

Prospects and Functionality of Bacterial Exopolysaccharides in Dairy Foods: A Review

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ABSTRACT

Lactic acid bacteria (LAB) produce exopolysaccharides (EPS), which are used to make fermented dairy products like dahi, yoghurt, cheese, fermented cream, and milk-based desserts. EPS synthesis by LAB varies greatly in terms of quantity, chemical compositions, molecular size, charge, presence of side chains, and molecule rigidity. Firmness and creaminess are two main sensory qualities that influence customer preference for dairy products. EPSs can perform as texturizers and stabilisers, raising the viscosity of a final product and boosting the rigidity of the casein network by binding hydration water and interacting with other milk constituents such as proteins and micelles. As a result, EPS can help to reduce syneresis and improve product stability. The application of EPS in a dairy food matrix remains a challenge in order to better meet customer demand for appealing, flavorful, and even healthier products.

KEYWORDS

Exopolysaccharides, Dairy products, Polysaccharides, Lactic acid bacteria

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INTRODUCTION

Polysaccharides may function as thickening agents, stabilizers, emulsifiers, gelling agents and water-binding agents in different dairy and food products. The plant and algae derived polysaccharides like starch, galactomanan, alginate, carrageenan and pectin are still dominating in global hydrocolloids market (Freitas *et al*, 2011) while xanthan gum is the only bacterial exopolysaccharide (EPS) which significantly accounted (approximately 11%) in global hydrocolloids market (Sworn, 2010). EPS is an alternative source of plant and algae derived hydrocolloids. The microbial EPS have distinctive rheological properties as it has ability to make viscous solutions at very low concentrations (Becker *et al*, 1998).

Several species of lactic acid producing bacteria (LAB) are also capable of synthesizing EPS into their environment like milk (De.Vuyst and Degeest, 1999; Ricciardi and Clementi, 2000). LABs are widely used in several applications in food, chemical and pharmaceutical industries. The EPS have huge commercial value as it has unique industrially and physico-chemical properties. The EPS has broadened industrial application because LAB produced a wide range of EPS with different composition and functionality. The EPS synthesized in situ by the food associated LAB possessing 'Generally Recognized as Safe' status (Laws *et al*, 2001). These EPS synthesizing strains of LAB are widely used for the production of various fermented milk products like dahi, yoghurt, cheese, milk-based desserts, acidophilus milk (Duboc and Mollet, 2001). The utility of distinct EPS is depending on their composition of monosaccharide, type of linkages present, molecular

weight and degree of branching (Illey *et al*, 2006). EPS produced by LAB has played an important role in improving the mouthfeel, texture and rheology of fermented food preparations (Doleyres *et al*, 2005). LAB is also capable of the production of a range of functional oligosaccharides. Oligosaccharides have wide industrial applications such as prebiotics, sweeteners, nutraceuticals, immune stimulators, humectants, etc. (Remaud-Simeon *et al*, 2000). The production of EPS by LAB depends on the various factors like medium composition, strain and growth conditions such as pH, temperature, incubation period and oxygen tension (Degeest *et al*, 2001). There is a need to enhance the EPS productivity from LAB to reduce the overall production cost and also produce customized functionality EPS for its commercial viability.

What are Exopolysaccharides (EPS)?

EPS are composed of sugar residues of high molecular weight polymers which are secreted by a microorganism into their surrounding environment. Basically, it is long chain polysaccharides and mainly produced extracellularly by bacteria and microalgae. It consists of branched and repeating units of sugars or derivatives of sugar such as glucose, galactose, mannose, N-acetyl galactosamine, N-acetylglucosamine and rhamnose in variable ratios. EPS are linked temporarily to the surface of the microbial cell and are released into their surrounding environment during growth as slime. This differentiates them from the structurally related capsular polysaccharides that remain permanently bound to the surface of microbial cells. EPS plays an essential role in shielding microbes from harmful conditions such as drying, lack of nutrients, poisonous chemicals, bacteriophages, osmotic stress and antag-

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onists (Looijesteijn *et al*, 2001). EPS plays a key role in the bacteria's initial adhesion and strong anchorage to solid surfaces, cation sequestration, formation of biofilms, cell recognition and pathogenicity. Generally, the bacteria that create it do not use the EPS as food, but *Streptococcus mutans* and *Streptococcus sobrinus* are capable of degrading the self-synthesized dextran and *Streptococcus mutans* also use the oligosaccharides that it produced.

Types of EPS

EPS is categorized into two distinct classes based on their chemical composition and biosynthesis mechanisms: Homopolysaccharides and Heteropolysaccharides.

Homopolysaccharides

Homopolysaccharides consist of repeated units of only one type of monosaccharide (mostly fructose or glucose) and are commonly formed by the action of glycosyltransferases such as dextran, levan, alternan, reuteran, etc. in large quantities from sugars.

Heteropolysaccharides

Heteropolysaccharides consist mainly of the same repeated units consisting of two or more monosaccharides such as galactose, glucose, rhamnose and fructose. There could be several linkages in one polysaccharide at the same time. Because of their involvement in sugar inter-conversions as well as sugar activation, sugar nucleotides play an important role in heteropolysaccharide synthesis.

The first heteropolysaccharide examined and identified in detail was the *Streptococcus thermophilus* EPS (Doco *et al*, 1990). Kefiran is a heteropolysaccharides, released by *Lactobacillus kefirianofaciens*, *L. kefirgranum*, *L. parakefir*, *L. kefir* and *L. delbrueckii subsp. bulgaricus*. It is water soluble and consists of the same amount of glucose and galactose monosaccharide (Micheli *et al*, 1999). EPS composition and properties are influenced by the medium's environmental conditions, biosynthetic pathways or rate of microbial growth.

Applications of EPS

The functions and properties of some bacterial exopolysaccharides are summarized in Table 1. Due to its structural diversity, rheological and physical properties, it has a broad range of implications in dairy, food and pharmaceutical industry.

Product rheology

EPS acts as a natural bio-thickening agent to prevent syneresis and enhances the rheology of fermented milk products by binding hydration water and interacting with other milk constituents, mainly minerals and proteins. However, the degree of thickening also depends on the composition and structure of EPS. Several problems of EPS such as low viscosity, fracturing of the gel or high syneresis (whey separation) that are commonly encountered during yoghurt processing can be solved by applying EPS (De.Vuyst and de.Ven, 1998). The physical and rheological properties of the EPS polymers are based on the chemical composition, molecular size, charge and existence of side chains, molecular rigidity and 3D structures. It is also responsible for the development of the final

product by the interaction between EPS and various components in food products. Several studies revealed that the rheological properties of fermented dairy products do not depend on the amount of EPS content in products (Guldager and Ipsen, 2006) , (Duboc and Mollet, 2001; De.Vuyst *et al*, 2007). Pleijsier *et al* (2000) studied the interaction between the charged polysaccharides and proteins. Neutral EPS increases the product's viscosity over time to about 10 times that of a control product produced with a non-EPS strain. But the product's viscosity with the charged EPS has been found to be comparable with the control product. By comparison, the negatively charged polysaccharide appears to improve the product storage modulus G or elasticity. Therefore, it is inferred that the linear neutral EPS contributes to the product's viscosity but not to the elasticity as it interacts weakly with the molecules of positively charged proteins and dissolves uniformly in the serum phase. On the other hand, negatively charged polysaccharides contribute to the elasticity, but not to the viscosity of the product, due to their interaction with the positively charged casein molecules through electrostatic interactions, increasing the strength of the network and thus increasing the elasticity modulus. They may not contribute to viscosity as they are poorly distributed in the serum phase. Predominantly, LAB responsible for the production of polysaccharides of various charges and composition which contributes apparently to the viscosity and elasticity of the fermented product (Duboc and Mollet, 2001).

Texturing agent

The most important textural property of fermented dairy and non-dairy products is their firmness and water holding capacity. These characters are related to the formation of gel structure and greatly influenced by the type of culture. The EPS producing strains reduces the firmness and cohesiveness of yoghurt as the amount of EPS increases in the product (Marshall and Rawson, 1999) , (Hassan *et al*, 1996). The EPS may interfere with the interaction of casein micelles which leads to the formation of less firm coagulum. Yoghurt microstructure studies reveal that the void spaces around the EPS producing bacteria in confocal laser scanning micrographs can influence the protein matrix's integrity (VanMarle and Zoon, 1995) (Hassan *et al*, 1995). Yoghurts made from ropy crops exhibited the highest water holding capacity which decreases syneresis susceptibility (Hassan *et al*, 1995). Similarly, lactobacilli derived polymers such as levan, dextran or reuteran are externally added in the case of bakery products and are responsible for increased water absorption of the dough and improved bread quality (Tieking *et al*, 2005).

EPS application in dairy foods

Dahi or Curd

Dahi or Curd is a common fermented Indian milk product containing 3.5-8% milk fat Prajapati and Nair (2008) . Presently, the health-conscious consumer's demand for low-fat, low-calorie and fat-free dairy products is rising day by day.

Table 1: Properties and functional attributes of some bacterial exopolysaccharides

Bacterial EPS	Polysaccharide Component	Bacteria strains	Properties	Applications
Dextran	Glucose	<i>L. mesenteriodes</i>	Non-ionic, good stability Newtonian, fluid behaviour	Foods, Pharmaceutical industry (Blood volume expander) and Chromatographic media
Alginate	Guluronic acid and mannuronic acid	<i>P. aeruginosa</i> and <i>A. vinelandii</i>	Gelling capacity, film forming	Food hydrocolloid and medicine (surgical dressings, wound management and controlled drug release)
Xanthan	Glucose, mannose and glucuronic acid	<i>Xanthomonas</i> spp.	High viscosity, Stable over a wide temperature, pH and salt concentrations ranges	Foods, petroleum industry, pharmaceuticals, cosmetics and personal care products
Curdlan	Glucose	<i>Rhizobium meliloti</i> and <i>Agrobacterium radiobacter</i>	Gel-forming ability, water insolubility, edible and non-toxic has biological activity	Foods, pharmaceutical industry, heavy metal removal and concrete additive
Cellulose	Glucose	<i>Acetobacter</i> spp.	Not soluble in most solvents and high tensile strength	Foods (indigestible fiber), biomedical (wound healing, tissue engineered blood vessels) and audio speaker diaphragms
Succinoglycan	Glucose and galactose	<i>Alcaligenes faecalis</i> var. <i>myxogenes</i>	High viscosity and acid stability	Food and oil recovery
Glucuronan	Glucuronic acid	<i>Sinorhizobium meliloti</i> M5N1CS and <i>Glucacetobacter hansenii</i>	Gelling and thickening capacity	Food and cosmetics products
Colanic acid	Fucose, glucose, glucuronate and galactose	<i>E. coli</i> , <i>Shigella</i> spp., <i>Salmonella</i> spp. and <i>Enterobacter</i> spp.	Gelling capacity	Cosmetics and personal care products

Source: Nwodo *et al* (2012)

However, milk fat is responsible for the product's taste, texture and body formation while after elimination of fat loss of those properties. EPS producing Lactobacilli strains functions in this sense as "bio-thickeners or bio-stabilizers" and also helps to minimize total solids in the product without affecting the sensory attributes (Behare *et al*, 2009). Based on microstructural studies, dahi produced with EPS strain was found to have a more porous and open structure with discontinuous casein matrix than the controlled dahi and thus showed increased water binding capacity (Praveen, 2000).

Yoghurt

Yogurt is a type of fermented milk produced by *L. delbrueckii bulgaricus* and *S. thermophilus* starter cultures at a ratio 1:1. These cultures have the ability to produce EPS in the yoghurt. *Streptococcus thermophilus* and *Lactobacillus bulgaricus* culture produces EPS in yoghurt in the range of 30 to 890 mg /L and 60 to 150 mg /L respectively (Bouzar *et al*, 1997). The polysaccharides synthesized by EPS producing strains have ability to minimize the quantity of added milk solids by improving the viscosity of product, texture, mouthfeel and also prevents syneresis during fermentation or storage. However,

EPS does not have any taste but it enhances texture of the end product which greatly acceptable by the consumers (Jolly *et al*, 2002). Yogurt manufactured with strains producing EPS have higher capacity for holding water which reduces the susceptibility of the product to syneresis. Yang *et al* (2010) reported that partial or full replacement of *L. delbrueckii subsp. bulgaricus* with *L. rhamnosus* JAAS8, an EPS producing culture resulted in significant increases in apparent viscosity of approximately 16% and 21% respectively. Studies conducted to elucidate the function of EPS in yoghurt texture showed that the presence of EPS producing strains found to be higher viscosity values than the non-EPS microorganisms (Amatayakul *et al*, 2005; Bouzar *et al*, 1997). Moreover, other findings also showed the contribution of high molecular mass polysaccharide and a stiff conformation that improves the viscosity of the yoghurt (De.Vuyst *et al*, 2007). However, there has been no any simple correlation between the viscosity and the amount of EPS produced in the yoghurt. Purwandari *et al* (2007) found a weak relationship between texture and concentration of EPS produced at different temperatures by using the *S. thermophilus* culture. Therefore, literature

has shown the diversified inferences about the product rheology and EPS concentration but authors agree with the fact that the interactions with caseins by pH value and EPS conformation are the keys point for improvement of the texture. Application of pure EPS in yoghurt has not yet been studied and findings about their function as a texturing agent are not very definite at present.

Cheese

The growth of *Lactobacillus* species was studied in the processing and ripening of semi-soft cheeses (Gouda), pressed cheeses (low-fat Cheddar cheeses) and blue-veined cheeses (Roquefort). EPS excreted with strains of *lactobacilli* such as *L. Helveticus*, *L. Bulgaricus*, *L. delbrueckii* and *L. Casei* increases water retention and enhances the overall quality of the cheese (Zisu and Shah, 2007). Several manufacturers used texture promoting or ropy culture as a stabilizer substitute for the production of low-fat cheese to resolve the functional defects. These textures promoting culture produced carbohydrate which imparts flavour, mouthfeel etc in the fermented milk. Further, Behare *et al* (2009) and Dabour *et al* (2005) also observed that viscosity of skim milk gel prepared by ropy strains *L. Lactis subsp Lactis* was enhanced as compared to non-ropy cultures. Perry *et al* (1997) observed that the moisture rises in the preparation of low-fat mozzarella while incorporating EPS producing starter cultures. This water retention ability is responsible for improving the texture of the cheese and allows for calorie reduction in the end product.

Kefir

Kefir is a slightly alcoholic, self-carbonated yoghurt-like fermented milk product and quite popular in Eastern European countries. It is prepared by Kefir grains which are an aggregation of EPS producing microorganisms such as *Lactobacillus* sp. (*L. kefirgranum*, *L. kefir*, *L. acidophilus*, and *L. parakefir*) with *Candida kefir*, *Saccharomyces* sp., *Acetobacter* sp., etc and embedded in a polysaccharide matrix also called Kefiran. *L. kefirifaciens* is responsible for the production of slimy polysaccharide named Kefiran in the center of the grain under anaerobic conditions which act as natural viscosifying agent and affects the texture of kefir. Kefiran is composed of D-Glc and D-Gal in ratio 1:1 and exists in grains with a proportion of about 45% (Micheli *et al*, 1999). This biopolymer consists of a branched hexa or heptasaccharidic unit. One or two residues are branched at the main chain composed of 5 monosaccharides. Rimada and Abraham (2006) used skim-milk gels with and without kefiran and compared their rheological behaviour. The mixture of skim milk with kefiran increased viscosity and raise visco-elasticity up to 300 mg/L of polysaccharide. It was concluded that the natural polysaccharide might be employed as an alternative thickening agent in dairy products.

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EPS as food additives

EPS produced by microorganisms mainly *Lactobacillus* species possess GRAS status and are allowed to be incorporated in food without labelling. EPS imparts highly desirable rheological properties to the food matrix like increased viscosity, improved texture and reduced syneresis that proves them as food additives.

Dextran, a polysaccharide produced by *Leuconostoc*, *Streptococcus* and *Lactobacillus* species is the first industrial polysaccharide which is used in confectionary to improve viscosity, moisture retention and inhibit sugar crystallization. It also acts as a gelling agent in gum and candies and can be used as a crystallization inhibitor in ice-creams. It provides viscosity and mouthfeel to the pudding mixes. It is a gel and hence can be used as a molecular sieve for purification and separation of macromolecules such as proteins, nucleic acids and polysaccharides. It can be safely consumed; therefore, it is used in clinical research and medical application as blood plasma extenders and also be used as a stationary phase in many chromatographic techniques. Xanthan is the second most important commercially available polysaccharides produced by *Xanthomonas campestris* and it was approved as a food additive in 1969. It is important in both food and non-food applications as in dairy products, drinks, confectionary, dressing, bakery products, syrups, pet foods as well as the cosmetic, pharmaceutical, paper, paint and textile industry. Due to its highly pseudoplastic and suspending properties, it possesses large industrial importance as a suspending and emulsifying agent. Gellan is a multifunctional gelling agent found from a non-pathogenic bacterium *Sphingomonas paucinibilis*.

CONCLUSION

Bacterial exopolysaccharides are produced by LAB and shows enormous diversity and functions. The diversity of EPS can be seen in the linkage bonds, monomeric compositions and associated conjugates. In addition to their natural protective role, EPS produced by LAB is found to shield the cell against technological stresses during manufacturing fermented milk products. EPS have characteristic physical properties, structural diversity and rheological properties which makes it widely useful in the pharmaceutical industry and dairy foods. But manufacturing cost is found to be the major constraint for the application of EPS at industrial scale. Hence, an alternate way for higher production of EPS and the optimization of the fermenting parameters is very necessary. Overall, rapid development and search for novel LAB probiotic organisms is an ongoing process and utilization of their prebiotic EPS products should be increased in dairy products to improve rheological and physical properties.

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