REVIEW

Food-grade heteropolysaccharides: ongoing research and future trends of biopolymers from lactic acid bacteria

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Abstract: Exopolysaccharides (EPSs) produced by lactic acid bacteria (LAB) play a major role in the manufacturing of fermented foods, especially fermented dairy products. Although the presence of heteropolysaccharides (HePSs) seems not to confer any advantage to cell growth or survival, in situ production typically imparts a desirable viscous, sometimes 'ropy' texture to fermented foods and milk products. These food-grade biopolymers are economically important for the food industry. Current challenges are to improve the yield of HePSs from LAB and to produce HePSs with a particular functionality on the basis of a better understanding of their biosynthesis and the relationship between their structure and function.

Keywords: heteropolysaccharides, exopolysaccharides, lactic acid bacteria, functional starter cultures

Introduction

Many bacteria, yeasts and fungi can produce polysaccharides, some of which have proved to be useful industrial products that can compete with plant and algae polysaccharides as well as synthetic products. Exopolysaccharides (EPSs) are long-chain polysaccharides consisting of repeating units of sugars or sugar derivatives, which may be assembled as capsular polysaccharides (CPSs) that are tightly associated with the cell surface, or secreted into the extracellular environment as slime material. The term EPS may be used to describe either type of extracellular polysaccharide (Sutherland 1972).

EPSs from microbial sources can be classified into two groups: homopolysaccharides (HoPSs), ie cellulose, dextran, glucan, mutan, pullulan, levan and curdlan; and heteropolysaccharides (HePSs), ie gellan, xanthan and most biopolymers from lactic acid bacteria (LAB).

The HoPSs from LAB consist of repeating units of only one type of sugar (D-glucose or D-fructose) and can be divided into two major groups: glucans and fructans (Monsan et al 2001). Well known examples of LAB HoPSs include dextrans and glucans produced by *Leuconostoc mesenteroides* and *Streptococcus mutans*, respectively. Dextran was the first microbial polysaccharide to be commercialised and to receive approval for food use. Although no longer employed as a food ingredient, it is used to prepare biochemical adsorbents of the Sephadex™ range.

The HePSs are composed of a backbone of repeating subunits, branched or unbranched, that consist of three to eight monosaccharides, derivatives of monosaccharides or substituted monosaccharides (De Vuyst and Degeest 1999; De Vuyst et al 2001).

The HePSs secreted by mesophilic LAB (*Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus paracasei*, *Lactobacillus sakei*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis*) and thermophilic LAB (*Streptococcus thermophilus*, *Streptococcus macedonicus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus acidophilus*) show large variations in composition and molecular mass, and probably in charge, spatial arrangement, rigidity and ability to interact with proteins. Despite these differences, the constituting monosaccharides are very similar; the monomers D-galactose and D-glucose and, to a lesser extent, L-rhamnose are usually present, although in different ratios. In a few cases, fucose, ribose, acetylated amino sugars and uronic acids, as well as

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other non-carbohydrate compounds such as phosphate, acetate and glycerol, are also present (De Vuyst and Degeest 1999; De Vuyst et al 2001). The wide biodiversity of HePSs from LAB with respect to molecular structure and size may be ascribed to the different biosynthesis routes of sugar nucleotides and genetic potential of these strains (De Vuyst et al 2003).

The present review will deal with HePSs produced by LAB (food-grade microorganisms), focusing on the research carried out to date, the industrial applications of HePSs and the future challenges.

HePS biosynthesis

Cellular HePS biosynthesis is an energy-demanding process that involves: enzymes for the production of sugar nucleotide precursors; a glycosyl 1-phosphate transferase that transfers the first sugar onto a phosphorylated carrier lipid; one or more glycosyltransferases (GTFs) that sequentially add new sugars to the growing repeating unit; and additional enzymes that participate in *eps* gene regulation, membrane translocation, and polymerisation and/or chain length determination (Broadbent et al 2003). These reactions commonly involve additional proteins with functions that are not unique to this process. As an example, there are housekeeping enzymes involved in the synthesis of molecules such as lipid carriers and sugar nucleotide precursors that are required for the assembly of other glycan-containing polymers, like peptidoglycan, teichoic acids and lipoteichoic acids (Delcour et al 1999).

Carbohydrates may enter the cell either in a phosphorylated state or as a free sugar, which has to be phosphorylated inside the cell prior to further degradation. A key intermediate linking the anabolic pathways of HePS production and the catabolic pathways of sugar degradation appears to be glucose 6-phosphate, from which the flux of carbon bifurcates between the formation of fructose 6-phosphate toward the products of glycolysis, ATP formation and cell synthesis, and toward the biosynthesis of sugar nucleotides, the precursor molecules of HePSs. Phosphoglucomutase (PGM), the enzyme responsible for the conversion of glucose 6-phosphate into glucose 1-phosphate, plays an important role in this flux divergence between the catabolic and anabolic pathways (Hugenholtz and Kleerebezem 1999; Degeest and De Vuyst 2000). Glucose 1-phosphate is another key metabolite in many of the metabolic pathways leading to the formation of the required sugar nucleotides that are formed from the glycolysis intermediate glucose 6-phosphate (Ramos et al 2001).

Several researchers have demonstrated a correlation among HePS production, the activity of the sugar nucleotide biosynthetic enzymes and the monomers present in the HePS repeating units, although differences between strains have been reported (Grobben et al 1996; Escalante et al 1998, 2002; Degeest and De Vuyst 2000; Boels et al 2001; Degeest, Janssens et al 2001; Degeest, Vaningelgem et al 2001; Mozzi et al 2001, 2003; Levander et al 2002).

For the mesophilic LAB strain *L. casei* (a species that is currently used in different probiotic fermented milks), the carbon source and the sugar metabolic pathways are important steps for HePS biosynthesis. The strain *L. casei* CRL 87 produced a HePS (composed of glucose, galactose and rhamnose, 1.7:1.0:2.5) from either galactose or glucose, the amount of polymer synthesised being 1.7 times higher for the former carbohydrate (Mozzi et al 2001). This microorganism has two alternative pathways for catabolising galactose: the tagatose 1,6-biphosphate pathway and the Leloir pathway (see Figure 1) (Chassy and Thompson 1983). Batch fermentations at controlled pH showed that galactose 1-phosphate was also a key metabolite for *L. casei* CRL 87 grown on galactose; the sugar uptake was mediated through an ATP-dependent permease system (Mozzi et al 2001). The Leloir enzyme UDP-galactose 4-epimerase played an essential role in the HePS production by this strain since a correlation between polymer synthesis and enzyme activity was observed (Mozzi et al 2003). These results were supported by the differential RNA expression of the *galE* gene that encodes for the Leloir enzyme (Torino et al 2002). A clear relationship between the activity of the PGM enzyme and biopolymer production by the thermophilic HePS-producing strain *L. helveticus* CRL 1176 (ATCC 15807) was observed. The microorganism was able to synthesise HePSs (glucose:galactose, 2.0:1.0) from lactose, glucose and galactose as carbon sources. The enzyme activity increased 2.2-fold for cultures grown on lactose at pH 4.5 compared with pH 6.2; nevertheless, for optimal HePS production, a high activity for both PGM and galactose 1-phosphate uridyltransferase is required (Torino, Mozzi et al unpub).

A similar correlation between the activity of HePS-biosynthesis enzymes, ie PGM, UDP-galactose 4-epimerase and UDP-glucose pyrophosphorylase, and HePS production was reported by Degeest and De Vuyst (2000) for the yoghurt strain *S. thermophilus* LY03. The authors also demonstrated that the activity of the precursor-producing enzyme UDP-*N*-acetylglucosamine 4-epimerase, which converts UDP-*N*-acetylglucosamine into UDP-*N*-acetylgalactosamine, is responsible for the presence of *N*-acetylgalactosamine in the HePS repeating units (Degeest, Vaningelgem et al 2001).

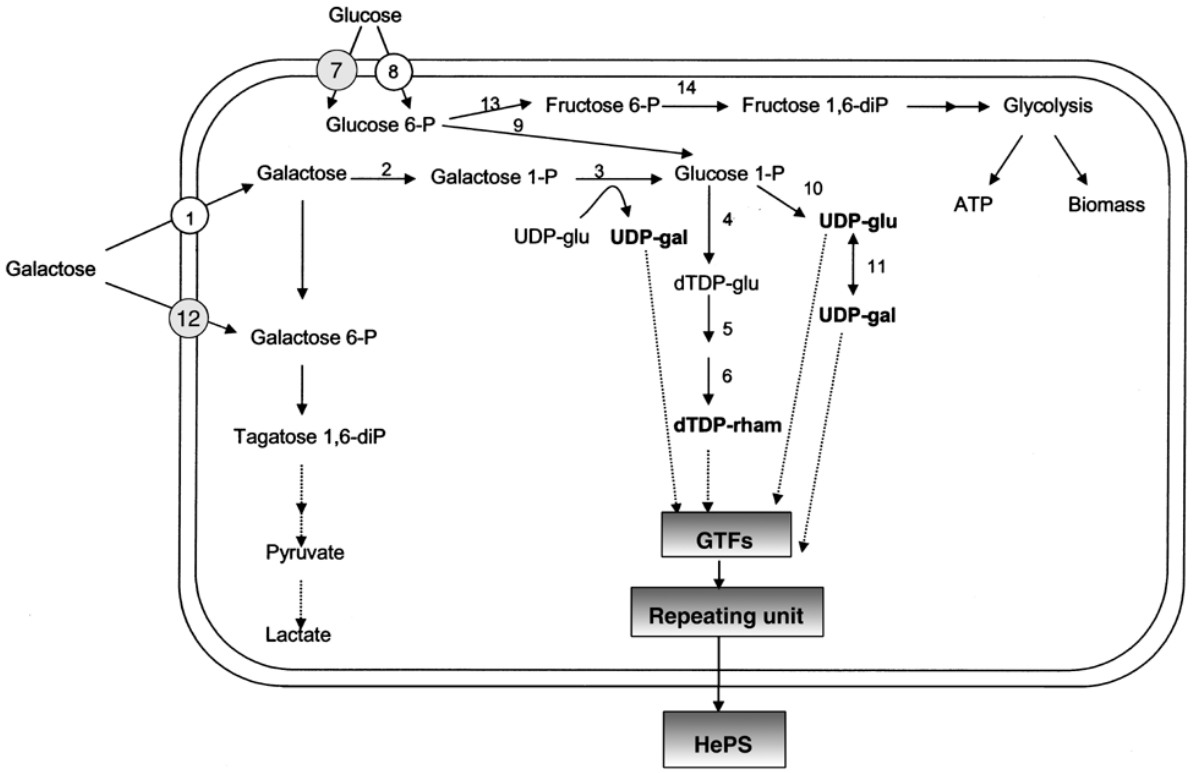

Figure 1 Pathways for the metabolism of galactose and glucose leading to the synthesis of HePS in the mesophilic lactic acid bacterium strain *Lactobacillus casei* CRL 87. 1 and 8: ATP-dependent permease; 2: galactokinase; 3: galactose 1-phosphate-uridyltransferase; 4: dTDP-glucose-pyrophosphorylase; 5: dTDP-glucose 4,6-dehydratase; 6: dTDP-rhamnose-synthetic enzyme system; 7: phosphoenolpyruvate-glucose-phosphotransferase; 9: α-phosphoglucomutase; 10: UDP-glucose-pyrophosphorylase; 11: UDP-galactose 4-epimerase; 12: phosphoenolpyruvate-galactose-phosphotransferase; 13: phosphoglucose isomerase; 14: fructose 1,6-biphosphatase. GTFs: glycosyltransferases; HePS: heteropolysaccharides; UDP-glu: uridine diphosphate-glucose; UDP-gal: uridine diphosphate-galactose; dTDP-rham: thymidine diphosphate-rhamnose. Sugar nucleotide precursors of HePSs are in bold.

The GTFs are also key enzymes for HePS biosynthesis. They catalyse the transfer of sugar moieties from activated donor molecules (sugar nucleotides) to specific acceptor molecules, thereby forming glycosidic linkages. Considering the structural diversity of the HePSs produced by LAB, it is assumed that these bacteria contain a large pool of specific GTFs, which may have multiple specificities for the donor and the acceptor sugar molecules (Stingele et al 1999). Functional assays of GTFs seem to be a prerequisite for future HePS and oligosaccharide bioengineering to produce polymers with different sugar compositions and hence with diverse texturising properties. Promising tools to produce different linkages within HePSs include gene shuffling with GTFs and stimulating their heterologous expression, which could allow adding other sugars at strategic positions to generate HePSs with new properties (Jolly et al 2002). Furthermore, a better insight into the HePS polymerisation and export mechanisms, still poorly understood, might allow optimisation of HePS production (Jolly and Stingele 2001).

Enhancement of HePS production by process parameters

One of the main disadvantages of LAB HePSs is the small amount of polymer synthesised by these microorganisms, which usually varies from 25 to 500 mg/L. The highest production levels reported so far were obtained for the mesophilic strains *L. rhamnosus* 9595M (1755 mg/L) (Bergmaier et al 2001) and *L. sakei* 0-1 (1375 mg/L) (van der Berg et al 1995). The production of HePSs by LAB may be improved through proper handling of the culture conditions. The main factors having a strong effect upon the synthesis of biopolymers are: temperature; fermentation period; pH; stirring; medium composition (carbon and nitrogen sources); and the presence of certain components such as salts (ie citrate), ions (ie manganese, calcium) (Gancel and Novel 1994; De Vuyst and Degeest 1999; Ricciardi and Clementi 2000), vitamins (Grobben et al 1998) and bases such as adenine. Adenine was the only non-essential component, which stimulated both the culture growth and

HePS production by *L. helveticus* CRL 1176 in a simplified chemically defined medium (Torino, Hébert et al unpub). Mozzi et al (1996, 2003) reported optimal HePS production by *L. casei* CRL 87 in both milk and broth cultures when they were grown at suboptimal temperatures (30 °C) and at a constant pH of 5.0. In *L. helveticus* CRL 1176, the incubation temperatures (37 °C and 42 °C) were not the main factor affecting HePS production when the organism was grown in batch milk cultures. On the contrary, the environmental pH played a key role in biopolymer formation. In fact, the production of HePSs in milk (247 mg/L) and broth (280 mg/L) cultures at pH 4.5 was 5.0-fold (Torino et al 2001) and 2.9-fold (Torino and Font de Valdez unpub) higher than at pH 6.2, respectively. The regulating effect of environmental pH on polymer formation by *L. helveticus* CRL 1176 was confirmed in chemostat fermentation; the highest HePS production (46 mg/L) was found at pH 4.5 and the lowest (24 mg/L) at pH 6.2, using lactose as the growth-limiting substrate (5 g/L) at a dilution rate (D) of 0.1 h^{-1} (Torino and Font de Valdez unpub). These results indicate that the hypothesis of Sutherland (1972), which suggests that higher HePS synthesis occurs at suboptimal growth conditions, is not applicable for *L. helveticus* CRL 1176 grown at different pH values (4.5 and 6.2) in chemost cultures, where the cells are at the same physiological state for a given steady-state.

For *L. delbrueckii* subsp. *bulgaricus*, another thermophilic HePS-producing strain, the best pH for polymer formation was coincident with the optimum for culture growth (pH 6.0) (Petry et al 2000). These apparent controversial results show that culture conditions should be optimised for each HePS-producing strain to yield efficient production.

Several authors developed new methods for EPS determinations and quantifications as well as culture media formulations to optimise biopolymer formation (eg Bergmaier et al 2001; Macedo et al 2002). As an example, HePS production by *L. casei* strains was enhanced in the presence of galactose and manganese. These compounds not only increased HePS yields but also markedly improved the ropy character of the cultures (Mozzi et al 1995, 1997).

Recently, EPS-overproducing *L. delbrueckii* subsp. *bulgaricus* strains were isolated using a chemically induced mutagenesis protocol for metabolic study (Welman et al 2003). The strains with enhanced HePS yield exhibited a global improvement in glycolysis accompanied with a mucoid and/or ropy phenotype.

HePS production by LAB occurs at different growth phases. Polymer synthesis by thermophilic LAB appears to be growth-associated as it was reported for the thermophilic HePS-producing strain *L. helveticus* CRL 1176 in both milk (Torino et al 2001) and broth cultures (Torino and Font de Valdez unpub). Similar primary metabolite kinetics have also been demonstrated for *S. thermophilus* LY03 (Degeest and De Vuyst 1999). In contrast, HePS production by mesophilic LAB strains seems to be improved under conditions less favourable for bacterial growth (Cerning et al 1992; Mozzi et al 1996). From this point of view, EPSs from LAB may be considered as minor products diverted away from glycolysis rather than as secondary metabolites (Pham et al 2000).

Degradation of HePSs produced by LAB has been reported by several authors (Cerning 1990; Cerning et al 1992; Gancel and Novel 1994; Mozzi et al 1996, 2003; Degeest et al 2002). The presence of different glycohydrolases in cell extracts of the strain *L. rhamnosus* R responsible for degrading the HePSs was demonstrated (Pham et al 2000). Degeest et al (2002) also reported that the HePS breakdown in *S. thermophilus* LY03 cultures upon long fermentations was due to glycohydrolase-type enzymes displaying endo-activity. Further characterisation of these glycohydrolases is necessary to prevent biopolymer breakdown in their industrial exploitation (Degeest et al 2002).

The ropiness of fermented milks seems to be more dependent on the molecular interactions among the HePSs and certain components of the medium, such as proteins and the bacterial surface, than on the amount of HePS produced. Thus, the loss of ropiness in stirred or long-fermented dairy products might be related to the breaking-off of these molecular associations (Torino et al 2000).

Genetic and metabolic engineering for improving HePS production

HePS genetic determinants can be located either on plasmid or chromosomal DNA. The genes involved in HePS biosynthesis are always organised in gene clusters that show significant conservation in their organisation and sequence (Jolly and Stingele 2001). Genes or gene clusters involved in the biosynthesis of HePS from LAB have been reported for *S. thermophilus* (Stingele et al 1996; Low et al 1998; Bourgoin et al 1999; Almirón-Roig et al 2000), *L. helveticus*

(Jolly et al 2002), *Lactoc. lactis* (van Kranenburg et al 1997, 1999), *L. delbrueckii* subsp. *bulgaricus* (Lamothe et al 2002) and more recently for strains of the *L. casei* group (*L. casei*, *L. paracasei* and *L. rhamnosus*) (Provencher et al 2003). The HePS gene clusters appear to be divided into four regions: the first one contains regulation genes; the second codes for proteins involved in determining polymer chain length; the third region contains genes similar to those coding for GTF enzymes (specifically required for the biosynthesis of the HePS repeating unit); and the fourth region encompasses genes involved in transport and polymerisation (Provencher et al 2003).

Based on the available genetic information, genetic modification of *eps* genes should lead to HePSs with a different repeating unit or with a different chain length, which would provide a tool for obtaining new HePSs with different rheological properties (Jolly et al 2002). There is considerable interest in the metabolic and genetic engineering of LAB strains as a strategy to either enhance HePS production or produce tailor-made HePSs with desired specific properties for new functional fermented foods.

A key consideration in formulating a strategy for the overproduction of HePSs by LAB is the large proportion of carbon that flows to lactate and other non-polysaccharide products. Strategies that have been applied to achieve the re-routing of carbon to overproduce specific products, ie HePSs, include engineering single and multiple genes and whole pathways of redox reactions and global control systems (Hols et al 1999). To ensure that excessive carbon will not be diverted into unwanted metabolites via the pyruvate pathway, it would also be relevant to include the control factors in the pathways of sugar degradation and EPS formation (de Vos 1996).

Levander et al (2002) showed that it is possible to increase HePS production by altering the levels of enzymes of the central carbohydrate metabolism. Higher levels of both PGM (formerly described) and UDP-glucose pyrophosphorylase enzymes, which leads to the formation of the sugar nucleotide UDP-glucose, led to a proportional increase in the yields of HePSs produced by *S. thermophilus* LY03, although no effect could be seen from overexpressing either enzyme alone. The overexpression of the *fbp* gene in *Lactoc. lactis* NIZO B40 caused by using the nisin-controlled expression system (encoding for the enzyme fructose biphosphatase (FBPase) that catalyses the conversion of fructose 1,6-biphosphate into fructose 6-phosphate) resulted in an increased HePS synthesis using fructose as the carbon source (Looijestein et al 1999). The authors indicated that the limiting factor for both HePS production and bacterial growth was low FBPase activity. Boels et al (2001) claimed that UDP-galactose 4-epimerase activity was not the main factor for EPS biosynthesis while the level of UDP-glucose pyrophosphorylase played a key role in the biosynthesis of intracellular UDP-glucose and UDP-galactose in the strain *Lactoc. lactis* NIZO B40. However, increased UDP-glucose pyrophosphorylase activity did not significantly increase HePS production by this strain. More recently, the same authors studied the effect of modulating the activity of different enzymes on the metabolic branching point between glycolysis and sugar nucleotide biosynthesis on HePS formation by *Lactoc. lactis* NIZO B40. They concluded that increased concentrations of sugar phosphates or sugar nucleotides did not significantly affect HePS production (Boels, Kleerebezem et al 2003). It is clear that more knowledge on the regulation of EPS biosynthesis for further improvement of the HePS synthesis, either for in situ production or for its use as food additives, is required.

Alternative methods for improving the production of LAB biopolymers could involve cloning the entire *eps* gene cluster on a single plasmid with a high copy number (Welman and Maddox 2003). Boels, van Kranenburg et al (2003) have reported for the first time that homologous overexpression of a complete *eps* gene cluster in *Lactoc. lactis* increased the HePS production. A ninefold elevation in HePS plasmid copy number led to an almost threefold increase in the *eps* expression level, resulting in an almost fourfold increase in HePS production by *Lactoc. lactis* NIZO B40.

However, the market application of genetically modified strains – in which entire operons or clusters of genes associated with EPS production have been cloned – would be subject to regulatory controls and public acceptance of these type of products.

HePS structure and function

Progress can now be made to determine the influence of the amount and the structure of HePSs on the texture and stability of fermented foods. To date, several HePS structures from LAB have been determined, which seemed to be strain-specific (Laws and Marshall 2001).

The advantage of using HePSs from LAB is their thickening power, which influences the rheology and texture of many fermented food products at low concentrations. These biopolymers show large variations in composition and molecular mass, and may vary in charge, spatial

arrangements, rigidity and the ability to interact with proteins (Duboc and Mollet 2001), thus making it difficult to interpret the EPS structure–function relationships. It has been postulated that stiffer chains are required for obtaining high intrinsic viscosities, which are more likely with β (1–4) linkages (Tuinier et al 2001), and which in turn will lead to a higher consistency of the EPS solutions. The degree of branching as well as the HePS molecular mass may also contribute to the stiffness of the polymer, these being the main factors contributing to the intrinsic viscosity of a polysaccharide (Faber et al 1998; Tuinier et al 2001).

Recently, Petry et al (2003) proposed that the ratio between the high and low molecular mass fractions is crucial for the texturising properties of HePS from *L. delbrueckii* subsp. *bulgaricus*. Ruas-Madiedo, Hugenholtz et al (2002) and Ruas-Madiedo, Tuineir et al (2002) proved that the viscosity of stirred fermented milks was strongly related to the intrinsic viscosity of the HePSs produced by different strains of *Lactoc. lactis* subsp. *cremoris*. As the molar mass and the polymer stiffness determined the intrinsic viscosity of the HePSs, these molecular characteristics affected the viscosity of stirred fermented milks. Moreover, the length and composition of branches strongly affected the rheological properties of the HePSs (Vincent et al 2001).

Using HePSs with identical structures but different molecular masses, De Vuyst et al (2003) also observed a positive correlation between the molecular mass of different HePSs produced by *S. thermophilus* strains and the consistency of the HePS solutions. It was suggested that complex interactions among the bacterial cells, the protein network and the HePSs play an important role in texture build-up (van Marle and Zoon 1995; Duboc and Mollet 2001; Hassan et al 2002).

These contributions indicate that no clear correlation between HePS concentration and the apparent viscosities of the products can be established, which is of particular importance to predicting the performance of polysaccharides in a given food product. Beyond the structure-based relationships recently obtained from the information available on HePSs from LAB (Vincent et al 2001), molecular modelling is developing as an important tool for unravelling the link between macroscopic properties (such as rheology) and the capacity of the HePSs to interact with other molecules present in the medium. The next step should be to determine the correlations between atomic structural properties and physicochemical properties of a particular EPS in the complex food matrix, as well as the interactions among EPSs, bacterial cells and proteins, and the influence of different parameters such as pH or heat treatment on these relationships.

Biological and ecological functions

Information about the physiological role of HePSs in LAB is almost completely lacking. It is assumed that these biopolymers have some kind of biological function but are not essential for bacterial growth, as was demonstrated with spontaneous HePS-negative mutants (Schellhaas 1983). Most proposed functions of EPSs are generally of a protective nature such as protection against adverse environmental factors. However, it has been claimed that they play a role in adhesion and in biofilm formation in most natural environments where the biofilm is the prevailing microbial lifestyle (Watnick and Kolter 2000). Thus, EPSs may play an important role protecting against dehydration, macrophages, protozoa, antibiotics and toxic compounds (Weiner et al 1995; Roberts 1996).

Looijestein et al (2001) studied the tolerance of the non-HePS-producing strain *Lactoc. lactis* subsp. *cremoris* MG1614 and a HePS-producing isogenic variant of the same strain to several antimicrobial factors. The presence of cell-associated HePSs and HePSs in suspension (as slime) resulted in an increased tolerance to copper and nisin. The cell-associated HePS also protected the bacteria against the cell wall-degrading enzyme lysozyme and bacteriophages.

Virulent phages cause the most significant problems in the dairy industry worldwide. Commercial HePS-producing *Lactococcus* strains are increasingly used in milk fermentations, and it is well known that extensive use of a particular culture can lead to failures due to phage attack. It has been suggested that EPSs play a role in bacteriophage infection either by avoiding the phage attack (Forde and Fitzgerald 1999) or by favouring it acting as a receptor (Cerning 1990). It remains to be seen whether the structure of some HePSs produced by LAB play a role in phage sensitivity. Indeed, the phage infection process in *Lactococcus* started with the adsorption of the phage tail to sugar components of the cell wall such as galactose or rhamnose (Forde and Fitzgerald 1999), which are also present in some HePSs. The authors demonstrated that the hydrophilic HePS produced by the strain *Lactoc. lactis* subsp. *cremoris* MG1363 masked certain cell-surface receptors, resulting in a dramatic decrease in bacteriophage adsorption. On the other hand, Moineau et al (2002, cited in Broadbent et al 2003) reported that the presence of capsular EPSs in the strain *S. thermophilus* MR-1C was not sufficient to protect the cells against phage infection. Recently, Deveau et al (2002)

reported the isolation of eight different lactococcal phages that infect two HePS-producing strains used in different North American buttermilk factories. The derivatives obtained by curing the *eps*-encoding plasmid from these phage-sensitive strains were still phage-sensitive and adsorbed phages to the same degree as the parental strains. Hence, from the information available it is not apparent why EPSs may serve as a target for phage adsorption sometimes, and act as a barrier at other times. It is clear that the process of phage infection is too complex to rely solely on EPS production.

Technological applications

Initially, the research on EPSs of bacterial origin was focused on Gram-negative bacteria such as *Azotobacter vinelandii*, *Xanthomonas campestris*, *Sphingomonas paucimobilis* and *Rhizobium meliloti*, microorganisms that are not recognised as food-grade, and therefore their use in food systems is restricted. As a result, attention was later focused on GRAS (generally recognised as safe) microorganisms such as LAB, traditionally used for food fermentation (Ricciardi and Clementi 2000; Laws et al 2001).

HePS-producing LAB are a typical example of functional starter cultures, a new generation of starter cultures that have at least one inherent functional property that may contribute to the technological, nutritional or health properties of a fermented food (Leroy and De Vuyst 2004). LAB-biopolymers (Table 1) have found their most important applications in the dairy industry by improving the texture, rheology and 'mouthfeel' of fermented milk products, mainly yoghurts, eliminating the use of food additives to achieve new market demands. Although having no taste of their own, HePSs from LAB increase the time that milk products spend in the mouth and hence improve the perception of the bouquet (Duboc and Mollet 2001).

For many years, dairy processors have exploited the differences among ropy HePS-positive LAB to produce a variety of fermented milks with unique properties, as well as to control syneresis in yoghurt, a practice that is particularly widespread in countries where the addition of stabilisers is prohibited (Cerning 1995; Duboc and Mollet 2001). Within the food industry, yoghurt manufacture remains the most important commercial application for HePS-producing LAB; in dairy foods some attempts have been made to introduce HePS-producing strains as culture adjuncts to improve low-fat and partly-skim milk cheeses (Broadbent et al 2001). However, HePSs often accumulate in the cheese whey, thereby increasing the viscosity, which retards the efficiency of membrane processing and slows down concentration of the whey proteins during the drying processes (Petersen et al 2000).

Consumer demand for reduced-fat foods resulted in the development of low-fat and non-fat mozzarella cheese, but fat removal has several undesirable effects on the physical properties of cheese. Specifically, this kind of cheese becomes tough and rubbery, it loses its pliability rapidly during cooling and more heat is required for melting compared with other cheeses (McMahon and Oberg 1998). Studying cheese microstructure, the authors observed that the channels that formed in the protein matrix contained entrapped fat globules, and these channels became much

Table 1 Examples of most common heteropolysaccharides from lactic acid bacteria involved in the production of fermented foods

Lactic acid bacteria	*Monomer composition*	*Fermented food*	*Reference*
Lactococcus lactis subsp. *cremoris* SBT 0495	Glc:Gal:Rha (2.0:2.0:1.0)	Buttermilk, kefir, dahi, villi	(Nakajima et al 1992b)
Lactococcus lactis subsp. *cremoris* NIZO B891	Glc:Gal (3.0:2.0)	Buttermilk, kefir, dahi, villi	(van Casteren et al 2000)
Lactobacillus casei CRL 87	Glc:Gal:Rha (1.7:1.0:2.5)	Fermented milks	(Mozzi et al 2001)
Lactobacillus rhamnosus C83	Glc:Gal (2.0:3.0)	Kefir	(Vanhaverbeke et al 1998)
Lactobacillus paracasei 34-1	Gal:NAcGal (3.0:1.0)	Fermented milks	(Robijn, Wienk et al 1996)
Lactobacillus helveticus CRL 1176	Glc:Gal (4.0:2.0)	Low-fat cheeses	(Torino et al unpub)
Lactobacillus helveticus TN-4	Glc:Gal (3.0:3.0)	Low-fat cheeses, kefir	(Yamamoto et al 1995)
Lactobacillus delbrueckii subsp. *bulgaricus* RR	Glc:Gal:Rha (1.0:5.0:1.0)	Yoghurts, fermented milks	(Gruter et al 1993)
Lactobacillus delbrueckii subsp. *bulgaricus* 291	Glc:Gal (3.0:2.0)	Yoghurts, fermented milks	(Faber et al 2001)
Lactobacillus acidophilus LMG 9433	Glc:Gal:NAcGlc (3.0:1.0:1.0)	Fermented milks, kefir	(Robijn, Gutiérrez Gallego et al 1996)
Streptococcus thermophilus LY03	Glc:Gal:NAcGal (1.4:3.2:1.0)	Yoghurts, cheese, fermented milks	(Degeest, Vaningelgem et al 2001)
Streptococcus thermophilus SY89 and SY102	Glc:Gal (2.0:2.0)	Yoghurts, cheese, fermented milks	(Marshall et al 2001)
Streptococcus thermophilus Sfi6	Glc:Gal: NAcGal (1.0:2.0:1.0)	Yoghurts, cheese, fermented milks	(Stingele et al 1996)
Streptococcus macedonicus Sc136	Glc:Gal:NAcGlc (3.0:2.0:1.0)	Cheese	(Vincent et al 2001)

narrower in low-fat mozzarella cheese with less space available for water retention. To increase moisture content without adversely affecting the functionality of cheese, it is important that the HePS produced by the LAB culture adsorbs tightly to the bacterial cells rather than being released into the whey. Many studies have been carried out using different combinations of HePS-positive and HePS-negative starter cultures (Merrill et al 1994; Perry et al 1997; Low et al 1998). The HePS from *S. thermophilus* MR-1C, which has a novel octasaccharide repeating unit composed of galactose, rhamnose and fucose (5:2:1), significantly increased moisture retention in low-fat mozzarella cheese, mainly due to the capsular EPS formation.

Another interesting HePS is kefiran, a slimy polysaccharide composed of glucose and galactose in an equimolar ratio (Micheli et al 1999; Rimada and Abraham 2001), produced by the microbiota (homo- and heterofermentative LAB, yeasts and acetic acid bacteria) that are present in kefir grains. The product of milk fermentation by these grains is known as 'kefir', a traditional, self-carbonated, slightly alcoholic fermented milk from Eastern Europe (Roginski 1999; Tamime and Robinson 1999).

A novel application of EPS-producing LAB is in bakery products. In recent years some studies have been carried out on this subject, especially relating to in situ EPS production by LAB in sourdough in order to replace hydrocolloids currently used as texturising or prebiotic additives in bread production (Korakli et al 2002; Tieking et al 2003). The incorporation of a sufficient amount of EPSs into the dough may also result in an improved texture build-up by softening the dough's gluten and increasing the specific volume of the resultant bakery product, as well as extending the product's shelf life (De Vuyst et al 2001). To date, the EPSs involved in sourdough fermentation belong only to the HoPS type.

Physiological benefits of HePSs

Certain HePSs produced by LAB are claimed to have beneficial physiological effects. It is speculated that the increased viscosity of HePS-containing foods may increase residence time in the gastrointestinal tract and, coupled with a low degradability of HePSs (Ruijssenaars et al 2000), might therefore be beneficial for a transient colonisation by probiotic bacteria (German et al 1999). Another health benefit is the potential generation of short-chain fatty acids upon degradation of EPSs in the gut by the colon microflora, which would provide energy to epithelial cells and may play a role in the prevention of colon cancer (Cummings and Englyst 1995). Studies with animal models showed the immunostimulatory (Hosono et al 1997), antitumoral (Kitazawa et al 1991) and cholesterol-lowering effects (Nakajima et al 1992a) of some HePS-producing strains.

Recently, Vincent et al (2001) determined the structure of a HePS produced by the strain *S. macedonicus* Sc136 that contains an internal backbone of lacto-*N*-tetraose and lacto-*N*-neotetraose. The same trioses have been identified in the structure of several human milk oligosaccharides, which are important for healthy infant nutritional status. Further research on this topic would be necessary before employing HePSs or HePS-producing LAB in functional foods.

Conclusions

The low amounts of HePS produced by LAB reduce their potential use as food-grade ingredients. However, current interest in glycobiology and the application of new analytical methods have stimulated research on LAB polysaccharides (De Vuyst and Degeest 1999; Ricciardi and Clementi 2000; De Vuyst and Marshall 2001; Laws et al 2001; Ruas-Madiedo, Hugenholtz et al 2002). There is a gap between our knowledge of the structure of HePSs and the ability to predict their physical properties, and thus their functionality in a food matrix. Further characterisation of HePS functionality, structure and genetics will allow food processors to select or even construct functional HePS-positive cultures with unique and predictable properties. As this research continues, the food industry can expect to see more widespread application of EPS-producing cultures and LAB biopolymers in ways that offer new opportunities to target applications for improved health benefits in the direction of consumer-specific nutritional demands. This will provide additional innovation in dairy products for years to come.

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