. The concentration of lactulose has important implication on growing rate of bifidobacteria in milk.

3. The increase in the concentration of lactulose over 3% in milk demonstrates the prevention of growing of bifidobacteria in milk.

References

1. Dairy technology: principles of milk properties and processes/P.Walstra, T.J.Geurts, A.Noomen [et al]. – New York [etc]: Marcel Dekker, 1999. XV, 727 p.: ill, tab.
2. Gibson, G., Roberfroid, M.B. (1995) Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics'. Journal of Nutr., 125, 1401–1412.
3. Lewis, S.J., Freedman, A.R. (1998) Review article: the use of biotherapeutic agents in the prevention and treatment of gastrointestinal disease. Aliment. Pharmacol.Ther., 12, 807–822.
4. Mizota, T. (1996) Lactulose as a growth promoting factor for Bifidobacterium and its physiological aspects. International Dairy Federation, Bulletin 313, 43–48.
5. Modler, H.W. (1994) Bifidogenic factors – Sources, Metabolism and Applications. International Dairy Journal, 4, 383–407.
6. Saxelin, M., Grenow, B., Svensson, U., Fonden, R., Reniero, R., Mattila-Sandholm, T. (1999) The technology of probiotics. Trends of Food Science Technology, 110, 387–392.
7. Shin, H.S., Lee, J.H., Petska, J.J. and Ustunol, Z. (2000) Growth and viability of commercial *Bifidobacterium spp.* in skim milk containing oligosaccharides and inulin. Journal of Food Science, 65, 884–887.
8. Strohmaier, W. (1998) Lactulose: Status of health-related applications. International Dairy Federation, Bulletin 9804, 262–271.
9. Vuyst, L. (2000) Technology aspects related to the application of functional starter cultures. Food Technolology& Biotechnology, 38(2), 105–112.
10. Vuyst, L., Avonts, L., Makras, L. (2004) Probiotics, prebiotics and gut health// Functional foods, ageing and degenerative disease. – Cambridge: Woodhead Publishing Limited, – 771 p.