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Lactic Acid Bacteria Exopolysaccharides in Foods and Beverages: Isolation, Properties, Characterization, and Health Benefits

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Abstract

Exopolysaccharides produced by lactic acid bacteria are a diverse group of polysaccharides produced by many species. They vary widely in their molecular, compositional, and structural characteristics, including mechanisms of synthesis. The physiochemical properties of these polymers mean that they can be exploited for the sensorial and textural enhancement of a variety of food and beverage products. Traditionally, lactic acid bacteria exopolysaccharides have an important role in fermented dairy products and more recently are being applied for the improvement of bakery products. The health benefits that are continually being associated with these polysaccharides enable the development of dual function, added-value, and clean-label products. To fully exploit and understand the functionality of these exopolysaccharides, their isolation, purification, and thorough characterization are of great importance. This review considers each of the above factors and presents the current knowledge on the importance of lactic acid bacteria exopolysaccharides in the food and beverage industry.

INTRODUCTION

Lactic acid bacteria (LAB) have been closely associated with humans throughout history. They impart a myriad of desirable and positive effects on the fermented foods that they inhabit, including improvement of sensory attributes and product safety and quality (Holzapfel & Wood 2014). Exopolysaccharides (EPSs) are an important metabolite produced by many LAB species. EPSs are a diverse group of long-chain, high-molecular-mass polysaccharides that vary in their composition and physiochemical characteristics (Ruas-Madiedo et al. 2009). Some authors define EPSs as those polymers that are loosely associated with the cell envelope or released into the extracellular medium, whereas others use the term solely to describe those polysaccharides that have no covalent attachment to the bacteria and exist freely in the surrounding environment (Badel et al. 2011, Kleerebezem et al. 2010). The ecological role of EPSs for the producing bacterium remains unclear and may be multifaceted, such as providing protection and enabling persistence in various niches (biofilm formation), or they may be associated with carbon catabolism (Schwab et al. 2007, Walter 2008, Zannini et al. 2016). Traditionally, EPSs were not believed to be used by the producer strain as an energy source, but this may not be the case for all species (Baruah et al. 2017, Gänzle & Schwab 2009).

LAB EPSs have traditionally been known to play a role in the rheological and sensory characteristics of fermented dairy products such as cheese and yogurt. Many cultures used as starters or adjuncts in these products produce EPSs that can positively influence product characteristics such as texture and sensory qualities (Mende et al. 2016). These technofunctional aspects of EPSs are generally related to their ability to bind water and retain moisture (Costa et al. 2010). In addition, use of EPS-producing cultures has garnered interest from the cereal and bakery industry, where their hydrocolloidal nature is being studied as a natural replacement for commercial food gums such as hydroxypropylmethylcellulose (HPMC) (Galle et al. 2012). The increasing recognition of the health-associated benefits of these polymers enables the production of added-value, functional products that are in line with consumer demand for natural and healthy products containing fewer additives (Welman 2009).

EXOPOLYSACCHARIDES: THEIR SYNTHESIS, ISOLATION, AND CHARACTERIZATION

EPSs can be divided into heteropolysaccharides (HePSs) and homopolysaccharides (HoPSs), depending on the composition of the main chain and their mechanisms of synthesis. In general, HePSs are constituted by more than one monosaccharide type and are synthesized intracellularly, whereas HoPSs consist of only a single type of monosaccharide and are produced externally to the cell by an enzyme secreted by the bacterium (Badel et al. 2011). The characteristics of LAB HoPSs and HePSs are outlined in **Table 1**.

Heteropolysaccharides

HePSs contain more than one type of monosaccharide in the sugar polymer chain, typically D-glucose, D-galactose, and L-rhamnose, but other monosaccharides (e.g., fructose, fucose, mannose, N-acetylglucosides, and glucuronic acid) and noncarbohydrate moieties (e.g., phosphate or acetyl groups) can be present (De Vuyst & Degeest 1999, London et al. 2014, Werning et al. 2012). In general, the number of different monosaccharides in the HePS can range from two to eight (Werning et al. 2012). HePSs are produced by members of the genera *Lactobacillus*, *Lactococcus*, *Streptococcus*, and *Bifidobacterium*. The number of different constituent monosaccharides (e.g., noncarbohydrate groups) that can be present, the variety of linkage types between the monomers, and the potential for synthesis of a linear or branched polymer mean that there is great diversity in

Table 1 Characteristics of heteropolysaccharides and homopolysaccharides produced by lactic acid bacteria

Heteropolysaccharides	Homopolysaccharides
Contain more than one type of monosaccharide	Contain one type of monosaccharide
Major monosaccharides: glucose, galactose, and rhamnose	Major monosaccharide: glucose or fructose
α and β links present	α or β link present
Typically branched	Typically linear or branched
Molecular mass: 10^4–10^6 Da	Molecular mass: $>10^6$ Da
Mainly produced by *Lactobacillus*, *Lactococcus*, *Bifidobacterium*, and *Streptococcus*	Mainly produced by *Lactobacillus*, *Leuconostoc*, *Oenococcus*, and *Weissella*
Produced from intracellular intermediates	Produced extracellularly from sucrose or starch
Produced in relatively low amounts: milligrams per liter	Produced in relatively high amounts: grams per liter
Presence of noncarbohydrate groups	Absence of noncarbohydrate groups
Can contain charged groups	Typically carries no charge
Primarily associated with immune modulation	Primarily associated with prebiotic capacity

the structure of HePSs that can be produced by LAB. In addition, even within strains of the same species, the monosaccharide content of the produced HePSs can vary. For example, analysis of the polymer composition of HePSs produced by a number of strains of *Lactococcus lactis* showed variation in the sugar composition, ranging from three to six different monosaccharides (Suzuki et al. 2013).

HePSs are produced by the polymerization of sugar-nucleotide precursors (e.g., UDP-glucose and UDP-galactose) that are formed within the cell. These sugar nucleotides are not wholly involved in EPS production and are used in the synthesis of a variety of polysaccharides within the cell; the enzymes involved in their formation also have important housekeeping functions (Welman & Maddox 2003) (**Figure 1**). In contrast, the formation of EPSs from these precursors is controlled by glycosyltransferase genes that, along with genes involved in regulation,

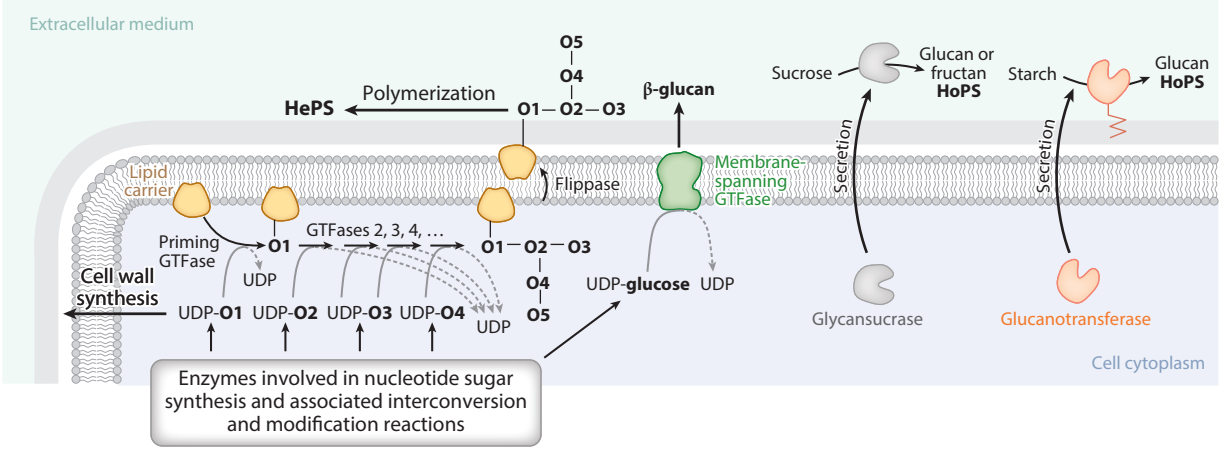

Figure 1

Mechanisms of synthesis of heteropolysaccharides (HePSs), homopolysaccharides (HoPSs), and β-glucan in lactic acid bacteria (LAB). HePSs are synthesized via the action of intracellular glycosyltransferases. In contrast, HoPSs are formed by the action of secreted glycansucrase or glucanotransferase enzymes. Abbreviations: GTFase, glycosyltransferase; UDP, uridine diphosphate; O1–O5, glycoside 1–glycoside 5.

chain-length determination, and polymerization and export, are generally located together in an operon and form what is termed an EPS biosynthesis cluster (Werning et al. 2012). Variability in the glycosyltransferase genes present in this operon is a driver of the large diversity of HePSs that are produced by LAB (Kranenburg et al. 1997, Stingele et al. 1996). The exact mechanisms of polymerization, chain-length determination, and export from the cell are yet not fully understood, but a flippase enzyme may be involved in the transfer of the lipid-bound polymer to the external side of the plasma membrane (Werning et al. 2012).

HePSs are synthesized in low amounts, in the range of milligrams per liter of culture, and the molecular mass of HePSs ranges from 4×10^4 to 9×10^6 Da (Ruas-Madiedo et al. 2009). Mozzi et al. (2006) analyzed 31 HePS-producing mesophilic and thermophilic LAB and found that yields ranged from 10–166 mg/L (Mozzi et al. 2006). The yield and molecular properties of HePSs can be affected by a number of factors, including medium composition (e.g., carbon, nitrogen, and inorganic nitrogen sources), growth conditions (e.g., pH, temperature, and oxygen), and incubation time (De Vuyst & Degeest 1999, Ruas-Madiedo et al. 2009). Under optimized culture conditions, Ismail & Nampoothiri (2010) obtained a yield of 1.2 g/L HePS from a strain of *Lactobacillus plantarum*.

Homopolysaccharides

HoPSs are polymers of a single monosaccharide, either glucose or fructose, and are termed glucans or fructans, respectively. They are synthesized extracellularly from sucrose by the action of a single enzyme known as a glycansucrase (Monsan et al. 2001). The glycansucrases that synthesize glucans and fructans are glycoside hydrolases (GH) and are grouped into the GH70 and GH68 families, respectively (Leemhuis et al. 2013a, van Hijum et al. 2006). HoPSs are produced by a number of LAB genera, including *Lactobacillus*, *Streptococcus*, *Leuconostoc*, *Oenococcus*, and *Weissella* (Dimopoulou et al. 2016, van Hijum et al. 2006). HoPS production has not been identified in bifidobacteria (Monsan et al. 2001). HoPSs can be further classified based on the predominant glucosidic or fructosidic linkage that occurs in the polymer backbone. Both dextran and reuteran are α-glucan HoPSs; however, whereas dextran contains predominantly α-(1→6) linkages between the glucosyl units, reuteran contains predominantly α-(1→4) linkages. Similarly, inulin and levan are fructan HoPSs but consist of β-(2→1) and β-(2→6) linkages, respectively (Monsan et al. 2001).

Synthesis of HoPSs is relatively direct when compared to HePSs, as it does not involve sugar nucleotide precursors and is mediated by a glycansucrase enzyme that is encoded by a single gene (Badel et al. 2011). The α-glucan HoPSs synthesized in this manner are distinct from the so-called β-glucans that are synthesized by some genera of LAB. β-glucans are produced by some strains of *Pediococcus*, *Oenococcus*, and *Lactobacillus*. In contrast to α-glucan synthesis, β-glucan formation is more comparable to HePS production in that the polymer is built intracellularly by a single glycosyltransferase that uses UDP-glucose as the repeating unit (Werning et al. 2012). Sucrose is not a substrate for this enzyme. Although both α-glucans and β-glucans have been described in LAB, only β-fructans have been identified (Ruas-Madiedo et al. 2009).

The majority of HoPS-producing LAB produce a single glycansucrase enzyme, but some contain more than one glycansucrase gene and therefore may synthesize more than one type of HoPS. For example, the well-studied prototype dextran-producing bacterium *Leuconostoc mesenteroides* NRRL B-512F encodes both a glucansucrase and a fructansucrase and has been shown to produce levan in addition to dextran (Kang et al. 2005, Maina et al. 2008). In general, the molecular mass of HoPSs is greater than 10^6 Da (Ruas-Madiedo et al. 2009). For instance, dextran synthesized by *L. mesenteroides* NRRL B-512F ranges from 6.2 to 7.1×10^6 Da, whereas dextran

produced by *Weissella cibaria* MG1 varies from 5×10^6 to 4×10^7 Da (Cerning 1990, Wolter et al. 2014). HoPSs are produced in amounts higher than are HePSs, in the range of grams per liter of culture, generally reaching values of up to 10 g/L. It is noteworthy that for use in food applications, production amounts of 10–15 g/L are required to be economically viable (De Vuyst & Degeest 1999). *L. mesenteroides* NRRL B-512F, which is used to produce dextran at industrial levels for food applications, yields under 20 g/L (Ruas-Madiedo et al. 2009). Higher levels are obtainable from some LAB species, particularly strains of *Weissella*, some of which are notable for very efficient production of dextran. Such hyperproducer strains include *Weissella confusa* Cab3, which has been shown to produce dextran at 34 g/L, and *W. cibaria* MG1, from which 36 g/L dextran is obtainable (Shukla & Goyal 2011, Shukla et al. 2014, Zannini et al. 2013). Similar to HePS production, optimization of media composition and culture conditions is required to attain maximum HoPS yield (Ruas-Madiedo et al. 2009).

In the presence of certain acceptor sugars, glycansucrases can form low-molecular-mass oligosaccharides (OSs) from sucrose (Korakli & Vogel 2006). Their synthesis occurs via successive transfer of glucosyl or fructosyl units to the acceptor rather than the glucan or fructan polymer, with the formation of glucooligosaccharides (GOSs) or fructooligosaccharides (FOSs), respectively. OS synthesis can lead to a decreased yield of high-molecular-mass glucan or fructan EPSs. For example, in the presence of maltose, dextransucrase from *W. cibaria* MG1 synthesizes panose and glucosylated panose with a degree of polymerization up to 14 (Galle et al. 2010).

Recently, the presence of an enzyme in strains of *Lactobacillus reuteri* that can produce α-glucan EPSs using starch and maltodextrins, rather than sucrose, as a glucose donor has been identified (Leemhuis et al. 2013b). The enzyme is a cell-associated transglycosylating (GH70) 4,6-α-glucanotransferase that synthesizes α-glucan polymers containing a significantly increased proportion of α-(1→6) linkages compared to the substrate starch molecules. In *L. reuteri* 121, the gene encoding this enzyme is located adjacent to the glycansucrase gene that encodes reuteran HoPS production from sucrose (Bai et al. 2016).

Production, Isolation, and Purification of Exopolysaccharides

Screening for EPS production typically involves the use of a solid growth medium supplemented with additional growth factors or nutrients to promote EPS production. For example, screening for HoPS production typically involves variations on the supplementation of de Man Rogosa and Sharpe (MRS) media with sucrose. Other monosaccharides such as fructose or maltose may be included (Di Cagno et al. 2006, Lynch et al. 2014, Schwab et al. 2008, Van der Meulen et al. 2007). After bacterial growth, the appearance of a slime or mucoid phenotype is indicative of HoPS production. As the synthesis of HePSs is linked to bacterial growth and metabolism, screening for these EPSs involves the use of media or reconstituted skim milk supplemented with additional carbon sources such as glucose or lactose (in the case of dairy isolates) (Mende et al. 2012a, Mozzi et al. 2006). HePS-producing colonies appear shiny on solid media, and the EPSs may form a string if an inoculation loop is used to examine the colony (Mozzi et al. 2001); however, because HePS production levels are generally less than those of HoPSs, HePS synthesis may not be as obvious in the absence of further investigation. Molecular biology employing degenerate primers has also been used as a rapid means to screen for HoPS production; however, the presence or absence of an identifiable gene does not always correlate with the phenotype (Van der Meulen et al. 2007).

If the desire is to further study and characterize the EPSs or simply to exploit them as a food ingredient, the initial aim may be to maximize their production, followed by isolation from

the bacterial culture. The bacterium is typically grown in a liquid medium or milk of a similar composition to that used for screening. Unlike the medium used for screening, the medium for production can be further optimized to maximize EPS yields. For example, because of the fastidious nature of LAB, supplementation of media with micronutrients, such as additional carbon and nitrogen sources, or vitamins may be advantageous (Mende et al. 2012a).

There is no single optimal solution for maximizing EPS yields. This is due to the strain-dependent nature of EPS production, with variations due to strain characteristics such as adaptation to different niches and environmental conditions, sugar uptake, gene regulation, and enzyme activity (Leroy & De Vuyst 2016). An important consideration regarding the growth medium for EPS production is the presence of carbohydrates that could interfere with subsequent EPS quantification and characterization. These include glucomannans that are present in yeast extract and peptone. Although the aim of subsequent purification procedures is to remove such carbohydrates, their avoidance by employing a semi-defined media is preferential (Ruas-Madiedo & de los Reyes-Gavilán 2005).

Adjustment of process conditions such as temperature and pH may also lead to higher EPS levels. Because of the coupling of HePS synthesis to cell growth and carbon flux, temperature modification has been shown to have varying effects on EPS production (De Vuyst & Degeest 1999); however, in general, a lower temperature is better for HoPS synthesis, as glycansucrases have a lower optimum temperature range (Grosu-Tudor & Zamfir 2014, Kang et al. 2009). Control of reaction pH, although more difficult to achieve, could also result in higher EPS yields. For example, the HoPS enzyme of *W. cibaria* has been found to have a pH optimum in the range of pH 5–6 (Kang et al. 2009). Use of suitable agents to buffer the pH drop during fermentation or continuous readjustment of pH in a bioreactor could potentially be used to maintain the reaction pH within the optimum range for the enzyme for a longer period of time, thus increasing EPS yield.

Methods for the production and isolation of EPSs were recently reviewed by Leroy & De Vuyst (2016), who provided a more in-depth view of recent developments on this topic. For the isolation and recovery of EPSs, a liquid medium is typically employed. EPSs can be recovered using variations of a general method involving precipitation from the medium, followed by purification of the recovered polysaccharide. The amount of purification performed is dependent on the desired final purity (Ruas-Madiedo & de los Reyes-Gavilán 2005). Depending on the medium, prior to isolation it may be desirable to introduce certain pretreatment steps. For example, a heating step has been used to inactivate any endogenous enzymes (Shao et al. 2014). An initial centrifugation separates the bacterial cells and coagulated proteins from the medium. In addition, proteins can be precipitated at this stage using trichloroacetic acid (TCA) (Shao et al. 2014) or, alternatively, with proteases (London et al. 2014); however, some choose to perform this step after the EPSs have been separated from the medium (Polak-Berecka et al. 2015). EPSs are removed from the medium via precipitation with cold ethanol (Malang et al. 2015, Shao et al. 2015), although acetone has also been employed (Garai-Ibabe et al. 2010, Ismail & Nampoothiri 2010). Following precipitation, the EPSs are dissolved in distilled water. In addition, protein removal with TCA may be performed at this stage. Ultrafiltration has also been applied (Polak-Berecka et al. 2015). In general, the dissolved EPSs are then dialyzed against water to remove low-molecular-mass contaminating carbohydrates. A dialysis membrane with a 12–14-kDa cutoff has been used for this purpose (Wang et al. 2010). Subsequently, the EPSs are lyophilized, which permits stable storage. Additional purification steps such as size-exclusion chromatography (SEC) or ion-exchange chromatography have been performed, but such steps are mainly important if the EPSs are to be subsequently characterized (Fontana et al. 2015, Li et al. 2014, Shao et al. 2014).

Exopolysaccharide Quantification and Characterization

The most straightforward method for quantifying EPSs is to measure the dry weight of the purified polysaccharide. This, however, is not very accurate, particularly when small amounts of EPSs are produced (HePSs) and especially if impurities remain (Lynch et al. 2014, Mende et al. 2016). The most commonly used method to determine EPS yield is the measurement of reducing sugars by the colorimetric phenol-sulfuric acid method (Hongpattarakere et al. 2012, London et al. 2014, Mende et al. 2012a,b, Wang et al. 2014). Again, the presence of contaminating carbohydrates (from growth medium) can interfere with this method. Fractionation of the EPSs with SEC prior to quantification can improve the specificity of the quantification (Mende et al. 2016, Notararigo et al. 2013).

Given the diversity of possible sugar components, the stereo-specificity of linkages, and the possibility of charged groups, coupled with the potential for linear or branched structures, the range of molecular conformations possible is large, making the characterization of EPSs complex. However, the properties and behavior of EPSs in food systems are determined by their composition, molecular structure, and average molecular mass; thus, such properties must be investigated to fully understand their role and exploit their functionality and food applications (Freitas et al. 2011). A suitably purified polymer is an important factor for reliable characterization (Mende et al. 2016). In addition, the complexity of the methods employed depends on the physiochemical complexity of the polysaccharide and the desired level of information. Although the structure of HePSs suggests that their characterization represents a greater challenge than that of HoPSs, the apparent structural simplicity of the latter is deceptive, as these EPSs may show a wide distribution in terms of molecular mass, length of branch chains, if present, and density of branches (Maina 2012, Mende et al. 2016).

The molecular mass of EPSs is typically determined by high-performance SEC (HP-SEC) coupled with a refractive index detector or, more recently, a multi-angle light-scattering detector (Bounaix et al. 2009, Garai-Ibabe et al. 2010, Miao et al. 2016, Polak-Berecka et al. 2015). Gel permeation chromatography has also been used for this purpose but provides lower resolution (Freitas et al. 2011, Malang et al. 2015). Suitable molecular mass standards are required for size determination. The monosaccharide composition has been primarily determined by high-performance, anion-exchange chromatography coupled with pulsed amperometric detection (Costa et al. 2012, Lynch et al. 2014, Shao et al. 2014) or gas chromatography (GC). An initial hydrolysis is required to degrade the polysaccharide to its constituent monosaccharides, which are then detected. GC also allows the determination of linkage type and monosaccharide confirmation; however, an initial methylation step is required. In this way, the linkages present in the polymer are determined by methylation of all free hydroxyl groups prior to hydrolysis, after which the resulting monosaccharides are converted to partially methylated alditol acetates, which are detected by GC-mass spectrometry (Freitas et al. 2011). The type of linkage present, particularly for HoPSs, can more simply be determined by applying enzymes that are specific to the linkages present in the polysaccharide. The resultant release of constituent monosaccharides indicates the presence of a linkage in the polymer that is targeted by the enzyme (e.g., hydrolysis of a polymer by a dextranase suggests the presence of α-1,6 linkages, i.e., the EPS may be a dextran) (Schwab et al. 2008). The presence of a charge on the EPSs has been determined by anion-exchange chromatography, carbohydrate gel electrophoresis, or zeta-potential measurements with latex beads (Costa et al. 2012; Mende et al. 2012b, 2016). To obtain information about functional groups, including the presence of charged moieties such as phosphate and sulfate groups, Fourier transform–infrared spectroscopy has been employed (Pan & Mei 2010, Shao et al. 2014, Wang et al. 2014).

Nuclear magnetic resonance (NMR) spectroscopy represents the single analytical tool from which the most information about carbohydrate molecular structure can be gained and can provide

sufficient information to determine all the structural features of a polysaccharide (Maina 2012). NMR allows the elucidation of the interactions of carbon and hydrogen atoms with adjacent atoms and chemical groups, enabling the determination of their relative position in the structure and thus the polymer configuration (Freitas et al. 2011).

Quantification of EPSs in foods presents a challenge because the complex nature of food matrices can interfere with the analysis. Typically, EPSs have been measured as equivalently released monosaccharides. That is, upon hydrolysis, the EPSs are broken down into their constituent monosaccharide units, e.g., glucose. It is this released glucose that is measured and used to determine the original level of EPS present. The food substance that does not contain EPSs is used as a control for correction of background carbohydrates in the matrix (Leroy & De Vuyst 2016). Katina et al. (2009) developed an enzyme-assisted method for the determination of dextran in sourdough. This involved the application of dextranase and amyloglucosidase to hydrolyze in situ produced dextran, which was then measured as equivalent glucose using a specific assay kit. An alternative approach, as performed by Tamani et al. (2013), is to precipitate carbohydrates from the food matrix and then measure the content with the phenol-sulfuric acid method (Tamani et al. 2013). Acid hydrolysis is an alternative to using enzymes (Galle et al. 2010, Juvonen et al. 2015), particularly for HePSs, where there is limited availability of suitable enzymes specific to the many linkage types that can be present in these polysaccharides (Enikeev 2012). These approaches can be imprecise, and it is important to use the appropriate controls to account for interfering background carbohydrates in the matrix. In addition, the recovery is generally lower than 100 percent, which must be taken into account (Katina et al. 2009).

Several methods allow the direct visualization of EPSs within the food matrix; although such methods are qualitative rather than quantitative, they allow visualization and confirmation of EPS production in situ. Confocal laser scanning microscopy (London et al. 2015, Zhang et al. 2015) and scanning electron microscopy (London et al. 2014) are most commonly employed, particularly in dairy fermentation research, as they confirm in situ production without destroying the microstructure of the food and allow observation of the interaction of the EPSs with other food constituents (Costa et al. 2010, London et al. 2015).

TECHNOFUNCTIONAL PROPERTIES OF EXOPOLYSACCHARIDES: ROLES IN FOOD PRODUCTS

The technofunctional role of EPSs in foods is generally related to their ability to act as hydrocolloids and to bind water and retain moisture within the product. Such properties are a function of the molecular characteristics of the polysaccharide, such as composition, molecular mass, chain conformation, and presence and density of branching, and, therefore, intrinsic viscosity, [η]. [η] represents the space occupied by a polymer in solution and can be considered as a parameter describing the thickening capacity of a polysaccharide. A high [η] usually indicates larger molecules with stiff chains, whereas a low [η] relates to smaller molecules with flexible chains (Mende et al. 2016). EPSs have applications in many food sectors but primarily in the dairy and cereal areas. Increasing recognition of their health-associated benefits enables the production of added-value, functional products that are in line with consumer demand for natural and healthy alternatives containing fewer additives.

Dairy Products

In the dairy fermentation industry, LAB EPSs are technologically important for their rheological and textural effects on products, e.g., they have been used in reduced-fat (RF) and half-fat cheeses

to improve the textural and rheological properties of these products (Awad et al. 2005, Costa et al. 2010). Milk fat plays an important role in cheese structure because the membrane that surrounds the milk-fat globules presents a hydrophilic surface to the surrounding protein matrix and acts as a noninteracting filler that prevents complete coalescence of the proteins. This results in pools of serum being distributed with fat globules throughout the curd matrix. If the fat content of the cheese is lowered, the number of these pools and the moisture-to-protein ratio of the cheese decrease (Broadbent et al. 2001). Thus, the removal of fat results in a denser protein network, which affects the cheese texture (Mozzi et al. 2006). Therefore, an important strategy for improving the functional properties of low-fat cheese is to increase its moisture content sufficiently to provide a moisture-to-protein ratio that is comparable to its full-fat counterpart (Broadbent et al. 2001). Cheese made with EPS-producing starters has higher moisture levels, with a microstructure similar to full-fat cheeses (Hassan & Awad 2005). The EPS-producing LAB that have typically been studied and employed in dairy products are strains that are traditionally associated with dairy fermentations and that typically synthesize HePSs. Awad et al. (2005) examined the effect of using a ropy *L. lactis* subsp. *cremoris* strain (JFR1) on the textural, melting, and sensory characteristics of RF Cheddar cheese. Use of this EPS producer generated RF cheese with a higher moisture level than the control and similar textural and melting properties to that of the full-fat type (Awad et al. 2005). Binding and water retention by the EPSs were suggested to be the reasons for the observed positive effects. The mechanistic role of EPSs was shown by electron microscopy observations, which found that RF cheese made with strain JFR1 had a more porous protein network compared to RF controls; a three-dimensional network of EPSs was observed within large pores (Awad et al. 2005).

Similar findings were observed by Dabour et al. (2006) who produced half-fat Cheddar cheese with a number of capsular and ropy *L. lactis* subsp. *cremoris* strains, including JFR1. All strains increased cheese yield and moisture levels; however, it was found that strain JFR1 resulted in ripened or aged cheese with a very open structure and slightly weak texture, thus suggesting it may be more suited to producing RF unripened or soft cheeses (Dabour et al. 2006). Application of an *L. lactis* subsp. *cremoris* strain cured of its EPS phenotype demonstrated the importance of EPSs in retaining moisture, as half-fat cheese produced with the EPS-producing parental strain had a significantly higher moisture content and yield (Costa et al. 2010). Few studies have examined the use of HoPS EPSs in cheesemaking. Lynch et al. (2014) used a dextran-producing strain of *W. cibaria* as an adjunct culture and its associated dextran EPSs as bio-ingredients in the manufacture of Cheddar cheese. Although not statistically significant, increased moisture levels were observed in all cheeses when compared with the control cheeses containing only the starter strain (Lynch et al. 2014).

In fermented milk, such as yogurt, as is the case with cheeses, EPSs can affect the formation of the casein-gel structure by acting as a filler and as nuclei for the formation of serum channels and large pores containing bacterial cells, EPSs, and milk serum (Hassan 2008). Important factors influencing the function of EPSs in yogurt are understood to be their molecular characteristics and their ability to interact with milk proteins. For example, the level of EPS-EPS or EPS-protein interactions is responsible for the formation of long strands in yogurt, i.e., ropiness. The intrinsic viscosity of EPSs depends on molecular mass and chain stiffness, with no clear effect of the chemical composition. However, it has been shown that certain β-linkages in the backbone lead to stiffer chains than do α-linkages (Laws et al. 2001). The polymer-like behavior of the serum phase in yogurt containing EPSs increases the product consistency and viscosity (Hassan 2008). In general, EPSs with a high molecular mass and a stiff backbone with few branches are responsible for a creamy texture with high viscosity, whereas flexible, highly branched EPSs with low molecular mass lead to thin products with low viscosity (Gentès et al. 2011).

Cereal-Based Products

LAB EPSs can be exploited to positive effect in cereal-based products, particularly through the application of sourdough technology (Moroni et al. 2009). Heterofermentative LAB are predominant in sourdough, and many strains produce HoPSs. Of 140 sourdough isolates tested for EPS production, 20% produced HoPSs from sucrose. In addition, it is postulated that every sourdough contains at least one EPS-producing *Lactobacillus* strain (Tieking & Gänzle 2005). Species of other LAB genera, such as *Leuconostoc* and *Weissella*, can also constitute the EPS-producing community (Björkroth et al. 2014, Galle et al. 2010).

Research with LAB EPSs in the baking industry has focused on their potential to replace commercial compounds such as HPMC, guar gum, and xanthan gum. These hydrocolloids are extensively employed for their positive influence on product rheology, texture, and shelf-life; however, as additives, their use in a product requires labeling. In contrast, the application of LAB EPSs, when produced in situ by starter or adjunct cultures, does not require labeling and therefore is seen as positive in an industry in which consumers continually demand products with fewer additives (Waldherr & Vogel 2009). The potential beneficial effects of the EPSs in dough and bread include increased water absorption of the dough, better dough rheology and machinability, increased loaf volume, and decreased bread staling rate (Arendt et al. 2007, Waldherr & Vogel 2009).

Because of their water-binding capacity and interaction with structure-forming components such as gluten and starch, EPSs can positively impact baking and bread quality. Dextran produced during wheat sourdough fermentation was shown to increase loaf volume and improve crumb softness compared to sourdough without dextran (Di Cagno et al. 2006). Similarly, Katina et al. (2009) demonstrated a 10% increase in sourdough loaf volume and a 25% to 40% increase in crumb softness through in situ dextran production by a *W. confusa* strain. Galle et al. (2012a) found an increased wheat bread volume of up to 35% when supplemented with 20% *W. cibaria*–fermented sourdough, compared to control wheat bread with glucose alone. The incorporation of sourdough also resulted in a significant decrease in crumb hardness and a reduced staling rate (Galle et al. 2012). The mechanisms by which HoPSs improve loaf volume are not fully understood, but they are postulated to support the gluten network, thus improving gas retention (Lacaze et al. 2007). Staling of bread is a complex process resulting from starch degradation and migration of moisture from starch granules to interstitial spaces, followed by subsequent loss and thus drying and firming of the crumb. HoPSs may slow the staling process by binding water and preventing moisture loss, thus retarding starch crystallization (Waldherr & Vogel 2009).

In celiac disease, which affects approximately 1% of the world's population, complete avoidance of gluten is the only treatment for sufferers. Because the gluten network is an important structure-forming component of baked goods, gluten-free (GF) products are often of low quality and poor texture and exhibit poor crumb and crust characteristics and mouthfeel (Moroni et al. 2009, Waldherr & Vogel 2009). In addition, GF bread is characterized by decreased loaf volume, early staling, and a lower content of dietary fiber compared to conventional wheat and rye products (Arendt et al. 2007). The technofunctional properties of HoPSs mean that they represent an important tool in the development of GF products of high quality and nutritional value. Schwab et al. (2008) prepared GF breads with a sorghum sourdough addition. This sorghum sourdough was produced by fermentation with either *W. cibaria* or *L. reuteri* strains. Both strains produced EPSs and OSs during fermentation. Breads fermented with *W. cibaria* were less firm than those fermented with *L. reuteri*, and although the FOSs produced by the latter strain were digested by *Saccharomyces cerevisiae*, GOSs produced by *W. cibaria* were detected in the bread, thus potentially increasing the prebiotic content of the GF product (Schwab et al. 2008). Similarly, Galle et al.

(2010) demonstrated EPS and OS production by *W. cibaria* strains in sorghum sourdough. The levels of EPSs produced [~0.8% (w/v)] were suggested to have the potential to replace hydrocolloids, which are generally used at 0.3% (w/v). In addition, these strains produced low amounts of acetate during the fermentation (its presence at high concentrations causes off-flavor and compromises crumb structure), indicating the suitability of such strains as starter cultures (Galle et al. 2010). Thus, it is important to consider the metabolism of potential starter strains in combination with the food substrate (e.g., cereal or flour base) and also the role or effect of metabolites other than EPSs when assessing the suitability of strains for a particular fermentation application. For example, the substrate flour can have an effect on the levels of in situ–produced EPSs as a result of the flour's nutritional content and ability to support the growth of the starter strain (Wolter et al. 2014).

The majority of strains that have been applied in cereal-based products synthesize HoPSs. This may, in part, be because high concentrations of polysaccharides are generally required to obtain an effect, and such levels of HePSs are difficult to produce, as they are synthesized by strains in much lower amounts. Nevertheless, Galle et al. (2011) showed that use of a HePS-producing strain of *Lactobacillus buchneri* in sorghum sourdough resulted in decreased resistance to deformation. The strain was observed to be extremely ropy, which has been postulated to be the reason for its effects on dough rheology (Galle et al. 2011).

Apart from their application in bakery products, HoPS producers are promising for application in other cereal-based products, such as functional beverages. Zannini et al. (2013) fermented barley malt wort with dextran-producing *W. cibaria* and showed that EPSs and OSs were synthesized in this matrix and significantly influenced the rheological behavior of the wort (Zannini et al. 2013).

In Situ Production Versus Application as a Purified Ingredient

Purification of EPSs lends itself to application as a bio-ingredient in food products. Thus, EPSs could be applied in a known concentration and at a specific time point, which would enable a more controllable process compared to in situ production because the level of in situ–produced EPS depends on the fermentation conditions and substrate. In addition, in vitro production and isolation of EPSs for use as a bio-ingredient may allow wider application of HePSs, which are produced in much lower amounts than are HoPSs. Currently, up to 5% (w/w) dextran addition is allowed under European law (Eur. Comm. 2000).

Di Cagno et al. (2006) demonstrated an improvement in wheat bread volume when dextran produced by a strain of *W. cibaria* was added as an ingredient at 0.5% (w/w) (Di Cagno et al. 2006). Similarly, incorporation of such levels of dextran in a recipe containing low-protein wheat flour significantly improved the volume, hardness, and shelf-life of the low-protein breads produced (Zannini et al. 2014). Regarding the ex situ addition of EPSs in GF baking, Rühmkorf et al. (2012) compared the quality of GF breads prepared with a buckwheat and rice flour mix and supplemented with different *Lactobacillus* EPSs (levan and dextran) or HPMC [1% (w/w) addition]. All supplements increased the specific volume and reduced the crumb hardness to some extent; however, the moisture content, baking loss, and crumb hardness were improved significantly by dextran produced by a strain of *Lactobacillus curvatus* (Rühmkorf et al. 2012).

Although the ex situ application of EPSs presents a number of advantages, their use as a bio-ingredient, despite their natural source, necessitates that they are listed on the product label as an additive. Thus, there is potential to negatively impact the product, as consumer demand for products with fewer additives is increasing. In addition, isolation and purification of EPSs for use as a bio-ingredient could lead to increased production costs. In contrast, in situ production of EPSs does not necessitate labeling or additional expenditure (Waldherr & Vogel 2009).

HEALTH BENEFITS OF EXOPOLYSACCHARIDES

There is increasing evidence that EPSs from LAB have potential to influence human host health and that dietary consumption of such polymers may, for example, modulate immune function or levels of beneficial bacteria in the gastrointestinal tract. In addition, certain EPSs such as β-glucans may have a cholesterol-lowering effect and therefore be beneficial for cardiovascular health. In general, HePSs can be considered primarily as effectors of host function (immune, antioxidant), whereas HoPSs are more associated with microbial modulation (prebiotic). It is clear that the compositional (e.g., linkage type, presence of charge groups) and structural (e.g., molecular mass, branching) natures of this diverse group of polymers are important factors in determining their effects on host health.

Exopolysaccharides with Prebiotic Potential

A prebiotic is defined as "a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health" (Blatchford et al. 2013, p. 110). Bifidobacteria and lactobacilli are considered the main microbial targets of prebiotic action (Gibson 2004). One of the best known and widely commercialized prebiotics is inulin [β-(2→1) fructan] and its associated FOSs. The metabolism of these carbohydrates produces a variety of products, such as short-chain fatty acids (SCFAs), gases, and organic acids, that can have varying effects on the host, such as providing energy to intestinal colonocytes, inhibiting harmful bacteria (Wong et al. 2006), and modulating host metabolism (Delzenne et al. 2011).

LAB EPSs are of particular interest for their potential prebiotic functionality. In a batch-culture fermentation system designed to simulate transit through the large intestine, Olano-Martin et al. (2000) demonstrated the prebiotic potential of dextran and oligodextrans, which are associated low-molecular-mass OSs. Low-molecular-mass oligodextrans elicited a bifidogenic effect similar to the well-known prebiotic FOS and also resulted in decreased levels of undesirable bacteria such as bacteroides and clostridia. The utilization pattern of the oligodextrans and dextran in the batch cultures was shown to be dependent on their molecular mass, indicating the preferential utilization of lower-molecular-mass OSs by the microbiota. It was observed that higher-molecular-mass dextrans persisted for longer through the colonic model than lower-molecular-mass OSs (Olano-Martin et al. 2000). The preferential utilization of lower-molecular-mass OSs and dextrans has been observed previously, particularly for lactobacilli. Although bifidobacteria express a number of extracellular glycosyl-hydrolases, few lactobacilli contain similar extracellular enzymes, relying on OS transporters. Thus, although lactobacilli preferentially utilize di-, tri-, and tetrasaccharides, bifidobacteria preferentially metabolize OSs (Gänzle & Follador 2012, Hu et al. 2013, van den Broek et al. 2008). Sarbini et al. (2011) found that 1-kDa dextrans (linear and branched) gave rise to significant increases in *Bifidobacterium* populations and high selectivity for this genus, suggesting that the low molecular mass (i.e., versus 70-kDa dextran) meant more nonreducing ends, which are susceptible to attack by various exo-acting α- and β-glycosyl-hydrolases produced by colonic bacteria, per unit of mass. Similarly, fructan HoPSs have been shown to stimulate bifidobacterial populations in both the simulated gut environment and pure culture; in contrast, lactobacilli were unable to utilize such fructans as a carbon source in pure culture (Dal Bello et al. 2001, Korakli et al. 2002).

The ability of the intestinal microbiota to utilize various polysaccharides as prebiotics is dependent on the presence of the necessary hydrolytic enzymes specific to as large a variety of linkage types as possible (i.e., bacterial glycosyl-hydrolases) as well as the presence of transport systems for assimilation of the constituent saccharides (e.g., mono/oligosaccharide transporters) (Salazar

et al. 2009). Sanz et al. (2005) examined the influence of disaccharide structure (i.e., linkage type and composition) on prebiotic selectivity in vitro, demonstrating that the presence of certain compositions, e.g., α-(1→2)- and α-(1→6)-linked glucobioses, is more indicative of prebiotic potential. Thus, considering that the majority of LAB EPSs with demonstrated prebiotic potential are HoPSs and associated OSs, it is possible that the more complex composition of HePSs is more difficult for the gut microbiota to degrade, limiting their prebiotic potential (Ryan et al. 2015). Indeed, Cinquin et al. (2006) demonstrated that HePSs from *Lactobacillus rhamnosus* RW-9595M were not metabolized by infant microbiota, had no prebiotic effect, and caused a reduction in levels of lactobacilli. In addition, HePSs are generally produced in much lower amounts than are HoPSs, making their application in these studies more difficult. However, Salazar et al. (2008) demonstrated the prebiotic effect of HePSs isolated from a number of bifidobacteria strains. The authors observed that the bifidogenic effect of the EPSs was dependent on the producer, but the effects were similar to those found with inulin, as were the levels of SCFA. They also suggested that beneficial shifts in SCFA profiles toward butyrate and propionate resulted from increases of other intestinal members via cross-feeding mechanisms, the beneficial effects not being solely due to increased bifidobacteria levels. Thus, EPSs produced by certain members of the intestinal microbiota, e.g., bifidobacteria, may act as fermentable substrates for, and cause shifts in, other intestinal populations (Salazar et al. 2008, 2016).

Immune Modulation and Exopolysaccharides

Although prebiotic activity is generally associated with HoPSs, EPSs with immunogenic properties have been shown to be HePSs. This topic has recently been reviewed in detail by Laiño et al. (2016). Early studies demonstrated the activation of macrophages in mice and induction of cytokine production [interferon-γ (IFN-γ) and IL-1] by phosphorylated HePSs produced by *L. lactis* subsp. *cremoris* KVS20 (Kitazawa et al. 1996). The phosphate groups on the HePSs were demonstrated to be important effectors of this immunostimulation (Kitazawa et al. 1998). Examination of the mitogenic activity (ability to stimulate immune cell proliferation) of HePSs produced by two strains of *Lactobacillus delbrueckii* subsp. *bulgaricus*, differing only in the presence of phosphate groups, showed that only the phosphorylated polysaccharide was mitogenic, whereas dephosphorylation of this polysaccharide resulted in a loss of mitogenic activity (Kitazawa et al. 1998). This phosphorylated EPS increased macrophage phagocytic activity, and, for thioglycolate-induced macrophages, augmentation with EPS increased the macrophages' cytostatic activity against tumor cell lines. The presence of EPS alone had no effect on the tumor cells. In addition, dephosphorylation of the EPS also resulted in reduced phagocytosis and cytostatic activity of the macrophages (Kitazawa et al. 2000). Another study using the same EPS demonstrated increased mRNA expression for several cytokines in a macrophage cell line, which was not seen with a neutral (absence of phosphate groups) polysaccharide (Nishimura-Uemura et al. 2003). In concordance with earlier studies, Makino et al. (2006) observed that these same phosphorylated EPS stimulated mouse splenocytes, leading to significantly increased IFN-γ production, and, moreover, orally administered EPS augmented natural killer cell activity. Furthermore, oral administration of yogurt fermented with the *L. delbrueckii*–producer strain to mice showed a similar level of immunomodulation. Such effects were not replicated with the administration of yogurt fermented with a traditional yogurt starter culture (Makino et al. 2006). The importance of the presence of charged groups in eliciting an immunogenic effect is also highlighted by the observation that chemically phosphorylated dextran (a HoPS) demonstrated enhanced activity as a murine splenocyte mitogen when compared to the neutral, native dextran (Sato et al. 2004).

The immunomodulating capacity of kefiran, produced by *Lactobacillus kefiranofaciens*, has been demonstrated by oral administration to mice followed by measurement of immunoglobulin (Ig) and cytokine levels over a number of days. Kefiran induced a gut mucosal response, enhancing IgA production in both the small and large intestine. Serum levels of interleukin 6 (IL-6), IL-4, IFN-γ, and IL-10 increased compared to those of controls, indicative of an immune response (Vinderola et al. 2006). Stimulation of macrophage activity has also been demonstrated in vitro for HePSs produced by *Lactobacillus paracasei* and *L. plantarum*. Both EPSs stimulated macrophage proliferation, and proinflammatory cytokine release increased proportionately to increasing dosages of the EPSs (Liu et al. 2011).

In contrast to the above findings, anti-inflammatory or immunosuppressive properties were demonstrated for the EPS produced by *L. rhamnosus* RW-9595M (Bleau et al. 2010). Compared to the parental strain, *L. rhamnosus* ATCC9595, which stimulated production of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and IL-6 in macrophages, isogenic strain RW-9595M, which displayed increased EPS production compared to the parental strain, had immunosuppressive properties and induced high levels of the anti-inflammatory cytokine IL-10 without affecting TNF-α or IL-6 levels. In addition, the EPS from strain RW-9595M suppressed the immune response of macrophages stimulated with the proinflammatory parent strain. Hydrolysis of the EPSs affected the degree of cytokine induction, suggesting that different cytokine signaling pathways may be activated according to the molecular mass of the polysaccharide (Bleau et al. 2010). It is possible that the presence of the EPS layer may mask surface antigens that are exposed on the parental strain, a suggestion proposed by Fanning et al. (2012) to explain the stronger immune response elicited by an EPS-negative isogenic variant of *Bifidobacterium breve* UCC2003 when compared to the EPS-producing strain. Similarly, the immunosuppressive effects of EPS produced by a strain of *Bifidobacterium longum* were demonstrated by Wu et al. (2010). In addition, EPS pretreatment of macrophages prevented LPS-induced growth inhibition and release of TNF-α (Wu et al. 2010). Recent research suggests that the upregulation of Toll-like receptor (TLR) negative regulators, in particular, modulation of TLR-4, could be a mechanism through which LAB EPSs elicit anti-inflammatory activity (Laiño et al. 2016, Wachi et al. 2014).

Pathogen Antagonism

Ruas-Madiedo et al. (2006) showed that HePSs isolated from certain probiotic LAB were unable to prevent binding of pathogenic bacteria to mucus. Indeed, the EPSs inhibited the binding of the producing probiotic strains while promoting pathogen adherence (Ruas-Madiedo et al. 2006). It was postulated that the polymers may have directly adhered to intestinal mucus and then competitively inhibited the adhesion of the probiotics or bound to the probiotic surface, masking adhesion molecules, and that components on the pathogen surface might have bound specific EPSs that were able to adhere to mucus. The EPSs from the same probiotic strains were, however, found to negate the cytotoxic effect of bacterial toxins on eukaryotic cells (human Caco-2 enterocytes and rabbit erythrocytes) in vitro (Ruas-Madiedo et al. 2010). All EPSs prevented the detachment of Caco-2 monolayers when co-incubated with *Bacillus cereus* supernatants and helped maintain cell integrity. Kefiran was shown to elicit a similar protective effect on Caco-2 cells exposed to *B. cereus* toxins (Medrano et al. 2008). This protective effect was not observed with dextran, indicating that composition and structure of the EPS are important factors in mediating their effects (Medrano et al. 2009). In addition, kefiran reduced the cytopathic effects when *B. cereus* cells (rather than toxin-containing supernatant) were applied to enterocyte monolayers. Although it did not antagonize adhesion of the strain, kefiran modified the distribution of adhered bacteria on the monolayers. The authors suggested that kefiran may have a masking or decoy role, with

bacterial cells or their toxins binding to the EPSs rather than the natural ligands or receptors on the enterocyte surface (Medrano et al. 2009). EPSs produced by *Lactobacillus acidophilus* have been demonstrated to inhibit the biofilm formation of a number of pathogens, including enterohemorrhagic *Escherichia coli* and *Salmonella enterica* serovar Enteritidis (Kim et al. 2009).

Few studies have examined the antagonistic potential of HoPSs. As stated, the protective effects of kefiran on *B. cereus* toxicity were not replicated with dextran. This is in agreement with Wang et al. (2010) who demonstrated that reuteran and levan HoPSs produced by *L. reuteri* strains decreased the ability of enterotoxigenic *E. coli* to bind erythrocytes, a property not observed with commercial dextran or dextran produced by *W. cibaria*. In addition, there was no correlation between molecular mass and antiadherence activity, suggesting that a degree of structural specificity is involved (Wang et al. 2010). Indeed, dextran synthesis has been shown to reduce aggregation and biofilm formation for some LAB species (Nacher-Vazquez et al. 2017). In a piglet model, reuteran perfusion was also found to reduce the adhesion of enterotoxigenic *E. coli* to the intestinal mucosa and the associated fluid loss (Chen et al. 2014). In contrast to the findings of Wang et al. (2010), an early study found that dextran inhibited the binding of certain pathogens to pulmonary cells, albeit nonspecifically, as other neutral polysaccharides had similar effects, with low-molecular-mass dextran the most effective (Barghouthi et al. 1996).

Antioxidant Potential

HePSs for certain LAB species have also been demonstrated to exhibit antioxidant capacity, including the ability to scavenge hydroxyl and superoxide anion radicals in vitro while stimulating increased levels of antioxidant enzymes (catalase and superoxide dismutase) in vivo (Liu et al. 2011, Pan & Mei 2010).

The antiproliferative nature of certain LAB EPSs has been examined in recent studies, highlighting potential applications for their anticancer effects; however, results are preliminary. Oxidative stress and damage are believed to play a crucial role in cell transformation and cancer pathogenesis, and it is thought that the antiproliferative potential of EPSs may be related to their antioxidant activity (Mates 2000). Li et al. (2014b) demonstrated the antiproliferative potential of HePS fractions produced by *Lactobacillus helveticus* on BGC-823 gastric cancer cells in vitro. It has been observed that the EPS fraction with the highest antiproliferative activity also has the strongest free-radical scavenging activity (Li et al. 2014a,b). More recently, Wang et al. (2014) showed the anticancer potential of purified cell-bound HePS from a strain of *L. plantarum* on a number of tumor cells lines. Significant inhibition was observed against both BGC-823 and HT-29 cells when compared to the control antitumor compound fluorouracil (Wang et al. 2014).

Cholesterol-Lowering Potential

The cholesterol-lowering potential of LAB EPSs, particularly β-glucans, has been investigated, as it is known that β-glucans derived from oats can positively influence cardiovascular health. The exact mechanism by which EPSs reduce cholesterol levels is not fully understood, but they may bind cholesterol and promote its excretion, or indirectly increase conversion to bile through stimulation of increased numbers of microbes with bile salt hydrolase activity, with a resultant reduction in bile levels (Welman 2009).

Mårtensson et al. (2005) observed that an oat-based product, fermented with β-glucan-producing *Pediococcus parvulus* 2.6 (equivalent to 3.5 g β-glucan per day), reduced total cholesterol levels in humans when compared to a control group. A prebiotic effect on bifidobacteria levels was also demonstrated. Interestingly, the authors found no significant cholesterol-lowering effects

with oat-based fermented products that did not contain the β-glucan-producing *P. parvulus* 2.6 strain, although the LDL-cholesterol-lowering potential of oat β-glucan has been demonstrated in humans (Mårtensson et al. 2005, Wolever et al. 2011). In contrast, a study that fed live *P. parvulus* 2.6 (both the ropy and nonropy isogenic variant) and its purified EPS to hypercholesterolemic mice found no changes in blood lipids under any feeding regime (Lindstrom et al. 2012). Lindstrom et al. (2012) hypothesized that the hypocholesterolemic effect observed by Mårtensson et al. (2005) was achieved as a synergistic effect between oats and the EPS and/or *P. parvulus* 2.6, or that the β-glucan produced by *P. parvulus* 2.6 had a different chemical structure compared to those β-glucans that had previously been reported to exert hypocholesterolemic effects.

The potential of a number of *L. delbrueckii* subsp. *bulgaricus* strains to remove cholesterol from in vitro culture media has been demonstrated. All strains showed a capacity for removing cholesterol from MRS broth, with the level of EPS production correlating with the strain's ability to sequester cholesterol. The presence of cholesterol also appeared to affect the level of EPSs produced by the strains. In addition, heat-killed cells were able to remove cholesterol, suggesting that the binding or adsorption to the cell surface was the mechanism of action (Tok & Aslim 2010).

FUTURE EXPLOITATION OF EXOPOLYSACCHARIDES IN FOODS: POTENTIAL AND CHALLENGES

The future exploitation of LAB EPSs in the food industry offers great potential, particularly with food producers and consumers alike increasingly demanding clean-label products. Further expansion of their application will be assisted by continued research in a number of areas. Foremost, research into methods to produce these polysaccharides on an industrial scale will broaden their application. This is particularly the case for HePSs, as increased production would unlock their potential, particularly related to their health benefits. Continued research in this topic, especially into the mechanism of action of EPSs and the particular properties (molecular and structural) that promote modulation of health, will help to further substantiate the benefits being associated with these polymers. This will advance future functional and added-value food product development. The targeted design of novel EPSs is also an area of increasing interest that has the potential to lead to new functionalities and a broader application.

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