Indian J Microbiol (Jan–Mar 2012) 52(1):3–12
DOI 10.1007/s12088-011-0148-8

REVIEW ARTICLE

Potentials of Exopolysaccharides from Lactic Acid Bacteria

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Received: 6 February 2009 / Accepted: 4 November 2009 / Published online: 15 February 2011

Abstract Recent research in the area of importance of microbes has revealed the immense industrial potential of exopolysaccharides and their derivative oligosaccharides from lactic acid bacteria. However, due to lack of adequate technological knowledge, the exopolysaccharides have remained largely under exploited. In the present review, the enormous potentials of different types of exopolysaccharides from lactic acid bacteria are described. This also summarizes the recent advances in the applications of exopolysaccharides, certain problems associated with their commercial production and the remedies.

Keywords Lactic acid bacteria · Exopolysaccharides · Probiotic · Prebiotic · Oligosaccharides

Introduction

Lactic acid bacteria are known through ages for their wide applications in food, pharmaceutical and chemical industries. But recently lactic acid bacteria (LAB) have aroused interest for their ability to secrete extracellular polysaccharides [1, 2]. These extracellular polysaccharides or 'exopolysaccharides' (EPS) have immense commercial value because of their industrially useful physico-chemical properties. Capacity of LAB to produce a broad range of EPS with variable composition and functionality is widening their industrial applications. Food associated lactic acid bacteria are granted the status of 'generally recognised as safe' (GRAS) and are discovered to be the suitable candidates for the production of functional EPS [3]. These safe LAB which produce in situ novel functional EPS can escape the rigorous toxicological testing and marketing of this EPS holds huge prospects. These EPS producing strains of LAB are used for the production of yoghurt, cheese, acidophilus milk and milk based desserts [4]. The utility of various EPS are dependent on their monosaccharide composition, type of linkages present, degree of branching and molecular weight [5]. EPS derived from LAB play crucial role in improving the rheology, texture, mouth feel of fermented food formulations and conferring beneficial physiological effects on human health, such as antitumour activity, immunomodulating bioactivity and anticarcinogenecity [6]. LAB can also produce a variety of functional oligosaccharides. Oligosaccharides have huge industrial applications as prebiotics, nutraceuticals, sweeteners, humectants, drug against colon cancer, immune stimulators etc. [7]. The total yield of EPS produced by the LAB depends on the composition of the medium, LAB strain and growth conditions like temperature, pH, oxygen tension and incubation period [8]. The need of the day is to improve the productivity of EPS from LAB, reduce the cost of production for commercial viability and to produce custom made EPS with the desired functionality. The modulation of biochemical properties of EPS require a thorough understanding of its biosynthetic pathway and the relation between the structure of EPS and the functional effect provided by them after incorporation into the food matrix.

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Lactic Acid Bacteria

Lactic acid bacteria are gram positive rods or cocci, non spore forming, acid tolerant, low GC containing, catalase negative, anaerobic or micro-aerophilic bacteria characterised by their ability to ferment sugar to lactic acid. The commonly known LAB are *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus* and *Streptococcus*. Some other lesser known genera of LAB are *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Teragenococcus*, *Vagococcus* and *Weisella*. Because of the unique capacity of lactic acid fermentation, LAB are employed for prolonging the shelf life, enhancing the gustatory and nutritional value, imparting appetizing flavour and texture to the food. Some LAB produce proteinaceous antimicrobial compounds bacteriocins which inhibit the growth of Gram-positive pathogenic and spoilage bacteria and used as food additives. The LAB are evidenced to be good probiotics as they accelerate the growth of beneficial microbial gut flora and have been proven effective against diarrhoea, irritable bowel disorder, allergies, stimulation of immunity, lactose intolerance [9]. Some strains of LAB divert a minute fraction of fermentable sugars for the biosynthesis of exopolysaccharides (EPS). On the basis of the hexose fermentation pathways, LAB genera have been classified into two categories. Homofermentative LAB comprising the group I, catabolize one mole of glucose under limited oxygen in the Embden–Meyerhof–Parnas (EMP) pathway to yield lactic acid only. The members of this group are *Lactococcus, Enterococcus, Streptococcus, Pediococcus* and lactobacilli as *L. acidophilus*, *L. delbrueckii*, *L. helveticus*, *L. salivarius*. Heterofermentative LAB forming the group II, use the pentose phosphate pathway or pentose phosphoketolase pathway to degrade its substrate liberating the end product ethanol and lactic acid. The members of this group are *Leuconostoc, Oenococcus, Weissella* and lactobacilli as *L. brevis*, *L. buchneri*, *L. fermentum* and *L. reuteri*.

Exopolysaccharides (EPS)

EPS are long-chain polysaccharides produced extracellularly mainly by bacteria and microalgae. EPS consist of branched, repeating units of sugars or sugar derivatives. These sugar units are mainly glucose, galactose, mannose, N-acetylglucosamine, N-acetyl galactosamine and rhamnose, in variable ratios. EPS are not permanently attached to the surface of the microbial cell and are secreted into their surroundings during growth as loose slime. This distinguishes them from the structurally similar capsular polysaccharides, which remain permanently attached to the microbial cell surface. EPS play vital role in protection of the microbes from adverse conditions as desiccation, nutrient shortage, toxic compounds, bacteriophages, osmotic stress and antagonists [10]. EPS play key role in initial adhesion and firm anchorage of the bacteria to solid surfaces, cation sequestration, biofilm formation, cellular recognition and pathogenecity. Generally the EPS are not utilized as food by the bacteria producing it, but, *Streptococcus mutans* and *Streptococcus sobrinus* are capable of degrading the dextran self synthesized and *Streptococcus mutans* also utilizes the oligosaccharide produced by it.

EPS from microbial sources can be classified into two groups based on their monosaccharide composition and biosynthetic pathway [11]. The homopolysaccharides (HoPS) embody dextran, mutan, alternan, reuteran, pullulan, levan, inulin, curdlan etc. and heteropolysaccharides (HePS) comprise gellan, xanthan, kefiran. Homopolysaccharides consist of identical monosaccharides, D-glucose or D-fructose and can be divided into two major groups: glucans and fructans [12]. By contrast, heteropolysaccharides from LAB have repeating units showing very little structural similarity to one another.

Homopolysaccharides

Glucansucrase produces glucans as dextran (α-1,6 osidic bond), mutan (α-1,3 osidic bond), alternan (α-1,6 and α-1,3 osidic bond) and reuteran (α-1,6 and α-1,4 osidic bond). Similarly, fructansucrase produces levan (*β*-2,6 osidic bond) and inulin- type (*β*-2,1 osidic bond) of fructans.

Dextran

Dextransucrase secreted by *Leuconostoc mesenteroides* hydrolyses sucrose to produce dextran. Dextrans are a class of homopolysaccharides composed of α-1,6 glycosidic linkages in the main chains and α-1,2, α-1,3 and α-1,4 branched glycosidic linkages [13]. The degree of branching involving α-1,2, α-1,3 and α-1,4 linkages in dextrans vary according to the origin of dextransucrase. Native dextrans, the partially degraded dextrans and their derivatives have immense commercial applications in food, pharmaceutical and chemical industries as adjuvant, emulsifier, carrier and stabilizer [14]. Soluble dextrans synthesised by dextransucrase elaborated from the strain *Leuconostoc mesenteroides* NRRL B-512F are the most widely used. Presence of 95% linear linkages makes this dextran water soluble, which have immense applications [15]. FT-IR, ^{1}H and ^{13}C NMR and 2D ^{1}H, ^{13}C NMR spectral analysis of EPS produced by *L. mesenteroides* NRRL B-640 confirms that its a highly linear dextran with $\alpha(1 \rightarrow 6)$ linkages [16]. Dextran from *Leuconostoc dextranicum* NRRL B-1146 is branched and has a web like porous structure as revealed

by scanning electron micrograph [17]. Dextrans are used for the matrix preparation of chromatography columns such as Sephadex. Clinical dextrans of molecular size 40–100 kDa are used as therapeutic agents to restore blood volume in case of casualties [18]. They are also used for synthesizing dextran sulphate for blood coagulation prevention and blood flow facilitation. As the larger molecular weight dextrans can act as osmotic agents, they are used to treat hypovolemia. Iron dextran is used to treat iron deficiency anaemia. Dextrans come handy in microsurgery to reduce the risk of free tissue transfer loss. They are used as lubricant in eye drops and to increase blood sugar levels. Dextrans have also found use in veterinary medicines. Use of dextrans have ramified into paper, metal-plating processes [19] and enhanced oil recovery [20]. They are used as food syrup stabilizers and dough improvers [21]. Cyclodextran, a cyclic oligosaccharide derived from dextran has utility as cariostatic, anti-HIV and anti-ulcer agent [22]. Dextrans are also used in biosensors for different biointeraction analysis with surface plasmon resonance. Dextrans provide a stabilising coating for protecting metal nanoparticles against oxidation [23]. Dextran coating on biomaterials to prevent undesirable protein absorption is being explored to improve their biocompatibility [24]. Dextran therapy has side effects like anaphylaxis, volume overload, pulmonary oedema, cerebral oedema, acute renal failure and platelet dysfunction. However, the immense application possibilities of dextrans in industry and medicine, score over these few negative aspects.

Alternan

The strains producing alternansucrase are *Leuconostoc mesenteroides* NRRL B-1355, NRRL B-1501 and NRRL B-1498. The exopolysaccharide alternan, produced by alternansucrase contains alternating α-1,6 and α-1,3 glucosidic linkages, with some degree of α-1,3 branchings. Due to its unique structure, alternan has high solubility, low viscosity and remarkable resistance to enzymatic hydrolysis. Alternan is commercially exploited as low viscosity bulking agent and extender in foods and cosmetics. Extracellular alternanase depolymerises alternan to oligosaccharides. These alternan oligosaccharides are used as low-glycemic sweetener in confectionaries [25] and as prebiotics [26].

Reuteran

Reuteran is a water soluble glucan produced by reuteransucrase. It has 70% α-1,4 linkage, also α-1,6 glycosidic bonds and 16% 4,6-disubstituted α-glucosyl units at the branching points and molecular weight of 40 MDa [27]. It is elaborated by *Lactobacillus reuteri* strain LB 121, *Lactobacillus reuteri* strain ATCC 55730 and *Lactobacillus reuteri* strain 35–5 have been reported to produce reuteran. Because of water solubility, it is used in bakery [28].

Levan

Levan is a fructan having β-2, 6 osidic bonds with β-2, 1-linked side chains [29]. Levansucrase catalyzes the transfer of D-fructosyl residues from fructose to yield levan. LAB producing levan are *Steptococcus salivarius, Steptococcus mutans*, *Leuconostoc mesenteroides* NRRL B-512F, *Lactobacillus sanfranciscensis* LTH 2590 and *Lactobacillus reuteri* LB 121. Levan is a peculiar polysaccharide as it has a relatively low intrinsic viscosity than similar high molecular weight molecules. Levan does not gel or swell in water at room temperature. Levan from *L. sanfranciscensis* LTH 2590 exhibits prebiotic effects [30]. Levan has attracted attention for its antitumor properties [31], cholesterol-lowering properties and application as an eco-friendly adhesive. Levan also holds promise as bio thickener in food industry [21].

Inulin-type

Inulin-type EPS are fructans or fructooligosaccharides containing β-1,2 osidic bonds. *Lactobacillus johnsonii* NCC 533 produces high molecular mass inulin from sucrose by using an inulosucrase enzyme. *Streptococcus mutans* strain JC2, *Leuconostoc citreum* CW28 and *Lactobacillus reuteri* 121 are some other LAB which produce inulins. These are nondigestible and function as prebiotics in humans and animals. Inulins type fructooligosaccharides synthesize butyrate, which nourish the enterocytes, prevent pathogenic adherence and decrease pH of lumen [32]. Inulin-type fructans can be employed as vehicles for targeted drug delivery in treating colon cancer [33]. Gelling tendency of inulin in aqueous solution while retaining the food texture and stability makes it a desirable ingredient in food products.

Heteropolysaccharides

Biosynthesis and secretion of the heteropolysaccharides (HePS) from the LAB occur at different phases of growth. The amount and type of the HePS is regulated by growth conditions [2]. Structurally, HePS may be ropy or mucoid. Under optimal culture condition, 0.15–0.6 gl^{-1} HePS yield occurs [1]. The molecular mass of these HePS polymers ranges between 1.0×10^4 and 6.0×10^6 Da [2]. The producer strain, composition of growth medium and culture conditions as temperature, pH, oxygen tension and

turbidity influence monomer composition of the HePS and variations in glycosidic linkages [2, 8]. A chemically defined medium containing carbohydrates, amino acids, vitamins, nucleic acid bases and mineral salts is suitable for studying the effects of nutrients on growth, metabolic pathways and biosynthesis of HePS by LAB as media containing complex constituents like beef extract, peptone, and yeast extract interfere with the monomer and structure of the HePS [8].

Kefiran

It is a water soluble heteropolysaccharide produced by *Lactobacillus kefiranofaciens, L. kefirgranum, L. parakefir, L. kefir* and *L. delbrueckii* subsp. *bulgaricus*. Kefiran consists of approximately equal proportions of glucose and galactose [34]. Microscopic observation of kefir grains reveal that kefiran encapsulates LAB, acetic acid bacteria and yeasts involved in the fermentation. Kefiran improves visco-elastic properties of acid milk gels. Ability of kefiran to form edible transparent films are being explored [35]. Kefiran is reported to have antimicrobial and wound healing properties, ability to lower blood pressure and cholesterol in serum, also capacity to retard tumour growth [36]. Kefiran also serves as an oral antigen, inducing gut mucosal response [37]. It is reported to confer protective immunity, maintain intestinal homeostasis, enhance IgA level at both the small and large intestine level and influence the systemic immunity through the release of cytokines into the blood [35].

Applications of EPS

EPS improve rheology, texture, stability and mouth feel of fermented milk products. Problems like low viscosity, gel fracture or high syneresis (whey separation), which are frequently encountered during yoghurt manufacture can be solved by the application of EPS [2]. Texture profile analysis showed that hardness, consistency, adhesiveness, chewiness, relaxation and modulus were significantly lower in cheese fermented by EPS-producing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* sp. *bulgaricus* culture [38]. Some of the major applications of EPS with their linkages are listed in Table 1. Cheese manufactured using the EPS-producing culture becomes smooth, creamy, moist and soft while the one made without the EPS producing strain turns dry and granular. The EPS producing lactic starter cultures improve the sensory attributes of various reduced and low-fat cheese varieties. It has been established that EPS producing cultures enhance consumer acceptability of Karish, a traditional Egyptian variety of cheese and fruit or vegetables fortified cheese by improving their spreadability and creaminess [38]. Such products which have high consumer demand are conventionally produced by adding fat, sugar, protein or stabilizers like pectin, starch, alginate or gelatine content. Ban on the addition of stabilizers in yoghurt by European Union [2] and health consciousness amongst consumers make EPS a viable alternative. Though it is tasteless, EPS from LAB prolong the retention time of milk product in the mouth, enhancing its delicacy. EPS produced by *Lactobacilli* favourably influence a host of properties of bread by facilitating water absorption, softening the gluten content of the dough, improving the structure build-up, increasing specific volume of loaf, retarding bread staling and prolonging shelf life [39]. EPS produced by sourdough LAB improve dough rheology, bread texture and can substitute the expensive hydrocolloids used to improve bread texture [30].

Since the EPS stay longer in the gastrointestinal tract, they facilitate colonization of the tract by probiotic bacteria like *Bifidobacteria* and *Lactobacilli*. These prebiotics alleviate lactose intolerance, enhance immunity against pathogens, reduce mutagenic enzymes like β-glucuronidase, nitroreductase and chologlycine hydrolase [40]. Levan produced by *L. sanfranciscensis* LTH2590 is metabolized by *Bifidobacteria* [30]. In addition to role as food additives and prebiotics, LAB synthesized EPS have been implicated as anti-tumour agent, immuno-stimulator and blood cholesterol lowering agent. To improve the specificity of drug release in colon cancer treatment, certain types of EPS are used as drug conjugates, coatings and matrix agents which act as substrates for the colon microflora [41]. Some EPS derived from LAB possess B-cell mitogen activity, ability to modify some macrophages and splenocyte functions [42].

Uses of Exopolysaccharides Derived Oligosaccharides

Oligosaccharides are sugar polymers of degree of polymerization between 2 and 10. The presence of acceptors as maltose, lactose and cellobiose in the nutrient medium along with sucrose leads to synthesis of oligosaccharides along with EPS. Engineering of the EPS biosynthetic pathway also yields oligosaccharides. The acceptor reactions of dextransucrase from *Leuconostoc*, *Lactobacillus* and *Streptococcus* have the potential for synthesis of a wide range of oligosaccharides [43]. Prebiotic oligosaccharides are noncarcinogenic, acid resistant, nondigestible, low calorific compounds and they are used as nutraceuticals. A huge number of LAB synthesized oligosaccharides have immense prebiotic value as modulators of gut microflora composition [44]. The oligosaccharides fermented by *Leuconostoc mesenteroides* are readily catabolized by *Bifidobacteria* and *Lactobacilli*, promoting their

Table 1 Applications and linkages of functional exopolysaccharides and oligosaccharides from lactic acid bacteria

EPS	LAB producing EPS	Linkage	Uses	References
Dextran	*Leuconostoc mesenteroides* NRRL B-512F *Leuconostoc mesenteroides* NRRL B-640 *Leuonostoc mesenteroides* B-742 *Leuconostoc mesenteroides* B-1355 *Leuconostoc mesenteroides B-1299* *Streptococcus mutans 6715*	α-1,6 glycosidic linkages in main chain and α-1,2, α-1,3 and α-1,4 branched glycosidic linkages	As adjuvant, emulsifier, carrier and stabilizer in food and pharmaceutical industries, plasma substitute, matrix of chromatography column, anticoagulant, paper industry, metal-plating processing, for enhanced oil recovery, biomaterials	Remaud-Simeon et al. [7] Goulas et al. [14] Naessens et al. [18] Purama and Goyal [15] Walsh et al. [19] Padmanabhan and Kim [20] Sengupta et al. [24] Iliev et al. [5]
Alternan	*Leuconostoc mesenteroides* NRRL B-1355 *Leuconostoc mesenteroides* NRRL B-1501 *Leuconostoc mesenteroides* NRRL B-1498	α-1,6 and α-1,3 glucosidic linkages, with some α-1,3 branchings	Prebiotics, sweetener in confectionaries, low viscosity bulking agent and extender in foods	Cote [26] Leathers et al. [25]
Reuteran	*Lactobacillus reuteri* strain LB 121 *Lactobacillus reuteri* ATCC 55730 *Lactobacillus reuteri* strain 35-5	α-1,4 linkage, also α-1,6 glycosidic bonds	Used in bakery	Tieking and Ganzle [39] Kralj et al. [27]
Levan	*Steptococcus salivarius* *Steptococcus mutans* *Leuconostoc mesenteroides* NRRL B-512F *Lactobacillus sanfranciscensis* LTH 2590 *Lactobacillus reuteri* LB 121	*β*-2, 6 osidic bonds *β*-2,1-linked side chains	Prebiotic, antitumor property, hypocholesterolaemic, agent, eco-friendly adhesive, Bio-thickener in food industry	Yoon et al. [29] Korakli et al. [30]
Inulin	*Lactobacillus johnsonii* NCC 533. *Streptococcus mutans JC2*, *Leuconostoc citreum CW28* *Lactobacillus reuteri 121*	*β*-1,2 osidic bonds	Prebiotics, nourishes gut mucosal cells and inhibits pathogens, for targeted drug delivery against colon cancer, substitute of fat in food products	Buchholz and Seibel (2003) Sartor [32]

Table 1 continued

EPS	LAB producing EPS	Linkage	Uses	References
Kefiran	*Lactobacillus kefiranofaciens* *Lactobacillus kefirgranum* *Lactobacillus parakefir* *Lactobacillus kefir* *Lactobacillus. delbrueckii* subsp. *bulgaricus*	Glucose and galactose monomers form variable osidic bonds	Improves visco-elastic properties of acid milk gels, antimicrobial and wound healing properties, ability to lower blood pressure and cholesterol in serum, capacity to retard tumour growth, enhance immunity of gut	Micheli et al. [34] Medrano et al. [37] Vinderola et al. [36]
Oligo-saccharides	*Leuconostoc* sp. *Lactobacillus* sp. *Streptococcus* sp.		Prebiotic, nutraceutical, alternative of antibiotics, food additives, humectant, prevention of colon cancer, treatment of chronic constipation, reduce lipid level in blood, in skin cosmetics	Yoo et al. [45]. Wang et al. [46] Remaud-Simeon et al. [7]

luxurious growth. Conversely, *Salmonella* and *Escherichia coli* are unable to utilize the oligomers, hence, their proliferation is inhibited. This manifests into the domination of *Bifidobacteria* and *Lactobacilli* in the intestinal flora. Selected fructo-oligosaccharides (FOS) and gluco-oligosaccharides (GOS) hold promise as alternatives to antibiotics against *Salmonella* and *E. coli*. Oligosaccharides are used as food additives in soft drinks, cookies, cereals, candies and dairy products. They can also be used as low sweetness humectants [45]. Oligosaccharides yield short chain fatty acids like propionate or butyrate on fermentation, which play decisive role in the prevention of colon cancer. Some of the major applications of oligosaccharides are listed in Table 1. Isomaltooligosaccharides (IMO) and oligodextrans are promising prebiotics. IMO have been used for treatment of chronic constipation and hyperlipidemia occurring as complications of maintenance haemodialysis [46]. FOS significantly enhances the efficiency of different cytotoxic drugs administered for human cancer treatment [47]. Fermentation of FOS in the colon creates low pH environment which hinders the proliferation of pathogenic bacteria and promotes mineral absorption [48]. When consumed as food supplement, the nondigestible oligosaccharides inhibit the secretion of very low density lipoproteins (VLDL) which reduce the cholesterol level in blood [49]. Oligosaccharides are also marketed as additives of skin cosmetic products [7].

Exopolysaccharide Biosynthesis

Biosynthesis of bacterial EPS is a complex process involving large number of enzymes and regulatory proteins. The genes encoding the proteins required for EPS synthesis are of plasmid origin in the mesophilic LAB strains like *Lactococcus* [50] and chromosomally located in thermophilic strains of *Streptococcus* and *Lactobacilli* [3]. HoPS are synthesized by extracellular or cell-wall bound glycansucrases using sucrose as substrate [12]. Glucosyltransferases catalyse the transfer of D-glucopyranosyl units from sucrose to acceptor molecules, forming glycosidic bonds. The mechanism of HePS biosynthesis is more complex as compared to HoPS. The repeating units forming HePS are synthesized in the cytoplasm using sugar nucleotides as precursor molecules. They are translocated across the cell membrane by glycosyltransferases, where polymerisation occurs by sequential addition of the sugar nucleotide units to the growing chain anchored on to a lipid carrier and finally, the EPS is released into the medium [2, 51]. The specific *eps* genes and housekeeping genes are involved in the sugar nucleotide biosynthesis. The biosynthetic pathway for EPS synthesis can be classified into four steps. These are (i) the reactions involved with sugar transport into the cytoplasm (ii) the synthesis of sugar-1-phosphates (iii) activation and coupling of sugars and (iv) export of the EPS. Each of these steps is crucial and can be manipulated to modify the amount and composition of the EPS. 2 gl^{-1} EPS is obtained from the highest producing strains of lactic acid bacteria [52].

Genetics of Exopolysaccharide Production

The *eps* gene cluster of *S. thermophilus* Sfi6 revealed a 15.25 kb region encoding 16 open reading frames. Within the 15.25 kb segment, a 14.52 kb region was identified

which encoded 13 genes (*epsA* to *epsM*), found vital for EPS synthesis. Homology search of the predicted proteins showed a high level of homology (40–68% identity) for *eps A, B, C, D* and *E* with the genes encoding capsular polysaccharides in *Streptococcus pneumoniae* and *Streptococcus agalactiae* [53]. The entire information needed for the biosynthesis of EPS by *Lactococcus lactis* NIZO B40 was encoded in a single 12 kb gene cluster located on a single 40 kb plasmid (*epsRXABCDEFGHIJKL*). The predicted gene products of 11 of the 14 genes were homologous in sequence to gene products involved in EPS, capsular polysaccharides, lipopolysaccharides and teichoic acid biosynthesis by other bacteria [54]. A recent study of the *eps* gene cluster of *Lactobacillus delbrueckii* subsp. *bulgaricus* revealed an 18 kb DNA region comprising 14 genes (*eps A* to *eps N*), with a similar genetic organization to other LAB [55]. A high level of similarity between the gene clusters of other LAB is now becoming clear. The genes in the clusters are oriented in one direction and transcribed as a single mRNA [56]. The genes of LAB synthesizing EPS are well conserved at 5′ end and appear to follow a similar trend of regulation, chain-length determination, biosynthesis of the repeating unit, polymerization and export [50, 56]. The nucleotide sequence of two open reading frames (ORFs) and three insertion sequences (IS) in the *eps* locus of *Streptococcus thermophilus* CNRZ368 are found almost identical to IS and ORF sequences from *Lactococcus lactis*. Sequence comparison of different *eps* loci of *S. thermophilus*, revealed a small constant region and a large variable region. These instances of high polymorphism are attributed to sequence replacement following horizontal transfers [57]. The often encountered variable and unstable HePS production result from DNA deletions, insertions and genomic rearrangements as observed in *Lactobacillus delbrueckii* subsp. *bulgaricus* and *S. thermophilus* [21, 53]. It is identified that *L. lactis* MG1363 *galU* gene, is involved in the conversion of glucose-1P into UDP-glucose [58].

Structural Modification of Exopolysaccharides

Large scale production of EPS from LAB is not feasible due to their poor energetics of metabolism. LAB being anaerobes, their energy conversion from carbohydrate is relatively inefficient compared to the aerobes [59]. This technical barrier must be overcome for cost effective production of EPS. For a significant increase in titre and yield of EPS, an integrated approach involving metabolic and molecular modelling and combinatorial techniques is required. Before manipulating the EPS synthesizing pathways, a thorough investigation of the regulatory points, energy availability, competition for precursors and substrates, gene expression and physico-chemical interactions is mandatory.

A strategy to produce the 'designer EPS' is by modification of the primary structure of the EPS by altering its physical properties. The desired structure can be obtained by enzymatic treatment at biosynthetic or post-biosynthetic stage. The polysaccharide hydrolases and lyases act endogenously or exogenously hydrolyzing the backbone and the terminal ends, respectively [60]. EPS resistant to enzyme degradation can be treated with mild chemicals to remove the acyl or pyruvyl-groups. The combined use of chemical and enzymatic method has been studied on *Lactococcus lactis* NIZO B40 EPS [61] and found that the combined approach of chemical and enzymatic treatment enhances the probability of obtaining the desired structures of EPS. Random mutagenesis is also a promising option to produce functional EPS. Mutation of *Lactobacillus. sakei* 0–1 has yielded a strain that produces an EPS with different monosaccharide composition [62]. Heterologous expression of EPS gene clusters is the tool for biosynthesis of EPS and oligosaccharides of interest [63]. EPS of novel structural features can be manufactured by shuffling the gene coding for glycosyltransferase. There is a huge possibility of regulating the formation of EPS by introducing new or existing glycosyltransferase gene into LAB. The genetic manipulation of glycosyltransferase could give rise to EPS with different linkages. Engineering the genes involved in export, polymerization and determination of chain length might also alter EPS structure. In some cases, it has been found that, selection of the carbon source plays a pivotal role in regulating the EPS structure. Structural study of the EPS produced by *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772 grown in continuous culture revealed that it consisted of glucose and galactose in the ratio 1: 2.4, when grown in fructose, and of glucose, galactose and rhamnose in a ratio of 1:7:0.8, when grown in a mixture of fructose and glucose [64]. However, this result is not universal for all LAB strains, as chemical composition of *Lactobacillus sake* strain 0–1 did not respond to variation in the type of carbon source.

Enhancement of the Exopolysaccharide Yield

Looking at the various utility of EPS from LAB, it is essential to raise the yield and improve efficiency of EPS. A crucial strategy to achieve this will be to precisely understand the interaction between different EPS configurations and components of food matrix at the molecular level. Modelling of carbon-metabolism and experimental studies of the regulation of glycolytic flux in the lactic bacteria is expected to enhance the yield of EPS. Response surface methodology based statistical experimental designs

have been reported to enhance glucan production from *Leuconostoc dextranicum* [65] and *Leuconostoc mesentroides* [66]. Being anaerobes, despite all metabolic engineering, LAB can not bulk synthesize the EPS. Therefore, it is essential to identify the functionally efficient EPS from the available lot. Screening the spontaneous diversity in wild LAB will increase the chances of picking genetically stable strains of LAB and discover numbers of glycosyltransferases capable of providing large quantities of functionally efficient EPS [12, 21, 59].

Negative Attributes of Exopolysaccharide

In some cases, EPS cause food spoilage. The synthesis of EPS by LAB during wine and cider fermentation, renders undesirable rheological properties to the products. Dental plaque that leads to dental caries is due to EPS synthesis by LAB. The biofilm formation and proliferation by LAB as a result of accumulation of EPS leads to biofouling [67]. Biofilm formation also causes many technical and hygiene problems in dairy industry like obstruction of heat flow, increased frictional resistance of the fluid and accelerated corrosion of surface causing heat and product loss [68].

Conclusions

Recent reports have revealed the huge potential of EPS and their derivative oligosaccharides from LAB in food, pharmaceutical and medical industries. But lack of optimum production processes has limited their contribution to the present day biopolymer market to a negligible fraction. The inefficient downstream processing causing reduced product recovery and consequent inflation in production cost is the limiting factor preventing the commercial exploitation of EPS. This problem can be solved by generating adequate knowledge of EPS biosynthesis and optimized bioprocess technology. Another factor which limits wide marketability of EPS from LAB is the distrust of consumers to these microbial products. Added to these, the prevailing stringent legislations limit the full marketing potential of EPS [53]. Before we can fully utilize this 'biofactory', an in depth as well as extensive study on the EPS structure, molecular organization of the *eps* gene clusters, enzymes involved in the biosynthetic pathways and regulating factors for expression of the EPS is required. To mass produce the EPS of interest with desired chain length, monosaccharide composition, branching pattern and linkage type, genetic manipulation of the LAB is needed. However, these genetically modified microbes and their products require legal approval before they are released into the market.

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