Romanian Biotechnological Letters *Vol. 18, No. 5, 2013*

ORIGINAL PAPER

Prebiotic potential of some exopolysaccharides produced by lactic acid bacteria

Received for publication, May 15, 2013
Accepted, September 26, 2013

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Abstract

The aims of this study were to investigate the digestibility and the stability of six exopolysaccharides (EPS) produced by lactic acid bacteria isolated from raw milk and traditional fermented dairy products, and to evaluate their prebiotic potential. Four homopolysaccharides (HoPS, composed of glucose solely) produced by Lactococcus lactis 1.8, Leuconostoc citreum 1.12, Leuc. citreum 2.8, and Weissella confusa/cibaria 38.2 and two heteropolysaccharides (HePS) produced by Streptococcus thermophilus ST 111 (repeating unit composed of galactose and rhamnose in a 5:2 ratio) and S. thermophilus LY03 (repeating unit with a galactose:glucose ratio of 4:1) were tested. No degradation of the six EPS was seen during in vitro digestions mimicking the passage through the upper gastrointestinal tract. The two Clostridium strains used in the present study as well as Anaerostipes caccae LMG 14662^T and Roseburia intestinalis LMG 14610^T could not metabolize any of the six EPS tested, while the two Bacteroides strains grew on all EPS and L. casei YIT 9029 grew on all HoPS. The HoPS of L. lactis 1.8 proved to be the most susceptible for degradation, particularly by Bifidobacterium pseudocatenulatum LMG 10505^T. The fermentation profile of L. lactis 1.8 EPS by B. pseudocatenulatum LMG 10505^T indicated the prebiotic potential of this substrate.

Key words: prebiotics, exopolysaccharides, fermented dairy products, lactic acid bacteria

Introduction

Lactic acid bacteria (LAB) constitute a heterogeneous group of industrially important bacteria that are traditionally used to produce fermented foods and beverages as well as fine chemicals such as lactic acid and dextrans (WOOD & HOLZAPFEL [1]; WOOD [2]). During the last few decades, development of the functional food concept and, more specifically, the application of certain LAB strains as probiotics, have created new perspectives for LAB research and human consumption, attracting the attention of both food scientists and health professionals (DE VUYST & al. [3]).

The application of functional foods to modulate the composition of the gut microbiota is considered both a safe and accessible strategy for the promotion of human gastrointestinal health (FOOKS & GIBSON [4]; RASTALL al. [5]). Throughout the years, gastrointestinal health research has focused on two main groups of functional food ingredients, namely probiotics and prebiotics. Probiotics are live microorganisms that, when consumed in an adequate amount as part of the food, confer a health benefit on the host (FAO/WHO, [6]). Prebiotics are non-digestible food ingredients that selectively stimulate the growth and/or activity of beneficial bacteria, such as bifidobacteria and lactobacilli, strains of which are

known to display health-promoting properties (GIBSON & ROBERFROID [7]; GIBSON & al.[8]). The prebiotic approach overcomes potential disavantages inherently associated with a probiotic strategy, such as the loss of the viability of probiotic strains either during manufacturing and product storage (formulation, cooling, etc.) or gastrointestinal transit (stomach acid, bile salts, and intestinal digestive enzymes) (SU & al. [9]).
Oligosaccharides are the most commonly used prebiotic food ingredients (DE VUYST & al. [4]; MACFARLANE & al. [10]). Among them, inulin-type fructans are by far the most studied and have therefore acquired a status of model prebiotics (BOSSCHER & al. [11]). Inulin-type fructans, such as inulin and oligofructose, escape upper gastrointestinal digestion and absorption and cause upon colon fermentation a numerical increase of the resident bifidobacterial communities, referred to as the bifidogenic effect, because of the β-fructofuranosidase activity of particular bifidobacterial species or strains on the β-1,2-linkages of these fructose polymers (GIBSON & al. [12]; PALFRAMAN & al. [13]; ROSSI & al. [14]). Also, bifidobacteria enhance large-intestinal production of short-chain fatty acids, in particular butyrate, referred to as the butyrogenic effect, through cross-feeding (BELENGUER & al., [15]; FALONY & al. [16]; DE VUYST & LEROY [17]) . Consumption of inulin-type fructans and other prebiotics has been linked with significant health benefits, more specifically in relation to their improvement of gut balancing and transit, influence on mineral absorption, lipid metabolism, putative anti-cancer properties, and immunomodulating effects (RASTALL & al. [5]; BOSSCHER & al. [11]).
It has been suggested that other food compounds such as exopolysaccharides (EPS) might be able to exert a similar prebiotic effect on the human intestinal microbiota, although scientific data on the potential of these compounds are scarce (RUIJSSENAARS & al. [18]; DAL BELLO & al. [19]; KORAKLI & al. [20]; CINQUIN & al. [21]; SALAZAR & al. [22, 23]). EPS from LAB are high-molecular-mass sugar polymers encompassing homopolysaccharides (HoPS) and heteropolysaccharides (HePS) (DE VUYST & VANINGELGEM [24]; DE VUYST & DE VIN [25]; RUAS-MADIEDO & al. 26]). HoPS are polymerized extracelluarly by cell wall-anchored glycansucrases that use sucrose as substrate and are composed of either glucose (α-*D*-glucans) or fructose (β-*D*-fructans). HePS are polymers of a repeating unit (an oligosaccharide of three up to eight monosaccharides) that is synthesized out of sugar nucleotides intracellularly and polymerized extracellularly. From a technological point of view, EPS-producing LAB contribute to the texture, rheology, and mouthfeel of fermented milk products (DE VUYST & VANINGELGEM [24]).
The aims of this study were to investigate the digestibility and the stability of six EPS produced by LAB isolated from raw milk and fermented dairy products and to evaluate the prebiotic potential of these polymers.

Materials and methods

Microorganisms and their maintenance

The following bacteria from human intestinal origin (unless stated otherwise) were used as to test their ability to degrade selected EPS: *Bacteroides thetaiotaomicron* LMG 11262, *Bacteroides fragilis* LMG 10263^T, *Bifidobacterium adolescentis* LMG 10502^T, *Bifidobacterium angulatum* LMG 11568 (sewage), *Bifidobacterium bifidum* LMG 11583, *Bifidobacterium breve* LMG 11084 (human blood), *Bifidobacterium dentium* LMG 10507, *Bifidobacterium longum* subsp. *infantis* LMG 11570, *Bifidobacterium pseudocatenulatum* LMG 10505^T, *Bifidobacterium pseudolongum* subsp. *globosum* LMG 11614 (bovine rumen), and *Clostridium perfringens* LMG 11264^T (cow); they were purchased from the Belgian Co-

ordinated Collections of Microorganisms/Laboratory for Microbiology Ghent (BCCM/LMG, Ghent, Belgium). Also, *Anaerostipes caccae* DSM 14662^T [Deutsche Sammlung von Mikro-Organismen und Zellkulturen (DSMZ), Göttingen, Germany], *Bifidobacterium animalis* subsp. *lactis* Bb12 (yoghurt; Technical Research Centre of Finland, Espoo, Finland), *Bifidobacterium longum* BB536 (Morinaga Industry Co. Ltd., Tokyo, Japan), *Clostridium clostridioforme* DSM 933^T (calf's rumen), *Lactobacillus casei* YIT 9029 (Yakult Honsha Co. Ltd., Tokyo, Japan), *Lactobacillus paracasei* subsp. *paracasei* 8700:2 [29], and *Roseburia intestinalis* DSM 14610^T (DSMZ) were tested. All strains were stored at -80°C in the appropriate medium [reinforced clostridial medium (Oxoid) for *Bifidobacterium* spp., *A. caccae* DSM 14662^T, and *R. intestinalis* DSM 14610^T; MRS (Oxoid) for *Lactobacillus* spp.; Wilkins-Chalgren anaerobe broth (Oxoid) for *Bacteroides* spp. and *Clostridium* spp.].
Solid culture media were prepared by adding 1.5% (w/v) of agar (Oxoid) to the respective media.

EPS material

Six EPS, namely four HoPS (composed of glucose solely) produced by *Lactococcus lactis* 1.8, *Leuconostoc citreum* 1.12, *Leuc. citreum* 2.8, and *Weissella confusa/cibaria* 38.2 (VAN DER MEULEN & al. [27]) and two HePS produced by *S. thermophilus* ST 111 (repeating unit composed of galactose and rhamnose in a 5:2 ratio; VANINGELGEM & al. [28]) and *S. thermophilus* LY03 (repeating unit with a galactose:glucose ratio of 4:1; DE VUYST & al. [29]), were used in this study. The EPS were isolated from the corresponding cultures by acetone precipitation, purified by extensive dialysis and freeze-dryed (VANINGELGEM & al. [28]).

Digestibility and stability

In vitro digestions of the six EPS were carried out in a fermentor to mimic the passage through the upper part of the gastrointestinal tract. The resistance to low pH, digestive enzymes (pepsin and pancreatin), and bile salts was tested. Therefore, dialyzed EPS were used as aqueous solutions in a final concentration of 10.0 g liter^{-1} (HoPS), 6.0 g liter^{-1} (HePS from *S. thermophilus* ST 111), and 1.2 g liter^{-1} (HePS from *S. thermophilus* LY03), the tested concentrations being dependent on the production yield obtained in filtrated mMRS medium (HoPS and LY03-HePS; VAN DER MEULEN & al. [27]) or milk medium supplemented with 50 g liter^{-1} of sucrose and 16 g liter^{-1} of lactalbumin hydrolysate (ST 111-HePS, VANINGELGEM & al. [28]). As controls, starch (Merck), amylopectin, dextran, and inulin (BENEO-Orafti, Tienen, Belgium) were used (10.0 g liter^{-1}). Digestions were carried out in duplicate in 2-liter Biostat B-DCU fermentors (Sartorius AG/B. Braun Biotech International, Melsungen, Germany) containing one liter of an aqueous polymer solution. The incubation temperature was kept constant at 37°C; the initial pH was set at 4.8. During the experiments, a pH profile mimicking the evolution of pH upon gastrointestinal transit through stomach and small intestine was imposed (MINEKUS & al. [30]), using 1 M solutions of HCl and $NaHCO_3$. The profile applied was as follows: 0 min, pH 4.8; 90 min, pH 1.7; 120 min, pH 1.7; 135 min, pH 7.0; and 300 min, pH 7.0 (Fig. 1).

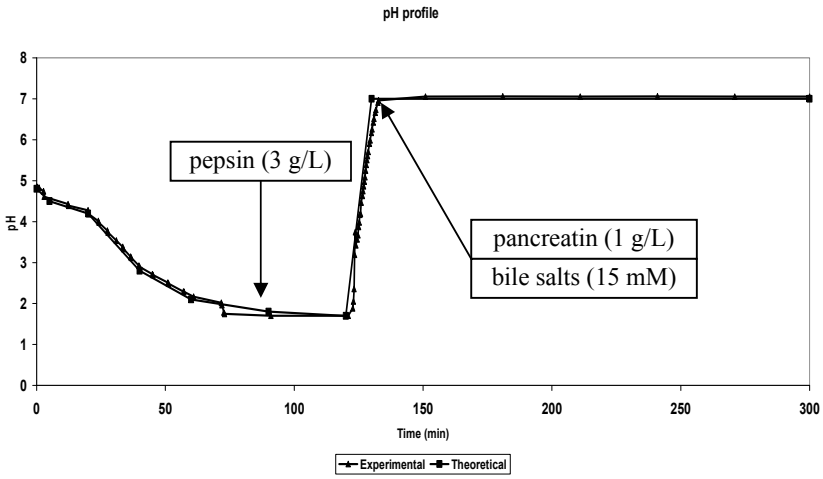


Fig. 1 Experimental design for the *in vitro* simulation of the gastrointestinal passage of EPS as to test their resistance and stability towards digestion. ▲, Experimental, ■, theoretical.

Temperature and pH were controlled on-line (MFCS/win 2.1; Sartorius AG/B. Braun Biotech). After 90 min, pepsin (Sigma-Aldrich) was added in a final concentration of 3 g liter^{-1}, and after 135 min pancreatin (Sigma-Aldrich) and bile salts (sodium salts of cholic acid and deoxycholic acid, Sigma-Aldrich) were added in a final concentration of 1 g liter^{-1} and 15 mmol liter^{-1}, respectively (Fig. 1). Samples were taken at regular time intervals to assess polymer degradation during incubation through high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (VANINGELGEM & al. [31]).

Prebiotic effect of exopolysaccharides

Fermentation studies were carried out in a medium for colon bacteria (MCB), designed to support growth of various human colon bacteria when supplemented with an adequate energy source (VAN DER MEULEN & al. [32].

Small-scale fermentations. Small-scale (10 mL) fermentations were carried out in duplicate in MCB supplemented with 5 g liter^{-1} of the EPS under investigation as the sole energy source. For monoculture fermentations with *A. caccae* DSM 14662^{T} and *R. intestinalis* DSM 14610^{T}, modified MCB (mMCB) was prepared by adding 6.8 g liter^{-1} of sodium acetate trihydrate to MCB (FALONY & al. [16]). Separately autoclaved EPS were added after sterilization of the media (autoclaving at 210 kPa and 121°C for 20 min). All strains tested were incubated (inocula of 2%, v/v) anaerobically at 37°C for up to 192 h in a modular atmosphere-controlled system (MACS; Don Whitley Scientific Ltd., West Yorkshire, United Kingdom) that was continuously sparged with a mixture of 80% N_2, 10% CO_2, and 10% H_2 (Air Liquide). Samples were taken after visual confirmation of growth. Bacterial growth was determined by measuring the optical density at 600 nm (OD_{600}). EPS degradation was monitored by gel permeation chromatography (GPC) (VAN DER MEULEN & al. [27]).

Intermediate-scale fermentations. Intermediate-scale fermentations with *B. pseudocatenulatum* LMG 10505^{T} were performed in 2-liter B-DCU fermentors containing 1.5 liters of MCB (inocula of 5%, v/v). The pH of the medium was adjusted to 6.3 before autoclaving at 210 kPa and 121°C for 20 min. After sterilization, separately autoclaved EPS were added. Anaerobic conditions were assured during fermentation experiments by continuously sparging the medium with a mixture of 90% N_2 and 10% CO_2 (Air Liquide).

The fermentation temperature was kept constant at 37°C. A constant pH of 6.3 was maintained by automatic control using 1.5-M solutions of NaOH and H_3PO_4. To keep the medium homogeneous, a gentle stirring of 100 rpm was applied. Temperature, pH, and agitation speed were controlled on-line (MFCS/win 2.1). Fermentations were followed up during 48 h; samples for further analysis were taken at regular time intervals. Bacterial growth was determined by spread-plating of the samples on MCB agar containing 9 g $liter^{-1}$ of lactose as the sole energy source and incubation for 24 h under anaerobic conditions (in MACS).

Concentrations of residual glucose and fermentation end-products were determined through high-performance liquid chromatography, as described previously (FALONY & al. [16]).

Analysis of the breakdown of the EPS into different oligosaccharide fractions was performed through HPAEC-PAD.

All fermentations were performed in duplicate. The results and figures presented onwards are representative for both fermentations.

Results and discussions

The ability of LAB to produce EPS has been extensively studied, due to their potential applications in food industry. More recently, the potential prebiotic effect of these polymers is under investigation (HARUTOSHI [33]). Raw milk and traditional fermented dairy products from Romania proved to be rich sources for EPS-producing LAB strains (VANINGELGEM & al. [31]; VAN DER MEULEN & al. [27]). In the present study, some of the EPS isolated from these strains have been investigated from the perspective of their potential prebiotic effect.

Digestibility and stability

HPAEC-PAD analyses of starch- and amylopectin-containing samples collected during the *in vitro* simulation experiments showed a substantial increase of glucose monomers in the samples after the addition of amylase-containing pancreatin, as shown for the experimental samples with starch (Fig. 2). No monomer accumulation was detected for dextran and inulin or for the EPS under investigation, as shown for the ST111-HePS (Fig. 2).

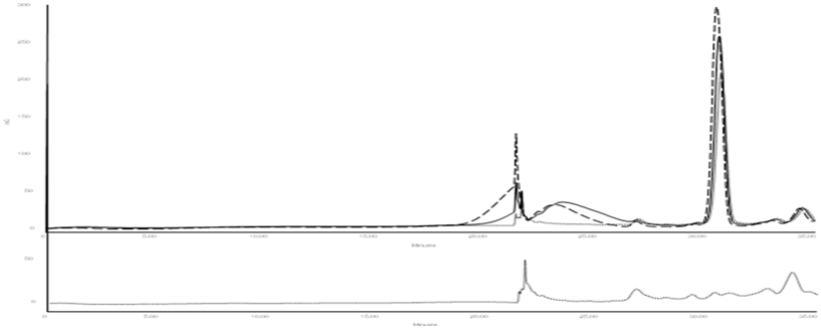


Fig. 2 HPAEC-PAD chromatograms of time-dependent samples of starch (upper graph) and ST 111-HePS (lower graph) collected during the *in vitro* simulation experiments of gastrointestinal passage after addition of pancreatin. ····, 180 min; —, 240 min; ----, 300 min.

The four HoPS tested in the present study seem to have a fiber effect, since none of them were degraded during the *in vitro* experiments simulating their gastrointestinal passage. Hence, they may reach the colon without being hydrolyzed or absorbed in the upper part of the gastrointestinal tract.

Prebiotic effect of exopolysaccharides

Small-scale fermentations (Fig. 3). Four *Bifidobacterium* strains out of ten tested, namely *B. angulatum* LMG 11568, *B. breve* LMG 11084, *B. dentium* LMG 10507, and *B. pseudocatenulatum* LMG 10505^T, were able to grow on the HoPS produced by *L. lactis* 1.8, while only one strain (*B. breve* LMG 1084) could grow on the HoPS produced by *Leuc. citreum* 2.8. Minor growth of *L. casei* YIT 9029 was found on all HoPS. None of the *Bifidobacterium* spp. and *Lactobacillus* spp. tested were able to grow on the HePS added as the sole energy source. Both *Bact. thetaiotaomicron* LMG 11262 and *Bact. fragilis* LMG 10263^T could metabolize all EPS, but *C. perfringens* LMG 11264^T and *C. clostridioforme* DSM 933^T were not able to metabolize the EPS used in this study.

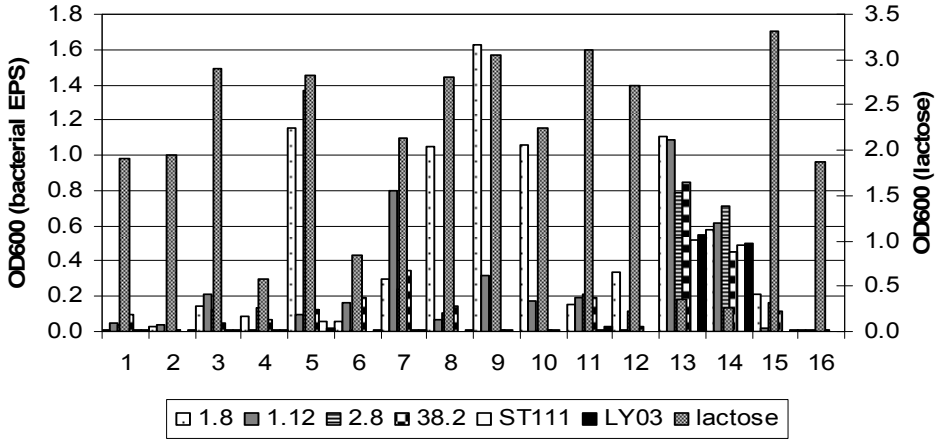


Fig. 3 Optical density at 600 nm of the cultures grown in MCB supplemented with lactose or six EPS. The strains used were 1 - *B. animalis* subsp. *lactis* Bb12; 2 - *B. longum* BB536; 3 - *B. adolescentis* LMG 10502^T; 4 - *B. bifidum* LMG 11583; 5 - *B. breve* LMG 11084; 6 - *B. pseudolongum* subsp. *globosum* LMG 11614; 7 - *B. infantis* LMG 11570; 8 - *B. angulatum* LMG 11568; 9 - *B. pseudocatenulatum* LMG 10505^T; 10 - *B. dentium* LMG 10507; 11 - *Lb. casei* YIT 9029; 12 - *Lb. paracasei* 8700:2; 13 - *Bact. thetaiotaomicron* LMG 11262; 14 - *Bact. fragilis* LMG 10263^T; 15 - *C. perfringens* LMG 11264^T; and 16 - *C. clostridioforme* DSM 933^T.

GPC analysis revealed a similar degradation pattern of the HoPS isolated from *L. lactis* 1.8 by the four positive *Bifidobacterium* spp., the digestion by *B. pseudocatenulatum* LMG 10505^T being most pronounced (Fig. 4).

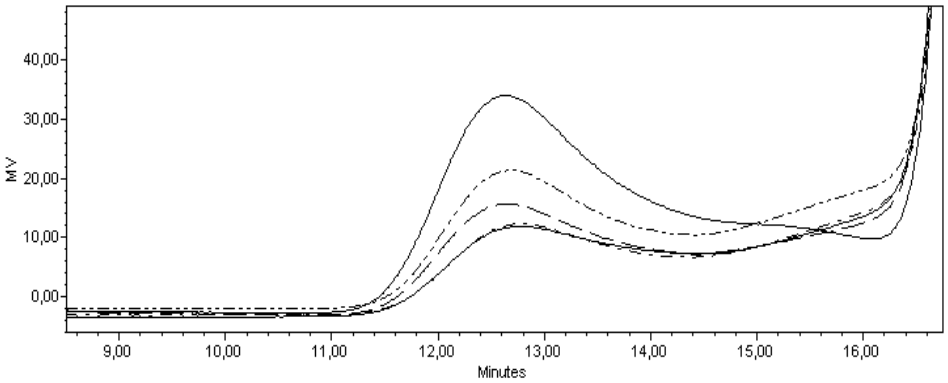
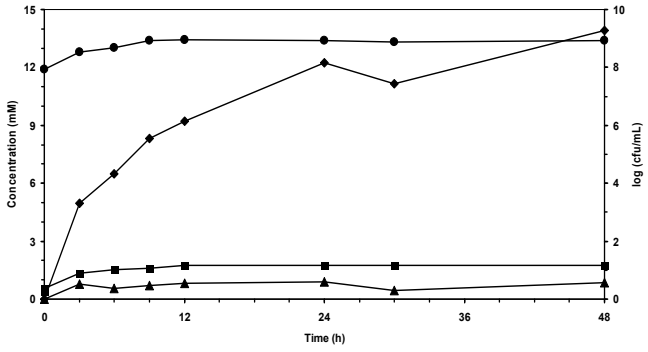


Fig. 4 GPC chromatograms of the EPS from *L. lactis* 1.8 before (—) and after fermentation with *B. angulatum* LMG 11568 (– ··· –), *B. breve* LMG 11084 (– – –), *B. dentium* LMG 10507 (····), and *B. pseudocatenulatum* LMG 10505^T (– · –).

Some *Bifidobacterium* strains (*B. angulatum* LMG 11568, *B. breve* LMG 11084, *B. longum* subsp. *infantis* LMG 11570, *B. pseudocatenulatum* LMG 10505^T, and *B. dentium* LMG 10507) were able to grow on one or more of these HoPS used as the sole energy source, as shown during the small-scale fermentation experiments. Such selective fermentation by beneficial colon bacteria, in particular bifidobacteria and lactobacilli, is a prerequisite for a prebiotic (FAO/WHO, [6]). Bifidobacteria represent a subdominant, functionally important bacterial group in the large-intestinal ecosystem of humans and many animals (MATSUKI & al. [34]; ZOETENDAL & al. [35]). *Bifidobacterium breve* and *B. longum* are predominant members of the bifidobacterial communities encountered in the colon of human infants (BENNO & al. [36]; BIAVATI & al. [37]), while *B. pseudocatenulatum* is the most common taxon inhabiting the human adult intestinal tract (MATSUKI & al. [38]). It has been suggested that *Bifidobacterium* spp. are linked with maintenance of general colon health and that the diversity and numbers of bifidobacteria provide a marker for the stability of the human intestinal microbiota (LEAHY& al. [39]; MAKRAS & DE VUYST [40]). Many attempts have been made to increase bifidobacterial numbers in the large intestine through direct consumption of certain beneficial strains and/or food ingredients that selectively stimulate the growth of these bacteria (RASTALL & al. [5]; GIBSON & al. [8]). The use of EPS from LAB for the stimulation of bifidobacterial growth has been suggested before (DAL BELLO & al. [19]; KORAKLI & al. [20]; SALAZAR & al. [22]; MARTENSSON & al. [41]).
None of the two clostridial strains tested during the present study could metabolize the EPS. In contrast, both bacteroidal species included in the experiments could grow on all EPS tested. Since *Bacteroides* species are very versatile saccharolytic bacteria, their capacity to metabolize bacterial EPS was expected (SALAZAR & al. [23]; SONNENBURG & al. [42]). They contribute to food digestion considerably but seem not to be competitive enough toward bifidobacteria because of their overall degradation of undigestible polymers (FALONY & al. [43]; VAN DER MEULEN & al. [32, 44]).

Intermediate-scale fermentations. Minor growth of *B. pseudocatenulatum* LMG 10505^T was found in MCB supplemented with the HoPS of *L. lactis* 1.8 as the sole energy source, which was reflected in the relatively low final concentrations of the metabolic end-products found after 24 h of fermentation (acetate, 12 mmol liter^{-1}; lactate, 0.9 m mol liter^{-1}) (Fig. 5). HPAEC-PAD analysis showed a slight increase in the concentration of intermediate-length polymer fractions, indicating the presence of oligosaccharides resulting from EPS degradation (Fig. 5). Neither *A. caccae* LMG 14662^T nor *R. intestinalis* LMG 14610^T were able to grow in mMCB supplemented with the HoPS of *L. lactis* 1.8 as the sole energy source (results not shown).

A



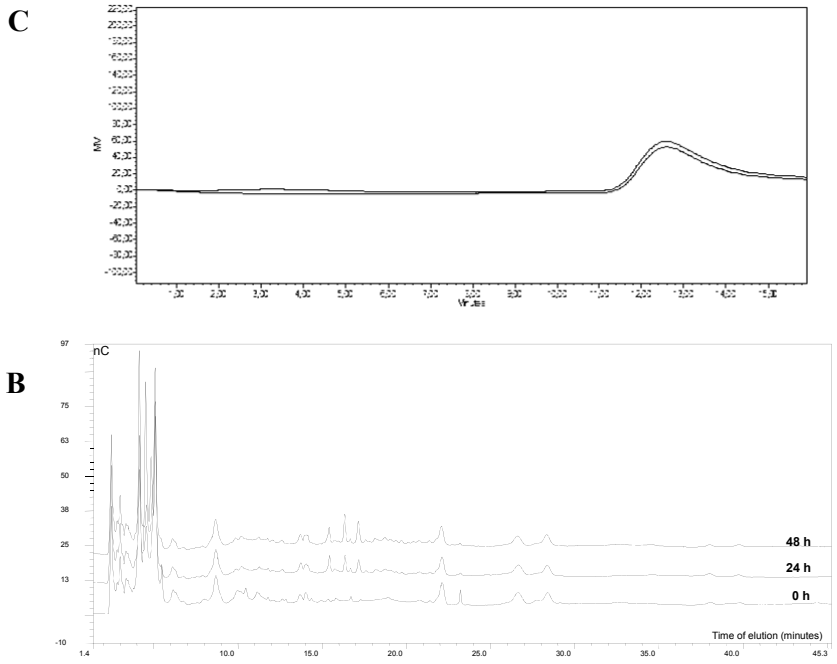


Fig. 5 Growth (as optical density at 600 nm) of *B. pseudocatenulatum* LMG 10505^T in MCB in the presence of EPS isolated from *L. lactis* 1.8 (A) and EPS degradation patterns as revealed by HPAEC-PAD (B) and GPC analysis (C). ●, Cell counts of *B. pseudocatenulatum* LMG 10505^T; ■, OD_{600}; ♦, acetate; ▲, lactate.

Up to now, the molecular characteristics that would differentiate common prebiotics from regular EPS are not fully understood (RUAS-MADIEDO & DE LOS REYES-GAVILAN [45]). It is expected that the presence of certain glycosidic linkages and their accessibility to glycolytic enzymes expressed in the appropriate colon inhabitants will influence the prebiotic potential of a substrate. In particular, β-linkages as in fructo-oligosaccharides, which are to be tackled by β-fructofuranosidases, favour bifidobacterial growth, as bifidobacteria possess these enzymes (FALONY & al. [16]; VAN DER MEULEN & al. [32,44]). The structures of the EPS tested in the present study mutually differ in more than one aspect, which did not allow relating their respective susceptibility to biological degradation with their primary structure. However, the HoPS that were partially degraded by several bifidobacterial strains appeared to possess a more diverse linkage pattern (α-1,2,4-; α-1,3-; α-1,4-; α-1-6-; and α-1,3,6-linkages) compared with those that were only metabolized by one particular strain (α-1,3-; α-1,6-; and α-1,3,6-linkages). This indicates that a diversity of α-linkages may facilitate EPS degradation (VAN CASTEREN & al. [46]).

The human large-intestinal ecosystem is a complex network of interdependent microbial communities characterized by extensive metabolic interactions (FALONY & DE VUYST [47]). Stimulation of colon bifidobacteria will result in an increase of large-intestinal lactate and/or acetate production, which will in turn serve as substrates for other bacteria, such as colon butyrate producers, as has been shown for resistant starch and inulin-type fructans (BELENGUER & al. [15]; FALONY & al. [16]; DE VUYST & LEROY [17]; BOURRIAUD & al. [48]; FLINT & al. [49]). Such cross-feeding interactions may be stimulated by EPS present in the human diet too.

Although the present results are promising, more detailed studies are needed to allow speculation on the potential *in vivo* prebiotic impact of the LAB EPS isolated. The complexity

of the colon ecosystem harbors enormous numbers of interdependent microorganisms, making it hard to evaluate the significance of *in vitro* studies in the real-life environment without additional *in vivo* experiments. Nevertheless, this study shows the potential of LAB EPS as prebiotics.

Acknowledgements

The authors acknowledge their financial support from the International Scientific and Technological Cooperation between Flanders and Romania of the Administration of Science and Innovation in Flanders (AWI-BWS02/04) and the Ministry of Education, Research and Youth in Romania and from the Research Council of the Vrije Universiteit Brussel, the Fund for Scientific Research – Flanders (FWO), and the Flemish Institute for the Encouragement of Scientific and Technological Research (IWT) in the Industry in Belgium. Part of this work was supported by the Research project TD_179 for young PhD students of the Romanian National Research Plan (PNII-RU). GF was a recipient of a PhD fellowship from the IWT.

References

1. B.J.J. WOOD, W.H. HOLZAPFEL, The genera of lactic acid bacteria, Vol. 2. Glasgow, UK: Blackie Academic and Professional (1995).
2. B.J.B. WOOD (1997) Microbiology of Fermented Foods. London, UK: Blackie Academic and Professional.
3. L. DE VUYST, L. AVONTS, E. MAKRAS, *Probiotics, prebiotics, and gut health.* C. REMACLE B. REUSENS, eds., Functional Foods, Ageing and Degenerative Disease Cambridge, UK: Woodhead Publishing, 2004, pp. 416-482.
4. L.J. FOOKS, G.R. GIBSON, Probiotics as modulators of the gut flora. *Brit. J. Nutr*., **88**, 39, 49 (2002).
5. R.A. RASTALL, G.R. GIBSON, H.S. GILL, F. GUARNER, T.R. KLAENHAMMER, B. POT, G. REID, I.R. ROWLAND, M.E. SANDERS, Modulation of the microbial ecology of the human colon by probiotics, prebiotics and synbiotics to enhance human health: an overview of enabling science and potential applications. *FEMS Microbiol. Ecol*., **52**, 145, 152 (2005).
6. FAO/WHO. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Report of a joint FAO/WHO expert consultation. World health organization, Geneva, Switzerland. (2001).
7. G.R. GIBSON, M.B. ROBERFROID, Dietary modulation of the human colonic microbiota - Introducing the concept of prebiotics. *J. Nutr.,* **125**, 1401, 1412 (1995).
8. G.R. GIBSON, H.M. PROBERT, J.A.E. VAN LOO, R.A. RASTALL, M.B. ROBRERFROID, Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutr. Res. Rev.*, **17**, 259, 275 (2004).
9. P. SU, A. HENRIKSSON, H. MITCHELL, Prebiotics enhance survival and prolong the retention period of specific probiotic inocula in an *in vivo* murine model. *J. Appl. Microbiol.,* **103**, 2392, 2400 (2007).
10. S. MACFARLANE, G.T. MACFARLANE, J.H. CUMMINGS, Prebiotics in the gastrointestinal tract. *Aliment. Pharm. Therap*., **24**, 701, 714 (2006).
11. D. BOSSCHER, J. VAN LOO, A. FRANCK, Inulin and oligofructose as prebiotics in the prevention of intestinal infections and diseases. *Nutr. Res. Rev*., **19**, 216, 226, (2006).
12. G.R. GIBSON, E.R. BEATTY, X. WANG, J.H. CUMMINGS, Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology,* **108**, 975, 982, (1995).
13. R.J. PALFRAMAN, G.R. GIBSON, R.A. RASTALL, Carbohydrate preferences of *Bifidobacterium* species isolated from the human gut. *Curr. Issues Intest. Microbiol.*, **4**, 71, 75 (2003).
14. M. ROSSI, C. CORRADINI, A. AMARETTI, M. NICOLINI, A. POMPEI, S. ZANOI, D. MATTEUZZI, Fermentation of fructoologosaccharides and inulin by Bifidobacteria: a comparative study of pure and fecal cultures. *Appl. Environ. Microb.,* **71**, 6150, 6158 (2005).
15. A. BELENGUER, S.H. DUNCAN, G. CALDER, G.HOLTROP , P. LOUIS, G.E. LOBLEY, H. J. FLINT, Two routes of metabolic cross-feeding between *Bifidobacterium adolescentis* and butyrate-producing anaerobes from the human gut. *Appl. Environ. Microb.,* **72**, 3593, 3599 (2006).

16. G. FANOLY, A. VLACHOU, K. VERBRUGGHE, L. DE VUYST, Cross-feeding between *Bifidobacterium longum* BB536 and acetate-converting, butyrate-producing colon bacteria during growth on oligofructose. *Appl. Environ. Microb.,* **72**, 7835, 7841, (2006).
17. L. DE VUYST, F. LEROY, Cross-feeding between bifidobacteria and butyrate-producing colon bacteria explains bifidobacterial competitiveness, butyrate production, and gas production. *Int. J. Food Microbiol.,* **149**, 73, 80 (2011).
18. H.J. RUIJSSENAARS, F. STINGELE, S. HARTMANS, Biodegradability of food-associated extracellular polysaccharide. *Curr. Microbiol.,* **40**, 194, 199 (2000).
19. F. DAL BELLO, J. WALTER, C. HERTEL, W.P. HAMMES, *In vitro* study of prebiotic properties of levan-type exopolysaccharides from lactobacilli and non-digestible carbohydrates using denaturing gradient gel electrophoresis. *Syst. Appl. Microbiol*., **24**, 232, 237 (2001).
20. M. KORAKLI, M.G. GANZLE, R.F. VOGEL, Metabolism by bifidobacteria and lactic acid bacteria of polysaccharides from wheat and rye, and exopolysaccharides produced by *Lactobacillus sanfranciscensis*. *J. Appl. Microbiol.,* **92**, 958, 965 (2002).
21. C. CINQUIN, G. LE BLAY, I. FLISS, C. LACROIX, Comparative effects of exopolysaccharides from lactic acid bacteria and fructo-oligosaccharides on infant gut microbiota tested in an *in vitro* colonic model with immobilized cells. *FEMS Microbiol. Ecol.* **57**, 226, 238 (2006).
22. N. SALAZAR, M. GUEIMONDE, A.M. HERMANDEZ-BARRACO, P. RUAS-MADIEDO, C.G. DE LOS REYES-GAVILAN, Exopolysaccharides produced by intestinal *Bifidobacterium* strains act as fermentable substrates for human intestinal bacteria. *Appl. Environ. Microb.,* **74**, 4737, 4745 (2008).
23. N. SALAZAR, P. RUAS-MADIEDO, S. KOLIDA, M. COLLINS, R. RASTALL, G. GIBSON, C.G. DE LOS REYES-GAVILAN, Exopolysaccharides produced by *Bifidobacterium longum* IPLA E44 and *Bifidobacterium animalis* subsp. *lactis* IPLA R1 modify the composition and metabolic activity of human faecal microbiota in pH-controlled batch cultures. *Int. J. Food Microbiol*., **135**, 260, 267 (2009).
24. L. DE VUYST, F. VANINGELGEM, *Developing new polysaccharides*. B. M. MC. KENNA eds., Texture in Food - Volume 2: Semi-solid Foods Cambridge, UK: Woodhead Publishing Ltd., 2003, pp. 275-320.
25. L. DE VUYST, F. DE VIN, *Exopolysaccharides from lactic acid bacteria.* H. KAMERLING eds., Comprehensive Glycosciences Oxford, UK: Elsevier Ltd., 2007, pp. 447-519.
26. P. RUAS-MADIEDO, N. SALAZAR, C.G. DE LOS REYES-GAVILAN, *Biosynthesis and chemical composition of exopolysaccharides produced by lactic acid bacteria.* M. ULLRICH Ed., Bacterial polysaccharides Norfolk, UK: Caister Academic Press, 2009, pp. 279-312.
27. R. VAN DER MEULEN, S. GROSU-TUDOR, F. MOZZI, F. VANINGELGEM, M. ZAMFIR, G.F. DE VALDEZ, L. DE VUYST, Screening of lactic acid bacteria isolates from dairy and cereal products for exopolysaccharide production and genes involved. *Int. J. Food Microbiol.,* **118**, 250, 258 (2007).
28. F. VANINGELGEM, R. VAN DER MEULEN, M. ZAMFIR, T. ADRIANY, A.P. LAWS, L. DE VUYST, *Streptococcus thermophilus* ST 111 produces a stable high-molecular-mass exopolysaccharide in milk-based medium. *Int. Dairy J.,* **14**, 857, 864 (2004a).
29. L. DE VUYST, F. VANDERVEKEN, S. VAN DE VEN, B. DEGEEST, Production by and isolation of exopolysaccharides from *Streptococcus thermophilus* grown in a milk medium and evidence for their growth-associated biosynthesis. *J. Appl. Microbiol.,* **84**, 1059, 1068 (1998).
30. M. MINEKUS, P. MARTEAU, R. HAVENAAR, J.H.J. HUIS IN'T VELD, A multicompartmental dynamic computer-controlled model simulating the stomach and small-intestine. *Altern. Lab. Anim.*, **23**, 197, 209 (1995).
31. F. VANINGELGEM, M. ZAMFIR, F. MOZZI, T. ADRIANY, M. VANCANNEYT, J. SWINGS, L. DE VUYST, Biodiversity of exopolysaccharides produced by *Streptococcus thermophilus* strains is reflected in their production and their molecular and functional characteristics. *Appl. Environ. Microb.,* **70**, 900, 912 (2004b).
32. R. VAN DER MEULEN, L. MAKRAS, K. VERBRUGGHE, T. ADRIANY, L. DE VUYST, *In vitro* kinetic analysis of oligofructose consumption by *Bacteroides* and *Bifidobacterium* spp. indicates different degradation mechanisms. *Appl. Environ. Microb.,* **72**, 1006, 1012 (2006).
33. T. HARUTOSHI, *Exopolysaccharides of Lactic Acid Bacteria for Food and Colon Health Applications*, Lactic Acid Bacteria - R & D for Food, Health and Livestock Purposes, Dr. J. MARCELINO KONGO ed., ISBN: 978-953-51-0955-6, InTech, DOI: 10.5772/50839. (2013)
34. T. MATSUKI, K. WATANABE, J. FUJIMOTO, T. TAKADA, R. TANAKA, Use of 16S rRNA gene-targeted group-specific primers for real-time PCR analysis of predominant bacteria in human feces. *Appl. Environ. Microb*., **70**, 7220, 7228 (2002).

35. E.G. ZOETENDAL, C.C. BOOIJNK, E.S. KLAASSEMS, H.G. HEILING, M. KLEEREBEZEM, H. SMITH, W.M. DE VOS, Isolation of RNA from bacterial samples of the human gastro-intestinal tract. *Nat Protocols,* **1**, 954, 959 (2006).
36. Y. BENNO, K. SAWADA, T. MITSUOKA, The intestinal microflora of infants - Composition of fecal flora in breast-fed and bottle-fed infants. *Microbiol. Immunol.,* **28,** 975, 986 (1984).
37. B. BIAVATI, P. CASTAGNOLI, F. CROCIANI, L.D. TROVATELLI, Species of the *Bifidobacterium* in the feces of infants. *Microbiologica,* **7**, 341, 345 (1984).
38. T. MATSUKI, K. WATANABE, R. TANAKA, M. FUKUDA, H. OYAIZU, Distribution of bifidobacterial species in human intestinal microflora examined with 16S rRNA-gene-targeted species-specific primers. *Appl. Environ. Microb*., **65**, 4506, 4512 (1999).
39. S.C. LEAHY, D.G. HIGGINS, G.F. FITZGERALD, D. VAN SINDEREN, Getting better with bifidobacteria. *J. Appl. Microbiol.*, **98**, 1303, 1315 (2005).
40. L. MAKRAS, L. DE VUYST, The *in vitro* inhibition of Gram-negative pathogenic bacteria by bifidobacteria is caused by the production of organic acids. *Int. Dairy J.,* **61**, 1049, 1057 (2006).
41. O. MARTENSSON, M. BIORKLUND, A.M. LAMBO, M. DUENAS-CHASCO, A. IRASTORZA, O. HOLST, E. NORIN, G. WELLING, R. OSTE, G. ONNING, Fermented, ropy, oat-based products reduce cholesterol levels and stimulate the bifidobacteria flora in humans. *Nutr. Res.,* **25**, 429, 442 (2005).
42. E.D. SONNENBURG, H. ZHENG, P. JOGLEKAR, S.K. HIGGINBOTTOM, S.J. FIRBANK, D.N. BOLAM, J.L. SONNENBURG, Specificity of polysaccharide use in intestinal *Bacteroides* species determines diet-induced mirobiota alterations. *Cell* **141**, 1241, 1252 (2010).
43. G. FALONY, T. CALMEYN, F. LEROY, L. DE VUYST, Coculture fermentations of *Bifidobacterium* species and *Bacteroides thetaiotaomicron* reveal a mechanistic insight into the prebiotic effect of inulin-type fructans. *Appl. Environ. Microb*., **75**, 2312, 2319 (2009).
44. R. VAN DER MEULEN, L. AVONTS, L. DE VUYST, Short fractions of oligofructose are preferentially metabolized by *Bifidobacterium animalis* DN-173 010. *Appl. Environ. Microb.,* **70**, 1923, 1930 (2004).
45. P. RUAS-MADIEDO, C.G. DE LOS REYES-GAVILAN, Methods for the screening, isolation, and characterization of exopolysaccharides produced by lactic acid bacteria. *J. Dairy. Sci.,* **88**, 843, 856 (2005).
46. W.H.M. VAN CASTEREN, C. DIJKEMA, H.A. SCHOLS, G. BELDMAN, A.G.J. VORAGEN, Characterisation and modification of the exopolysaccharide by *Lactococcus lactis* subsp. *cremoris* B40. *Carbohyd. Polym.,* **37**, 123, 130 (1998).
47. G. FALONY, L. DE VUYST, *Ecological interactions of bacteria in the human gut.* D. CHARALAMPOPOULOS, R.A. RASTALL eds., Prebiotics and Probiotics Science and Technology Heidelberg, Germany: Springer Verlag GmbH., 2009, pp. 641-682.
48. C. BOURRIAUD, R.J. ROBINS, L. MARTIN, F. KOZLOWSKI, E. TANAILLEAU, C. CHERBUT, C. MICHEL, Lactate is mainly fermented to butyrate by human intestinal microfloras but inter-individual variation is evident. *J. Appl. Microbiol.,* **99**, 201, 212 (2005).
49. H.J. FLINT, E.A. BAYER, M.T. RINCON, R. LAMED, B.A. WHITE, Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. *Nature Rev. Microbiol.*, **6**, 121, 131 (2008).