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The Use of Exopolysaccharide-Producing Cultures of Lactic Acid Bacteria to Improve the Functional Value of Fermented Foods

P. Semjonovs, J. Jasko, L. Auzina and P. Zikmanis
Institute of Microbiology and Biotechnology, University of Latvia,
Kronvalda Blv., 4, Riga, Lv 1010, Latvia

Abstract: The ability of food-grade Lactic Acid Bacteria (LAB) *L. reuteri* (strains MBI-C42 and MBI-C43) and *L. panis* strain MBI-V35 to produce Extracellular Polysaccharides (EPS) was detected and confirmed in this study. Alike so far acknowledged LAB *L. reuteri* LB 121 and *L. sanfranciscensis* DSM 20451, the strains under study in the presence of sucrose produced a complex of EPS consisting of glucans and fructans (at a ratio within a range 1:2.9, respectively) with a molecular mass about 55-867 kDa. The growth and acidification power of bifidobacteria and other constituent cultures of probiotic dairy starters (*L. acidophilus*, *Streptococcus thermophilus, L. bulgaricus*) were stimulated in the presence of exogenously added polysaccharides and above LAB were capable to assimilate these EPS as a sole carbon source. An increase of fructan content was confirmed during lactic acid fermentation of food substrates by EPS-producing strains, thus suggesting their potent application for an improvement of products towards synbiotic food.

Key words: Exopolysaccharides, lactic acid bacteria, fructans, probiotics, prebiotics, synbiotics, fermented foods

INTRODUCTION

The reasons for a widespread use of Lactic Acid Bacteria (LAB) are to make food durable, to improve its safety, flavour, appearance and texture as well as to enhance its physiological and hygienic value due to the presence of viable cells and valuable metabolites of LAB. On the other hand, steady growing demand for foods with health-promoting properties, so called functional foods (Vuyst, 2000; Leroy and Vuyst, 2004), requires a goal-directed involvement of appropriate strains and specific non-digestible ingredients selectively enhancing their growth which are generally acknowledged as probiotics and prebiotics (Roberfroid *et al*., 1998), respectively.

Products obtained by lactic acid fermentation processes therefore are of special importance for functional foods since the synbiotic (probiotic and prebiotic) properties could be obtained by an adequate combination of LAB and bifidobacteria (Schrezenmeir and Vrese, 2001; Gomes and Malcata, 1999) besides with prebiotic substances such as oligo and polysaccharides of plant and microbial origin (Rastall and Maitin, 2002). The use of any prebiotic substance for the enrichment of fermented products provides its delivery into human Gastrointestinal Tract (GIT) and hence, a stimulation of beneficial probiotic bacteria regardless of their origin (native GIT inhabitants or delivered by functional products). The overall prophylactic and therapeutic qualities of fermented functional foods substantially depend on the content of viable probiotic cells. Therefore, it is necessary to achieve a high cell count for probiotic bacteria at the stage of lactic acid fermentation of product by an addition of potent prebiotic substances. Fructose polymers and oligomers (Biedrzycka and Bielecka, 2004; Semjonovs *et al*., 2004, 2005, 2006; Semjonovs and Zikmanis, 2007) as well as Exopolysaccharides (EPS) of LAB should be considered as most appropriate additives for this purpose (De Bello *et al*., 2001; Korakli *et al*., 2002; Welman and Maddox, 2003). However, bifidogenic properties of prebiotics, being well manifested in GIT (Roberfroid *et al*., 1998; Biedrzycka and Bielecka, 2004; Roberfroid, 1996), remain not so evident at the stage of fermentation of synbiotic product with probiotics containing starters, particularly against a high background of basic carbon source, e.g. milk lactose. Besides, it is necessary to evaluate possible growth responses of basic starter cultures (for instance,

Corresponding Author: P. Semjonovs, Institute of Microbiology and Biotechnology, University of Latvia, Kronvalda Blv., 4, Riga, Lv 1010, Latvia

Lactobacillus delbrueckii sp. *bulgaricus* and *Streptococcus thermophilus* for yogurt production) to the prebiotic additives during fermentation process since varied relations between them could substantially affect overall sensory and technological properties. Above points obviously being essential to improve the qualities of synbiotic products to our knowledge remain almost uninvestigated at present. It is well-known, that some technologically important strains of LAB belong to the normal microflora of GIT. Namely, an exhaustive evidence is accumulated on the prophylactic and therapeutic efficiency of *L. acidophilus* (Gomes and Malcata, 1999) and *L. reuteri* (Cases and Dobrogosz, 2000) for both human and animals. Due to it *L. acidophilus* finds extensive technological and commercial application, particularly in dairy fermentations or as the preparations of frieze-dried viable cells (Tablets, Capsules etc.) (Gomes and Malcata, 1999). In turn, technological usage of *L. reuteri* remain somewhat limited at present due to the strict heterofermentative metabolism and weak proteolytic activity and, hence, quite limited growth potential in milk (Cases and Dobrogosz, 2000). However recently, several products containing *L. reuteri*, have been launched into the market by BioGaia (Sweden) (Speck *et al*., 1993). Nevertheless, the widespread occurrence and broad-spectrum protective properties of *L. reuteri* strains (Cases and Dobrogosz, 2000) strongly suggest to search new ways for further applications. Thus, *L. reuteri* is the major component of the gut lactobacilli microflora of humans, mammals and birds, i.e., the only enteric lactobacilli known to be indigenous such a broad spectrum of hosts (Cases and Dobrogosz, 2000). *L. reuteri* has been isolated from a variety of food products including meats and milk products, i.e. sausages (Speck *et al*., 1993; Kandler *et al*., 1980), lamb rennet paste, sheep milk and cheese (Dellaglio *et al*., 1981), sour dough (Vogel *et al*., 2002), fermented rice noodles and fermented cane molasses (Cases and Dobrogosz, 2000; Speck *et al*., 1993). The species' primary habitat, however, appears to be the GIT of humans and animals (Cases and Dobrogosz, 2000)and an unique traits of *L. reuteri* make it very important as a member of the human intestinal microflora (Cases and Dobrogosz, 2000; Mitsuoka, 1992). Besides, *L. reuteri* is capable to produce a wide spectrum of antimicrobial compounds (Cases and Dobrogosz, 2000), under conditions similar to those in the GIT as well as *in situ* during food fermentations (Vogel *et al*., 2002).

The ability of LAB to synthesize EPS (Dal Bello *et al*., 2001; Korakli *et al*., 2002; Welman and Moddox, 2003; Van Geel-Schutten *et al*., 1999; Tieking, 2005), should be considered as the other important chance to improve the qualities of product. In fact, to give a desired texture, mouthfeel. Taste perception and stability of yoghurts some natural texturizers (skimmed milk concentrate, whey powder, caseinate etc.) are frequently added to the milk. Milk solids may be also rised through the concentration of milk (evaporation, membrane filtration etc.) that represent unwanted extra-cost for the manufacturer (Vuyst, 2000; Leroy and Vuyst, 2004). Besides, a number of other stabilizers and viscosifiers of plant (starch, pectin, guar gum etc.), animal (gelatin) or microbial (xantan, alginate, gellan) origin are used for this purpose (Vuyst, 2000; Leroy and Vuyst, 2004; Welman and Moddox, 2003). From this viewpoint the production of EPS with LAB, particularly *in situ*, is a promissing alternative for the use of above texturizers. EPS produced by LAB have been claimed to have antitumor effects, to possess immunostimulatorory (Welman and Moddox, 2003) and prebiotic activity (De Bello *et al*., 2001; Korakli *et al*., 2002; Welman and Maddox, 2003)and to lover blood cholesterol level (Welman and Moddox, 2003). The formation of EPS by several strains of LAB has been frequently reported and finds application in dairy fermentation, particularly for the production of yogurt (Vuyst, 2004). Synthesis of glucose and fructose homopolysaccharides (glucans and levans, respectively) within non-food species of *Leuconostoc* and *Streptococcus* genera is well documented (Russ-Madiedo *et al*., 2005), however, the use of homopolysacharide producing strains remains quite restricted (*Leuconostoc*) or even impossible (e.g. *S. mutans*) for food fermentations.

At present only few studies concern in detail with the synthesis of glucan and fructan EPS by lactobacilli (Van Geel-Schutten *et al*., 1999; Tieking, 2005), however, this feature might be relatively widespread within the genus. Thus, at least 25% of *Lactobacillus* strains from pigs and ducks intestines were capable to form oligo- or polysaccharides (Tieking *et al*., 2005). Further exhaustive research for fructan-synthesising representatives of lactobacilli should be of special importance since such strains could possess probiotic properties. It was reported that eight species of lactobacilli - *L. reuteri*, *L. acidophilus*, *L. panis*, *L. pontis*, *L. mucosae*, *L. sanfranciscensis*, *L. crispatus* and *L. frumenti* are capable to synthesize fructans (to a very different extent), however, only first 5 species are most frequently isolated from GIT of humans and animals (Dal Bello *et al*., 2001; Van Geel-Schutten *et al*., 1999; Tieking, 2005; RussMadiedo *et al*., 2005; Tieking *et al*., 2005). In turn, fructans of both plant (Roberfroid *et al*., 1998; Biedrzycka and Bielecka, 2004) and microbial origin (Semjonovs *et al*., 2004; Dal Bello *et al*., 2001; Korakli *et al*., 2002) are well-known prebiotics, alike ß-glucans(Angelov *et al*., 2005), due to the nondigestible ß-glycosidic linkage

between carbohydrate units (Roberfroid *et al*., 1998; Biedrzycka and Bielecka, 2004; Angelov *et al*., 2005). The formation of extracellular ß-glucan has been shown in *L. brevis* G-77 and *Pediococcus damnosus* 2.6 (Martensson *et al*., 2005). The biosynthetic potential of *L. reuteri* LB 121 in regard to levan (150 kDa) and á-glucan (3500 kDa) formation from sucrose (3:1 ratio, respectively) has been investigated in considerable detail (Van Geel-Schutten *et al*., 1999). The formation of high-molecular mass (2000 kDa) levan by *L. sanfrancisciensis* during sourdough fermentation substantially improved dough's rheological properties and sensory qualities of bread (Vogel *et al*., 2002; Tieking, 2003), presumably towards qualities of functional food, since bifidogenic properties of corresponding thermo-and acid stable EPS have been demonstrated (Dal Bello *et al*., 2001; Korakli *et al*., 2002).

The objectives of this study, are to evaluate the fermentation potential of several EPS-synthesizing LAB in respect of various food-grade substrates, to assess the possibility of *in situ* fructan formation during fermentation of these substrates and to examine the growth of probiotic LAB starter cultures in the presence of extracellular polysaccharides.

MATERIALS AND METHODS

Cultures *Lactobacillus reuteri* LB 121 (LMG P18388) was obtained from the BCCM/LMG Bacteria Culture Collection with the agreement of depositor. *Lactobacillus* strains (*L. reuteri* MBI-C42, MBI-C43, *L. pontis* MBI-C40, *L. sanfranciscensis* DSM 20451, *L. panis* MBI-V35) and *Bifidobacterium lactis* MBI-OX1 were from the LU MBI Microorganism Collection, University of Latvia. The dairy starter culture *L. delbrueckii* sp. *bulgaricus* Lb-12 (hereafter *L. bulgaricus*) were from the Chr. Hansen (Denmark) as well as the combined dairy starter ABT-5. The individual constituent strains of ABT-5: *B. lactis* Bb-12, *L. acidophilus* La-5 and *Streptococcus thermophilus* were isolated by means of appropriate selective media as reported previously (Semjonovs *et al*., 2007). Bifidobacteria strains *B. angulatum* CCM 7093, *B. animalis* CCM 4988, *B. pseudocatenulatum* CCM 7094 and *B. breve* CCM 3763 were obtained from the Czech Collection of Microorganisms.

Media and growth conditions. The MRS growth medium (De Man *et al*., 1960) supplemented by 0.5 g L^{-1} L-cysteine was used for the maintenance and propagation of bifidobacteria, *S. thermophilus* and lactobacilli (*L.reuteri*, *L. acidophilus*, *L. bulgaricus*). The Sour Dough Medium (Medium 225, DSMZ) and *Lactobacillus* medium IV (Medium 859, DSMZ) were used for the maintenance of *L. sanfranciscensis* at 30°C and *L.panis* at 37°C, respectively (www.dsmz.de/microorganisms/main.php). L. pontis was maintained on the *Lactobacillus* medium III (Medium 638, DSMZ) (www.dsmz.de/microorganisms/main.php).

The synthesis of Exopolysaccharides (EPS) by *L. reuteri* (LB 121 and other strains) was performed as described elsewhere (Van Geel-Schutten *et al*., 1999). EPS from *L. sanfranciscensis, L. panis* and *L. pontis* were obtained on above mentioned specialized media supplemented by sucrose up to total concentration of carbon sources 120.0 g^{-1}.

Acquitisation of exopolysaccharides. After cultivation (72 h, semianaerobic conditions) EPS preparations were isolated and purified by repeated precipitations with ethanol as described elsewhere (Van Geel-Schutten *et al*., 1999). Obtained EPS preparations were used either a sole carbon source (120.0 g^{-1}) in the MRS medium of basic (glucose eliminated) composition or as a supplement (20.0 g^{-1}) to the modified (lactose 45.0 g^{-1} instead of glucose) MRS medium.

Peeled barley, rolled oats, rolled rye, buckwheat groats, split peas, white beans (all from A/S Dobeles Dzirnavnieks, Latvia) were grounded and flour mashes (60.0 g^{-1}) were prepared in tap water. The thermal and enzymatic (alpha-amylase) treatment of mashes were performed as described elsewhere (Bekers *et al*., 2005). For preparations of milk and soymilk media, reconstituted (75.0 g^{-1}) dry skimmed milk (A/S Valmieras piens, Latvia) or instant soymilk (Alma-Sinta, Latvia) (75.0 g^{-1}) were used. Apple and carrot (local market, Latvia) squashes were prepared by the mechanical squiser. All food substrates were supplemented by 100.0 g^{-1} sucrose, as well as by 3.0 g^{-1} triptone from casein (Difco, USA) and 3.0 g^{-1} yeast extract (Sigma, Germany), to facilitate the growth of LAB and bifidobacteria. Media were sterilized by autoclaving (121°C, 15 min), possible liberation of additional reducing sugars were checked and found to be negligible.

Fermentations were carried out under semianaerobic conditions (BBL Gas Pak 150™ System, USA) at the appropriate temperature for each strain as mentioned above. Two percent of stationary phase culture (24-48 h) grown in the appropriate medium was used as an inocula for fermentations. The concentration of cells in inocula was checked by OD_{550} measurements (Shimadzu UV-vis, Japan) and equalized if necessary.

Other fructan sources. Inulin from dahlia tubers (Sigma, Germany), fructooligosaccharide preparation Nutraflora FOS® (Twinlab, USA), food-grade inulin from chicory Raftiline ST (Orafti, Belgium), food-grade oligosaccharides Raftilose L60/75 (Orafti, Belgium) and

Table 1: Characteristics of exopolysaccharides synthesized by lactobacilli on sucrose as a substrate

Culture	EPS average molecular mass, kDa			An average glucan/fructan ratio	EPS produced, g L^{-1}
	Total EPS	Glucan[a]	Fructan[a]		
L. reuteri MBI-C42	114	114	95	1/0.8	6.0
L. reuteri MBI-C43	66	95	55	1/2.1	5.4
L. panis MBI-V35	867	867	867	1/2.9	
	345	415	345	1/1.5	8.6
	137	79	165	1/1.2	

[a]Apparent average molecular mass calculated from the changes of glucose/fructose ratio within the fraction of total EPS

Table 2: Acidification power of *Lactobacillus reuteri* LB 121 on different food substrates in response to supplementation with sucrose and á-amylase treatment, during 24 h fermentation

Food substrate	Co-substrate and á-amylase treatment					
	Control[a]		Sucrose 120.0 g L^{-1},without á-amylase treatment		Sucrose 120.0 g L^{-1}, á-amylase treatment	
	Titrable acidity,°T	pH	Titrable acidity,°T	pH	Titrable acidity,°T	pH
Oat mash	8.6	5.46	42.2	3.90	65.0	3.65
Barley mash	16.8	4.80	70.2	3.64	77.4	3.56
Pease mash	24.4	4.89	74.9	3.69	72.2	3.83
Buckwheat mash	17.5	5.68	70.2	3.77	75.9	3.89
Bean mash	25.0	4.76	49.5	4.12	54.1	4.12
Rye mash	15.0	4.98	58.0	3.63	75.2	3.83
Soymilk [b]	22.2	6.18	24.8	6.02	ND	
Milk [b]	26.0	5.84	83.2	4.17	ND	
Carrot squash [b]	95.8	3.69	79.5	3.76	ND	
Apple squash [b]	67.7	3.99	68.3	3.97	ND	

[a] without supplemantation by sucrose and á-amylase treatment; [b] without á-amylase treatment.

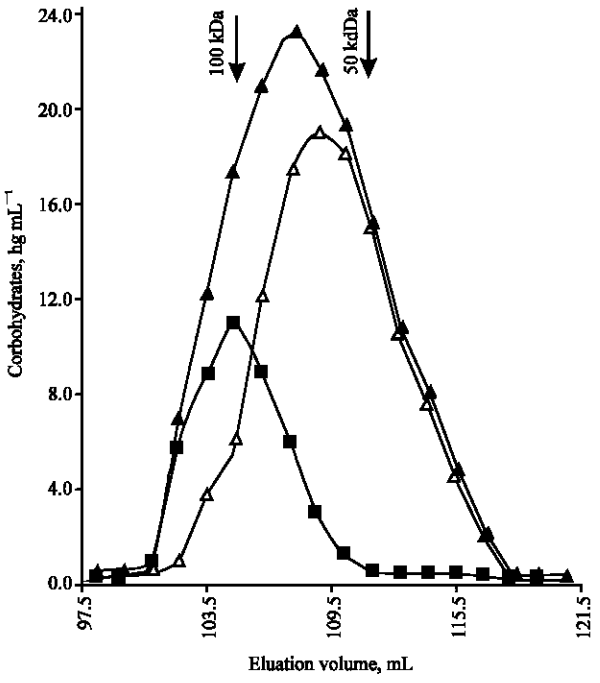

Fig. 1: Gel-filtration profile of the exopolysaccharide from *Lactobacillus reureri* MBI-C43 under conditions as described in Materials and Methods.Apparent profiles of glucose (■) and fructose (▲) polymers as calculated from the content of total carbohydrates (◊) and the estimated ratio between glucose and fructose in the fraction

based substrates as well as milk (Table 2 and 3) revealed certain strain specifity in regard to the acidification power of EPS-producing LAB. Thus, all *L. reuteri* strains appeared compatible with the apple and carrot squashes as well as (except *L.reuteri* LB 121) with the raffinose containing substrates, however the fermentations of soymilk were less pronounced and only *L. reuteri* MBI-C42 demonstrated an acceptable acidification power (Table 2 and 3). In some contrast to the *L. reuteri* LB 121, the strains MBI-C42 and MBI-C43 were capable to ferment milk, however the efficiency of process strongly depended on the presence of yeast extract and tryptone in the medium. The bean and pease mashes and the apple squashes appeared as suitable food matrices for *L. panis* and *L. pontis* whereas other employed substrates fall behind the efficiency of fermentation achieved by *L. reuteri* strains (Table 2 and 3).

Sucrose as a common substrate is essential for both glucan and fructan EPS production by LAB (Welman and Moddox, 2003; Van Geel-Schutten *et al*., 1999; Russ- Madiedo *et al*., 2005). However, to provide satisfactory levels of EPS synthesis the fermentation medium, such as milk or substrates of plant origin, requires the sucrose supplement due to its absence or a small content in basic substrates. Such a supplement substantially improved the fermentation of majority of substrates (Table 2), however, did not affect or even inhibited the acidification of apple and carrot squashes,

Table 3: Formation of titrable acids by fructan-producing *Lactobacillus* strains on different food substrates supplemented with sucrose (120.0 g L^{-1}), during 48 h fermentation

	Titrable acidity, °T				
Foodsubstrates	*L. reuteri* MBI-C42	*L. reuteri* MBI-C43	*L. panis*	*L. pontis*	*L. sanfranciscensis*
Oat mash	148.2	132.6	27.5	35.5	75.8
Barley mash	108.4	143.1	20.6	38.9	81.7
Pease mash	125.5	181.9	61.5	72.1	58.6
Buckwheat mash	125.3	139.4	46.6	42.1	80.4
Bean mash	159.5	117.5	70.2	51.6	91.3
Rye mash	132.3	182.3	19.3	37.3	56.6
Soymilk [a]	68.7	17.9	22.7	29.2	28.2
Milk [a]	144.1	188.9	53.0	44.4	82.2
Carrot squash [a]	192.2	182.4	36.2	65.0	97.8
Apple squash [a]	120.1	144.7	102.8	98.0	103.2

[a]Without á-amylase treatment

Table 4: Formation of fructans during 48 h fermentation of food substrates treated with á-amylase and supplemented with sucrose 120.0 g L^{-1}

	Final concentration of fructans, mg/g dry mass of substrate		
Substrate culture	Ray mash	Carrot squash	Milk
L. reuteri LB 121	7.7	9.9	6.2
L. reuteri MBI-C42	22.0	10.7	8.4
L. reuteri MBI-C43	26.1	10.1	9.3
L. panis MBI-V35	7.3	1.9	3.2
L. sanfranciscensis DSM 20451	9.4	5.6	3.5

Table 5: The influence of various exogenously added fructan supplements on the *Bifidobacterium lactis* Bb 12 biomass formation in the MRS-lactose medium, during 24 h

Fructan supplements	Biomass, g L^{-1}
None	0.38
Levan (*Z. mobilis*)	0.81
Inulin (Sigma)	0.48
Nutraflora FOS	0.72
Raftiline	0.76
Raftilose	0.73
EPS of *L. reuteri* LB 121 (from sucrose)[a]	1.15
EPS of *L. reuteri* LB 121 (from raffinose)[b]	0.82

[a]EPS of *L. reuteri* LB 121 containing fructan and glucan complex, according to Van Geel-Schutten *et al.* (1999); [b] EPS of *L. reuteri* LB 121 containing individual fructan, according to Van Geel-Schutten *et al.* (1999)

levan (ß-2,6-polyfructan) obtained by sucrose fermentation with *Zymomonas mobilis* 113S (Bekers *et al.*, 2005) were used throughout the study.

Analytical determinations. Gel-filtrations of EPS preparation were performed using the column Amerscham Biosciences XK (1.6×70 cm) packed with Sepharyl S-400 HR gel (Pharmacia Biotech) and 0.1 M NaCl as the mobile phase at a flow rate 0.75 mL min^{-1} and 2 min interval for each fraction. Dextrans (Pharmacia, Sweden) of varied molecular mass (100-4000 kDa) were used to obtain the calibration curve for EPS molecular mass determination. The composition of low molecular mass carbohydrates in culture liquids was analyzed by High-Pressure Liquid Chromatography (HPLC) using a Jordi Gel GBR column (0.8×30 cm) and Agilent 1100 refractive Index detector. The column temperature was kept at 35°C and dimethylsulfoxide was used as the mobile phase at a flow rate of 0.6 mL min^{-1}. The growth of cultures in the MRS medium was monitored by spectrophotometric measurements of biomass concentration at 550 nm as described previously (Semjonovs and Zikmanis, 2007; Semjonovs *et al.*, 2005). Concentration of total carbohydrates was estimated by the anthron method (Morris, 1948), content of glucose and fructose was determined by the enzymatic assay with the Enzytec™ D-glucose D-fructose kit (Scil Diagnostics GmbH, Germany).

EPS composition after acid hydrolysis (H_2SO_4 (1 mol L^{-1}), 100°C, 2 h) was analyzed by High-Pressure Liquid Chromatography (HPLC) using a Zorbax Carbohydrate column (0.46×25 cm) and Agilent 1100 refraction detector. The column temperature was kept at 30°C and acetylnitrile aquatic solution (70:30) was used as the mobile phase at a flow rate of 1.2 mL min^{-1}. The concentration of fructans was specified by selective procedures of enzymatic analysis as described elsewhere (Dysseler *et al.*, 1999). Titrable acidity (°T) was determined by alkaline titration (0.1 N NaOH) of samples, using phenolphthalein as an indicator as described previously (Semjonovs and Zikmanis, 2007; Semjonovs *et al.*, 2005). Each experiment was performed at least in triplicate and data are presented as averages, where standard deviation did not exceed 10% of the mean.

RESULTS AND DISCUSSION

Alike recently reported exopolysaccharide-producing bacteria *Lactobacillus reuteri* LB 121 (Van Geel-Schutten *et al.*, 1999) and *L. sanfranciscensis* strains (Tieking *et al.*, 2003). The strains under study were found to be promising producers of EPS (Fig. 1; Table 1). The range of EPS concentrations in the growth medium (6-8.6 g^{-1}) was comparable with that o bserved for *L. reuteri* LB 121 (9-9.8 g^{-1}) and *L. sanfranciscensis* (Van Geel-Schutten *et al.*, 1999; Tieking, 2005). These EPS preparations appeared as mixtures of high-molecular

mass glucose- and fructose- polymers since the glucose/fructose ratio varied across all fractions of eluent during gel-filtration procedures. The calculated apparent average molecular mass (Fig. 1, Table 2) were found to be close for both glucose- and fructose-polymers. Contrary to the *L. reuteri* strains MBI-C42 and MBI-C43 the EPS preparation from L.panis MBI-V25 displayed 3 fractions of varied molecular mass (Table 1) where the fraction of highes molecular mass appeared as more fructan-rich, however undistinguishable from glucan in this respect (867 kDa for both polymers).

In addition to high-molecular mass EPS noticeable amount (3 - 15 %) of total carbohydrates was formed in the fermentation medium. Thus, the profiles of HPLC chromatography (Fig. 2 a, b) displayed the presence of 1-kestose and nystose as main Fructooligosaccharides (FOS), however in different proportions depending on strains under study. The relatively low concentration (=0.1) g^{-1}) of high-molecular mass carbohydrate in growth medium of *L. pontis* MBI-C40 was insufficient to isolate EPS in preparative amounts, however fractions of low-molecular mass carbohydrate polymers (1100-5000 kDa) and FOS (1-kestose and nystose) were represented in the fermentation medium (Fig. 2 a). The same compounds were characteristic of *L.reuteri* LB 121 as could be expected from the previously reported data for this strain (Van Geel-Schutten *et al*., 1999) and 1-kestose was found as the main constituent of the FOS fraction (Fig. 2b). The prebiotic and bifidogenic properties of these low-molecular mass compounds have been well-documented (Roberfroid *et al*., 1998; Biedrzycka and Bielecka, 2004; Marx *et al*., 2000) and, therefore, their contribution should be considered in overall estimates of EPS-producing LAB. On the other hand, to realize *in situ* the synthesis of prebiotic substances in order to obtain synbiotic products by means of lactic acid fermentations compatibility between growth requirements of EPS-producing strains and properties of substrates, i.e., potent food matrices, should be assessed.

It has been shown that the biosynthesis of EPS by *L. reuteri* LB 121 as well as by other LAB (Welman and Moddox, 2003; Van Geel-Schutten *et al*., 1999) is a growth associated process and proceeds concomitantly with the production of organic acids (principally lactate) and, therefore, could be evaluated by the acidification power and/or biomass formation for each particular strain and substrate under study. Comparative estimates of various food substrates, including cereal-based (oat, barley, rye, buckwheat), raffinose-containing (beans, pease, soymilk), fruits (apple) and vegetable (carrot)-
perhaps, due to the high content of other rapidly assimilated carbon sources.

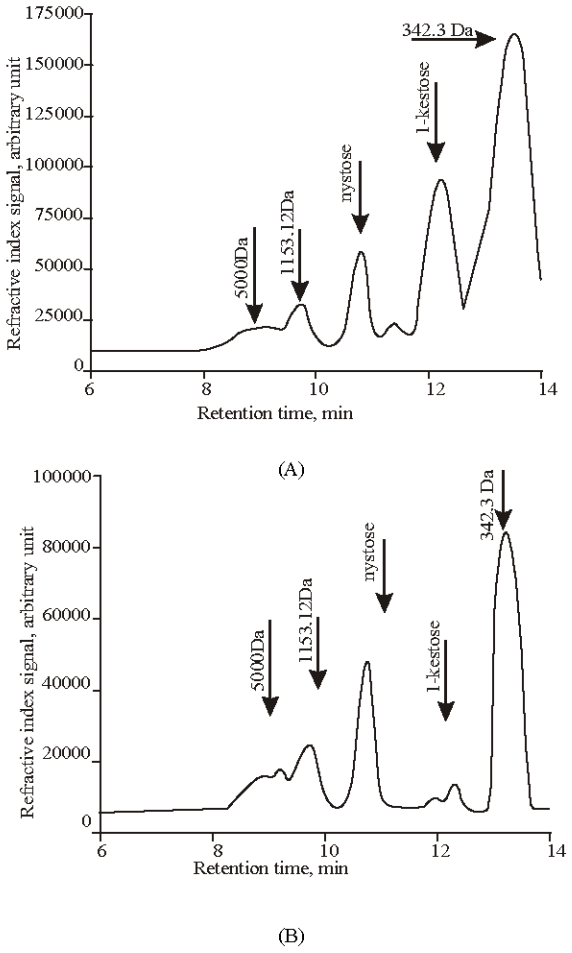

Fig. 2 a, b: HPLC profiles of fructan-containing oligo- and polysaccharides from the growth medium of *Lactobacillus reuteri* LB 121 (a) and *Lactobacillus pontis* (b) under conditions as described in Materials and Methods

The use of cereal-based substrates are considered as a promising alternative to fermented dairy products due to a high nutritional value and the presence of both soluble and insoluble dietary fibers (Angelov *et al*., 2005; Martensson *et al*., 2005; Bekers *et al*., 2001). The treatment of oat-based substrate by á-amylase was shown to enhance the growth of LAB keeping the same level of functionally important ß-glucan (Bekers *et al*., 2005; Bekers *et al*., 2004). As shown in Table 2, the treatment with á-amylase markedly improved the efficiency of fermentation of cereal-based and raffinose-containing substrates.

In repeatedly performed fermentations of food substrates it was determined whether those strains producing EPS in the modified MRS and containing the sucrose supplement also produce EPS during fermentation of milk and food substrates of plant origin. For all

Table 6: The influence of exopolysaccharydes from *L. reuteri* LB 121 and *L. panis* MBI-V35 (sintesized on sucrose) on the growth and acidification power of *Bifidobacterium* during 48 h fermentation in the MRS-lactose medium

	Carbon source in the medium									
	Lactose[a]		*L. reuteri*LB 121 EPS[a]		*L. panis* EPS[a]		*L. reuteri* LB 121 EPS[b]		*L. panis* EPS[b] strains	
Bifidobacterium	Biomass, g L^{-1}	Titrable acidity, °T	Biomass, g L^{-1}	Titrable acidity, °T	Biomass, g L^{-1}	Titrable acidity, °T	Biomass, g L^{-1}	Titrable acidity, °T	Biomass, g L^{-1}	Titrable acidity, °T
B. lactis Bb 12	0.99	122.4	0.26	52.3	0.66	56.1	1.14	137.6	1.14	145.1
B. lactis OX-1	2.10	213.2	0.44	56.2	0.35	58.2	2.42	224.3	2.63	236.1
B. angulatum	0.11	37.3	0.11	38.4	0.40	73.5	0.15	38.7	0.18	46.5
B. brevis	0.11	38.4	0.10	33.5	0.06	43.8	0.13	41.2	0.25	45.1
B. longum	0.11	37.8	0.36	58.3	0.29	68.5	0.27	58.4	0.25	52.6
B. animalis	0.97	142.1	0.34	56.4	0.30	64.6	1.10	160.8	1.07	179.7
B.pseudoca-tenulatum	0.11	39.2	0.10	35.2	0.11	42.1	0.13	44.2	0.19	50.2

[a] carbohydrate as a sole carbon source;[b] carbohydrate as a supplement (20.0 g L^{-1}) to the MRS-lactose medium

Table 7: The influence exopolysaccharides (EPS) from lactic acid bacteria on the formation of biomasas by common probiotic dairy starter cultures during 24 h in the modified MRS medium

	Biomass, g L^{-1}			
Carbon source	*B. lactis* Bb12	*L. bulgaricus*	*L. acidophilus*	*S. thermophilus*
Lactose (Control)	0.36	1.37	0.40	1.13
L. reuteri MBI-C42				
EPS from sucrose:[a]	0.38	0.73	0.62	0.67
Lactose + EPS [b]	0.56	1.60	0.76	0.60
L. reuteri MBI-C43				
EPS from sucrose:[a]	0.35	0.76	0.54	0.46
Lactose + EPS [b]	0.50	1.54	0.62	0.38
L. panis MBI-V35				
EPS from sucrose:[a]	0.23	2.94	0.28	0.87
Lactose + EPS [b]	0.47	2.81	0.11	1.08
L. sanfranciscensis DSM20451				
EPS from sucrose:[a]	0.37	1.24	0.58	0.96
Lactose + EPS [b]	0.50	2.37	0.72	0.87
L. reuteri LB 121				
EPS from sucrose:[a]	0.10	3.78	0.12	2.38
Lactose + EPS [b]	1.12	2.44	0.94	1.11
EPS from rafinose:[a]	0.06	3.19	0.16	0.84
Lactose + EPS [b]	0.80	1.45	0.91	0.80

[a] EPS as a sole carbon source in the modified MRS medium; [b] EPS as a supplement (20.0 g L^{-1}) in the MRS- lactose medium

evaluated EPS-producing strains (Table 4) *in situ* production of fructose polymers at levels ranging from 1.9-27.7 mg g^{-1} of dry mass of substrate was demonstrated. These findings confirm the possibility to enrich of lactobacilli fermented food by prebiotic substances using the EPS-producing strains of LAB, i.e., to advance towards qualities of synbiotic products. Detected levels of fructose polymers could reflect the total amount of EPS in characteristic proportion of the particular strain (Table 1), which however, might be somewhat affected due to the possible operation of extracellular glucan- and/or fructan-hydrolases. Nevertheless, observed concentration of EPS were comparable and even exceeded those which have been reported for *in situ* production of EPS during sourdough fermentations (Tieking *et al*., 2003) by LAB.

The ability of any prebiotic substance maintain its influence over the Gastrointestinal Tract (GIT) is of primary importance to improve the microbial balance by the growth stimulation of probiotic LAB and bifidobacteria within the native microflora of GIT (Roberfroid *et al*., 1998; Biedrzycka and Bie lecka, 2004). On the other hand, it is quite obviously to assume that synbiotic products which contain highest contents of probiotic LAB could better contribute into these overall beneficial effects.

Recently we have shown that several fructan compounds of microbial or plant origin, such as levan from *Zymomonas mobilais* (Semjonovs *et al*., 2004, 2005, 2006; Semjonovs and Zikmais, 2007) or oligomers from Jerusalem artichoke tubers (Semjonovs *et al*., 2007) can be utilized as a sole carbon source and promote the growth of bifidobacteria and probiotic LAB even on the background of relatively high concentration of other carbohydrates, such as lactose (40-48 g^{-1}) in milk. The preparations of EPS obtained from LAB exhibited similar properties in this respect (Table 5-7), however some characteristic qualities were observed for particular strain and EPS under study.

Thus, the EPS preparation from *L. reuteri* LB 121 which contains both glucan and fructan polymers when synthesized on sucrose as a carbon source (Van Geel-Schutten *et al*., 1999) was more efficient in comparison with the individual fructan polymers (synthesized on raffinose) from this strain, possibly, due to the more pronounced activity of extracellular glycosidases of *Bifidobacterium lactis* Bb 12 (Table 5).

Besides, some other constituent cultures of traditionally used dairy starters were capable to assimilate EPS either a sole carbon source or as a supplement to the lactose-containing MRS medium (Table 7). Both EPS from *L. reuteri* LB 121 obtained on sucrose or raffinose as the substrates markedly enhanced the growth of *L. bulgaricus* and *S. thermophilus* as compared to the lactose-containing medium, alike EPS from *L. panis* in respect to *L. bulgaricus*. In a similar way EPS from *L. sanfranciscensis* appeared as more efficient carbon source in regard to *L. acidophilus* (Table 7). All the EPS under study stimulated the growth of *L. bulgaricus* in the presence of lactose whereas the growth of *S. thermophilus* remained unaffected or even inhibited in the presence of EPS from *L. reuteri* MBI-C42 and *L. reuteri* MBI-C43 (Table 7). Observed differences in growth responses suggest that a necessary balance between *S. thermophilus* and *L. bulgaricus* could be affected by the addition of these EPS to the dairy substrates and/or due to the biosynthesis of EPS in situ and have to be considered to achieve desired functional qualities of products. For instance, the optimal growth of *S. thermophilus* is required to maintain the level of L(+)-lactic acid as an essential functional quality of yogurt (Tamime and Robison, 1999). However, the well-known probiotic relationships between *S. thermophilus* and *L. bulgaricus* (Semjonovs *et al*., 2007; Tamime and Robison, 1999; Zourari *et al*., 1992) could help to maintain the necessary balance during dairy fermentation by combined starters.

The above results suggest that EPS- producing LAB should be considered as potent constituents of starters to perform fermentation of various fruits-, vegetables-, cereal-, pulses-based or lactose containing food substrates. These cultures are capable to synthesize *in situ* fructan polymers from sucrose as a co-substrate and therefore could contribute into synbiotic qualities of the products of lactic acid fermentation. However, possible strain-specific effects of EPS from different sources in respect o f prebiotic starter cultures should be considered and remain to be elucidated during further study.

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