IN VITRO ASSESSMENT OF PREBIOTIC UTILIZATION BY DAIRY LACTOBACILLI

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Abstract


The new consumers’ demand for healthy products on the market enhances intensive researches on lactic acid bacteria (LAB) and prebiotics. Individually and in combinations (in a synbiotic form), they can be effective microbial modulators, with a positive impact on human health and wellbeing. Thus, the selection of successful combinations LAB strain-prebiotic is a milestone in the development of new functional foods and products. With this aim, the potential of 33 Bulgarian LAB strains to growth in the presence of galactooligosacharide (GOS), glucooligosacharide (GLOS) and fructooligosacharide (FOS) were estimated. Thirty-three original LAB strains, from traditional Bulgarian dairy products have been identified and pre-selected on the base of widely accepted in vitro criteria for probiotics. The in vitro assessment of prebiotic’s utilization was evaluated in a mini-plate model system with a modified MRS broth. The major part of tested LAB was able to growth in medium containing 10% v/v GLOS (69–76% of the tested strains) and GOS (88–99.82%) as a single carbon source. About the FOS, approximately 67% of the tested strains utilized it partially, and only 3% of them – completely. A higher metabolic activity has been proved for LAB strains belonging to the species Lactobacillus plantarum and Lactobacillus brevis. A strict correlation has been reported between the ability of different Lactobacillus plantarum to ferment GOS and their β-galactosidase activity. The results revealed that the oligosaharide utilization patterns are strain-specific and they do not dependent from the origin of the tested LAB. Obtained data is promising for further development of new synbiotic formulas.

Key words: prebiotics, oligosaccharides: FOS, GOS, GLOS, dairy lactobacilli
Abbreviations: LAB – Lactic acid bacteria; GOS – Galactooligosacharide; GLOS – Glucooligosacharide; FOS – Fructooligosacharide

Introduction

Probiotics are live microorganisms (mainly Lactobacillus and Bifidobacteria) which administered in adequate amounts confer a health benefit to the host (FAO/WHO, 2003). Lactobacilli are currently added to a variety of functional foods and several studies have demonstrated their beneficial properties in human health (Reid et al., 2011). Prebiotics are defined as “non-digestible food ingredients that beneficially affects host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon” (Gibson and Roberfroid, 1995; Gibson et al., 2004). The probiotic-prebiotic combination, called synbiotic, can have a synergistic effect, resulting in promoting the growth of beneficial bacteria in the colon, such as Lactobacillus and Bifidobacterium spp. (Rycroft et al., 2001a; Cardelle-Cobas et al., 2011). In addition, the vitality and survival of the newly added probiotic strains can be improved (Schrezenmeir and de Vrese, 2001). The use of prebiotics may lead to a significant increase in mineral absorption, reduction in serum lipid levels, decreased production of putrefactive substances, and inhibition of intestinal pathogens (Cummings and Macfarlane, 2002). Some prebiotic carbohydrates are currently

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available in the market, such as fructooligosaccharides, lactulose, inulin and galactooligosaccharides (Rastall, 2010). In recent years, an important step in the development of synbiotics is the selection of suitable probiotic strains, able to utilize single oligosaccharide or a mixture of oligosaccharides. In general, oligosaccharide (prebiotic) utilization patterns of probiotics are species- or strain-dependent and varied, depending on the degree of polymerisation or complexity of the prebiotics (Kaplan and Hutkins, 2000).

The aim of this study was to assess the capacity of pre-selected as candidate-probiotic dairy lactobacilli to utilize different prebiotics.

Materials and Methods

Oligosaccharides substrates: Three different prebiotics were used – GOS – galactooligosacharide (TOS-P, Yakult, Japan), FOS – fructooligosacharide (Raftilose P95, Orafti, Belgium) and GLOS – glucooligosacharide (BioEcolians, Solabia, France).

LAB strains and growth conditions: Thirty-three Lactobacillus strains, isolated from traditional dairy products – katak- 4 strains, white brined cheeses- 22, and yogurt- 7 strains, were studied. They are previously identified and pre-selected as LAB strains with beneficial properties (Tropcheva and Danova, 2011). For the assay, all lactobacilli were pre-cultured twice in MRS broth (Merck, USA) at 37°C and 42°C. The washed cells of exponential LAB cultures were inoculated (at 10 % v/v) in a modified MRS broth, containing an indicator (bromcresol purple) and 10% v/v of the particular oligosaccharide, added aseptically. The assays were done in triplicate for each LAB strain, using micro-plates and the changes in media’s indicator during the cultivation were monitored at 24 and 48 h.

Results

In the present study, the ability of 33 Bulgarian lactobacilli to utilize prebiotics was estimated. The selected original strains differ in their species affiliation and origin. They were identified as Lactobacillus brevis (4 strains from “katak”), Lactobacillus plantarum (25 strains from cheese and yogurt), Lactobacillus delbrueckii subsp. bulgaricus (4 strains from yogurt) and possess different probiotic features. The ability of lactobacilli to utilize GOS, FOS and GLOS was evaluated by the color change of the dye indicator, during the cultivation in modified MRS broth for 24 and 48 hours. Thus, the strains were divided into

![Fig. 1. In vitro assessment of dairy lactobacilli for prebiotics utilization in a model mini-plate system with modified MRS broth](image-url)
In Vitro Assessment of Prebiotic Utilization by Dairy Lactobacilli

107

three groups: (1) completely, (2) partial and (3) non-utilization to each of the tested oligosaccharides (Figure 1). The different Lactobacillus species and strains showed variable biochemical profiles (Figure 1 B, C, D) and therefore different ability to grow in the presence of tested oligosaccharides (Figure 1A). Best utilization rates were observed in terms of GOS and GLOS and the lowest in the FOS respectively (Figure 1). The representatives of the species L. plantarum are characterized by a higher rate (in terms of the number of strains) and a shorter growth time in a media with oligosaccharides (Figure 1D).

Discussion

The obtained results are among the first reported for the LAB microflora of the still insufficiently studied traditional Bulgarian dairy products like “katak” and white brined cheese. Two strains L. plantarum, isolated from homemade yogurt, which are capable to grow in GOS, GLOS, and partially in the presence of FOS are of interest because in this way they can complement the representatives of the species L. bulgaricus, which are very limited as regards their metabolic characteristics. In a further development of products, such cooperation would be very useful, because, even within this study, L. bulgaricus demonstrate a good growth rates only in the presence of GLOS (Figure 2B). In general, for the entire tested LAB, carbohydrate utilization patterns were strain-dependent (Figure 1 B, C, D). While, for the commercially available probiotic strains, Kaplan and Hutkins (2000) observed a species–specific dependent FOS fermentation (Kaplan and Hutkins, 2000). The ability to metabolise GOS, as a single carbon source is also a specific property of the group of LAB and only few organisms inhabiting the gastrointestinal tract possess the enzymes needed to hydrolyze GOS (Macfarlane et al., 2007). Our results with L plantarum from cheese (data not shown), confirmed a reported correlation between β galactosidase activity and GOS fermentation (Pennacchia et al., 2006). According to the reported high β-galactosidase activity for the group of tested strains of L. plantarum, it is not surprising that they are able to ferment GOS. For the other tested LAB species (L. bulgaricus and L. brevis), which did not show the ability to utilize GOS or a slower growth rates, there is a need of more analyses, because this can result both from the lack of this enzyme, but also on other factors such as lack of a transport system in order to hydrolyze these oligosaccharides into the cell by β-galactosidases.

Conclusion

The present study showed, that the selected 33 candidate-probiotic Lactobacillus strains were capable to utilize the tested oligosaccharides – GOS, FOS and GLOS, but a diversity in the growth/acidity rate varied among the species, strains and substrates. These data will advantage a further selection of suitable synbiotic pairs, in which the prebiotic would benefit the specific probiotic strain.

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References


