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Overview of the most important characterization of exopolysaccharides produced by probiotics bacteria and their biological function

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Abstract: *Microbial exopolysaccharides (EPS), used as food and medicinal additives, are high molecular weight polymers made by reducing sugars produced during the growth cycle of various strains of bacillus and lactic acid bacteria (LAB). The exact role of EPS depends on structural unit and environmental conditions of producing microorganisms that protect them in foods against environmental stresses. It is well established that probiotic bacteria have beneficial influence on health of their host. The probiotic effects ascribed to LAB as the major probiotics are resulted not only from the action of whole microorganisms and cell wall components, but also from extracellular polysaccharides. Physicochemical properties of EPS make the commercialization of these products possible. However to date, the mechanisms responsible for their biological effects are still poorly understood and the exact function of these metabolites remained relatively unknown, although by increasing researches for isolation and identification of EPS and their newly applications, these compounds are being used as reproducible natural sources in food industries. This review attempts the role of EPS in bacterial physiology, biofilm formation and biosynthesis of exopolysaccharides from LAB. The most important applications of microbial EPS in food industry are also discussed briefly in this article.*

Keywords: *Microbial exopolysaccharides, Probiotics, Biofilm, Physicochemical*.

I. Introduction

Probiotic bacteria have the capability of producing extracellular polymers known as exopolysaccharides (EPS[1]). It has been suggested that the health interest of probiotic bacteria can be attributed to the production of EPS. But, the composition, structure and biological functions of EPS may greatly depend on the type of microorganism and environmental conditions (Vu et al 2009). Microbial metabolites are derived from a wide variety of sources: bacterial, fungal, algal and plant (Sutherland 1998). Despite the multitude of polysaccharides sources, the global market is dominated by polysaccharides from algae and higher plants (Leung et al 2006; Li et al 2006). The polysaccharides produced by microorganisms can be classified into three main groups according to their situation in the cell: first group, cytosolic polysaccharides, which provide a carbon and energy source for the cell; second group, polysaccharides that construct the cell wall, including peptidoglycans, techoid acids and lipopolysaccharides and third group, polysaccharides that are permeated into the extracellular environment in the form of capsules or biofilm, known as exopolysaccharides (EPSs). EPS is the main substance involved in biofilm formation and may achieve 50-90% of the total organic substances such as proteins, lipids and nucleic acids. Bacteria develop biofilms to protect the microbial community against environmental stress (Vu et al 2009). Exopolysaccharides are divided into two groups: (i) homopolysaccharides and (ii) heteropolysaccharides. Homopolysaccharides are made up of a single type of monosaccharides, like dextran or levan. Heteropolysaccharides are composed of several types of monosaccharides like xanthans or gellans, have complex structures and are usually synthesized inside the cell in the form of repeating units (Bergmaier 2002; Lahaye 2006). Most of the heteropolysaccharides are composed by bacterial exopolysaccharides. Microbial exopolysaccharides have different functions in the cell. For example, protect living cells against stress, competition, and biotic stresses that might include temperature condition, light intensity or pH. Accordingly, extracellular metabolites derived from acidophilic or thermophilic species and Archaea, aid in adaptation to extreme conditions. In spite of the wide variety of microbial extracellular metabolites with physicochemical properties that are industrially valuable, just two microbial extracellular metabolites are permitted for use as additives in the food industry of the United States and Europe: xanthan (30000 tons/year) and gellan. There exists a large variety of microbial extracellular metabolites produced by a normally large number of lactic acid bacteria (Cerning et al 1994). Most microbial extracellular metabolites were isolated from dairy products, but fermented meat also supplied a source for EPS-producing lactic acid bacteria strains (Makela 1992). Some EPS-producing lactic acid bacteria strains have been investigated in more

[1] Exopolysaccharides

detail, for example: *Lactococcus lactis* isolated from diverse Scandinavian ‘‘ropy’’ fermented-milk products like Viili (Kontusaari and Forsen 1987), *Lactobacillus kefiranofaciens* isolated from kefir grains (Toba et al 1986), *Lactobacillus delbrueckii* sub sp. *bulgaricus* isolated from various yogurts (Garcia-Garibay and Marshall 1991), and *Streptococcus thermophilus*, also isolated from yogurt (Doco et al 1990). The in situ synthesis of microbial extracellular metabolites by lactic acid bacteria cultures during milk fermentation improves the viscosity and texture of the fermented product since these biopolymers act as thickeners and emulsifiers or could also be fat replacers in low-caloric products. Furthermore, it seems that the synthesis of EPS could help the producing bacteria to survive detrimental environmental conditions and it has been proposed that these polymers could promote some benefits for human health (Ruas-Madiedo et al 2010). The production of intracellularly metabolites synthesized microbial extracellular metabolites by different lactic acid bacteria varies approximately from 50 to 350 mg.liter-1 when the bacteria are grown under unoptimized culture conditions (Cerning, 1990). Bacteria develop biofilms to protect the microbial community against environmental stress. It has been established that both pathogenic and commensal bacteria, generate biofilms in human mucosa. Bacterial infections are associated with biofilm formation and have a protective role. For example, biofilm-like communities of the gastrointestinal and female urogenital tracts contain beneficial lactic acid bacteria. It has been shown that the cell wall components of probiotic bacteria, such as peptidoglycans or teichoic acids play an important role in the activation of immune cells. By contrast, the role of EPS in modulation of the immune system is still unclear.

II. Probiotic bacteria

The term probiotic was derived from the Greek language “for life.” The FAO[2] of the United Nations defines them as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (FAO/WHO, 2002), Probiotic LAB thus represents a class of live food ingredients that exert a beneficial effect on the health of the host (Gibson et al 1995). Probiotic bacteria consist of species belonging to the families Bacteroides, *Saccharomyces cerevisiae*, *Bacillus subtilis*, *Nitrobacter* spp., *Nitrosomonas* spp., *Streptococcus faecalis*, Rhodobacter spp., *Fusobacterium*, *Butyrivibrio*, *Clostridium*, *Bifidobacterium*, *Eubacterium* and *Lactobacillus* spp., *Enteroccocus* spp. and *Escherichia coli* constitute less than 1% of all intestinal microorganisms (Tournut 1993; Klein et al 1998), whereas genera Lactobacillus and Bifidobacterium are the most common probiotics used (Crittenden et al 2003). LAB are the most important unrivaled probiotic microorganisms normally associated with human digestive system. These bacteria are Gram-positive, rod-shaped, non-spore-forming, and catalase-negative. They are devoid of cytochromes and are of non-aerobic habit but are aerotolerant, fastidious, acid-tolerant, and strictly fermentative; lactic acid is a major end-product of sugar fermentation (Axelsson 1993). Some of the known LAB and bifidobacteria are used as probiotic bacteria such as *Lactobacillus plantarum*, *L. rhamnosus*, *L. delbrueckii*, *L. acidophilus*, *L. casei*, *Lactobacillus* lactis sub sp. lactis, *Bifidobacterium bifidum*, *B. breve*, *B. longum*, *B. adolescentis*, *B. animalis*, etc (Anal and Singh 2007).

Probiotics preserves the balance within such complex ecosystems such as human intestine in many ways: proliferation prohibition of pathogens by competition between bacteria and pathogens for adhesion; production suppression of virulent factors by pathogens secreting bacteriocins; or modulation of the host immune system via interaction between probiotic bacteria and intestinal epithelial cells (Sengul et al 2010; Gill et al 2001). However, the effectiveness of probiotics is strain-specific and each strain may affect the host health trough different mechanisms (Marcinkiewicz et al 2007; Christensen 2002). For instance, lactic acid bacteria (LAB) diminish symptoms of lactose intolerance, reduce serum cholesterol, prevent diarrhea, enhance immune responses and anti-carcinogenic activities, alleviate allergies (Grandy et al 2010; Savilahti et al 2008). Lactic acid bacteria can even prevent or inhibit growth of pathogenic bacteria. All these effects depend on adhesion of bacteria and their survival in specific regions of the gastrointestinal tract, competition with pathogens, presence of harmful antigens in the environment and mucosal barrier function. Useful microorganisms in the bowel are increased by “prebiotics” which are defined as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and activity of one or a limited number of bacterial species already resident in the colon, and thus improving host health" (Gibson et al 1995). Most of the current prebiotics are low molecular weight except for inulin. As long carbohydrate chains are metabolized more slowly than the short ones, and polysaccharides thus exert prebiotic effects in more distal colonic regions compared to oligosaccharides, which are more rapidly digested in the proximal colon (Rastall 2003).

III. Structure of lactic acid bacteria derived exopolysaccharides

EPS is spattered in two composed: as a capsular exopolysaccharide which is associated with the cell surface or as slime exopolysaccharide secreted as free polymers to the environment (Whitfield 1998). Chemical

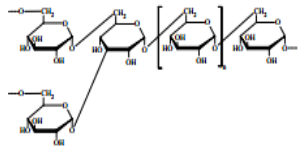
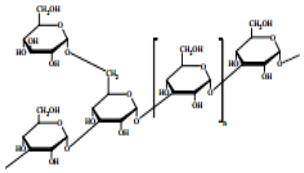
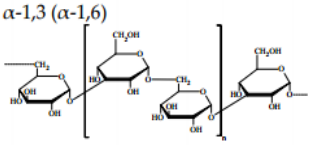
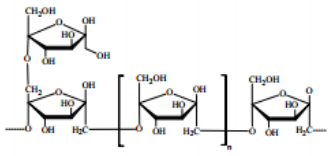
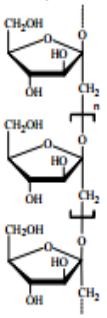
[2] Food and Agriculture Organization

structure of exopolysaccharide has been studied in details (Landersjo et al 2002; Robijn et al 1996). There are over 50 different EPSs derived from LAB described and they are mostly composed of repeated units of a certain number of diverse sugar residues or sugar derivatives (Gorska et al 2010).

3.1. Homopolysaccharides from LAB

Some LAB can produce EPS that are either spattered to the environment or attached to the cell surface forming capsules. EPS are classified into two groups: homo-EPS, consisting of a single type of monosaccharides (α-D-glucans, β-D-glucans, fructans, and others represented by polygalactan) and hetero-EPS, composed of different types of monosaccharides, mostly D-glucose, D-galactose, L-rhamnose, and their derivatives. The differences arise between the homopolysaccharides mainly because of the features of their primary structure such as the template of main chain bonds, molecular weight, and branch structure. Accordingly, homo EPS produced by LAB is presented in Table 1 (Cerning 1990).

Table 1. Homo EPS produced by LAB (Monsan et al 2001).

Homo-EPS	Main linkage (branching linkage)	Organism
α-D-Glucans *Dextran*	 α-1,6 (α-1,3)	*Le. mesenteroides NRRL B-512F* *Le.mesenteroides NRRL B-1355* *Le. mesenteroides dextranicum FPW-10* *Le. mesenteroides dextranicum* *Leuc. amelibiosum,* *Lb. curvatus*
Mutan		*Str. mutans* *Lb. Reuteri ML1* *Le. Mesenteroides NRRL B-1149* *S. sobrinus 6715*
Alternan	α-1,3 (α-1,6)  α-1,3 and α-1,6	*Leuc. mesenteroides* *S. gordonii CH1* *Le. mesenteroidesNRRL B-1355*
Fructans *Levan*	 β-2,6 (β-2,1)	*Lb. Sanfranciscensis LTH2590* *Lb. frumenti* *S. salivarius HHT* *S. salivarius 51* *S. salivarius SS2*
Inulin	 β-2,1 (β-2,6)	*S. mutansIngbritt A* *S. mutans JC-1* *S. mutans JC-2* *S. mutans BHT* *Le. Citreum CW28*

Lb., Lactobacillus; Le., Leuconostoc; S., Streptococcus

3.2. Heteropolysaccharides from LAB

HePSs[3] from LAB are produced in a greater variety with regard to monosaccharide composition, monosaccharide ratio, and molecular structure (monosaccharide components, ring forms, anomeric configurations, and stereo- and region-specific linkages) of the repeating unit, as well as the conformation and MM of the polymer (De Vuyst et al 2001). Hetero-EPS are polymerized repeating units mainly composed of D-glucose, D-galactose, and *L-rhamnose* the composition of the monosaccharide subunits and the structure of the repeating units are considered not to be species-specific, except in the case of *Lactobacillus kefiranofaciens* sub sp. *kefiranofaciens*. This species, isolated from kefir grain, a fermented dairy food from the North Caucasus region, produces large amounts of polysaccharides (Devos 1981). The main species producing heteropolysaccharides are mesophilic (e.g., *L. lactis*, *Lb. brevis*, *Lb. casei*, *Lb. paracasei*, *Lb. rhamnosus*, *Lb. sakei*) and thermophilic (e.g., *Lb. acidophilus*, *Lb. Delbrueckii* subsp. *bulgaricus*, *Lb. helveticus*, *S. macedonicus*, and *S. thermophilus*) LAB (De Vuyst et al 2001). The quantities of hetero-EPS produced by LAB vary greatly. EPS production is 50-350 mg/l for Str. *thermophilus*, 80-600 mg/l for *Lc. lactis* sub sp. *cremoris*, 60-150 mg/l for *Lb*. *delbrueckii* sub sp. bulgaricus, 50-60 mg/l for *Lb*. *casei* (Cerning 1990).

Nevertheless, the quantity of EPS produced by LAB is adequate to be exploited for in situ applications. LAB are 'generally recognized as safe' (GRAS) microorganisms, and LAB strain culture would be a useful method to produce EPS for food applications if the fermentation conditions using undefined media have been improved to maximize yields. However, a chemically defined medium containing a carbohydrate source, mineral salts, amino acids, vitamins, and nucleic acid bases is more suitable for investigating the influence of different nutrients on LAB growth and EPS biosynthesis. The total yield of EPS produced by LAB depends on the composition of the medium (carbon and nitrogen sources) and the growth conditions, i.e., temperature, pH, and incubation time. LAB could be grown in edible and safe culture media such as whey, and if fermentation conditions are optimized a high yield can be obtained (Garcia-Garibay and Marshall 1991).

IV. Production of exopolysaccharides

Exopolysaccharides are reproducible resources indicating an important class of polymeric materials of biotechnological value with a wide variety of potential applications (Kumar et al 2007). EPS are long-chain polysaccharides containing branched, repeating units of sugars or sugar derivatives such as glucose, fructose, mannose, galactose, etc., which is secreted into their surrounding environment during the bacterial growth. Moreover, microbial exopolysaccharides such as dextrans, xanthan, gellan, pullulan, yeast glucans, and bacterial alginates are potentially used in many industries as food additive including xanthan from Xanthomonas campestris and gellan from Pseudomona elode. Microorganisms are more suitable for EPS production than macroalgae and higher plants, since they display high growth rate and manipulate the provided environment for their growth resulting in enhanced microbial EPS (Parikh and Madamwar 2006). Across the wide variety of polysaccharide producing microorganisms, LAB have obtained special consideration due to the remarkable property of the polymers they synthesize and the fact that they do not carry any health risk which are GRAS. In gastrointestinal tract, EPS from LAB will remain stable in order to increase the colonization of probiotic bacteria. EPS is produced by LAB either as capsular polysaccharides (CPSs) or slime polysaccharides. The CPSs strongly bind with bacterial cell surface, while slime EPS is secreted into surrounding environment. These biopolymers are composed of one type of monosaccharide (homopolysaccharides) or repeating units of different monosaccharides (HePSs) with molecular weight ranging from 4.0×10^{4} to 6.0×10^{6} Da (Mozzi et al 2003).

4.1. Biosynthesis of exopolysaccharides from lactic acid bacteria

4.1.1. Homo EPS biosynthesis

Homo EPS are synthesized outside the cell by specific glycosyltransferase (GTF) or fructosyltransferase (FTF) enzymes (commonly named glucansucrases or fructan-sucrases). Homo-EPS producing LAB also use extracellular GTF enzymes to synthesize high-molecular mass α-glucans from sucrose. This process uses sucrose as a specific substrate, and the energy required for the process comes from sucrose hydrolysis. There is no energy requirement for EPS-production other than for enzyme biosynthesis because EPS synthesis by GTF or FTF does not involve active transport processes or the use of activated carbohydrate precursors. Therefore, large amounts of sucrose can easily be converted to EPS. Lb. sanfranciscensis produces up to 40 g/l levan and 25 g/l 1-kestose during growth in the presence of 160 g/l sucrose (Korakli et al 2003) [41].

4.1.2. Hetero EPS biosynthesis

Hetero-exopolysaccharides are not synthesized by extracellular enzymes, but are instead synthesized by a complex sequence of interactions involving intracellular enzymes. Exopolysaccharides are made by polymerization of repeating units, and these repeating units are made by a series of sugar nucleotides addition at

[3] heteropolysaccharides

the cytoplasmic membrane. The sugars are starting materials for the synthesis sequence. LAB strains can utilize different monosaccharides and disaccharides as energy sources, by some well-studied sugar uptake systems including primary transport systems, direct coupling of sugar translocation to ATP hydrolysis via a transport-specific ATPase; secondary sugar carrier systems, coupling of sugar transport to the transport of ions or other solutes, both as symport and antiport transport systems; and group translocation systems, coupling of sugar transport to phosphorylation by the phosphoenolpyruvate (PEP)-dependent phosphotransferase system (De Vuyst et al 2001). After the synthesizing a hetero-exopolysaccharides repeating unit, the unit is sent out through the cell membrane and becomes polymerized into the ultimate hetero-exopolysaccharides. Therefore, multiple enzymes and proteins are involved in the biosynthesis and secretion of hetero type exopolysaccharides, and the enzymes and proteins involved in these processes may not be inimitable to hetero-EPS anabolism. Sugars derived from the cell are converted into sugar nucleotides. Iintracellular monosaccharides are converted to sugar nucleotide substrates for polymerization reactions, including UDP (uridine diphosphate), dNTP (thymidine diphosphate), and GDP (guanosine diphosphate). Such polymerization reactions are catalyzed by glycosyl pyrophosphorylases.

Glucose-1P (Gal-1P) + UTP (uridine threephosphate) →UDP-Glu (UDP-Gal) + pyrophosphate

UDP-glucose is then converted to UDP-galactose by epimerases such as UDP-glucose-4-epimerase. This reaction is reversible.

UDP-glucose ↔UDP-galactose

Glycosidic linkages are formed on membranes in the cytoplasm. A sugar section is transferred to C55-polyprenyl phosphate, a carrier lipid and component of the membrane, by priming glycosyl transferases. This transfer intends to add a repeating unit to the hetero-EPS molecule. Disruption of the priming glycosyl transferase gene generates non EPS-producing mutants (Dabour et al 2006). Thus, structure priming glycosyl transferases are thought to be crucial for EPS biosynthesis. The addition of the repeating unit is completed by the operation of glycosyl transferase on the sugar residue attached to C55-polyprenyl phosphate. Therefore, the type and number of glycosyl transferases accessible determine the limited area of repeating units in hetero-exopolysaccharides. C55-polyprenyl phosphate is also involved in bacterial cell wall biosynthesis, and accordingly, cell wall biosynthesis and exopolysaccharides synthesis corrival for this substrate. The synthesized repeating unit is derived through the bacterial membrane, and is polymerized to become a hetero-exopolysaccharides (Fig. 1).

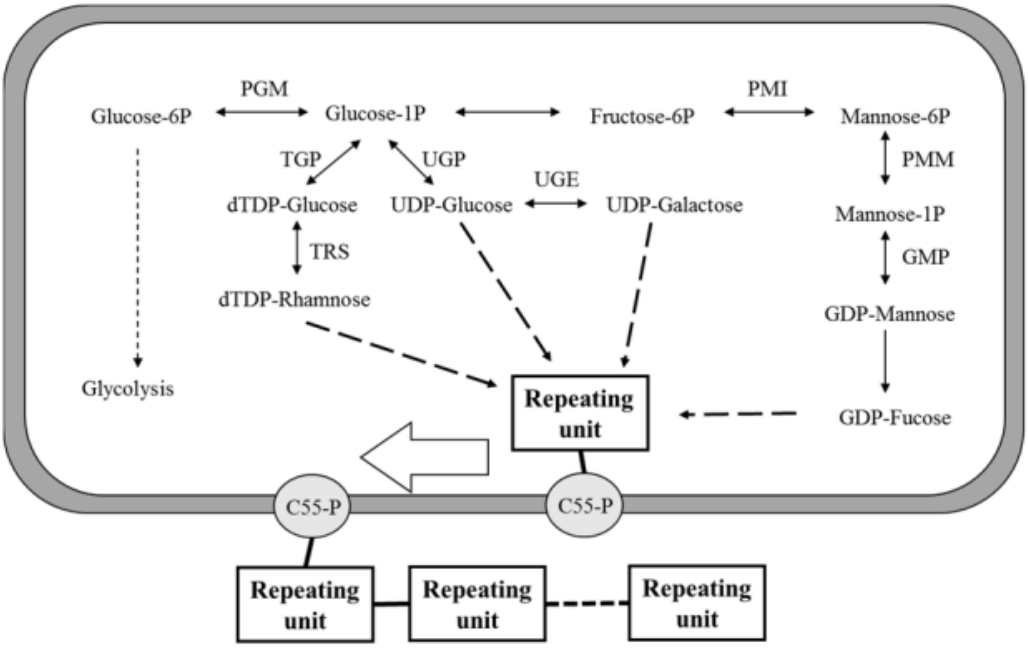


Figure 1. Outline of biosynthesis of hetero-exopolysaccharides.

PGM: α-phosphoglucomutase, UGP: UDP-glucose pyrophospholyrase, UGE: UDP-galactose 4-epimerase, TGP: dTDP-glucose pyrophospholyrase, TRS: dTDP-rhamnose synthetic enzyme system, PMI: phosphomannoisomerase

PMM: phosphomannomutase, GMP: GDP-mannose pyrophospholyrase

4.2. Genes encoding EPS production by LAB

Genetic information of both gram negative and gram positive bacteria illustrates that biosynthetic pathway of the heteropolysaccharides is controlled by several housekeeping genes and a cluster of EPS-related genes, which contain four functional regions involved in (i) regulation of EPS production, (ii) chain length termination, (iii) biosynthesis of EPS repeating unit, and (iv) polymerization and export of repeating units (Deng et al 2013). Production of microbial exopolysaccharides by mesophilic bacteria such as L. lactis subsp. cremoris and L. lactis subsp. lactis are usually associated with plasmids, and genetic inconstancy can be described by the loss of that plasmid. On the other hand, the genes encoding production of EPS by thermophilic lactic acid bacterias (*L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*) are located in chromosome and the genetic instability can be explained with the mobile elements or genomic instability such as elimination and rearrangements (Stingele et al 1999). In general, genes encoding EPS biosynthesis in both mesophilic and thermophilic lactic acid bacteria strains are organized in four functional regions: a central region containing genes for glyccosyltransferase which is essential for the accumulation of EPS repeating unit, two regions flanking the central region which show similarity to enzymes participating in polymerization and export, and a regulatory region located in the 59th end of EPS gene cluster (Degeest and De Vuyst 2000). Also, the chimeric structure of locus may involve in both horizontal transfer and genome exchanges within L. lactis and S. thermophilus (Degeest et al 2001). Similarly, results of Liu et al (2009) predicted horizontal gene transfer events between two strains which include the transfer of EPS biosynthesis genes from S. thermophilus to L. bulgaricus and gene cluster cbs-cblB (cg lB)-cys-E for the metabolisms of sulfur containing amino acids, transferred from L. bulgaricusto S. thermophilus. (Nga 2006), also, explained horizontal gene transfer system in S. thermophilus for EPS synthesis. A gen 32.5-kb variable locus of S. thermophilus CNRZ368 chromosome, known as locus, contains 25 open reading frames (ORFs) and 7 mobile elements. The 17 open reading frames (ORFs) are related to polysaccharide synthesis in many bacterial strains. The end 13.6-kb regions encoded seven mobile elements and EPS open reading frames which are relatively similar to EPS L of L. lactis NIZOB40. These results suggested that the 13.6-kb region may be procured from L. Lactis by horizontal transfer and that genetic exchanges within the S. Thermophilus strains would have accounted for the variation in the different EPS open reading frames. Moreover, the genetic locus of EPS in S. Thermophilus Sfi6 revealed a 15.25-kb region encoding 15 ORFs.

V. LAB derived exopolysaccharides and the immune-system

The health benefit of LAB have been attributed to the production of EPS (Makino et al 2006). LAB EPSs have been assumed to have immune stimulatory activity (Badel et al 2011; Laws et al 2001), antitumor effects (Vuong et al 2004; Looijesteijn et al 2001) or blood pressure and cholesterol lowering activity (Watnick and Kolter 2000; Byrd et al 2010). Exopolysaccharides decrease symptoms of lactose intolerance and prevents diarrhea (Costerton et al 1978). There have been reports that sugar polymers have antimicrobial attributes and help cure lesions (Herasimenka et al 2007; Kumon 2000). It has been also shown that some EPSs induce cytokine production, a behavior like lymphocytes B mitogens or changing functions of splenocytes (Sengul et al 2006; Hoiby et al 2010) also, EPS can reduce the symptoms of collagen-induced arthritis or diminish arteriosclerosis in mice. Orally administrated EPS-producing by LAB attenuate severity of colitis and may be a promising agent in remedy of inflame matory bowel disease (Burmolle et al 2010; Vu et al 2009). It seems that such wide diversity of EPS effects on the immune system results not only from strain specificity, but also from micro environmental impact on the EPS metabolism of probiotic bacteria. Nevertheless, it is still not clear whether EPS can be the ligand for pattern recognition receptors and also how the immune system can differentiate pathogenic bacteria from commensal flora. It is possible that EPS plays the role of signaling molecule in the mucosal immune system.

VI. Biofilm formation–the role of exopolysaccharides

There is an increasing interest among researchers concerning EPS, but the physiological role of these molecules is still not clear (Ruas-Madiedo et al 2006; Vinderola et al 2006; Hosono et al 1997). Most of this researches are related to biofilm formation and its role in bacterial ecology (Furukawa et al 2000; Kitazawa et al 1998). The term 'biofilm' was used for the first time in 1978 by Costerton et al (Grandy 2010). Studies on the role of EPS in biofilm formation are generally focused on pathogenic bacteria which are mostly Gram-negative species (Maeda et al 2004; Nakajima et al 1992). Less is known about microbial exopolysaccharides in gram-positive species. Exopolysaccharides fills the intracellular space between bacteria and together with proteins, nucleic acids and lipids, composes the structure of the biofilm matrix.

Microbial exopolysaccharides in biofilm protects bacterial cells from desiccation, phage attack, antimicrobial compounds, osmotic stress and predatory attack from protozoa (Furukawa et al 2000; Rodrigues et al 2005; Wu et al 2010). It helps bacteria to survive in deleterious and extreme conditions such as too low or too high temperatures or pH. Capsular polysaccharides (CPS) can promote the adherence of bacteria to biological

surfaces, thereby facilitating the colonization of various ecological niches (Ruas-Madiedo et al 2003; Landersjo et al 2002). EPS also can enable probiotics to survive in gastric acid and bile salts (Chabot et al, 2001). Biofilm produced by pathogenic bacteria makes them less susceptible to antibiotics and attacks by innate host defense. It plays an important role in many chronic bacterial infections (Bleau et al 2010; Sengul et al 2010). Biofilm formation and EPS production is under the control of regulatory pathway of QS. It has been suggested that QS allows bacteria to communicate and regulate the expression of genes which are required for synthesis of EPS in response to changes in bacteria density (Lebeer et al 2007).

VII. Conclusions

Usage of probiotic bacteria in various appliances like food industry, shrimp farms, and health industry has already created an impact in this area of research. Safety and the functional and technological characteristics of the probiotics are well appraised in this review. Antimicrobial substances like bacteriocins and polymeric substances like EPS, biosurfactants are widely used in seafood industry. Furthermore, LAB strains have also been reported for production of antioxidants which are able to scavenge the free radicals such as superoxide anions and hydroxyl radicals. Application of bacteriocins along with other non-thermal techniques paved the way for the emergence of new hurdle technologies to improve the shelf life of food. This review elaborates the ecology, biosynthesis, genetics, target sites and functionality of the bacteriocins alone or in combination with other hurdle technologies.

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