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Interaction of Probiotic Bacteria with Pathogens of *Enterobacteriacae* Family

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Abstract: Probiotic lactic acid bacteria were grown on erythritol, xylitol, sorbitol or lactitol and produced various derivatives: gal-erythritol, gal-xylitol and gal-sorbitol as prebiotics. Galactosyl derivatives of erythritol, xylitol and sorbitol were metabolised by *Lactobacillus* sp. This resulted in their antagonistic activity against the test microflora. No activity was observed in the presence of xylitol and erythritol. Gal-sorbitol obtained by enzymatic transglycosylation from lactose had the same abilities of inducing the antagonistic activity of lactic acid bacteria that lactitol had.

Key words: Antagonism, *Lactobacillus*, galactopolyols, polyols, interaction, LAB

INTRODUCTION

The antagonistic activity of Lactic Acid Bacteria (LAB) results from the acid-forming abilities of these microorganisms. Formation of lactic and acetic acid from carbohydrates leads to a decrease in the pH of a medium (Lee and Salminen, 1995) which inhibits the growth of many pathogenic and food-contaminating microorganisms. Glucose, galactose and lactose are known to be the best sources of carbon for LAB (Saarela *et al*., 2003). To construct a synbiotic (probiotic + prebiotic system) we need to find a prebiotic that will not be digested during its passage through the human alimentary tract. Its beneficial effect on the human health should rather be observed as the selective stimulation of growth and activity of probiotic cultures (Gibson and Roberfroid, 1995; Hugenholtz and Smid, 2002). Since galactosyl derivatives of polyols obtained during the hydrolysis of lactose (Klewicki, 2000) are dimers, they are not directly absorbed into the blood stream-absorption must be preceded by hydrolysis. Experiments carried out with lactitol have shown that such dimmers (lactitol has a similar structure to that of galactosyl derivatives of sorbitol, xylitol and erythritol) are not hydrolysed in the small intestine. Lactitol passes into the large intestine where it contributes to an increase in the population of *Bifidobacterium* sp., *Lactobacillus* sp. and *Streptococcus* sp. This, in turn, inhibits the growth of pathogenic microflora (Ballongue *et al*., 1997). An important property of a probiotic strain is its antagonistic activity in the presence of a prebiotic. This activity protects both a synbiotic-enriched food product and a human who eats such a product. To investigate the inter-strain antagonism o f probiotic lactic acid bacteria in the presence of gal-polyols as prebiotics, galactosyl derivatives: Gal-erithritol, galxylitol and gal-sorbitol were used.

MATERIALS AND METHODS

Microorganisms: *Lactobacillus acidophilus* (18 strains, denoted as: LOCK 0840, 0842, 0925-0940, 0942), *Lactobacillus casei* (15 cultures, denoted as: LOCK 0848, 0849, 0899-0911) and *Lactobacillus paracasei/casei* (13 cultures, denoted as: LOCK 0912-0924) were obtained from the Collection of Industrial Microorganisms of the faculty of Biotechnology and Bioengineering of National Technical University of Ukraine.

Escherichia coli ATCC 23922, *Salmonella typhimurium*, *Shigella sonnei* S, *Enterobacter cloacae* LOCK 0835, *Citrobacter ferundi* (heat-resistant strain, 10 min at 80°C, isolated from homogenised cottage cheese as biological fouling of this product) were used as test microorganisms.

Production and structure of galactosyl derivatives of polyols: The mixture (500 mL) of lactose (DMV International) and selected polyol (sorbitol, xylitol or erythritol from Cerestar) of a molar ratio 1:1.85 (dry matter 65% w v^{-1}) was subjected to the action of β-galactosidase EC 3.2.1.23 from *Kluyveromyces lactis* (Gist-brocades, now DSM, Delft, NL). The hydrolysis was carried out at 40°C for 5 h (gal-sorbitol, enzyme-19 500 U and gal-xylitol, enzyme-6500 U) or 6 h (gal-erythritol, enzyme- 13 000 U). One unit is defined as the amount of enzyme which releases 1 μmol glucose per

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min under the following standard conditions: 4.75% (w w^{-1}) lactose as substrate, pH = 6.5, 37°C, reaction time 30 min.

Galactosyl derivatives of polyols were isolated from the hydrolysates using chromatography (3.3×100 cm, packing: Dowex 50 W × 4-200 from Lancaster, mobile phase: Water at 5 mL min^{-1}, at 78°C).

Gal-polyols are dimers formed from galactose and polyhydroxyalcohol. In produced gal-polyols monosacharide and polyol were mainly held together by β-1,1 bonds (gal-erythritol, gal-xylitol, gal-sorbitol). In gal-sorbitol there were also β-1,6 bonds. In galerythritol β-1,2 bonds were present, while in galxylitol and gal-sorbitol β-1,3 bonds occured.

The purity of each gal-polyol (a sum of isomers contained in the dry matter of gal-polyol concentration) was over 95%. In purified galactosyl derivatives, glycerol (below 4%) and galactose (below 1%) were also found.

Antagonistic activity of LAB: Inter-strain antagonism activity was investigated on MRS medium (Merck) containing 1% polyol selected (erythritol, xylitol, sorbitol or lactitol) or their galactosyl derivatives by the agar slab method (Strus, 1998). The method is based on the observation of parallel growth of the strains (the indicator and the antagonistic ones). Strains of lactic acid bacteria (106 cells mL^{-1}) were introduced into MRS-agar medium and poured onto Petri dishes. Then, LAB were incubated at 37°C for 24 h, in an atmosphere of 5% (v v^{-1}) CO2. Next, from solidified MRS medium overgrown with LAB, 10 mm-diam. slabs were cut out and put on the prepared agar (Nutrient Agar, Merck) containing test microorganisms (105-106 cells mL^{-1}). The dishes were incubated at 37°C for 18 h. Following the incubation, the diameter of the test strain growth inhibition zone was measured, the slab diameter was subtracted and the result was given in mm.

RESULTS AND DISCUSSION

In the presence of erythritol and xylitol, no antagonistic activity of bacteria *Lb. acidophilus*, *Lb. casei* and *Lb. paracasei/casei* against the test strains as observed. The growth of LAB in the presence of these compounds was weak or there was no increase in biomass, which means that xylitol and erythritol were not metabolised. After galactosyl derivatives of the polyols were ntroduced into the cultivation medium, antagonistic activity was observed in all the species groups of the LAB strains used, both in the case of gal-erythritol (Table 1) and gal-xylitol (Table 2). The results indicate that the bacteria studied are capable of utilising galactose in metabolic processes after galactosyl derivatives

Table 1: Antagonistic activity of *Lactobacillus* sp. against the *Enterobacteriaceae* family bacteria in the presence of gal-erythritol as a source of carbon

	Test strain growth inhibition zone (mm)[a]		
Test strain	*Lactobacillus acidophilus* (18 cultures)	*Lactobacillus casei* (15 cultures)	*Lactobacillus paracasei/casei* (13 cultures)
Escherichia coli	4.7±1.1	5.0±1.0	3.6±1.3
Salmonella typhimurium	5.9±1.3	6.6±0.9	3.9±0.9
Shigella sonnei S	5.8±1.4	5.9±0.5	3.7±1.0
Enterobacter cloacae	6.7±1.3	7.5±1.3	4.7±0.8
Citrobacter freundi	6.2±1.3	5.8±1.0	4.8±0.5

[a]An average value for a given population of strains±the standard deviation

Table 2: Antagonistic activity of *Lactobacillus* sp. against the *Enterobacteriaceae* family bacteria in the presence of gal-xylitol as a source of carbon

	Test strain growth inhibition zone (mm)[a]		
Test strain	*Lactobacillus acidophilus* (18 cultures)	*Lactobacillus casei* (15 cultures)	*Lactobacillus paracasei/casei* (13 cultures)
Escherichia coli	4.3±1.6	5.3±1.8	4.2±1.9
Salmonella typhimurium	2.1±1.1	3.7±1.1	3.2±1.0
Shigella sonnei S	1.0±0.4	2.1±0.9	2.8±1.0
Enterobacter cloacae	5.1±1.5	5.6±1.7	4.9±1.9
Citrobacter freundi	4.8±1.8	5.2±1.6	5.2±1.4

[a]An average value for a given population of strains±the standard deviation

Table 3: The pH value[a] of MRS medium[b] after incubation of *Lactobacillus* sp. for 24 h in the presence of different carbon sources

	Strains		
Carbon source	*Lactobacillus cidophilus* (18 cultures)	*Lactobacillus casei* (15 cultures)	*Lactobacillus paracasei/casei* (13 cultures)
Galactose	4.48±0.09	4.35±0.05	4.33±0.06
Sorbitol	4.56±0.07	4.48±0.06	4.48±0.09
Gal-Sorbitol	4.37±0.04	4.28±0.05	4.25±0.07
Lactitol	4.48±0.20	4.23±0.11	4.24±0.10
Erythritol	6.90±0.12	6.88±0.07	6.92±0.06
Gal-Erythritol	4.61±0.05	4.43±0.09	4.45±0.10
Xylitol	6.99±0.04	6.74±0.08	6.89±0.16
Gal-Xylitol	4.76±0.07	4.60±0.08	4.58±0.14

[a]An average value for a given population of strains±the standard deviation
[b]Initial pH-7.04

introduced into the medium have been split. Galactose is transformed by LAB into lactic acid (homofermentation) or into lactic acid and acetic acid (heterofermentation). The amount of acids affects the pH of a culture (Gripon *et al*., 1991). Thus, in cultures containing galactose or the derivatives of galactose, lower pH values were observed than those found in cultures containing polyols (Table 3). In the media containing gal-xylitol and galerythritol, pH values were lower (4.43-4.76) than in the media containing xylitol (6.74-6.99) and erythritol (6.88-6.92). This resulted from the occurrence of acidic metabolites formed from the galactose contained in gal-polyols. In the tests in which only slight changes in

Table 4: Antagonistic activity of *Lactobacillus* sp. against the *Enterobacteriaceae* family bacteria in the presence of sorbitol, lactitol or gal-sorbitol as a sources of carbon

	Antagonistic strain								
	Lactobacillus cidophilus (18 cultures)			*Lactobacillus casei* (15 cultures)			*Lactobacillus paracasei/casei* (13 cultures)		
	S	L	G-S	S	L	G-S	S	L	G-S
Test strain	Test strain growth inhibition zone (mm)[a]								
Escherichia coli	2.8±0.8	6.0±1.1	5.8±1.2	6.3±1.0	7.9±1.1	9.6±1.5	5.9±1.2	5.4±1.1	7.9±1.6
Salmonella typhimurium	2.6±0.7	3.0±0.9	4.1±1.0	5.3±1.1	5.2±1.2	4.7±0.9	5.1±0.9	4.0±0.9	4.2±0.9
Shigella sonnei S	4.9±1.1	5.6±1.4	5.7±0.9	8.2±1.2	7.9±1.3	6.8±1.0	8.6±1.5	6.5±1.3	7.5±1.3
Enterobacter cloacae	2.9±1.0	5.3±1.1	5.6±1.2	4.7±1.0	7.4±1.3	6.2±1.0	3.8±0.9	5.4±1.1	6.9±1.0
Citrobacter freundi	1.4±0.2	4.0±0.9	5.7±1.1	2.0±0.4	6.2±0.9	7.6±1.4	2.7±0.7	4.4±0.7	6.3±1.4

S: Sorbitol, L: Lactitol, G-S: Gal-Sorbitol. [a]An average value for a given population of strains±the standard deviation

pH occurred (xylitol and erythritol), no antagonistic activity of lactic acid bacteria was observed. *Lb. casei* was the most active group of strains in the presence of gal-erythritol and gal-xylitol. A different situation was observed when sorbitol, lactilol or gal-sorbitol was introduced into the medium. Antagonistic activity in all the species groups of the LAB was distinct for all three compounds (Table 4). The activities in the presence of gal-sorbitol and lactitol were similar. Lactitol is a substance with a prebiotic potential (Sarela *et al*., 2003; Crittenden, 1999). It contains a β-1,4 bond while gal-sorbitol is a mixture of isomers in which β-1,1 and β-1,6 bonds prevail. Thus, the presence of different bonds between galactose and sorbitol does not have a significant effect on the capability of LABs to metabolise the compounds studied.

Lb. acidophilus manifests a substantially higher antagonistic activity in the presence of lactitol and gal-sorbitol than in the presence of sorbitol Shoem in Table 5. In this case too, galactosyl derivatives of the polyol, sorbitol, caused a greater reduction in pH than sorbitol itself. This indicates that the presence of galactosyl radical in the molecule is conducive to the production of acidic metabolits. Lactic acid is the main product of *Lb. acidophilus* (Kandler, 1983). However, lactic acid bacteria are capable of metabolising sorbitol thus media containing sorbitol had lower pH than those with xylitol or erythritol. This had an effect on the occurrence of antagonistic activity of lactic acid bacteria when sorbitol or its derivatives were a source of carbon.

The antagonistic activity of *Lb. casei* and *Lb. paracasei/casei* against *Salmonella typhimurium* and *Shigella sonnei* in the presence of sorbitol was higher then in the presence of the remaining carbon substrates. At the same time, the pH values of the media with sorbitol were higher than with its derivatives. This suggests that not only the acids but also other metabolites (in the presence of acids) inhibit the growth of the pathogens. Ethanol, acetaldehyde and hydrogen peroxide were found in the media with various carbohydrates as a source of carbon (Kandler, 1983; Vandenbergh, 1993). In order to determine individual metabolites in the media with gal-polyols further investigation is planned.

CONCLUSION

Galactosyl derivatives of erythritol, sorbitol and xylitol are metabolised by lactic acid bacteria. Galpolyols have the abilities of inducing the antagonistic activity of LAB against the test microflora. The activity is higher than in the presence of polyols-monomers. Gal-sorbitol obtained by en zymatic transglycolysation from lactose has the same properties of inducing the antagonistic activity of LAB as does lactitol.

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REFERENCES

Ballongue, J., C. Schumann and P. Quignon, 1997. Effect of Lactulose and Lactitol on Colonic Microflora and Enzymatic Activity. Scand. J. Gastroenterol., 32: 41-44.

Crittenden, R.G., 1999. Prebiotics. In: Tannock, G.W. (Ed.). Probiotics: A Critical Review. Wymondham: Horizon Scientific Press, pp: 141-156.

Gripon, J.C., V. Monnet, G. Lamberet, M.J. Des Mazeaut, 1991. Microbial Enzymes in Cheese Ripening. In: Fox P.F. (Ed.). Food Enzymol., London: Elsevier Applied Sci., 1: 131-218.

Gibson, G.R. and M.B. Roberfroid, 1995. Dietarymodulation of the colonic microbiota: Introducing the concept of prebiotics. J. Nutr., 125: 1401-1412.

Hugenholtz, J. and E.J. Smid, 2002. Nutraceutical production with food grade microorganisms. Curr. Opin. Biotechnol., 13: 497-507.

Kandler, O., 1983. Carbohydrate metabolism in lactic acid bacteria. Antonie van Leeuwenhoek, 49: 209-224.

Klewicki, R., 2000. Transglycosylation of a β-galactosyl radical, in the course of enzymatic hydrolysis of lactose, in the presence of selected polyhydroxyalcohols. Biotechnol. Lett., 22: 1063-1066.

Lee, Y.K. and S. Salminen, 1995. The coming of age of probiotics. Trends Food Sci. Technol., 6: 241-245.

Strus, M., 1998. A new method for testing antagonistic activity of Lactic Acid Bacteria (LAB) on selected pathogenic indicator bacteria. Med. Do´sw. Mikrobiol., 50: 123-130.

Saarella, M., K. Hallamaa, T. Mattila-Sandholm and J. Matto, 2003. The effect of lactulose lactitol and lactobionic acid on the functional and technological properties of potentially probiotic *Lactobacillus* strains. Int. Dairy J., 13: 291-302.

Vandenbergh, P.A., 1993. Lactic acid bacteria, their microbial growth. FEMS Microbiol. Rev., 12: 221-238.