In Vitro Fermentation of Galactosyl Derivatives of Polyols by *Lactobacillus* Strains

Elżbieta KLEWICKA[1] and Robert KLEWICKI[2]

[1]Institute of Fermentation Technology and Microbiology and [2]Institute of Chemical Technology of Food, Faculty of Biotechnology and Food Sciences, Technical University of Łódź, Łódź, Poland

Abstract

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Probiotic lactic acid bacteria belonging to the species *Lactobacillus casei*, *Lb. paracasei,* and *Lb. acidophilus* were cultivated in the presence of galactosyl derivatives of erythritol, xylitol, and sorbitol, as well as the polyols themselves: erythritol, xylitol, sorbitol, lactitol, and glucose as a reference. After 48-h incubation, the profile of the main metabolic products (lactic and acetic acids) and the amount of the studied bacteria biomass were determined. It was found that none of the bacteria studied metabolised erythritol or xylitol. In the presence of these compounds, no increase of metabolic activity of the lactic fermentation bacteria was observed. On the other hand, the *Lactobacillus* sp. bacteria effectively utilised galactosyl derivatives of polyols. The bacteria growth in the presence of gal-polyols was comparable to their growth on glucose, while the fermentation profile was determined by the carbon source used.

Keywords: polyols; prebiotics; *Lactobacillus*; growth response

Lactic acid bacteria belonging to the genus *Lactobacillus* are one of the predominant groups populating the human alimentary tract, and in particular the large intestine. The presence in the intestine of *Lactobacillus* strains with probiotic properties is related to the positive health effects induced by these bacteria (Gibson & Roberfroid 1995; Moura *et al.* 2007). Nevertheless, the induction of the probiotic effect is only possible with an appropriately high level of multiplication of these bacteria in the intestine. It is well-known that probiotic bacteria consumed in the form of food or as dietary supplements must overcome drastic conditions prevalent in the initial section of the alimentary tract before they reach the intestinal lumen. Thus, optimum or even stimulating conditions for the growth of these bacteria in the intestine should be created. Among the stimulators of probiotic lactic bacteria are prebiotics, *i.e.* saccharide compounds not at all or poorly digested by the human organism (Manning & Gibson 2004). On reaching the intestine, the above-mentioned substances can become carbohydrate nutrients for the bacteria of the genus *Lactobacillus* and *Bifidobacterium.* In functional foods, the most frequently used prebiotics are fructooligosaccharides (FOS), galactooligosaccharides (GOS), maltooligiosaccharides (MOS), xylooligosaccharides (XOS), soya oligosaccharides, and lactulose (Douglas & Sanders 2008). Galactosyl derivatives of polyols are a group of compounds not digested by the enzymes of the human digestive system. On reaching the intestine, they can stimulate the growth of the beneficial intestinal microflora. The aim of our study was to assess the growth ability of the selected probiotic cultures of *Lactobacillus* sp.

and to determine their fermentation profile in the presence of galactosyl derivatives of polyols.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The strains of lactic bacteria with probiotic properties of the genus *Lactobacillus* that were used in the studies are shown in Table 1. All the strains were obtained from the Collection of Industrial Microorganisms of the Institute of Fermentation Technology and Microbiology LOCK 105. *Lactobacillus* bacteria were stored in the form of frozen permanents. The bacteria strains were activated by a two-fold passage on the standard MRS medium (prepared using reagents from Merck) at 37°C in the presence of CO_2 (5%, v/v) for 48 hours. The inoculum was prepared by centrifuging (12 000 rpm) of the 24-h cultivation of the *Lactobacillus* bacteria; the sediment was suspended in physiological salt and adjusted to a density of 10^8 CFU/ml.

The experiments were done using modified MRS medium (without glucose). Instead of glucose, (1%, w/v), polyols (erythritol, xylitol, sorbitol, lactitol), or galactosylopolyols (gal-xylitol, gal-erythritol, gal-sorbitol) were added to the liquid MRS medium. The MRS medium was sterilised for 15 min at 12°C. After cooling, it was inoculated with *Lactobacillus* sp. in an amount of 1% (v/v) of the inoculum prepared. The incubation was carried out for 24 h at 37°C in the presence of CO_2 (5%, v/v).

The growth of the genus *Lactobacillus* bacteria was determined in the MRS (Merck) medium solidified with 1.5% agar using the standard plate method. After the inoculation, the bacteria were incubated for 48 h at 37°C in the presence of CO_2 (5%, v/v) and the colonies were counted. The experiments were carried out in triplicates. The results were given in CFU/ml.

Lactic and acetic acids assay. The total content of lactic and acetic acids was assayed by the enzymatic method with the use of the Boehringer Mannheim Biochemica (Germany) enzymatic tests according to the procedure presented.

Production and structure of galactosyl derivatives of polyols. The production of galactosyl derivatives of polyols used in the studies was carried out according to the procedure described by Klewicki and Klewicka (2004).

RESULTS AND DISCUSSION

In the studies, bacteria belonging to three species were used: *Lactobacillus acidophilus*, *Lactobacillus casei,* and *Lactobacillus paracasei* – two cultures of each species. The selection of particular strains for the studies on the fermentation ability of the galactosyl derivatives of polyols was based both on their high antagonistic activity investigated in the presence of these derivatives in relation to the bacteria belonging to the family *Enterobacteriaceae* (Klewicka & Libudzisz 2001; Klewicki & Klewicka 2004), and on the studies of antifungal properties of lactic fermentation bacteria in the presence of polyols (Klewicka 2007). The antagonistic activity of lactic fermentation bacteria results from their fermentative metabolism of saccharides.

These bacteria metabolise saccharides and their derivatives through homofermentation, heterofermentation, and mixed acid fermentation. Lactic acid, acetic acid, and other metabolites such as ethanol, formate, and CO_2 are the final metabolic products. A mixture of lactic and acetic acids can be formed through the homofermentative pathway in the case of a limited amount of saccharides in the growth medium, a decrease in pH, or a change in temperature. In such a case, the homofermentative pathway of pyruvate conversions is different

Table 1. *Lactobacillus* strains used in the study

Species	Strain code	Source
Lactobacillus acidophilus	LOCK 0927 LOCK 0933	dairy product
Lactobacillus casei	LOCK 0908 LOCK 0910	faeces
Lactobacillus paracasei	LOCK 0919 LOCK 0922	faeces

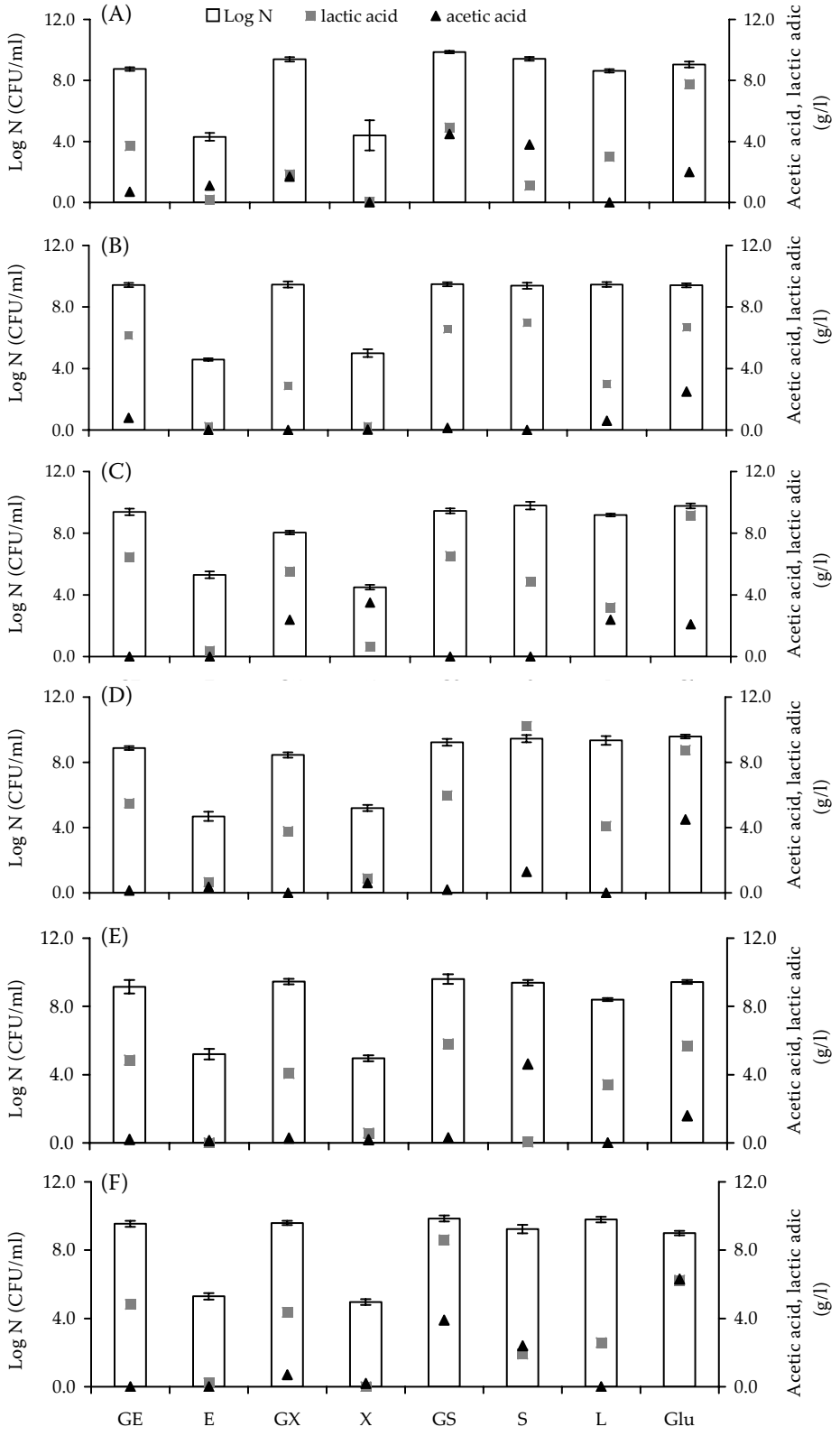

Figure 1. The growth of *Lactobacillus* strains on gal-xylitol (GX), xylitol (X), gal-erythritol (GE), erythritol (E), gal-sorbitol (GS), sorbitol (S), lactitol (L), and glucose (Glu) as a control at 1% carbohydrate substrate concentration after 24 hours: (A) *Lactobacillus casei* 0908; (B) *Lactobacillus casei* 0910; (C) *Lactobacillus paracasei* 0919; (D) *Lactobacillus paracasei* 0922; (E) *Lactobacillus acidophilus* 0927; (F) *Lactobacillus acidophilus* 0933

– pyruvate is transformed into lactic acid, formate, CO_2, and acetylCoA which in turn is transformed into acetic acid and ethanol (Hofvendahl & Hanh-Hagerdal 2000). In the case of classical homofermentation, glucose is transformed into lactic acid (Embden-Meyerhof-Parnas pathway) (Axelsson 1993).

On the other hand, lactic acid, acetic acid, ethanol, and CO_2 are the transformation products of glucose by lactic bacteria of heterofermentative metabolism, which makes use of the phosphoketolase pathway for this purpose (Kandler 1983). In the analysis of the acidic products of glucose metabolism, it was found that the strains *Lactobacillus casei* and *Lactobacillus paracasei* demonstrate the metabolic character of typical heterofermentation (according to their classification) (Axelsson 1993; De Vuyst & Neysens 2005). In the presence of glucose, these bacteria (used as a control sample) form a mixture of lactic and acetic acids in the amounts of (depending on the strain) 6.7 to 9.1 g/l and 2.1 to 4.5 g/l, respectively (Figure 1A–D). In the presence of polyols and their galactosyl derivatives in the growth medium, the strains *Lb. casei* and *Lb. paracasei* also form a mixture of these acids. However, depending on the polyol used and its galactosyl derivative, the concentrations and the reciprocal proportions of these products are varied. The most effective synthesis of lactic acid by these cultures giving the amount of 4.9 to 6.6 g/l took place when gal-sorbitol was used for all the cultures of *Lb. casei* and *Lb. paracasei* and sorbitol itself in the case of the strains *Lb. casei* 0910 and *Lb. paracasei* 0919, where the concentration of lactic acid was 7.0 g/l and 4.8 g/l, respectively. On the other hand, acetic acid in significant quantities was synthesised by only one strain, *Lb. casei* 0908, in an amount of 4.5 g/l in the presence of gal-sorbitol, and 3.8 g/l after sorbitol was used as the sole source of carbon. The growth of the bacteria in the presence of gal-sorbitol and sorbitol is comparable (8.6–9.5 log units) to the reference sample – glucose (7.5–9.7 log units). In the presence of lactitol, the growth of bacterial cells is comparable to or slightly lower than that of the reference sample (depending on the strain). However, in the samples containing lactitol, a significantly smaller amount of organic acids than that in the control sample was observed with definite predominance of lactic acid over acetic acid. The quantities of lactic acid ranged from 3.0 g/l to 4.1 g/l depending on the strain, *Lb. casei* or *Lb. paracasei.* The concentrations of acetic acid synthesised by this group of strains in the presence of lactitol ranged from 0.0 to 2.0 g/l. In the presence of erythritol and xylitol as a source of carbon in the cultivation medium, the bacteria of the species *Lb. casei* and *Lb. paracasei* did not show the ability of growth (the level shown in the diagram is the inoculation level) or the synthesis of acidic metabolites. The situation was different when the bacteria grew in the presence of the galactosyl derivatives of erythritol and xylitol (gal-erythritol and gal-xylitol). The bacteria growth ranging from 8.7 to 9.7 log units was found, which was comparable to the growth of these bacteria on glucose. In the presence of gal-erythritol, the strains *Lb. casei* 0908 and *Lb. casei* 0910 produced a predominant amount of lactic acid: strain 0908 – 3.7 g/l and strain 0910 – 6.1 g/l. Acetic acid was synthesised by both strains in the amount of about 0.7 g/l. In the presence of gal-xylitol the cultures of *Lb. casei*, despite the abundant growth of bacterial cells, were characterised by a low acidifying activity resulting in low concentrations of lactic and acetic acids (compared to other gal-polyol derivatives studied). The strain *Lb. casei* 0908 gave equal concentrations of lactic and acetic acids, in an amount of about 2.0 g/l, while the strain *Lb. casei* 0910 in the presence of gal-xylitol synthesised only lactic acid, in an amount of 3.0 g/l.

The studied strains *Lb. acidophilus* 0927 and *Lb. acidophilus* 0933 demonstrated in the presence of gal-polyols a growth ability related to the amount of biomass in the control sample containing glucose (Figures 1E–F). However, after using only polyols in the MRS medium, a growth comparable to the control sample was found in the case of sorbitol and lactitol. The bacteria *Lb. acidophilus* were not capable to grow in the presence of erythritol and xylitol as sole sources of carbon. The strain *Lb. acidophilus* 0927, in the presence of both galactosyl derivatives of polyols and glucose, demonstrates homofermentative metabolism. It synthesises lactic acid in a predominant amount and acetic acid in trace amounts (Figure 1E). Sorbitol and lactitol are polyols that can be utilised by the above-mentioned strain for the synthesis of organic acids. While in the presence of lactitol it demonstrates homofermentation with a typical set of metabolites: lactic acid (3.4 g/l) with or without minimal amount of acetic acid (0.05 g/l) in the presence of sorbitol, only acetic acid (in an amount of 4.6 g/l) can be identified in metabolic products. Presumably, in

the presence of sorbitol as the source of carbon, the metabolism process proceeds by mixed acid fermentation described by Hofvendahl and Hanh-Hagerdal (2000).

The strain *Lb. acidophilus* 0933 is capable of growing and metabolising gal-erythritol, gal-xylitol, gal-sorbitol, sorbitol, and lactitol (Figure 1F). In the case of gal-erythritol, gal-xylitol, and lactitol, a typical homofermentative metabolism was observed, where lactic acid amount (2.5–4.8 g/l) was predominant as compared to the slight quantity of acetic acid among acidic metabolites. However, in the presence of gal-sorbitol, sorbitol, and glucose (control), a mixture of organic acids was found. With gal-sorbitol, the amount of lactic acid was 8.6 g/l, whereas that of acetic acid was 3.9 g/l. The presence of sorbitol or glucose in the growth medium of the bacteria *Lb. acidophilus* 0933 induced equal amounts of lactic acid and acetic acid of about 2.0 g/l with sorbitol and 6.0 g/l with glucose. In this case, mixed acid fermentation is also involved.

The studied lactic fermentation bacteria belonging to two metabolic types – homofermentation and heterofermentation – are capable of utilising galactosyl derivatives of polyols as the source of carbon. These are effectively transformed into acidic products of metabolism (lactic acid and acetic acid). Based on the results obtained, it has been found that the type of carbon source determines the type of metabolism, and consequently, the composition of acidic products.

Lactitol is a synthetic compound with prebiotic properties documented (Crittenden 1999; Sarella *et al.* 2003). In lactitol, bonds between galactose and sorbitol of type β-1,4 were identified, while in galactosyl derivatives of polyols, bonds of type β-1,1, β-1,2, β-1,3 and β-1,6 were found (Klewicki & Klewicka 2004). The lactic acid bacteria studied effectively utilise gal-polyols; hence, they are capable of hydrolysing the above-mentioned bonds through an enzymatic pathway in order to cleave galactose. Galactose is the saccharide that is utilised by the bacteria studied as the first one when gal-polyols are decomposed.

CONCLUSIONS

Galactosyl derivatives of polyols such as erythritol, xylitol, and sorbitol are metabolised by lactic fermentation bacteria to lactic acid, a mixture of lactic and acetic acid, and even to acetic acid only. The composition of the final products of metabolism is determined both by the source of carbon metabolised and the individual abilities and preferences of particular lactic bacteria cultures. The gal-polyols used in the studies can be referred to as modern prebiotics stimulating both the growth and acidifying activity of the bacteria of the genus *Lactobacillus*.

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Corresponding author:

PhD Elżbieta Klewicka, Technical University of Łódź, Faculty of Biotechnology and Food Sciences, Institute of Technology Fermentation and Microbiology, ul. Wólczańska 171/173, 90-924 Łódź, Poland
tel.: + 48 42 631 34 86, fax.: +48 42 636 59 76, e-mail: klewicka@p.lodz.pl