European Journal of Nutrition & Food Safety
4(2): 63-86, 2014

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An Overview of Healthy and Functionality of Exopolysaccharides Produced by Lactic Acid Bacteria in the Dairy Industry

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Review Article

Received 18th September 2013
Accepted 18th November 2013
Published 2nd February 2014

ABSTRACT

Exopolysaccharides (EPS) synthesized by lactic acid bacteria (LAB) play a major role in the manufacturing of fermented dairy products such as yoghurt, drinking yoghurt, cheese, fermented cream, milk based desserts. The demand of consumers for natural dairy products with a smooth and creamy texture, low in fat and sugars, can be satisfied by a judicious use of LAB producing EPS. One of the major sensory attributes important for consumer preference of dairy products is firmness and creaminess. EPS's may act both as texturizers and stabilizers, firstly increasing the viscosity of a final product, and secondly by binding hydration water and interacting with other milk constituents, such as proteins and micelles, to strengthen the rigidity of the casein network. As a consequence EPS can decrease syneresis and improve product stability. Furthermore it has been reported that EPS can positively affect gut health. The heteropolysaccharides from both mesophilic and thermophilic lactic acid bacteria have received renewed interest recently. Nowadays, in regard to demand of modern consumers focusing towards safe and healthy food without additives, new perspectives of development appear for these biopolymers. The GRAS (Generally Recognized As Safe) and probiotic status of some lactobacilli give to them more preference for consumable EPS production. One of their most described applications is their utilization as texturing agents naturally synthesized in the

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fermented food products. A better understanding of the structure-function relationship of EPS in a dairy food matrix remains a challenge to further improve applications of EPS to better satisfy the consumer demand for appealing, tasty and even healthier products.

Keywords: Exopolysaccharide; fermented food; texturing agents.

1. EXOPOLY SACCHARIDES, BIOPOLYMERS FROM MICROBIAL ORIGIN

Polysaccharides are biopolymers widely distributed in nature. Their occurrence is well documented in all organisms' viz. animals, plants, fungi and bacteria. These are adequately implied in various biological functions such as storage of energy (starch), cell wall architecture (cellulose) and cellular communication (glycosaminoglycans). These macromolecules exhibit high molecular weights attaining sometimes several millions of Daltons. All these polysaccharides can be homopolymers or heteropolymers of neutral sugars (pentoses and hexoses) or anionic sugars (hexoses). They can or cannot be substituted by non-sugars compounds and attain linear or ramified final conformations. These features lead to exhibit specific behavior in solution correlated with different conformations such as spirals, sheet, single, double and triple helix [1]. Exopolysaccharides are located in the extracellular medium without covalent bounds with bacterial membrane Bacterial exopolysaccharides have been abundantly described since many years for their structural variability offering a large set of physico-chemical and biological properties. They are released in extracellular medium by Archebacteria and Eubacteria (both Gram positive and negative).

An alternative class of bio thickeners is that of microbial exopolysaccharides (EPS). Microbial exopolysaccharides are extracellular polysaccharides which are either associated with the cell surface in the form of capsules or secreted into the extracellular environment in the form of slime. Exopolysaccharides are located in the extracellular medium without covalent bounds with bacterial membrane they are referred to as capsular or slime exopolysaccharides, respectively [2]. EPS occur widely among bacteria and microalgae and less among yeasts and fungi [2,3]. EPS in their natural environment are thought to play a role in the protection of the microbial cell against desiccation, phagocytosis and phage attack, antibiotics or toxic compounds (e.g. toxic metal ions, sulfur dioxide, ethanol), predation by protozoans, osmotic stress, adhesion to solid surfaces and bio film formation, and also in cellular recognition (e.g. via binding to a lectin). It is not likely that EPS serve as a food reserve, since most slime-forming bacteria are not capable of catabolizing the EPS they produce [4]. In pathogenic bacteria, such as *Streptococcus pneumoniae* and *Streptococcus agalactiae*, capsular EPS and O-antigen lipopolysaccharides are involved in the immune response.

Examples of industrially important microbial exopolysaccharides are dextrans, xanthan, gellan, pullulan, yeast glucans and bacterial alginates [5,6,7]. Novel microbial biopolymers may fill a gap in the market-available polymers or may replace a traditional product in terms of the improved rheological and stability characteristics. Now, microbial polysaccharides represent only a small fraction of the current biopolymer market. Factors limiting the use of microbial EPS are their production, which requires a thorough knowledge of their biosynthesis and an adapted bioprocess technology and the high cost of their recovery. Xanthan is a microbial EPS approved in the food industry, mainly because of its unique rheological properties in foods and the possibility of low-cost production. It is produced in high amounts by *Xanthomonas campestris*, a phytopathogenic bacterium that is not

generally recognized as safe (GRAS). Recently, gellan from the phytopathogen *Sphingomonas elodea* has been introduced on the market too. Strains of GRAS, food grade microorganisms, in particular lactic acid bacteria that are able to produce EPS in large enough quantities could be the solution to many of the above-mentioned disadvantages. Most of the EPS-producing LAB strains studied in more detail were isolated from dairy products, e.g. Scandinavian ropy fermented milk products [8,9], various yoghurts [10,11], fermented milks [12], milky [13,14] and sugary [15] kefir grains. Also cheese [15] and fermented meat and vegetables served as a source of EPS-producing LAB strains.

Other examples of commercial applications have been also reported in non-food industries. Even if bacterial exopolysaccharides are limited to market niches unable to compete with other established polysaccharides from plant and algae, they could offer a better quality or a high level of purity. One of the best examples is bacterial cellulose, employed as stabilizer of emulsions in cosmetic industry, artificial skins for medical application or as acoustic membrane, etc. Its tensile strength is higher than the vegetal polymer [16]. Agro-Food industries are always looking for new products which encourage focusing on exopolysaccharides from Lactobacillus species.

The ultimate goal in research in this area will be to link gene functions and chemical EPS structure to physico-chemical behavior. This will allow selection and/or specific development of EPS-producing bacteria for optimized applications. Therefore it has to be kept in mind that the properties of an EPS in a fermented milk product may differ considerably from its properties in purified form. Nevertheless, determining the structure, composition and rheological properties of a purified EPS is necessary in a first step to investigate the molecular interactions with milk constituents thereafter. Hence, this review focuses on recent developments relative to exopolysaccharides and their perspectives in food industries

2. MOST PRODUCING BACTERIA

Many food grade microorganisms produce EPS, in particular lactic acid bacteria (LAB) [17,18], propionibacteria [18] and bifidobacteria [19,20].

Approximately 30 species of lactobacilli are described as EPS producers. Among them, the best known are *L. casei*, *L. acidophilus*, *L.brevis*, *L. curvatus*, *L. delbrueckii bulgaricus*, *L. helveticus*, *L. rhamnosus*, *L plantarum*, *L. johnsonii*, etc. They are principally cultivated between 30 and 37ºC on rich media as MRS (Man Rogosa Sharp), milk or milk derivatives; therefore, many strains of dairy LAB manufacture extracellular polysaccharides. These compounds may be produced as capsules (Cps) which are tightly associated with the cell wall or as a loose slime (ropy polysaccharide) that is liberated into the medium. The term exopolysaccharide (EPS) may be used to refer to both types of external polysaccharide [2]. In many LAB, the EPS+ phenotype is unstable and may be permanently lost following repeated cell transfer or after prolonged incubation [17]. Genetic studies have shown that genes for EPS production in *Lactococcus lactis* are encoded by plasmid DNA and can, therefore, be irreversibly lost during repeated culture transfer [21]. In contrast, genes responsible for EPS synthesis in *Strep. thermophilus* are located on the chromosome [22-25] and their unstable nature may be related to the proximity of insertion sequences [25]. The culture for these LABS is generally conducted without agitation due to their aero-anaerobic status. In general, *Lactobacillus sp*. are not the best polysaccharide producers compared to some soil bacteria, commonly cited as *Xanthomonas campestris*. More than 20000 tons of xanthan are consumed each year mainly as thickener [1]. The abundant literature validates that the amount of EPS depends on the carbon, nitrogen sources and physico-chemical

conditions for bacterial growth as temperature, pH, oxygen rate, etc. Specific carbon substrate differs from one species to another. Sucrose appears as the best source for various lactobacilli [26]. Generally the yield of production is under 1 gL^{-1} for homopolysaccharides, when culture conditions are not optimized and even lesser for the majority of HePSs (Heteropolysaccharides).

Milk derivatives (lactose based media) and MRS (glucose based medium) are the most employed media for EPS production by lactobacilli (Table 1). Complex media have also been experimented but interferences with extraction and/or polysaccharide analysis have often been detected. The presence of mannans and other complex carbohydrates in yeast extracts notably confirms that complex media were not appropriated for polysaccharide extraction and analysis [4]. Effectively, these compounds are difficult to eliminate even by dialysis or ultrafiltration. In consequence, synthetic, defined and mineral media were designed. Some of them could hold more than 30 components [27]. Their use implies high costs and takes long time for production of EPS. Therefore, if they become an interesting alternative to study polysaccharide composition, they are unprofitable at industrial scale. Moreover, bacteria produce less exopolysaccharides in such media. Supplementation of media could increase production. For example, addition of sugars in excess has a stimulating effect while thiamine decreases the rate of EPS in a *Lactobacillus delbrueckii bulgaricus* culture [27].

Table 1. Principle producers and culture conditions for polysaccharides production

Microorganisms	Media	Temperature (°C)	Time (h)	pH	Yield	References
L. rhamnosus 9595	M BMM[a]	32-37	72	6	~1000	[49]
L. delb. bulgaricus RR	Whey	37	24-28	6	95-110	[50]
L. rhamnosus	R BMM[a]	37	72	6	500	[28]
L. delb. bulgaricus	Milk	42	24	/	110	[51]
L. delb. bulgaricus	MRS	40	18	/	236	[52]
L. rhamnosus	GG Milk	37	20	/	80	[53]
L. delb. bulgaricus 291	Skimmed milk	37	22	/	80	[54]
L. casei CG11	BMM[a]	25	48	/	130	[18]
L. helveticus	Skimmed milk	37	60	5	730	[55]
L. delb. bulgaicus	Whey (protein free)	37	18	6	800	[56]
L. rhamnosus 9595	Whey permeate supplemented	37	24	6	2775	[29]
L. paracasei	BMM	32-37	72	6	80	[49]

BMM[a] BMM: Basal Minimal Medium.

Regulation at a constant pH promotes better yields of EPS (Table 1), indeed when acidification occurs due to lactate production, glycohydrolases are activated (approximately pH 5). When the maximum concentration of lactate is reached in the *Lactobacillus rhamnosus* culture, polysaccharide yields decrease due to enzymatic digestion. It corresponds generally from 24 to 48 h of fermentation [28]. The best yield of EPS production

is attributed to L. rhamnosus, a largely described probiotic microorganism. Its culture medium included herein yeast extract, salts and vitamins. Although, mutual interactions of these components resulted in increase of the biomass, precise mechanism of each component is not identified. This strain reported to synthesize approximately 2.7 gL^{-1} of polysaccharide, mainly composed of rhamnose and in lesser quantity of glucose and galactose [29,30]. Yogurt bacteria *Strep. thermophilus* and *L. delbrueckii bulgaricus* also produce exopolysaccharides but the low concentrations attained (100–800 mgL^{-1}) have not an economic interest even if they are introduced as interesting texturing agent for fermented milk.

3. BIOSYNTHETIC PATHWAYS LEADING TO EPS SYNTHESIS IN LAB

A key intermediate linking the anabolic pathways of EPS production and the catabolic pathways of sugar degradation appears to be glucose-6-phosphate, in which the flux of carbon bifurcates between the formation of fructose-6-phosphate toward the products of glycolysis, biomass and ATP formation and toward the biosynthesis of sugar nucleotides, the precursors of EPSs (Fig. 1). Phosphoglutcomutase (PGM), the enzyme involved in the conversion of glucose-6-phosphate to glucose-1-phosphate, potentially has an important role in the divergence of flux between these catabolic and anabolic pathways [31,32]. Glucose-1-phosphate serves as a branch point for the formation of the sugar nucleotides UDP–glucose and dTDP–glucose via the action of UDP–glucose pyrophosphorylase and dTDP–glucose pyrophosphorylase, respectively. Note that these sugar nucleotides are used to form a variety of polysaccharides in the cell and hence the enzymes associated with their formation are shared (often termed 'housekeeping enzymes'). Conversion of galactose to glucose-1-phosphate via galactose-1-phosphate (the Leloir pathway) is possible if the system is present in the cell (Fig. 1). The subsequent stage of EPS synthesis in LAB – assembly of the monosaccharide repeating unit is achieved by several EPS-specific enzymes, as identified initially in *Streptococcus thermophilus* S6 [23] and in *L. lactis* NIZO B40 [33]. This repeating unit is assembled on a C55-isoprenoid–lipid carrier molecule, which is attached to the cytoplasmic membrane of the cell [34] (Fig. 2).

The sugars are linked to form the repeating unit by the action of several gene products on the EPS gene cluster (glycosyltransferases) [35]. The mechanism of polymerization of the repeating unit in LAB and its subsequent export from the cell is unclear. The high level of homology between Gram-positive and Gram-negative organisms with respect to the repeating unit synthesis means that it is likely that a similar mechanism will occur at the level of EPS polymerization and export. A simple model for this involves the action of a 'flippase' to move the lipid-bound repeating units from the cytoplasmic face of the membrane to the periplasmic face [36]. Using the same analogy, a polymerase could catalyse the linking of the repeating units and an enzyme could uncouple the lipid-bound polymer and control chain length.

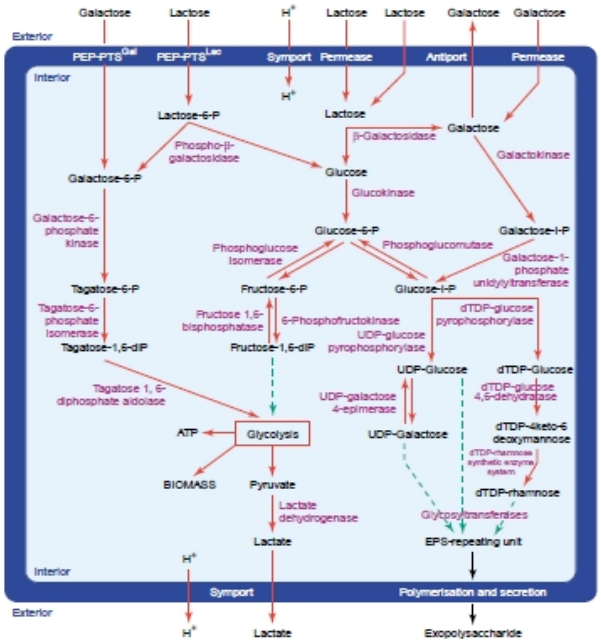

Fig. 1. Generalized diagram of the conversion of lactose, galactose and glucose to EPS and to glycolysis in lactic acid bacteria (glucose uptake not shown). In lactoseutilizing galactose-negative strains (e.g. *Lactobacillus delbrueckii* subsp. bulgaricus), galactose is not metabolized and is expelled from the cell via a lactose/galactose antiport system

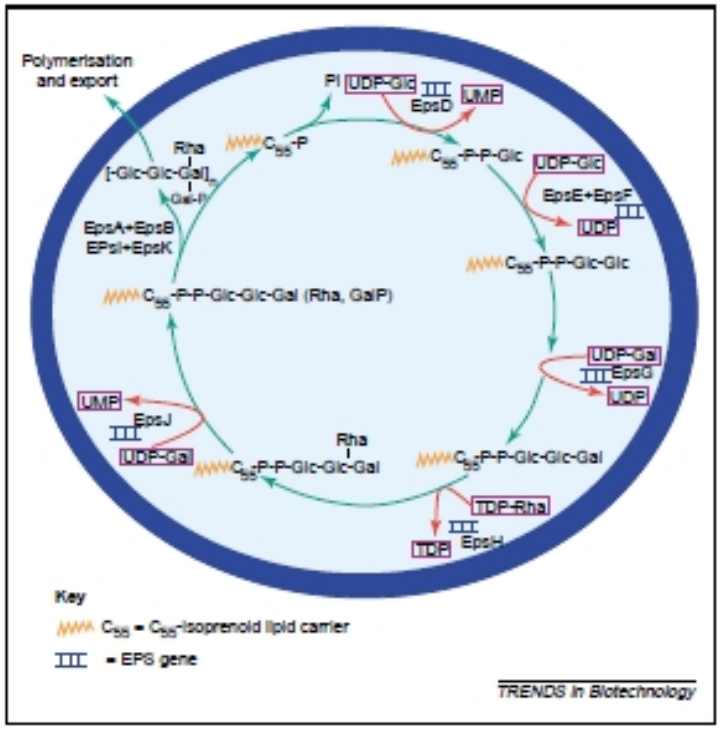

Fig. 2. Model of EPS biosynthesis in *Lactococcus lactis* NIZO B40. Adapted, with permission from [37]

4. CLASSIFICATION AND STRUCTURAL OF EXOPOLYSACCHARIDES FROM LACTIC ACID BACTERIA

Effectively, lactic acid bacteria (LAB) are well known as polysaccharide producers and one of these bacteria, *Leuconostoc mesenteroides* excretes dextran which is commercially exploited [38]. The low yields of polysaccharide production by the majority of LAB species is the main reason of their non-commercial exploitation. EPS from LAB can be subdivided into two groups: (1) Homopolysaccharides, consisting of four subgroups, namely (a) K-D-glucans, i.e. dextrans (*Leuconostoc mesenteroides* subsp. *Mesenteroides* and *Leuc. mesenteroides* subsp. *dextranicum*), mainly composed of K-1,6-linked glucose residues with variable (strain specific) degrees of branching at position 3, and less frequently at positions 2 and 4 and alternan (*Leuc. mesenteroides*) and mutans (*Streptococcus mutans* and *Streptococcus sobrinus*), both composed of K-1,3- and K-1,6-linkages; (b) L-D-glucans composed of L-1,3-linked glucose molecules with L-1,2-branches, produced by *Pediococcus* spp. and *Streptococcus* spp.; (c) fructans, mainly composed of L-2,6-linked D-fructose molecules, such as levan with some L-2,1-branching through the Ol site (*S. salivarius*); (d) others, like polygalactan, composed of structurally identical repeating units with di¡erent glycosidic linkages and (2) heteropolysaccharides produced by mesophilic (*Lactococcus lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *Lactobacillus casei*, *Lb. sake*, *Lb. rhamnosus*, etc.) and thermophilic (*L.acidophilus*, *L. delbrueckii* subsp*. bulgaricus*, *Lb. helveticus* and *Strep. thermophilus*) LAB strains. The latter group of EPS receives renewed interest, since they play an important role in the rheology, texture and body, and mouth feel of fermented milk drinks. For instance, the creamy, smooth texture is one of the aspects of the quality of yogurt which seems to be improved by the ability of the yogurt bacteria to produce EPS, even though only small amounts of EPS are being produced. In addition, generally, homopolysaccharides molecular weights range between 4.0×10^4 and 6.0×10^6 Da. These are produced at higher concentrations than heteropolysaccharides [39]. Homopolysaccharides yields can be several grams per liter contrary to these of Heteropolysaccharides, ranging between 50 and 200 mg/L. Homopolysaccharides secreted by Lactobacillus sp. contain glucose or fructose as sole monosaccharide and are classified as glucans and fructans respectively (Fig.3). These two subfamilies contain several polysaccharides with specific types of linkage, molecular mass, length and chemical conformation (Table 2). They have generally molecular masses as these described for reuteran (2.8×10^7 Da), levan (2×10^6 Da) or inulin-type fructan (10^7 Da) [40,41].

Table 2. Composition of homopolysaccharides produced by principle producing strains

Strain	Monosaccharide	Principal link on backbone	Polysaccharide type	References
L. hilgardii	D-Glcp	α-(1,6)	Dextran	[14]
L. fermentum	D-Glcp	α-(1,6)	Dextran	[40]
*L. reuteri*180	D-Glcp	α-(1,6)	Dextran	[40]
L. reuteri ML1	D-Glcp	α-(1,3)	Mutan	[40]
L. reuteri 121A	D-Fruf	α-(1,4)	Reuteran	[40]
*L. reuteri*LB121	D-Fruf	β-(2,1)	D-Fruf Inulin-type	[59]
L. sakei Kg15	D-Glcp	α-(1,6)	Dextran	[40]
L parabuchneri 33	D-Glcp	α-(1,6)	Dextran	[40]
L. reuteri 121	D-Fruf	β-(2,6)	Levan	[41]

Glucans are composed of a glucose polymer backbone with various degrees of branching depending on producing strains. Thus, dextrans from *Lactobacillus fermentum*, *L. sakei*, *L. parabuchneri* and *L. hilgardi* are α-(1,6) glucans ramified by glucose residues at position 3 and less frequently at positions 2 and 4. The sole homopolysaccharide from LAB present on industrial market is dextran synthesized by *Leuconostoc mesenteroides*. It finds specific applications as gel filtration compound (Sephadex) and as a blood plasma substitutes (Dextran 70) [38,42]. The second most described homopolysaccharide is "mutan", a linear EPS containing D-Glc residues linked by α-(1,3) glucosidic bonds (more than 50% of total linkages) associated with DGlc branched in α-(1,6). This water insoluble polymer is responsible for the adhesion of *Lactobacillus reuteri*, the producing bacteria, on teeth surface. At this time, mutan has not found any industrial application [38]. It is interesting to note the predominance of *L. reuteri* for homopolysaccharides production. Subspecies of *L. reuteri* are able to generate other types of EPS such as levan, inulin-type fructan, mutan, reuteran in a large range of molecular weights (Fig. 3).

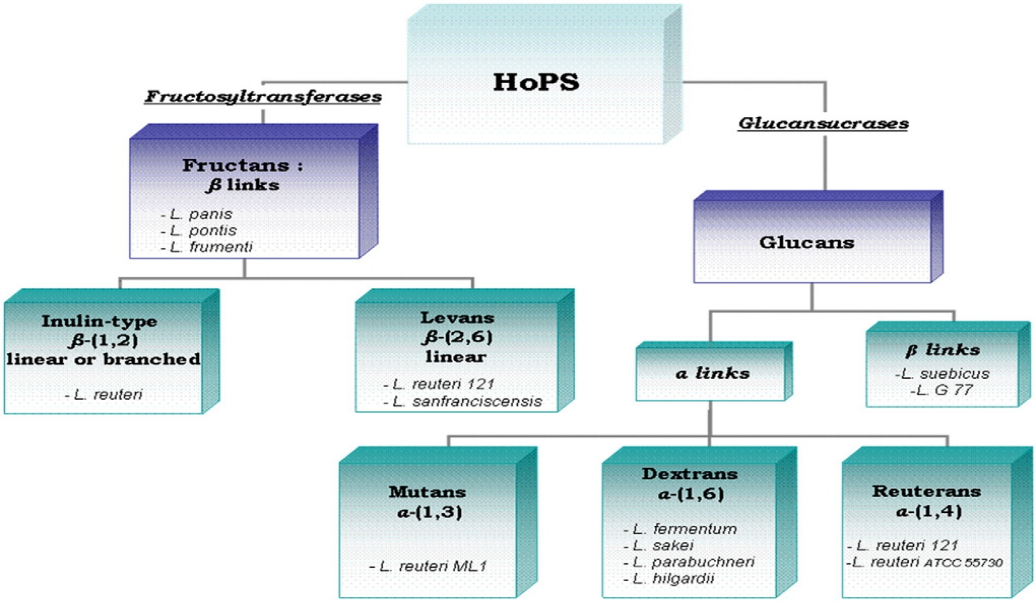

Fig. 3. Classification of homopolysaccharides produced by *Lactobacillus sp*

L. reuteri 121 has been found to be capable of several homopolysaccharides synthesis in. the same culture conditions [43]. This probiotic strain has been abundantly studied for its capacity to secrete β-(2,1) fructans (inulin like polysaccharide) recognized as prebiotic [41]. Inulin-type fructans show generally β-(2, 6) or β-(2,1) linkages when they are excreted by *L. reuteri* 121.

They are linear when *L. johnsonii* NCC 533 is the producer [44]. The soluble reuteran comprises of 70% α-(1,4) linkages and few α-(1,6) ones. It has found opportunities in baking industry in association with levan synthesized by *L. reuteriand* and *L. sanfranciscensis*. Indeed, their polysaccharides affect beneficially bread flavor, texture and shelf life of products derived from sourdough fermentation [45]. Levan consists in fructose as sole monosaccharide linked by β-(2,6) glycosidic bonds β-glucans production is often associated with wine or cider spoilage due to an increase of viscosity. Among the LAB identified as

responsible, different strains of Pediococcus, Oenococcus and Lactobacillus have been described [46,47]. For this last genus, *Lactobacillus suebicus* and Lactobacillus sp. G-77 are the strains identified as β-(1,3)-D-glucans producers [48]. However, another β-(1,3)-D-glucans, the curdlan produced by *Agrobacterium* sp. is authorized by Food and Drugs Administration as food additive (under the name Pureglucan) for its particular gelifying properties. Moreover curdlan and others bacterial β-(1,3)-D-glucans have been described as anti-tumor agents activating macrophages and white blood cells [46].

Heteropolysaccharides have a great variability in structures and on the contrary to homopolysaccharides, their concentrations in broth attain about hundreds mg per litre. Their molecular weights vary from 10^4 Da (*L. plantarum*) to 6×10^6 Da (*L. sakei* 0-1) [57,58]. EPS backbones of Lactobacillus spp. (*L. delbrueckii bulgaricus*, *L. rhamnosus* and *L. helveticus*) have repeating units composed of seven monosaccharides, where glucose, galactose and rhamnose are the main sugars (Table 3). Occasionally, amino-sugars as N-acetyl-D-glucosamine and N-acetyl-D-galactosamine as well as polyol (glycerol) are also present. Heteropolysaccharides may be partially anionic due to glucuronic acid and phosphate in their assembly. They are often highly branched with different types of linkages. Their appellations are also complex depending on the principal monosaccharide for example, galactoglucan or glucogalactan and further they also concern ratio of each sugar with names as rhamnoglucogalactan or galactoglucorhamnan (Fig. 4).

5. EXOPOLYSACCHARIDE YIELDS PRODUCED BY LACTIC ACID BACTERIA

The total yield of EPS produced by LAB depends on the composition of the medium (carbon and nitrogen sources) and the conditions in which the strains grow, i.e. temperature, pH and incubation time (Table 1). The production of intracellular synthesized EPS by different LAB strains varies roughly from 0.045 to 0.350 g l31 when the bacteria are grown under non-optimized culture conditions.

Optimal culture conditions result in EPS yields from 0.150 to 0.600 g, depending on the strain [17,18]. When a ropy strain of *Strep. thermophilus* is grown in association with a non-ropy strain of *Lb.delbrueckii* subsp. *bulgaricus* in milk, EPS production can reach quantities of almost 0.800 g [68,4]. An optimal carbon/nitrogen ratio in both milk and MRS media resulted in 1.1 gl31 with S. thermophiles LY03 [11,69,70]. With *Lb. sake* O-1, EPS yields of approximately 1.4 g131 is achieved [71]. These values are, however, as previously mentioned not comparable with the high yields obtained with dextran-producing LAB and Gram negative EPS producers such as *Xanthomonas campestris*.

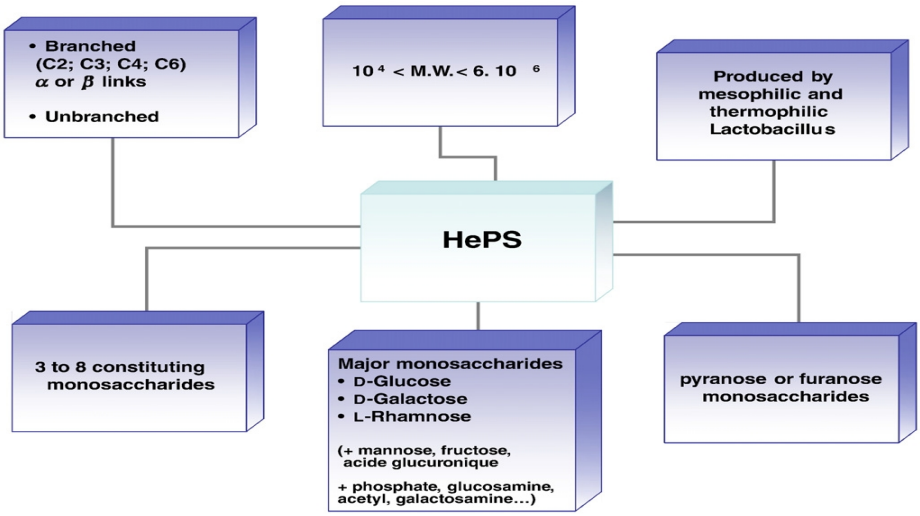

Fig. 4. Characteristics of Heteropolysaccharides produced by Lactobacillus sp

Table 3. Composition of different heteropolysaccharides produced by Lactobacillus strains

Strains	Principal monosaccharides	Substituent and/or secondary monosaccharides	References
L. acidophilus LMG 9433	Glc, Gal	GlcNAc+GluA	[57]
L. sakei 0-1	Glc, Rha		[32]
L. delb. subsp. bulgaricus 291	Glc, Rha Glc, Gal		[54]
LY 03, 24, 25	Glc, Gal Rha		[60]
NCFB2074	Glc, Gal		[61]
L. helveticus ATCC 15807	Glc, Gal, (Rha)	Phosphoric acid	[62]
L. paracasei 34-1	Gal	GalNAc, glycérol-3- P	[63,64]
L. pentosus LPS 26	Glc, Rha Glc	Man	[65]
L. plantarum EP 56	Glc, Gal	N-GlcNAc Glycerol,	[58]
	Glc, Gal, Rha	phosphoglycerol	
L. rhamnosus C83	Glc, Gal		[66]
GG	Gal, Rha	GlcNAc	[53]
RW-9595 M ou R	Glc, Gal, Rha		[28]
L. casei CG11	Glc, Rha		[15]
L. kefiranofaciens subsp. *kefiranofaciens*	Gal Glc,		[67]

6. ROLES OF EPS FOR BACTERIA

The physiological function of EPS from lactobacilli remains unclear in literature. Adhesive role is generally acknowledged, bacteria sticking onto the surface by polysaccharide secretion, fimbriae and pili. Furthermore EPS are described in final structure acquisition of biofilms [72] causing medical troubles and/or food spoilage [73-75].

Bacteria embedded in such a sticky matrix are protected against mechanical forces and anti-microbial compounds (notably during cleaning). Microorganisms in a biofilm can be 1500 times more resistant to antibiotics than planktonic cells implying higher required doses to

eradicate them, notably on medical tools as catheters or prostheses [76]. The slime layer also provides a protection against drying because a high rate of hydration permits the survival of cells even in extreme physical conditions [18]. Numerous authors venture to hypothesize that the bacteria able to form biofilms should synthesize polysaccharides. Among Lactobacillus species, *L. sakei* is well-known to generate a biofilm in food ingredients [74]. Experiments were led by Lebeer et al. [83] on *L. rhamnosus* GG, known to have a good attachment capacity on biotic and abiotic surfaces. A mutant for phosphotyrosine phosphatase expression implied in the regulation of EPS synthesis shown that biofilm formation seems to be EPS dependent in some culture media. Other observations revealed that the optimal medium for EPS production is not the medium where the adhesion rate for *L. rhamnosus* GG is the best. Same observations were obtained with another LAB well known to form biofilm: *Leuconostoc mesenteroides*. For it, evidence of proteins and nucleic acids implication were carried out [77]. Thus, biofilm are often associated to exopolysaccharides but their definite role in the matrix is not always evident. Another benefit is indirect. Effectively, polysaccharides facilitate communications between cells because they aggregate bacteria and decrease cell-to-cell distances. A favorable microenvironment is thus created to transfer genetic characters but also to protect bacteria against phages [78]. Moineau et al. [79] have studied the involvement of EPS into spread of phage infection. In industry, it represents the first cause of fermentation failure. Conclusions are still unclear but increasing of extracellular medium viscosity by EPS could slows down phage diffusion and then partially prevent viral expansion. The screening by the same authors of several *Lactococcus lactis* strains, EPS producers or not, revealed later than the EPS production does not confer a significant phage resistance phenotype [80].

Another putative role of EPS is their use by bacterial producers as carbon source. The expression by some strains of enzymes able to degrade their own EPS reinforces this hypothesis. More, during prolonged incubation (more than 48 h for LAB), the quantity of produced polysaccharides decreases, suggesting but not concluding about their function of carbon reserve [81] Nonetheless, only few species have all the necessary enzymes to degrade their polysaccharide [82] by release of glycohydrolases in their culture medium [4,39]. Apparently degraded products are not metabolized by producing bacteria which are not equipped with associated enzyme leading to monosaccharide release. This could imply consumption by other species in complex ecosystems. In all circumstances, this degradation is a paradox as EPS biosynthesis carried out with high energy consumption. To resume some authors described the EPS synthesis as a nutrient storage and others precised that bacteria can't use their entire EPS [82]. This apparent energetic paradox could be explained in future with mechanisms for polysaccharide molecular weight regulation in biofilm reorganization.

7. HEALTH PROPERTIES OF EPS

EPS from LAB have one of the largest technical potentials for development of novel and improved products such as low-milk-solid yogurts, low-fat yogurts, creamier yogurts, etc. [28]. Moreover, some polysaccharides may contribute to human health, either as non-digestible food fraction [84] or because of their anti tumoral [85,86], antiulcer [87], immune modulating [88,89] or cholesterol-lowering activity [90,9]. Therefore EPS from LAB have potential for development and exploitation as functional food ingredients with both health and economic benefits.

Many species of lactobacilli have been studied for their probiotic potential in food and more precisely in dairy products as *L. acidophilus*, *L. bulgaricus*, *L. lactis*, *L. plantarum*, *L. casei*, *L.*

reuteri, *L. rhamnosus*, *L. parasei*, *L. fermentum* and *L. helveticus*. Among each cited species, some strains are known as EPS-producers. Probiotics are live microorganisms which supply beneficial effects for host health when they are consumed in appropriate quantities, the prebiotic concept has emerged [84]. Prebiotics are non-viable and non-digestible ingredients stimulating gut microbiota. They are generally oligosaccharides, whose degrees of polymerization range between 2 and 20 monomers. They are metabolized by health beneficial bacteria and improve immunity to fight against pathogenic organisms. Ruas-Madiedo et al. summarize the effect of EPS from lactic acid bacteria in human physiology at different levels. Apart their prebiotic potentialities, as previously mentioned, EPS have been identified as blood cholesterolemia reducer, immunomodulation, antitumor and antiulcer agents [91].

Dal Bello et al. [92] have studied prebiotic properties of levan EPS produced by *Lactobacillus sanfranciscensis*. Experiments consisted in fermentation of levan used as sole carbone source by bacteria from freshly faces sampled on healthy humans. The number of bifidobacteria increased but the number of lactobacilli decreased. The bifidogenic effect is detected in vitro but the lack of experiments on human does not permit to conclude about health benefits. Numbers of publications are available on the same subject but most of them treated of in vitro investigations and the final prebiotic effect is not well appreciated. Moreover interactions between different bacterial populations are not studied and the global effect on this nutritional intervention is not considered on targeted species. Tieking et al. [45] have studied lactobacilli from intestines and detected glycosyltransferases described to produce levan and fructooligosaccharides. These biomolecules exhibit high potential as sugar substitute carrying glycaemia suppressive properties in addition to their prebiotic ones. However, further investigations are needed before to conclude definitively on the prebiotic status of these EPS from lactobacilli because drastic criteria are necessary to obtain this allegation. It is important for a prebiotic molecule to resist at the gastric acidity, hydrolysis by digestive enzymes and intestinal absorption (they must be digested but a sufficient quantity must be available in large bowel particularly). In addition, microbiota must ferment them and finally they have to stimulate selectively the growth and bacterial activity, to improve host health or well-being [93].

Roberfroid proposed a prebiotic index (PI) taking into account the number of bifidobacteria divided by the daily dose of consumed prebiotic. For the moment, two oligosaccharides answer to the totality of these criteria: inulin and fructooligosaccharides but their origins are not bacterial. EPS from lactobacilli and bifidobacteria are the most experimented when the prebiotic effect is studied. However, it is also interesting to correlate prebiotic effect with short chain fatty acids production such as acetic acid, propionic acid and butyric acid secreted by several lactic acid bacteria-producing EPS (*L. delbrueckii* subsp. *bulgaricus*, *Streptococcus salivarus* subsp. *thermophilus*, *Pediococcus damnosus* and *L. reuteri*) [94]. These metabolites have good functionalities in intestine. They decrease colonic pH resulting in an increase of mineral solubility, a decrease formation of secondary bile acids and a decrease of the proliferation of unwanted pathogens. More specifically these short chain fatty acids have been described as energy substrate for the colonocytes, playing a role in the prevention of ulcerative colitis and cancer and as cholesterol synthesis inhibitors. Indeed, benefits effects for patients are the mix of bifido genic effect and production of favorable metabolites, the latter being not included in PI calculation.

Polysaccharides from Lactobacillus sp. present other healthy effects. Kefiran can be classified as functional food because of its action at different levels on animals. Using doses about 100–300mg/kg on rats, kefiran reduces blood pressure, cholesterol and blood glucose

rates. Finally it seems to have a good influence on constipation [95,96]. Other properties were detected after oral administration of this polysaccharide as anti-inflammatory, anti-tumoural and stimulation of immunoglobulin secretion [97,98]. Heteropolysaccharide from *L. delbrueckii bulgaricus* and *L. acidophilus* present an antitumor activity and an enhancement of macrophage function. A secretion of tumor necrosis factor is improved by polysaccharide of *L. rhamnosus* [99]. Presence of rhamnose in heteropolysaccharides has also been proved to benefit against gastric ulcer. All these results appeal at the exploitation of heteropolysaccharides from lactobacilli and research of new active polysaccharides.

In addition to technological benefits, certain EPS produced by lactic acid bacteria are also claimed to have beneficial physiological effects on the consumer. It is speculated that the increased viscosity of EPS containing foods may increase the residence time of ingested fermented milk in the gastrointestinal tract an therefore be beneficial to a transient colonization by probiotic bacteria [103]. A further example of a suggested health benefit of some EPS is the generation of short-chain fatty acids (SCFAs) upon degradation in the gut by the colonic microflora. SCFAs provide energy to epithelial cells and some have been claimed to play a role in the prevention of colon cancer [100,101]. In vivo studied showed that EPS produced by *Lactococcus lactis* subsp*. cremoris* B40, *Lactobacillus sakei* 0-1, *Strep. thermophilus* SFi 20 and *L.helveticus* 59 were not degraded by fecal microorganisms [102]. Further health beneficial effects of EPS were postulated in the literature: e.g. an anti-tumor effect of EPS produced by Lactobacillus [85], a cholesterol-lowering effect by fermented milk viili [90], immune-modulatory effects from *Bifidobacterium adolescentis* M101-4 [89]. In fact, the same two trioses have been identified in the structure of several human milk oligosaccharides that are important for healthy infant nutrition. We are convinced that the future will show a shift from pure technical and texturing applications of selected EPS to more and more targeted applications of specifically developed EPS for improved consumer health benefits.

8. HOW TO FIND NEW SOURCES OF POLY SACCHARIDES?

Novel sources of polysaccharides are always looked for and studies tend towards LAB due to their GRAS status. It would be interesting to discuss methods to detect EPS-producing bacteria.

Slimy colonies on plates serve as a good indicator. Sometimes, identification of EPS producing bacteria is done by a simple test where a filament appears while a colony is lifted up with a loop. Staining methods were also developed. Congo red links by non-covalent bounds with β-(1, 3) and β-(1,4) linkages of polysaccharides. Ruthenium red is a polycationic stain capable of interactions with carboxylic groups of acidic polysaccharides. More recently, stained EPS has been directly observed by Hassan et al. in a hydrated dairy product by confocal scanning laser microscopy [104]. They succeed in employing fluorescent-labelled lectins and carbohydrate binding proteins. In nature, polysaccharides are sometime synthesized by bacteria aggregated in a biofilm following a stress, depending on the age of biofilm. Production is facilitated in a medium with a high ratio carbon/nitrogen. So, another strategy to find such molecules is to detect bacteria capable of elaborate a biofilm. Many methods have been developed as staining and microscopy. A recent method: the Bio Film Ring Test® permits to follow a biofilm formation with a numeric value. The principle behind this test is the culture of microorganisms in micro wells with magnetic particles. They aggregate more or less to form a spot according to their degree of embedding in the biofilm matrix, when a magnet is approached under the wells [105].

9. NOVEL APPLICATIONS OF EXOPOLYSACCHARIDES FROM LACTIC ACID BACTERIA

The intentional and controlled use of EPS from LAB as natural food additives or of functional starter cultures, i.e. strains producing interesting EPS, could result in a safe, natural end product, and may have an important impact on the development of novel food products (both fermented and non-fermented food products), especially food products with enhanced, rheological properties, improved texture and stability, and/or water retention capacity .For instance, low viscosity, gel fracture or high syneresis (whey separation) problems, which may occur during yogurt manufacture, can be solved in both ways. An EPS-producing *Strep. thermophilus* strain was also responsible for an increased moisture level in low-fat mozzarella cheese [24,106]. Since the use of lactic acid bacteria (GRAS microorganisms) is historically safe, production of in situ novel functional EPS means that toxicological testing will not be required and the products can be brought to the market more quickly.

10. PRODUCT EXAMPLES

Fermented dairy products have been well established worldwide as a fermentation which extends time for milk consumption. Exopolysaccharides produced by lactobacilli have been described for their indirect role on product rheology but also on its health benefit for the consumer. However, during fermentation of milk in association with other lactic acid bacteria, lactobacilli are firstly responsible for its acidification.

10.1 Yogurt

Yogurt is fermented milk by starter cultures of *L. delbrueckii bulgaricus* and *Strep. thermophilus* in ratio 1:1. Both the bacteria are EPS producers. Their production is ranging from 30 to 890 mg/L for *Streptococcus thermophilus* and from 60 to 150 mg/L for *Lactobacillus bulgaricus* [51,67,107]. Their activities are often described as synergistic for an improvement of texture and a decrease of syneresis. *L. delbrueckii bulgaricus* grows first and then produces metabolites, needed by *Strep. thermophilus* which liberate formic acid and CO2, stimulating lactobacilli growth in return. *L. bulgaricus* is responsible for the acidity favorable to caseins coagulation and *Strep. thermophilus* is described for aroma liberation [108]. Experiments to elucidate exopolysaccharides role in the yogurt structure were carried with non EPS-producing strain and EPS-producing strain. Even if the yogurt was stirred, the viscosity values were always higher in presence of EPS-producing microorganisms [109,51]. Polysaccharides contribute to boost the viscosity and reach a more favorable texture. EPS do not possess taste of their own in the final product but improve texture perception by consumers [110]. It avoids the addition of stabilizers, which are forbidden especially in European countries. Other results show the importance of having a polysaccharide with high molecular mass and a stiff conformation in order to obtain an increase in viscosity [99]. Caseins form a gel and polysaccharide was found in pores when structure was investigated with confocal scanning laser microscopy. Such observation shows that exopolysaccharides are incompatible with proteins aggregates in fermented milk [11]. Hassan et al. [112] found that capsule-forming non ropy cultures improved the textural and rheological properties of fermented milk Purwandari et al. [108] have examined EPS-producing *Strep. thermophilus* at different temperatures and have concluded that there is a weak relation between texture and EPS concentration [108]. Thus, different conclusions are found in literature concerning presence and concentration of EPS in yogurt for the appreciation of rheology. Nonetheless authors are agreed with the fact, that interactions with caseins according to pH value and

conformation of EPS are the key point of texture improvement. Implication of pure EPS has not been yet studied and at present, conclusions concerning their role as texturing agent are indirect. Introducing an EPS producing bacteria, instead of purified EPS, to study the role of EPS in yoghurt is pertinent only if the role of other released metabolites is taken in account. Important factors influencing function of EPS in yogurt are their molecular characteristics (molecular weight, degree of branching, radius of gyration, charge and so on) and their ability to interact with milk proteins (whey protein-casein complex in yogurt).

Because ropiness decreases curd firmness, it is expected to increase syneresis. Although Folkenberg et al. [113] confirmed this hypothesis, other researchers reported decreased syneresis in the ropy yogurt [4,107,109,125]. The reason for the disagreement on the effect of EPS producing cultures on syneresis is because of differences in the methods used for measuring syneresis [109,113].

10.2 Kefir

Kefir as yogurt takes an important place in Eastern European countries. This beverage is prepared from fermented milk with a complex consortium of bacteria and yeasts. Kefir grains correspond to aggregates of microorganisms embedded in a polysaccharidic matrix. Several species of microorganisms are used in its preparation, such as Lactobacillus sp. (*L. acidophilus*, *L. kefirgranum*, *L. kefir*, and *L. parakefir*) Candida kefir, Saccharomyces sp., Acetobacter sp., etc. The polysaccharide named kefiran is produced by *L. kefiranofaciens* in the centre of grain under anaerobic conditions in presence of ethanol produced by yeasts and lactic acid secreted by lactobacilli. Proportion of kefiran in grains is about 45% and it is composed of D-Glc and D-Gal in ratio 1:1 [114]. This biopolymer has a branched hexa- or heptasaccharidic unit. One or two residues are branched at the main chain composed of 5monosaccharides [95,96] kefiran is resistant to enzymatic degradation [115]. Major role of kefiran in grains is described as a protection of microorganisms against desiccation. Indeed, grains can be dried and employed again for other fermentations of milk. Rimada et al. performed comparisons of rheological behavior of skim-milk gels with and without kefiran. Kefiran is the ‘‘glue’’ of the grains and confers a slimy texture to the product. In the traditional method, kefir grains are removed by passing through a sieve after about 24 h of fermentation at room temperature. After this first fermentation, the milk can either be consumed or used to further inoculate fresh, pasteurized milk which is then fermented by the cells in suspension. With this method, it is possible to produce set-style type kefir. For industrial production, selected starter cultures are used in order to reach a balance between the different species. Furthermore, the process is easier, controllable and more reproducible when the fermentation process is split in two phases: first one with LAB fermentation (biosynthesis of lactic acid, flavors) and the second one with yeasts (production of ethanol and flavors) [116]. The mixture with kefiran offers a viscosity and viscoelasticity improved up to300 mg/L of polysaccharide. This natural polysaccharide might be employed as an alternative thickening agent in dairy products.

10.3 Cheese

Evolution of Lactobacillus sp. growth was studied on maturation of semi soft cheeses (e.g. Gouda), pressed cheeses (e.g. low fat Cheddar cheeses) and blue-veined cheeses (e.g. Roquefort). Heteropolysaccharides liberated by lactobacilli strains such as *L. delbrueckii bulgaricus*, *L. helveticus* and *L. casei* promote water retention and improve the overall texture of cheese in avoiding alteration of the structure.

Lactobacilli starter cultures contribute in the acidification because of lactic acid liberation, necessary at the beginning of ripening. Their proteolytic activities with the synthesis of secondary metabolites are favorable for other species development [117]. The key step is the strain selection because deterioration of flavor is induced by a stronger acidification. Cheese elaboration depends on associations with other strains and also on presence or absence of charges on exopolysaccharides produced. A neutral one interacts more slowly with caseins. Perry et al. [106] noticed that moisture increases when EPS-producing starter cultures were employed in the manufacture of low fat mozzarella. This water retention capacity is responsible for the texture improvement of cheeses and permits reduction of calories in the final product. The difficulty to attribute these observations to exopolysaccharides or capsular polysaccharides comes from the studies, conducted directly in the final product.

Several studies highlighted the positive effect of EPS producing cultures on the physical and functional properties of reduced-fat Cheddar cheese [118-123,]. A 33% reduced-fat Cheddar cheese had similar textural characteristics as a full fat cheese if made with a ropy strain of *Lactococcus lactis* ssp. *cremoris* (JFR1). Furthermore, the changes in the texture of full-fat cheese and reduced-fat cheese made with the EPS-positive strain JFR1 followed the same pattern. Unlike reduced-fat cheese made with no EPS, hardness of both full-fat cheese and reduced-fat cheese made with the JFR1 culture increased during the first month of ripening. Due to the improvement in texture of EPS-containing reduced-fat Cheddar cheese, panelists did not detect any differences in texture between full fat and reduced-fat cheeses [119].

Capsule-forming *Strep. thermophilus* used as an adjunct in making reduced-fat Cheddar cheese also improved the texture of reduced-fat Cheddar cheese [119,124]. However, the effect of such a culture was less pronounced than that produced by highly ropy strains of *L. lactis* ssp. *cremoris*. Although Dabour et al. [120] found that EPS producing cultures had no effect on fat recovery in cheese; Rynne et al. [124] reported improved milk fat recovery when such cultures were used. Rynne et al. [124] found also that the ropy EPS increased the efficiency of half-fat Cheddar cheese-making by improving the rennet coagulation properties, reducing cheese making time, and increasing moisture.

Hassan et al. [125] concluded that proteolysis and pH of base cheese are the most important factors influencing process cheese characteristics. Exopolysaccharides act as nuclei for the formation of large pores in base cheese. Such pores reduce the rigidity of cheese. However, when cheese is melted during process cheese making, the importance of such pores is minimized and changes in base cheese at the molecular level such as proteolysis and calcium removal due to reduction in pH seem to be more important [125]. The effect of EPS on reduced- fat process cheese characteristics is limited compared with changes that take place during the first month of the base cheese ripening Karish cheese made with the EPS-positive culture was softer, less adhesive and chewy, and more cohesive than the control cheese [126,127]. The moisture level in the EPS positive cheese was 3% greater than that in the control cheese [128]. This is a major factor responsible for the improvement in the textural characteristics of EPS-positive cheese. A moderately ropy strain of *Streptococcus thermophilus* produced Feta cheese with an undesirable slippery texture such texture was not produced in the acid-coagulated cheese (Karish) by the same strain. This was because of differences in the characteristics of rennet curd and acid-coagulated curd. Unlike rennet curd, acid curd does not show immediate syneresis after cutting [129]. The capsular type of EPS would be recommended for making rennet-coagulated soft cheeses because it does not cause ropiness. The capsular and moderately ropy EPS can be used successfully in making acid-coagulated soft cheese unless the viscosity of the whey is a concern, in which

case only the capsular type should be used. Moreira et al. [130] increased moisture retention and improved melt ability, textural, and proteolytic properties of a soft cheese, Quartirolo by using EPS-producing cultures.

CONCLUSION

The demand of consumers for natural dairy products with a smooth and creamy texture, low in fat and sugars, can be satisfied by a judicious use of LAB producing EPS. Exopolysaccharide-producing cultures can be used to improve the physical properties of dairy products. The selection criteria of such cultures depend on the physical characteristics desired in the product. However, the main drawback limiting their exploitation in food and other industries is the low yield of production which is not yet resolved. EPS from this genus have been well described for their texturing and biological roles notably in dairy products but these studies have not been conducted with purified EPS prior to texturing milk products. EPS are often studied directly in dairy products and seldom as a purified compound. Nonetheless healthy effects of exopolysaccharides are discussed alike to commercial prebiotics such as FOS (Fructo-OligoSaccharides) in food application. Its production could be significantly improved by bacterial fermentation and its application as a prebiotic could be attained after their chemical, physical or enzymatic degradations in order to increase their activity. The viability of this commercial ignition will be dependent on biotechnological developments from high yields producing strains obtaining with all desirable properties linked to their use in food area.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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