UTILIZATION OF DIFFERENT PREBIOTICS BY LACTOBACILLUS SPP. AND LACTOCOCCUS SPP.

Tanya Mandadzhieva, Tzveteslava Ignatova-Ivanova, Stanimir Kambarev, Iliya Iliev, and Iskra Ivanova

1Sofia University, Department of General and Applied Microbiology, Sofia, Bulgaria
2Shumen University, Department of Functional Biology, Shumen, Bulgaria
3Plovdiv University, Department of Biochemistry and Microbiology, Plovdiv, Bulgaria

Correspondence to: Iskra Ivanova
E-mail: vitanova@biofac.uni-sofia.bg

ABSTRACT

Different non-digestible di- and oligosaccharides act as prebiotics. Common prebiotics in use include inulin and other fructo-oligosaccharides (FOS), galacto-oligosaccharides (GalOS), xylo-oligosaccharides, gluco-oligosaccharides (GOS), lactulose, etc. The principal concept is that these sugars have a selective effect on the microbiota that results in an improvement in the health of the host, leading to an increase in health-promoting organisms such as different kinds of Lactic acid bacteria (LAB). With our latest results we describe the effects of utilization of different prebiotics upon specific LAB-strains, their antimicrobial activity and the changes in the metabolites.


Keywords: prebiotics, utilization of oligosaccharides, lactic acid bacteria, probiotics, health-promoting organisms

Introduction

Dietary modulation of the human gut flora has been carried out for many years. One approach to health maintenance and disease control is the use of dietary bacterial and carbohydrate supplements that aid the host’s indigenous bacterial communities form a barrier against invading pathogens. This comprises the use of prebiotics (“live organisms, which when administered in adequate amounts, confer a health benefit on the host”) and prebiotics.

Gibson and Roberfroid (2, 3) defined prebiotics as “non-digestible food ingredients that beneficially affect host health by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improve health”. For a dietary substrate to be classified as a prebiotic, at least three criteria are required: (a) the substrate must not be hydrolyzed or absorbed in the stomach or small intestine, (b) it must be selective for beneficial commensal bacteria in the colon such as bifidobacteria, (c) fermentation of the substrate should induce beneficial luminal/systematic effect within the host.

Several studies have shown that the ability of lactobacilli and bifidobacteria to ferment prebiotic carbohydrates is both strain and substrate specific (4, 5, 6). In addition, it is not clear which prebiotics are the most suitable substrates for selective growth of specific strains.

Materials and Methods

Bacterial strains and culture conditions

Ten new isolates from Bulgarian dairy products determined as Lactobacillus spp. and seven strains Lactococcus spp. from the Bulgarian national collection for microorganisms and cell cultures were cultivated and studied on different modified media. Also strains Lactobacillus delbrueckii B7, Lactobacillus fermentum B5 (7), Lactobacillus acidophilus 504 and Lactobacillus sakei S161 were used in this study. All strains were cultivated overnight (16-18h) as follows: on MRS (for lactobacilli) and M17 (for lactococci) at 37 ºC and 30 ºC.

Carbohydrates used in this study

Four different types of commercially available carbohydrates were studied. FOS (Raftilose P95 from Orafti, Belgium) contained 5% of glucose, fructose and sucrose. GalOS (TOS-P from Yakult, Japan), GOS (Bioecolians from Solabia, France) contained 6% of glucose and leucrose. Lactulose (Lactulose Crystals EP, Viccio, Italy) contained lactulose 97.5%, galactose 0.5%, lactose 0.5%, epilactose 0.5%, tagatose 0.5%, fructose 0.5%. Glucose (purity 99%, Merck, Germany) was used as a control as well as lactose (Merck, Germany). Each carbohydrate was sterilized on 0.3 μm sterile filter (Sartorius), and pH was not adjusted. All examinations were performed at least twice.

Fermentation

Lactobacilli were routinely grown in MRS broth (Merck) (8) and lactococci in M17 broth (Merck). Overnight grown cells were washed twice in saline (0.85% NaCl solution) and 10% of the bacterial suspension (10⁷ cfu/mL) was used to inoculate modified MRS broth medium (pH 6.8) and M17 broth medium containing 3% glucose, 3% lactose, 3% lactulose, 3% GOS, 3% FOS or 3% GalOS. The anaerobic fermentations were performed for lactobacilli in 100 mL glass bottles at 37 ºC for 48 h (BBL® Gas Pak anaerobic system Envelopes, Becton Dickinson).
Analytical assays

Microbial growth

Bacterial growth was measured by a turbidimetric method at 650 nm and calibrated against cell dry-weight using a spectrophotometer (UV/Vis Shimadzu, Japan). For each experiment, data was analyzed using Excel statistical package. The OD readings and standard deviations were calculated from duplicate samples from two separate experiments.

Analysis of metabolites

Lactic acid was determined by a UV method, enzymatically with L-lactate dehydrogenase and D-lactate dehydrogenase (commercially available kit, Boehringer Mannheim GmbH, code 10 139 084 035).

Acetic acid was determined by a UV method, enzymatically with acetyl-CoA synthetase, citrate synthase, malate dehydrogenase (commercially available kit, Boehringer Mannheim GmbH, code 10 148 261 035).

Ethanol was determined by a UV method, enzymatically with alcohol dehydrogenase and aldehyde dehydrogenase (commercially available kit, Boehringer, code 10 176 290 035).

Analysis of carbohydrates

To detect the utilization of oligosaccharides, modified media containing 0.05 % L-cysteine, 1.5 % agar, 30 mg bromcresol purple per litre (5) was used.

Oligosaccharides were analyzed by HPLC using a Symmetry C18 column (4.6 × 150 mm) and Waters 1525 Binary HPLC Pump. For detection Waters 2414 Refractive Index Detector was used. The products were identified in the chromatograms as described by Remaud-Simeon et al. (9).

Sugar (residual glucose, fructose, galactose and oligosaccharides in fermentation broth after fermentation) were determined by HPLC using Zorbax carbohydrate column (4.6 × 150 mm, Aglient, USA), analytical guard column Zorbax NH2 (4.6 × 12.5 mm), and a mobile phase 75/25 acetonitrile/water. Breeze Chromatography Manager Software (Waters, Milford, USA) was used for data treatment.

Antimicrobial activity assay

Antimicrobial assay was performed as previously described by the well diffusion method (1). After adjusting the pH to 6.5 by NaOH, the activity of the collected samples (60 μL) was checked against E. aerogenes ATCC 13048 on LB (Luria-Bertani) agar medium (Sigma, St Louis, MO, USA) and against L. innocua CIP 80.11 (ENITIAA, École Nationale d’Ingénieurs des Techniques des Industries Agricoles et Alimentaires, Nantes, France) on BH (Brain Heart) agar medium (Biokar Diagnostics, Beauvais, France). The plates were incubated overnight at 37 °C.

Results and Discussion

Most of the strains from both species utilized mono- di- and trisaccharides after 16 to 18 hours and changed the colour of the media from red to yellow first around the colonies (Fig. 1 – lactobacilli) and after 24 to 36 hours the whole media was

<table>
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yellow (Fig. 1). Some strains did not change the colour even after a longer period of time.

In the presence of long-chain sugars such as FOS, GOS and GalOS all new isolates, both lactococci and lactobacilli, did not change the colour of the media.

The growth of the studied *Lactobacillus delbrueckii* B7, *Lactobacillus fermentum* B5, *Lactobacillus sakei* S161 and *Lactobacillus acidophilus* 504 in modified MRS broth supplemented with different carbohydrate sources was evaluated in terms of maximum optical density at 600 nm, during 48 h fermentation. The kinetics of growth on glucose were used as control. All strains fermented lactulose, FOS, GOS and GalOS in a different manner. GOS was fermented by the four studied strains. GalOS was metabolized intensively by strains *Lactobacillus delbrueckii* B7, *Lactobacillus fermentum* B5, *Lactobacillus acidophilus* 504.

Residual oligosaccharide analysis showed different preferences for the types of degree of polymerization (DP) of the studied oligosaccharides (Fig. 3). GOS rich in DP5 (56%) and DP4 (24%), was better consumed by strain *Lactobacillus delbrueckii* B7. *Lactobacillus fermentum* B5 was unable to use DP6 (7%) and DP7 (7%) from GOS.

GalOS were hydrolyzed to the same extent by strains *Lactobacillus delbrueckii* B7 and *Lactobacillus fermentum* B5. The latter hydrolyzed oligosaccharides with DP4 more than strain B7.

**Production of lactic acid, acetic acid and ethanol**

The fermentation pattern depends on the physiological conditions of the growing cells. Homofermentative *Lactobacillus delbrueckii* and *Lactobacillus acidophilus* ferment hexoses via glycolysis with 90% of the glucose being metabolized to lactic acid. When cultivated on different types of oligosaccharides, the studied strains *Lactobacillus delbrueckii* B7 and *Lactobacillus acidophilus* 504 produced different amounts of acetic acid, lactic acid and ethanol (Table 1). When the fermentation end-products using oligosaccharides were compared to those observed on glucose, the main effect was that the production of lactic acid was lower while that of acetic acid and ethanol increased. The fermentation pattern for heterofermentative *Lactobacillus sakei* S161 and *Lactobacillus fermentum* B5 differ in the produced acetic acid when they were cultivated in the presence of oligosaccharides (Table 1).

**Antimicrobial activity**

The supernatants obtained after fermentation of the 4 studied strains on MRS-glucose, MRS-GaLOs, MRS-GOS and MRS-FOS were tested for their antimicrobial activity after pH adjustment to 6.5 against *L. innocua* and *Enterobacter aerogenes*. The heterofermentative *Lactobacillus sakei* S161 showed good antimicrobial activity when cultivated in MRS with fructooligosaccharides (Fig. 3). None of the lactococci strains showed any antimicrobial activity.

The antimicrobial activity determined against *Enterobacter aerogenes* after cultivation on oligosaccharides also indicated that the system of uptake of unusual sugars influenced the production of antimicrobial substances in a specific way. The mechanism of this stimulation remains unclear. The activity found against some bacteria in this study could enable prebiotic oligosaccharides to be protective in the colon. However, it is unclear whether sufficient amounts could be delivered to have any significant effect.

The question about how lactic acid bacteria use oligosaccharides is very interesting and not very well understood because of their strain-specific properties. But the potential that they have as a connection between prebiotics and human health needs to be explored in the future.

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**Table 1**

<table>
<thead>
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<th>Oligosaccharide</th>
<th>Acetic Acid</th>
<th>Lactic Acid</th>
<th>Ethanol</th>
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<tr>
<td>MRS-glucose</td>
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**Fig. 1.** Utilization of disaccharides by *Lactobacillus* spp. – after 24 and 36 hours.

**Fig. 2.** Degree of polymerization (DP).

**Fig. 3.** Inhibitory activity of strain *Lactobacillus sakei* S161 cultivated on *L. innocua* CIP 80.11 and *Enterobacter aerogenes* ATCC 13048.
Acknowledgements

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REFERENCES