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Heteropolysaccharides from Lactic Acid Bacteria: Current Trends and Applications

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Abstract

Exopolysaccharides produced by lactic acid bacteria have tremendous value in the development of new functional foods. Different types of exopolysaccharides produced vary in monomer composition, molecular weight and structure. This review focuses on the heteropolysaccharides produced by lactic acid bacteria. The huge diversity of heteropolysaccharides presents several applications in food industry. Here we report heteropolysaccharides produced by lactic acid bacteria along with their characteristics and applications.

Keywords: Lactic acid bacteria (LAB); Heteropolysaccharide (HePS); Exopolysaccharide (EPS); Homopolysaccharide (HoPS)

Introduction

Lactic acid bacteria (LAB) produce a wide variety of exopolysaccharides (EPS) and oligosaccharides that have immense food applications [1]. EPS can be used as prebiotics. Prebiotics are food ingredients that cannot be digested other than by some specific bacteria and are involved in selectively stimulating the growth of probiotics to benefit the health of the host. Oligosaccharides are carbohydrate polymers of monomeric residues with degrees of polymerization (DP) between 2 and 10 linked by O-glycosidic bonds they can also be the degradation product of their related EPS.

LABs have aroused interest for their ability to secrete extracellular polysaccharides which are either associated with the cell surface in the form of capsules (Capsular exopolysaccharides) or secreted into the extracellular environment in the form of slime (Slimy exopolysaccharide) [2]. The physiological roles of these exopolysaccharides in the microbial host are not yet completely understood, but they are involved in protection against dehydration, pathogenicity, biofilm formation and quorum sensing [3]. Lactic acid bacteria (LAB) belonging to the genera *Leuconostoc*, *Lactobacillus*, *Pediococcus*, Weissella and *Streptococcus* produce variety of exopolysaccharides synthesized by extracellular glucansucrases using sucrose as the glucosyl donor or by intracellular enzymes and later exports them outside the cells [4-6].

These EPS have immense commercial applications because of their physico-chemical properties [7]. The exopolysaccharides from LAB are of two types;

Homopolysaccharides (HoPS): Polymers composed of a single type of sugar residue.

Heteropolysaccharides (HePS): Polymers composed of a variety of sugar residues, which may or may not be in equal proportions.

The most widely used polysaccharides (e.g., agar, alginate, starch, pectin, mannan, heparin) for various industrial applications are chiefly produced from algae, plant and animal sources. Certain EPS such as xanthan gum largely used in industry comes from only bacterial source. The bacterial EPS have uniform quality whereas the plant and algal EPS have variable composition, lacks consistency and a uniform quality is not always achieved.

In the case of microbial polysaccharides, reliable quantities can be easily achieved by bioprocess engineering. Bacteria belonging to GRAS (Generally Regarded as Safe) such as *lactobacilli*, have the exemption from any risk in food and human health applications [8].

Heteropolysacchrides from Lactic acid bacteria

The HePS from LAB have received renewed interest similar to HoPS as HePS also play an important role in the rheology, texture, body and mouthfeel of fermented milk drinks [8]. In general the HePS from LAB display high ropiness in the fermentation medium and their yield are low [9]. The heteropolysaccharides are produced by mesophilic LAB such as *Lactococcus lactis* subsp. lactis, *Lactococcus lactis* subsp. cremoris, *Lactobacillus casei*, *Lactobacillus* sake, *Lactobacillus rhamnosus* and *Lactobacillus plantarum* [2,9]. In addition several thermophilic LAB such as *Lactobacillus acidophilus*, *Lactobacillus delbrueckii*, *Lactobacillus helveticus* and *Streptococcus thermophilus* are also known to produce HePS [2,9].

Composition and biosynthesis of heteropolysaccharides

Microbial heteropolysaccharides (HePS) are complex polymers composed of repeated units of 3 to 8 monosaccharides which could be either branched or unbranched. The monosaccharides present in HePS are D-glucose, D-galactose and L-rhamnose and are in different ratios. However, there are other monosaccharides such as fucose, mannose, ribose, fructose, N-acetylated monosaccharides (N-acetyl glucosamine and N-acetyl galactosamine) or substituted monosaccharides (glycerol, glucuronic acid, acetyl groups and phosphates) also occur in HePS [10]. Several enzymes responsible for epimerization, decarboxylation,

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dehydrogenation etc are involved in the biosynthesis and secretion of HePS. The four major consecutive steps involved in HePS biosynthesis in LAB, involve sugar transport in the cytoplasm, synthesis of sugar 1-phosphate, polymerization of repeating unit precursor and finally HePS export outside the cell [9,11]. The sugar nucleotides, derived from sugar-1-phosphates, play an essential role in HePS biosynthesis due to their role in sugar activation, which is necessary for monosaccharide polymerization as well as the sugar inter-conversions [2,9]. The schematic representation of the pathway involving the biosynthesis of HePS is illustrated in Figure 1.

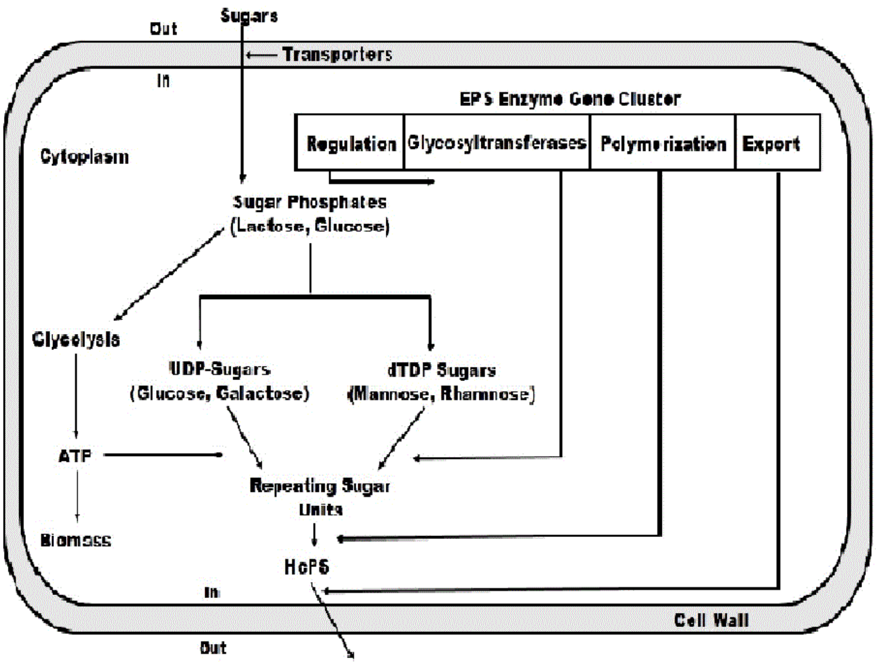

Figure 1: Schematic representation of the pathway involving the biosynthesis of HePS.

Microbial HePS are produced intra-cellularly and its formation is controlled by complex eps cluster gene encoding for HePS forming enzymes [12]. These genes form functional regions such as polymerization, chain length determination, export of HePS and regulation of gene cluster. These regions are responsible for variability in molecular weight and composition of HePS [12]. HePS formed by *Lactobacillus casei* LC2W isolated from skim milk was composed of glucose (57.8% mol%), rhamnose (27.7%, mol%) and galactose (14.5%, mol%), respectively. The linkage patterns of sugar residues were determined by methylation analysis and partial acid hydrolysis which included: Terminal-Rhamnose-1→(18.4%), →3-Rhamnose-1→(15.7%), Terminal-Glucose-1→(21.7%), →4-Glucose-1→(16.3%), →,,6-Glucose-1→(16.3%) and →,6-Galactose-1→(11.9%). 1D and 2D NMR analysis revealed that both O-acetyl and N-acetyl were linked to 3-Rhamnose at O-2 position [13]. The monosaccharide composition of HePS from several lactic acid bacteria has been reported in Table 1.

Strain	Source	Composition	Mw (Da)	Application	References
Lactobacillus plantarum C88	Chinese tofu	Galactose and glucose with a molar ratio of 1:2.	1.15×10^6	Antioxidant activity as well as prebiotic	[25]
Lactobacillus plantarum TISTR 875	Marine Fish, Thailand	Glucose and galactose (1:2)	-	Prebiotic	[21]
Lactobacillus suebicus CUPV225 and CUPV226	Ropy natural ciders of Basque Country (Spain)	Glucose, galactose, N-acetylglucosamine and phosphate	-	Improvement of rheology in fermented products.	[15]
Lactobacillus plantarum KF5	Tibet Kefir	Mannose, glucose and galactose moieties in an approximate ratio of 1: 4.99: 6.90	-	Emulsifier in food industry	[33]

*Lactobacillus plantarum*RJF4	Rotten Jack fruit	Glucose and Mannose	-	Antioxidant activity as well as prebiotic	[24]
Lactobacillus helveticus MB2-1		Galactose, glucose and mannose in a molar ratio of 1.00:1.69:3.54	2.0×10^5	Antioxidant activity as well as prebiotic	[34]
Lactobacillus plantarum 301102S	Mutant strain	Glucose and mannose (molar ratio, 1:2)	-	Prebiotic	[30]
Lactobacillus casei LC2W	Skim milk	Glucose (57.8% mol %), rhamnose (27.7%, mol %) and galactose (14.5%, mol %)	-	-	[13]
Lactobacillus plantarum YW11	Tibetan-Kefir	Glucose, galactose and N-acetylated sugar residues (molar ratio of 2:71:1)	1.1×10^5	Viscosifier of skim milk	[31]
Lactobacillus plantarum YW32	Tibetan-Kefir	Mannose, fructose, galactose and glucose (molar ratio of 8.2:1:4:1:4.2)	1.03×10^5	Antioxidant, antibioflim activity	[22]
Lactobacillus plantarum SKT109	Tibetan-Kefir	Fructose and glucose (molar ratio of 3:1)	2.1×10^6	Viscosifier of fermented milk	[23]
Lactobacillus paraplantarum BGCG11	Artisanal cheese	Glucose (86.6%), mannose (6.2%), galactose (4.1%), and rhamnose (3.1%)	-	Immunomodulatory activity	[18]
Leuconostoc mesenteroides strain NTM048	Green peas	Glucose and fructose	1 to 4×10^4	Enhance the mucosal barrier and influence the systemic immune response	[1,35]

Table 1: Heteropolysaccharide (HePS) from lactic acid bacteria and their properties.

Structure of heteropolysaccharides from Lactic acid bacteria

The average molecular weight (Mw) of HePS can be evaluated by size exclusion chromatography (SEC) [12]. The structure and molecular weight of HePS has great impact and that decides their application. The molecular weight of HePS varies considerably with its type and the producer strain. Generally, the molecular weight varies in the range from 10^4 to 10^6 Da [12]. The molecular weight of HePS determines its viscosity and the texture of the matrix they are dissolved in. It is quite common for a single strain to produce HePS of two different molecular weights [12].

Lactobacillus rhamnosus E/N produces a HePS consisting of four rhamnose, two glucose and one galactose residues with a pyruvate substituent [14]. *Lactobacillus rhamnosus* E/N grown separately in media containing glucose, maltose, galactose, sucrose or lactose produced HePS of molecular weights in the range 195-11130 kDa. However, in the case of galactose, lactose and sucrose an additional low molecular weight fraction of 45 kDa was observed. *Lactobacillus rhamnosus* E/N on growing separately in the media containing glucose or maltose showed single molecular weight fraction of 200 and 500 kDa, respectively. The composition of HePS remained same for all different molecular weight fractions but showed different physicochemical and rheological properties [14].

Lactobacillus suebicus CUPV225 and CUPV226 isolated from two ropy natural ciders of Basque Country (Spain) produced HePS that consisted of glucose, galactose, N-acetylglucosamine and phosphate present in different molar ratios [15]. Both strains produced HePS of two different molecular weights. It was observed that low molecular weight HePS is primarily produced when the strains were grown in medium containing pentose sugars such as ribose and xylose [15]. The glycosyl transferase encoding genes were detected by PCR for both *Lactobacillus suebicus* CUPV225 and CUPV226 [15].

Applications of heteropolysaccharides from lactic acid bacteria

HePS produced by food-grade lactic acid bacteria is an important source of natural alternatives to commercial additives from plant or animal origin. HePS like HoPS have reproducible physico-chemical properties and possess immunostimulatory, anti-tumour and antioxidant activities (Table 1). Some major applications of HePS produced from LAB are listed below.

Immuno-stimulant: Bacterial EPSs would be associated with their various physiological activities, such as anti-tumor, anti-viral, anti-inflammation, inducer of interferon production, platelet aggregation inhibition and colony stimulating factor (CSF). For example, the EPSs produced by marine Vibrio and Pseudomonas sp. have antitumor, antiviral and immunostimulating activities [16]. The HePS from LAB have also demonstrated immunostimuliant activity. *Leuconostoc mesenteroides* NTM048 produces a HePS that contains glucose and fructose units and its size ranges from 10 to 40 kDa [17]. The *in vitro* study revealed that NTM048 HePS induced total and antigen-specific IgA production by Peyer's patch cells and influenced T helper (Th1 and Th2) cell-mediated response in splenocytes. The study showed that NTM048 HePS enhanced the mucosal barrier and influenced the systemic immune response [17]. *Lactobacillus paraplantarum* BGCG1, a probiotic strain which was isolated from a soft, white, artisanal cheese, produced a high molecular weight HePS which was responsible for the ropy phenotype and immunomodulatory activity of the strain [18]. The HePS produced consisted of glucose (86.6%), mannose (6.2%), galactose (4.1%) and rhamnose (3.1%). The strain was capable of producing HePS of two different molecular weights [18].

Prebiotic activity: Prebiotics confers health benefits by promoting the growth of beneficial bacteria (probiotics) in the intestinal tract [19] and support the inhibition of the growth of lesions, such as adenomas and carcinomas in the gut and thus reduce the risk factors involved in colorectal diseases [20]. Bacterial EPSs exhibited high resistance to human gastrointestinal digestion and selective enhancement of beneficial bacteria colonized in the colon function in the similar way as the other commercial prebiotics, such as fructooligosaccharides, galactooligosaccharides and inulin by acting as carbon source for the growth of probiotic bacteria [21]. In this review we have reported the prebiotic potential of HePS from LAB in term of gut enzyme resistance and selective stimulation of probiotic bacteria. The HePS produced by *Lactobacillus plantarum* YW32 displayed anti-biofilm activity on several pathogenic bacteria and antitumor activity against human colon cancer HT-29 cells [22]. This HePS showed a web-like microstructure on scanning electron microscopic analysis which facilitated its application in foods in order to improve the rheological properties. The HePS was also thermally stable having a high degradation temperature (283.5°C) as observed by Thermogravimetric analysis. The HePS from *Lactobacillus plantarum* SKT109 isolated from Tibet Kefir contained fructose and glucose monomer units in an approximate molar ratio of 3:1 and the biopolymer had an average molecular weight of 2.1×10^6 Da [23]. Cell viability tests of the HePS showed that it specifically inhibits the cancer cell lines such as Pancreatic cancer cell line (MiaPaCa2) and Colon carcinoma cell line (DLD2) and is non-toxic to normal cell lines such as mouse fibroblast cell line (L929 cells) [24]. The HePS (LPC-1) from *L. plantarum* C88 exerted a significant antioxidant activity against H_2O_2-induced injury in Caco-2 cells *in vitro* [25]. Another HePS from *Lactobacillus plantarum* TISTR 875 isolated from Marine Fish (Table 1) dominantly promoted the growths of probiotics like bifidobacteria and lactobacilli under highly competitive condition of the human colon [21]. All of these above findings suggest that the HePS from LAB can be potentially used as a prebiotic ingredient in food industry.

Cryoprotectant: The lyophilization using cryoprotectants (one or more substances which protect cells membranes against the effects of exposure to low temperature) is the most satisfactory method for the long term preservation of microbial cultures [26]. The HoPS like dextran as cryoprotectants have been used for preparing bacterial suspensions before drying [27]. HePS can also be used as cryoprotectant. Several strains of *Oenococcus oeni* isolated from different types of wine were evaluated for the production of exopolysaccharides using Grape juice medium [28]. Most of the isolated strains of *Oenococcus oeni* produced HePS and possessed eps gene cluster in their genome [28]. The HePS produced were capsular in nature and concentration of HePS formed was less than 0.1 g/L. In the presence of sucrose the *Oenococcus oeni* strains produced HoPS like β-glucan, dextran and levan [28]. The capsular HePS and dextran proved to be effective cryoprotectants for the *Oenococcus oeni* strains as observed during revival after freeze drying. The ability to produce capsular HePS and dextran in the presence of sucrose helps certain strains *Oenococcus oeni* to naturally survive for longer time as compared to non-producing strains [28].

Antioxidant: The EPS from LAB are also proved to be beneficial in food and fermentation industries for antioxidant activity. The HePS has shown antioxidant properties comparable to the potent antioxidant ascorbic acid [24]. The composition analysis of HePS is of great interest when investigating its antioxidative activity. The antioxidant activity of several HePS from LAB has been evaluated as shown in Table 1 and it has been found that the monosaccharide constituent might have great influence on antioxidant activities of HePS [24]. It has been also observed that more is the content of neutral monosaccharides such as D-galactose, fucose, arabinose and mannose, the higher will be the antioxidant activities. Particularly the heteropolysaccharides containing the neutral D-galactose molecule has shown the antioxidant activities. Although there is an exception; like HePS from *Lactobacillus plantarum* RJF4 does not possess any D-galactose molecule but has mannose as neutral monosaccharide. However HePS from *Lactobacillus plantarum* RJF4 has shown DPPH free radical scavenging activity (measurement of antioxidant activity) of 22.63% and D-galactose containing HePS from *Lactobacillus plantarum* C88 and *Lactobacillus plantarum* YW32 demonstrate higher DPPH free radical activities of 55.23% and 64.5%, respectively [2,22]. Apart from antioxidant activity, HePS from *Lactobacillus plantarum* RJF4 (consisting of glucose and mannose) has displayed the cholesterol-lowering ability and the inhibition of α-amylase enzyme which could result in anti-diabetic role [24]. In addition to this another novel cell bound HePS from *Lactobacillus plantarum* 301102 showed antimutagenic activity on 3-amino-, 4-dimethyl-5H-pyrido indole [29].

Viscosifier: The application of EPS from in varying industrial areas is mainly due to their rheological properties that allow the formation of viscous solutions at low concentrations (0.05–1.0%) and stability over wide temperature, pH and ionic strength ranges [30]. The EPS which exhibited a non-Newtonian and pseudo-plastic behavior can be used as viscosifier in food and pharmaceutical industry [2,23]. The HoPS from LAB are extensively used as commercial viscofier although several HePS from LAB are also reported. HePS produced by *Lactobacillus plantarum* YW11 isolated from Tibetan-Kefir was composed of glucose and galactose along with N-acetylated sugar residues in a molar ratio of 2:71:1 [31]. The molecular mass of YW11 HePS was 1.1×10^5 Da and it showed high viscosity in skim milk at low temperature and acidic pH [31]. However, HePS produced by another strain of *Lactobacillus plantarum* YW32 also isolated from Tibetan-Kefir consisted of mannose, fructose, galactose and glucose in a molar ratio of 8.2:1:4.1:4.2 and had a molecular weight of $1.0^3 \times 10^5$ Da [31]. It has been observed that the HePS with high molecular weight (higher than 10^4 Da) possessed viscosifying ability [2,23]. It has reported that the high molecular weight of EPS also contributed in improving the texture of fermented milk by cross-linking of the EPS into a network structure with the proteins during fermentation [32]. This observation is correlated with the present findings. Another HePS from *Lactobacillus plantarum* STK109 with an average molecular weight of 2.1×10^6 Da improved the rheology of the fermented milk without causing post-acidification during storage [23]. It also enhanced the flavor of fermented milk by increasing the concentration of characteristic flavoring compounds such as 3-hydroxy-butanone, 1-pentanol, 2-nonanone and 2-ethyl-1-hexanol. HePS from *L. plantarum* STK109 at a same time also eliminated the compounds such as dimethyl sulfone, dimethyl trisulfide and octanoic acid which had characteristic filthy flavors [23].

Future prospects for heteropolysaccharides from Lactic acid bacteria

The current trend of food industry is to reduce the use of chemical additives. Natural food additives such as HePS can become highly valuable due to its wide array of physicochemical properties. The cost of producing HePS can further be reduced by in situ production in different food matrices using efficient HePS strains. With further research on HePS its novel functionalities and applications can be revealed.

Conclusion

There has been a marked increase in the use of HePS produced by LAB in the last decade. The enormous variation in the composition and structure of HePS allows it to be a versatile microbial product for diverse applications. The only drawback of HePS as compared with the more studied HoPS, is that the ability of HePS production in some strains is variable at the genetic level. Nonetheless HePS helps in survival of probiotic LAB in harsh environment such as of the human GI tract. The application of HePS as a viscosifier and texture enhancer is widely known. The HePS also confers the immunomodulating effects, cholesterol lowering effect and inhibits α-amylase, which are some of its health beneficial functions. However the majority of LAB produce a very low level of HePS as compared to HoPS therefore optimized methodologies are required for enhanced HePS production and for their recovery from fermentation broth.

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