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Review

Exopolysaccharides from probiotic bacteria and their health potential

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ABSTRACT

Exopolysaccharides (EPS) are extracellular macromolecules excreted as tightly bound capsule or loosely attached slime layer in microorganisms. They play most prominent role against desiccation, phagocytosis, cell recognition, phage attack, antibiotics or toxic compounds and osmotic stress. In the last few decades, natural polymers have gained much attention among scientific communities owing to their therapeutic potential. In particular the EPS retrieved from probiotic bacteria with varied carbohydrate compositions possess a plenty of beneficial properties. Different probiotic microbes have unique behavior in expressing their capability to display significant health promoting characteristics in the form of polysaccharides. In this new era of alternative medicines, these polysaccharides are considered as substitutes for synthetic drugs. The EPS finds applications in various fields like textiles, cosmetics, bioremediation, food and therapeutics. The present review is focused on sources, chemical composition, biosynthetic pathways of EPS and their biological potential. More attention has been given to the scientific investigations on antimicrobial, antitumor, anti-biofilm, antiviral, anti-inflammatory and immunomodulatory activities.

Contents

1. Introduction

Microbial polysaccharides are extracellular polymeric substances either soluble or insoluble that are synthesized by bacteria, yeast, algae, fungi etc. are considered to be value added substances and exploited for different purposes [1,2]. EPS are metabolic by-products of microorganisms [3]. They are high molecular weight compounds composed of carbohydrates (sugar residues), substituted with proteins, DNA, phospholipids and non-carbohydrate substituents such as acetate, glycerol, pyruvate, sulfate, carboxylate, succinate and phosphates [4–6]. According to the definitions of WHO and Food and Agriculture Organization of

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the United Nations (FAO), 'Probiotics are live micro-organisms, which when consumed in adequate amounts confer ahealth benefit on the host' [7,8]. Among microbial polysaccharides, EPS produced by probiotic lactic acid bacteria (LAB) have been chosen for various applications because they are generally regarded as safe (GRAS) and utilized for biological activities in-vitro as well as in-vivo conditions [9,10].

Microbial polysaccharides have interesting and attractive characteristics and are exploited in food, cosmetic and pharmaceutical industries as bio-flocculants, bio-absorbents, and drug delivery agents [11,12]. Since polysaccharides are more advantageous compared to synthetic polymers, they are used in green synthesis of silver nanoparticles [13]. The shape and size of a nanoparticle is greatly influenced by association of metal ions with hydroxyl groups of EPS. EPS are biodegradable, non-toxic, bio-compatible and abundantly present in natural sources [14]. The most prominent EPS producing LAB are *Lactobacillus*, *Lactococcus*, *Bifidobacterium*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *Enterococcus* and *Weissella* sp. [15,16]. Among several microorganisms consumed by the human beings, probiotic bacteria have the ability to survive in the presence of bile, low pH, gastric juices and colonize in the epithelial layer of the gastrointestinal tract [17,18]. Use of antibiotics reduces the antagonistic activity of normal microbial flora against the pathogenic microorganisms [19,20]. Probiotics have the capacity to act against antibiotics which creates immune suppression if ingested regularly [21]. They boost the immune system to fight against the diseases initiated by the pathogenic microorganisms [18,22]. Therefore, probiotic EPS are supplemented in the treatment of human disorders such as inflammatory bowel diseases, autoimmune diseases, colon cancer, gastric ulcers, cardiovascular diseases and obesity [23,24]. EPS are extensively studied in the field of pharmacological applications as anticoagulants, anti-allergic, antithrombotic, immunomodulatory, blood cholesterol lowering, nutraceuticals etc. [25–27]. EPS has strong water binding ability, water retention capacity, immense swelling and gelation potential [2]. EPS like xanthan, sphingan, alginate, cellulose promotes biofilm formation on the bacterial cell surfaces as a protecting barrier [28].

The EPS produced by *Lactobacillus acidophilus*, *Lactobacillus gasseri*, *Lactobacillus plantarum* and *Lactobacillus rhamnosus* isolated from various sources have been reported to possess antitumor as well as antioxidant activities [29,30]. Recent studies on EPS produced by *Lactobacillus plantarum* C70 and *L. plantarum* RJF4 of different origin revealed its antidiabetic activity by inhibiting α-glucosidase and α-amylase enzymes under in-vitro condition [1,31]. The EPS extracted from *Lactobacillus* sp. Ca6 had shown to possess in-vivo dermal wound healing property in male wistar rats [32]. The EPS of *Lactobacillus* strains have the ability to stimulate innate immune response and EPS from *Lactobacillus plantarum* JLK0142, *Leuconostoc citreum*, *Lactobacillus johnsonii* 142 and *Bifidobacterium* sp. contain significant immunomodulatory activity [10,33,34]. It was observed that EPS produced by *Bifidobacterium* sp. reduce cholesterol level in diet induced obese mice [35]. The present review is focused on EPS produced by probiotic bacteria and their sources, structure and classification of EPS, physical properties, biosynthetic pathways and various biological potential of EPS reported in recent years. The current review holds upper edge over the most recent review articles [36,37] as it discusses various captivating aspects of recent researches conducted on novel probiotic EPS obtained from fermented dairy and non-dairy food products. Diverse health promoting characteristics of EPS were discussed in depth covering the up to date investigations on this subject. The most fascinating feature is the inclusion of the most recent exploration on in-vitro anti-diabetic activity of EPS since the research on this attribute is much debated and delayed due to its polysaccharide nature. The impact of varied physical properties of EPS on its biological activity is explored in detail.

2. EPS secreting probiotic bacteria and their sources

Probiotic strains can be obtained from dairy based and non-dairy based sources as depicted in Fig. 1. Dairy based sources include milk and milk products whereas non-dairy based sources include cereals, fruits and vegetables. Although dairy based sources are studied for their potential health benefits, they are unsuitable for people who suffer from lactose intolerance, high cholesterol, and are allergic milk proteins [38]. In non-dairy based sources, the nutritional content of fruits, vegetables and cereals such as minerals, vitamins, dietary fibers, antioxidants and other therapeutic properties helps in the survival of enormous probiotic strains [39]. Any food based sources that contain fermentable sugars have the potential to support the growth of probiotics. In recent years to identify novel probiotic LAB, fermented foods were also employed since they possess many advantages like extended shelf life, enhanced flavor and texture of the final product [27]. Probiotic bacteria can also be isolated from animal origin (meat and meat products). Fermented food products contain probiotic bacteria which are employed as starter cultures in their production. The EPS retrieved from the probiotics isolated from fermented foods also exerts considerable benefits along with good probiotic properties and striking functional characteristics [40]. A detailed list of EPS producing bacterial sources with their biological properties is displayed in Table 1.

Fig. 1. Different sources of probiotic bacteria.

3. Structure and classification of EPS

Probiotic bacteria produce EPS extracellularly above the cell envelope as capsules or slime layer (Glycocalyx) [41]. Polysaccharides accumulated inside the cells are known as intracellular polysaccharides. Capsular polysaccharides are closely attached to the bacterial cell surface which offers protection to bacterial cells against phagocytosis, bacteriophage attack, desiccation and osmotic stress [15]. On the other hand, EPS produced as slime layers are less closely attached to the cells which can be removed easily. EPS are synthesized throughout the logarithmic or in late logarithmic or in stationary phase. However, maximum production occurs only during late logarithmic phase than in stationary phase [4]. The quantity of EPS produced varies with strains, medium composition, and culture conditions such as pH, temperature and carbon/nitrogen ratios. The EPS secreted by probiotics differ by monosaccharide composition, charge, linkage, presence of repeated side-chains and substitutions [16,42]. In general, EPS are classified into homopolysaccharides (HoPs) and heteropolysaccharides (HePs). HoPs are either branched or unbranched and composed of either glucose or fructose and are categorized into α-D-glucans (Ex. Dextran, Mutan, Alternan, and Reuteran), β-D-glucans, fructans (Ex. Levan and Inulin)

Table 1
EPS producing probiotic bacteria with their source/s and biological properties.

S. no.	Probiotic bacteria	Source/s	Biological properties	References
1.	*Enterococcus faecium* WEFA23	Healthy infant's feces	Antioxidant activity and strong inhibition against the adhesion of *Listeriamonocytogenes* CMCC54007 on HT-29 cells	[131]
2.	*Lactobacillus* sp. Ca6	Gastrointestinal tract (GIT) of indigenous poultry	Antimicrobial activity and sensitive to several antibiotics The wound healing activity of EPS-Ca6 was assessed using excision wound model in rats	[132] [32]
3.	*Lactobacillus gasseri* FR4	Native chicken	In-vitro antioxidant, antibacterial against food borne pathogens and anti-biofilm activity of EPS	[97]
4.	*Lactobacillus plantarum* KX041	Chinese Paocai	Possessed the immune activity, DPPH/ABTS radicals scavenging activities and DNA damage productive effect	[133]
5.	*Pediococcus pentosaceus* M41	Marine source (low water activity dried fish)	Antimicrobial activity, antioxidant activity, antitumor activity, α-amylase and α-glucosidase inhibitions	[107]
6.	*Lactobacillus* strains	Pulp of the durian (*Durio zibethinus*) fruit	Antimicrobial, antioxidant and reduces cholesterol	[134]
7.	*Bacillus tequilensis* FR9	GIT of free range chicken *Gallus gallus domesticus*	Higher antioxidant activity	[66]
8.	*Lactobacillus plantarum* C70	Camel milk	Antioxidant and cytotoxic activities against colon cancer and breast cancer lines	[31]
9.	*Enterococcus faecium* (BDU7)	"Ngari" (traditional fermented fish of Manipur)	Strong DPPH and superoxide radical scavenging ability (in-vitro)	[135]
10.	*Lactobacillus plantarum* YO175 and OF101	Traditional fermented cereal beverage	Antioxidant activity	[30]
11.	*Lactobacillus acidophilus* (LA1)	Infant feces	Antioxidant activity by suppression of malondialdehyde and nitric oxide serum levels In-vivo antitumor against Ehrlich ascites carcinoma (EAC) cells	[100]
12.	*Lactobacillus gasseri*	Human vagina	L-EPS of *L. gasseri* strains inhibit proliferation and induce apoptosis in HeLa cells in strain dependent manner	[29]
13.	*Lactobacillusacidophilus* 606	Human feces	Cell bound EPS inhibited the proliferation of HT-29 colon cancer cells by directly affecting cell morphology and not the cell cycle	[136]
14.	*Lactobacillus plantarum* MTCC9510	Curd	Antitumor activity and immunomodulatory activities	[105]
15.	*Lactobacillus plantarum* WLPL04	Healthy female breast milk	Inhibits the adhesion of *E. coli* O157:H7 to HT-29 cells, antitumor activity and anti-tumor activity against pathogens	[137]
16.	*Lactobacillus paracasei* M7	Human breast milk	Antioxidant, anti-biofilm and hypocholesterolemic activity	[115]
17.	*Enterococcus faecium* MC13	Gut of fish	In-vitro anti-biofilm activity against *Listeria monocytogenes*	[124]
18.	*Lactobacillusplantarum* JLK0142	Fermented dairy tofu	Improvement of the intestinal immunoglobulin A(IgA) content and the serum levels of the cytokines, IL-2 and TNF-α	[73]
19.	*Leuconostoc citreum* L3C1E7	Pico cheese	Suppresses allergen-specific IgE synthesis and may alleviate Th2-mediated allergic symptoms	[138]
20.	*Lactobacillus plantarum* HY	Home-made Sichuan pickle	Antioxidant activity and α-amylase inhibitory activity	[129]
21.	*Lactobacillus plantarum* LRCC5310	Kimchi	In-vitro anti-viral activity against Rota virus induced diarrhea and regulates inflammatory response	[139]
22.	*L. plantarum* 86, *Weisellaconfusa* AI10, *Pediococcusparvulus* AI1, *Weisellacibaria* 142	Traditional Indian fermented foods including dhokla batter, idli batter, dahi, vegetables such as carrot, cabbage, turmeric, cucumber and tomato	Antibacterial activity *E. coli*	[40]
23.	*Lactobacillusplantarum* 70,810	Chinese Paocai	c-EPS significantly inhibited the proliferation of HepG-2, BGC-823, especially HT-29 tumor cells	[106]
24.	*Lactobacillus plantarum* H31	Pickled cabbage	Reduce α-amylase activity and up-regulation of GLUT-4, AKT-2 and AMPK gene expression in insulin-resistant HepG2 cells	[118]
25.	*Lactobacillus helveticus* LZ-R-5	Tibetan kefir	In-vitro immunomodulatory activity	[140]
26.	*Lactobacillus plantarum*	Tunisian traditional fermented food	EPS induced gene expression in immunity and antioxidant responses in fish	[141]
27.	*Lactobacillus bulgaricus* subsp. *delbrueckii*	Traditional Bulgarian yoghurt	EPS activated NK cells, with the contribution of INF-γ, IL-12, IL-18 cytokines via MyD88-driven signaling in mice	[142]
28.	*Leuconostoc mesenteroides*	Traditional fermented sourdough samples	Levan up-regulates anti-inflammatory cytokine IL-4	[143]

and polygalactans [43]. HePs are made up of D-glucose, D-galactose and rare sugars such as L-rhamnose, mannose, arabinose and fucose. In some cases, *N*-acetylglucosamine, *N*-acetylgalactosamine or glucuronic acid are also present. They are designated as gellan, xanthan and kefiran [44]. The molecular weight of HoPs is greater than 10^6 Da [45] whereas for HePs it ranges from 10^4 and 6.0×10^6 Da [25]. The schematic classification of EPS is displayed in Fig. 2. Tables 2 and 3 represent the complete list of HoPs and HePs with their probiotic source, composition and potential industrial application/s.

4. Physical properties of EPS

EPS produced by LAB enhances the texture, mouth-feel and stability of food products which greatly contributes to the significant progress in production of novel food products. Microbial EPS have enormous functional effects in food processing like viscosifiers, bio-thickeners, emulsifiers, stabilizers, etc. which depends on temperature, pH and ionic strength [46,47]. Thermal stability of EPS is a key feature in dairy industries, where it is used for its emulsifying and flavor retaining activities. The purified EPS samples recovered from *Leuconostoc citreum*-BMS (bovine meat sausages), *Leuconostoc mesenteroides*-TMS (Turkey meat sausages), *Pediococcus pentosaceus*-DPS (date palm sap) and *Leuconostoc pseudomesenteroides*-CM (cow milk) showed high thermostability with the melting points higher than 224 °C which could be exploited in thermal processed foods [47]. The EPS produced by *Streptococcus thermophilus* CRL1190 with potential gastro-protective role, could be used as a healthy food grade additive in the dairy industry for various reasons such as good water and oil holding capacity, high aqueous solubility, thermal stability, antioxidant, emulsifying and flocculating activities [48]. With its high water solubility, emulsifying activity and thermal stability, the EPS retrieved from *Leuconostoc citreum* N21 could be employed in food processing as a food additive [49]. A novel EPS produced by probiotic strain *Enterococcus faecium* F1 is being explored for food and pharmaceutical applications in industries due to

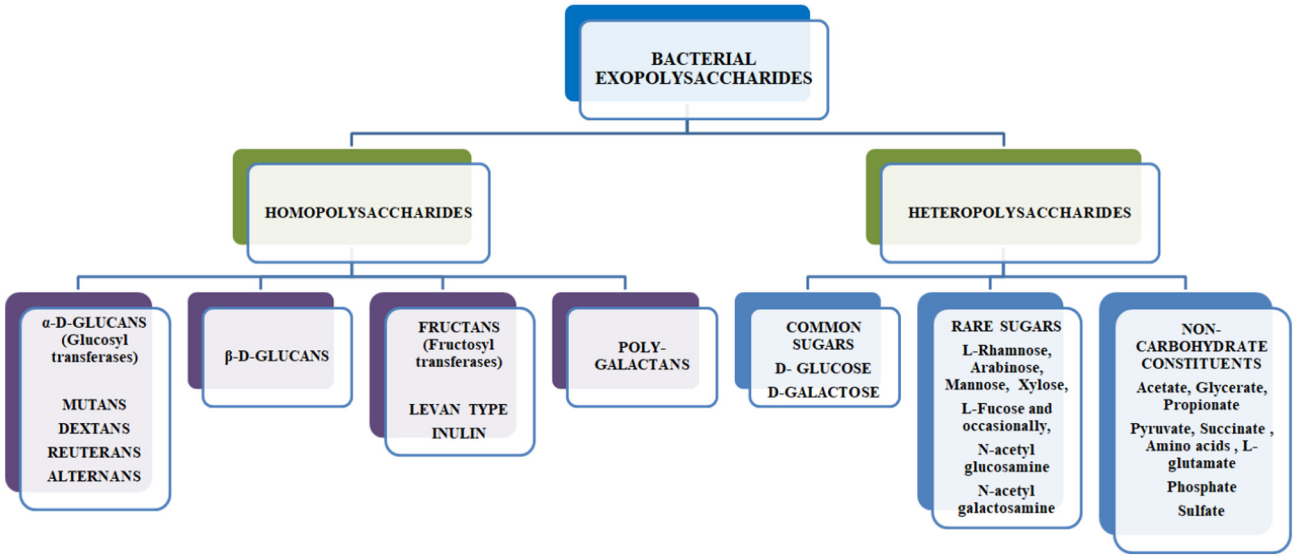

Fig. 2. Classification of bacterial exopolysaccharides.

its potential emulsifying, thermal stability and other physicochemical characteristics [50]. In-vitro evaluation of flocculation, emulsification, solubility and other functional properties provides promising results on the EPS produced by *Lactobacillus rhamnosus* which could be used for commercial purposes [51]. *Leuconostoc pseudomesenteroides* XG5 isolated from home-made wine produced a dextran which has high viscosity, making it ideal as thickening or viscosifying agent. Also it has high water solubility and water holding capacity which provides convincing characteristics to be used as hydrocolloids and stabilizer [52].

5. Biosynthetic pathways of EPS

Biosynthesis of EPS in probiotic bacteria occur in two ways, intra and extracellular as represented in Fig. 3. HoPs are generally produced by extracellular biosynthetic pathway whereas HePs are synthesized by intracellular or extracellular pathways. Extracellular biosynthesis of HoPs is mediated by specific extracellular glycosyl transferases and fructosyl transferases during the polymerization process [53,54]. The sugar residues are cleaved into monomeric units outside the cell and assembled extracellularly into a polymer with the help of glycosyl transferases. Sucrose is utilized as a specific substrate which gets cleaved into glucose and fructose and polymerized into glucan and fructan by glucan sucrase and fructan sucrase [55] (Fig. 3). Glucan sucrases are further classified into dextran sucrases (dextran), mutan sucrases (mutan), reuteran sucrases (reuteran) and alternan sucrases (alternan) whereas fructan sucrases are divided into levan sucrases (levan) and inulin sucrases (inulin) respectively [27]. These polymerized HoPs are directly released to the extracellular environment [55].

In intracellular biosynthetic pathway, the sugar residues are transported into cell, converted to different monomeric units, polymerized partially and attached to a membrane bound isoprenoid lipid carrier [56]. At this stage necessary modification of the polymer occurs and then the polymer is transported outside and assembled as polysaccharides. HePs synthesis by intracellular pathway is relatively complex which involves various enzymes, carriers and transporter proteins encoded by genes of chromosomal or plasmid origin [57]. The four groups of enzymes involved in the biosynthesis of bacterial EPS are listed in the Table 4. The HePs synthesis relies on Wzx/Wzy pathway wherein except polymerization, all other processes takes place inside the cytoplasm (Fig. 3). Sugar transportation, sugar nucleotide synthesis,

Table 2
Homopolysaccharides from probiotic bacteria and their applications.

S. no	EPS	Organism	Monomer	Chemical structure	Applications
1.	Dextran	*Leuconostoc mesenteroides*, *Lactobacillus reuteri*, *Lactobacilluscasei*, *Lactobacillussakei*, *Lactobacillusfermentum*, *L. parabuchneri*	Glucose	Linked by α-1,6 glycosidic bonds; some 1,2-, 1,3- or 1,4-bonds are also present in some dextran	As adjuvant, emulsifier, carrier and stabilizer in food, pharmaceutical industries, plasma substitute, matrix of chromatography column, anticoagulant, paper industry, metal plating processing, for enhanced oil recovery and biomaterials
2.	Mutan	*Streptococcus mutans S. sobrinus*, *Lactobacillus* sp., *Leuconostoc* sp.	Glucose	α-1,3-D-Glucan	–
3.	Alternan	*Leuconostoc mesenteroides*	Glucose	α-1,3 and α-1,6 glycosidic bonds	Prebiotics, sweeter in confectionaries, low viscosity, bulking agent and extender in foods
4.	Reuteran	*Lactobacillus reuteri* ATCC 55730	Glucose	α-1,4 glycosidic bonds	Used in bakery
5.	Curdlan	*Alcaligenes faecalis*	Glucose	β-1,3-D-Glucan and α-1,3-β-D-glucans	Starter culture
6.	Levan	*Bacillussubtilis*, *Streptococcus salivarius*, *Streptococcusmutans*	Fructose	β-2,6 glycosidic bonds	Prebiotics, antitumor property, hypocholesterolemic agent, adhesive, bio-thickener in food industry
7.	Inulin-type	*Streptococcusmutans*, *Lactobacillusreuteri*	Fructose	β-2,1 glycosidic bonds	Prebiotics nourishes gut mucosal cells and inhibits pathogens, for targeted drug delivery against colon cancer and substitute of fat in food products
8.	Poly-galactans	*Lactococcus lactis* subsp. *lactis* H414 *Lactobacillus delbrueckii* subsp. *bulgaricus* (CRL 406 and 142)	Galactose	Pentameric repeating unit of galactose	–

Table 3
Heteropolysaccharides from probiotic bacteria and their applications.

S. no	Organism	Sugar composition	Molecular weight	Molecular ratio	Applications	References
1.	*Lactobacillus plantarum* JLK0142	Glucose and galactose	1.34×10^5 Da	2.3:1.06	Useful as food adjunct or clinical immunomodulatory agent for use in functional foods or in medicines	[73]
2.	*Lactobacillus johnsonii* 142	D-Glucose and D-galactose	1.0×10^5 Da	1:4	–	[34]
3.	*Enterococcus faecium* MC13	Galactose and glucose	2.0×10^5 Da	–	Strong emulsifying and flocculating agent	[124]
4.	*Streptococcus thermophilus* CC30	Glucose and galactose	58 to 180 kDa	1:1	Useful as emulsifier in food industry	[144]
5.	*Lactobacillus helveticus* MB2-1	Glucose, mannose, galactose, rhamnose and arabinose	1.83×10^5 Da	3.12:1.01:1.00:0.18:0.16	–	[145]
6.	*Lactobacillus plantarum* C70	Arabinose, mannose, glucose and galactose	3.8×10^5 Da	–	Improving the texture and rheological properties of various food systems	[31]
7.	*Lactobacillus plantarum* WLPL04	Xylose, glucose and galactose	6.61×10^4 Da	3.4:1.8:1	Development of functional food	[137]
8.	*Bacillus tequilensis* FR9	Glucose, arabinose, galactose, mannose and xylose	–	–	Stabilizer and thickener in food and dairy industries	[66]
9.	*Enterococcus faecium* WEFA23	Mannose, glucose and galactose	2.50×10^4 Da	1.38:1.00:1.42	Development of therapeutics and functional food	[131]
10.	*Lactobacillus gasseri* FR4	Glucose, mannose, galactose, rhamnose, and a small fraction fucose	1.86×10^5 Da	–	Used in food industries as an antioxidant agent, viscosifying agent and antimicrobial agent.	[97]
11.	*Lactobacillus plantarum* JLAU103	Arabinose, rhamnose, fucose, xylose, mannose, fructose, galactose and glucose	12.4 kDa	4.05:6.04:6.29:5.22:1.47:5.21:2.24:1.83	Used as a natural antioxidant or functional additive in food industry	[146]
12.	*Lactobacillus casei* WXD030	Glucose, glucosamine, and mannose	37.37 kDa	1.4:1.1:1	Used as adjuvant and to develop subunit vaccines	[75]

repeating unit synthesis, and polymerization of the repeating units formed in the cytoplasm are the four main steps of the synthesis of HePs. The entry of substrates depends on the type of sugars and occurs via active or passive transport or phosphoenol pyruvate-phosphotransferase transport system [4,9]. The glucose molecule which enters the cytoplasm is phosphorylated by hexokinase (group 1 enzyme) into glucose-6-phosphate which further gets converted to glucose-1-phosphate by the action of phosphoglucomutase. The

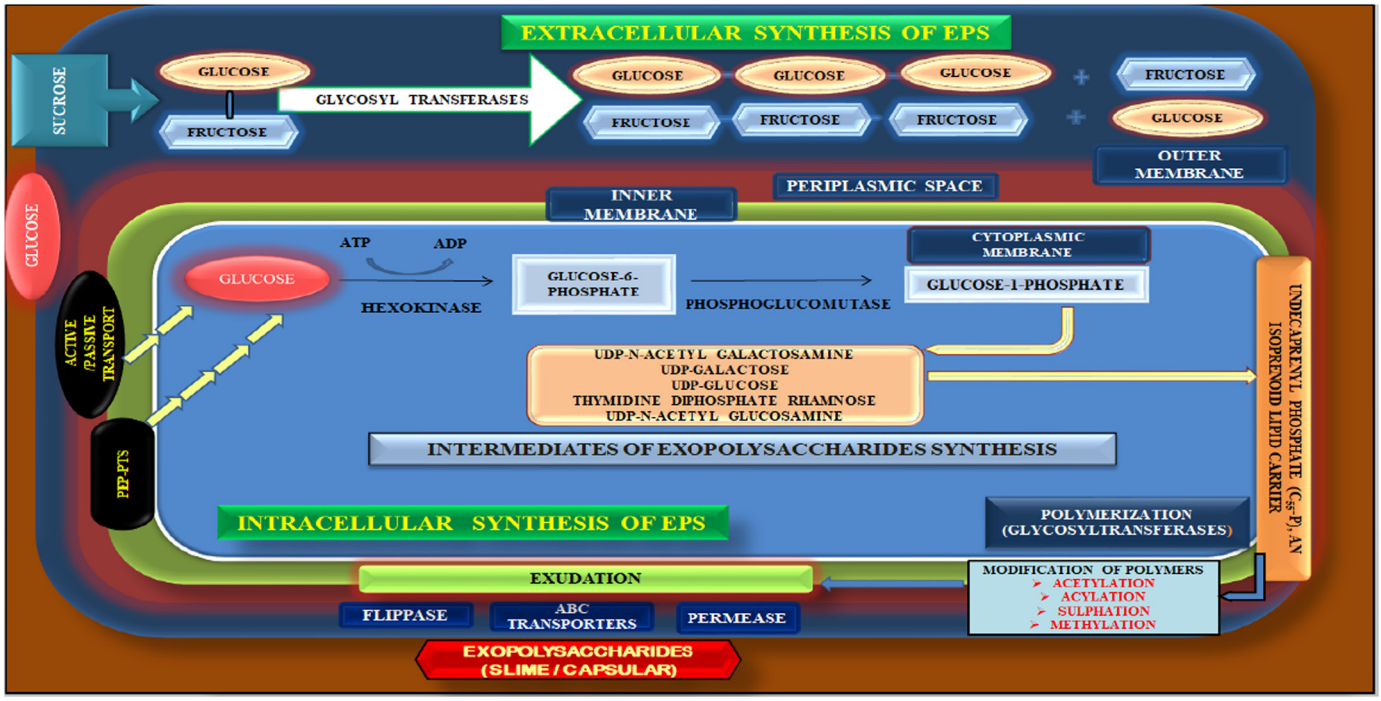

Fig. 3. Biosynthetic pathway of EPS.

enzyme belong to group 2, uridine-5′-di-phosphate (UDP)-glucose pyrophosphorylase catalyzes conversion of glucose-1-phoshphate to UDP-glucose (key molecule in EPS synthesis) [58]. Direct precursors for bacterial EPS biosynthesis are formed intracellularly from intermediates of the central carbon metabolism. The precursors and donor monomers for the biosynthesis of most of the repeating units are sugar nucleotides such as nucleoside di-phosphate sugars (NDP-glucose), nucleoside di-phosphate sugar acids (GDP-mannuronic acid), and nucleoside diphosphate sugar derivatives (UDP-glucose, UDP-*N*-acetyl glucosamine, UPD-galactose and deoxythymidine di-phosphate (dTDP)-rhamnose) [59,60]. In the Wzx/Wzy dependent pathway, these sugar nucleotides considered as individual repeating units are attached to undecaprenyl di-phosphate anchor (C55 lipid carrier) located at the inner membrane, by glycosyl transferases (group 3 enzyme) and translocate across the cytoplasmic membrane by a Wzx protein (flippase) [26,61,62]. The carrier lipid is identified as isoprenoid alcohol, and its terminal alcohol group is attached to a monosaccharide residue through a pyrophosphate bridge. At this stage, polysaccharides may be modified by different enzymatic activities such as acetylation, sulphation and methylation. Thus, these modified polymers are released at the external surface of the bacterial cell either as capsular polysaccharides or as slime layer (EPS) by the action of group 4 hydrophobic enzymes like flippase, permease or ABC transporters [6,63].

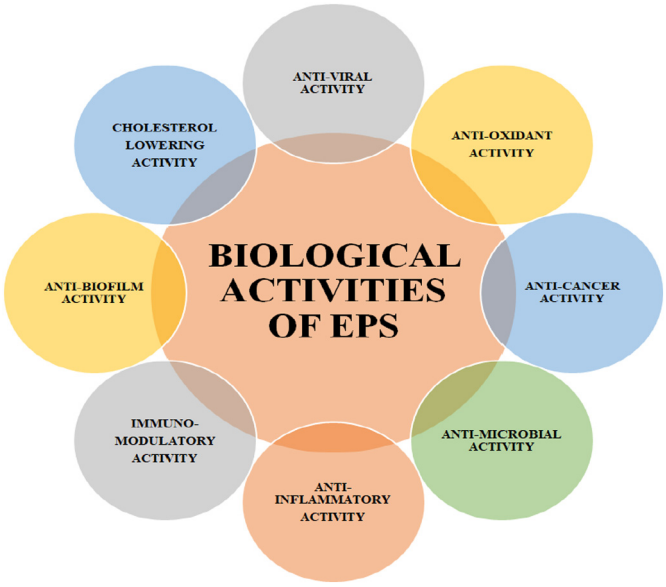

Fig. 4. Biological activities of EPS.

6. Health potential of EPS

The biological potential of EPS depends on its chemical nature which is influenced by fermentation conditions employed in the cultivation of probiotic bacteria [55,64]. EPS from different sources are influenced by monosaccharide composition, glycosidic linkage, chemical modifications, etc., that encompasses the specific conformation, expanded chain, molecular weight etc. [17]. The occurrence of these constituents contributes to the health promoting activities of EPS. The EPS produced by probiotic bacteria have gained much importance in therapeutic applications such as antimicrobial, immunomodulatory, anti-inflammatory, antioxidant, anti-tumor, anti-viral, anti-diabetic, anti-ulcer and cholesterol lowering activities [25,27] (Fig. 4).

6.1. Antimicrobial activity

Several studies reports LAB are able to produce many antimicrobial compounds that include bacteriocins, diacetyl, organic acid, carbon dioxide and some other low-molecular weight substances, such as reuterin, reutericyclin and antifungal peptides [65]. Apart from these compounds, EPS produced by LAB has the ability to express antagonistic effect towards the pathogenic bacteria which is well recorded by several researchers. The EPS producing *Lactobacillus rhamnosus* isolated from human breast milk showed strong anti-bacterial activity against pathogenic *E. coli* and *Salmonellatyphimurium* under in-vitro condition [51]. EPS from probiotic bacterium *Bifidobacterium longum* impairs the cell division rather than inhibiting the growth of pathogenic bacteria [10]. The HePs from *Lactobacillus gasseri* was investigated for its anti-bacterial activity on various test pathogens which revealed that it has inhibited *Listeria monocytogenes* MTCC 657 to a greater extent [97]. EPS from *Lactobacillus* sp.Ca6 inhibited the growth of *Micrococcus luteus* and *Salmonella enterica* with zone of inhibition of around 14 and 10 mm, respectively [32]. EPS-C70 from camel milk exhibited 2 to 3 log reduction against tested food-borne pathogens with initial population of 9 log CFU/ml and highest inhibition was observed against *Staphylococcusaureus* and *E. coli* [31].

6.2. Immunomodulatory activity

Various LAB and their extracellular polysaccharides were discovered to modulate the host immune system by boosting the proliferation of T/B lymphocytes, natural killer (NK) cell tumoricidal activity, mononuclear cell phagocytic capacity, mitogenic activity, inducing cytokines and collectively increases the host immune defense against pathogen [67]. Most importantly, the immunomodulatory activities of EPS synthesized by LAB are controlled by the physicochemical properties such as average molecular weight, monosaccharide composition, water solubility, electric charges and stereochemistry [68]. Some researchers have postulated that EPS of small molecular weight and/or negative charges are strong immunomodulators whereas a neutral polymer with larger molecular weight is weak immunomodulators and possess immunosuppressive activity [69]. EPS are employed as immunomodulators in

Table 4
Enzymes involved in biosynthetic pathway of EPS.

S. no.	Group	Enzyme	Mode of action	References
1.	Group-1	Hexokinase	Intracellular enzymes converts glucose to glucose-6-phosphate	[43]
2.	Group-2	Uridine-5′diphosphate (UDP)-glucose pyrophosphorylase	Catalyzes conversion of glucose-1-phosphate to UDP-glucose (key molecule of EPS synthesis)	[147]
3.	Group-3	Glycosyl transferases (GTFs)	Transfers sugar nucleotides to a glycosyl carrier lipid	[55]
4.	Group-4	Wzx protein (flippase), permease and ABC-transporters	Involved in the polymerization of the macromolecules and situated outside the cell membrane and cell wall Translocate individual repeating units attached to UDP-C55 lipid carrier across cytoplasmic membrane	[61]

regulating both innate and adaptive immunity. These exopolysaccharides improves antibody mediated immunity produced by B lymphocyte which is accountable for specific recognition and elimination of antigens [70]. They play a vital role in reducing the chance of infections, prevent gastrointestinal tract cancers and inflammatory diseases, such as inflammatory bowel disease (IBD) [51]. The modulation of cytokines has been promoted by these immunomodulators which leads to the change in the regular behavior of immune system [71]. *Lactobacillus johnsonnii* 142 and its corresponding EPS isolated from intestine of mice with experimentally induced IBD promoted the differentiation of dendritic cells, triggered the production of cytokines, and preferentially induced Th-2 immune response [72]. EPS isolated from *Lactobacillus plantarum* JKL0142 stimulated the immunomodulatory activity of RAW 264.7 macrophage cells in cyclosphamide induced immunosuppressed mice [73]. In-vivo studies conducted in treated female Swiss albino mice revealed that EPS produced by wild type *Weissella confusa* has stimulated highest production IgM and IgG antibodies whereas mutant type produced more IgA antibodies [74]. Subcutaneous administration of EPS from *Lactobacillus casei* in mice has promoted humoral and cellular immune responses by increasing serum antibodies, T cell proliferation, enhanced expression of cytokines, and up-regulation of dendritic cell maturation [75].

6.3. Anti-inflammatory activity

In general, inflammation is the process of restoration of normal tissue and its function in response to infection and tissue injury that occurs in host. The prolonged inflammatory action leads to serious inflammatory diseases and cancer [76]. The microorganisms initiate inflammation through endotoxin lipopolysaccharide (LPS) which targets Toll-like receptor-4 (TLR-4) to promote inflammatory gene expression [77]. LPS generates the production of mediators and cytokines such as nitric oxide (NO) and pro-inflammatory cytokines like IL-1β, IL-6, and tumor necrosis factor (TNF-α) and suppress the secretion of anti-inflammatory cytokines such as IL-10 [78]. The excessive production of pro-inflammatory cytokines via NF-κB activation results in both acute and chronic inflammation causing inflammatory diseases such as tissue injuries, asthma, IBD and rheumatoid arthritis. Microbial biomolecules activate macrophages which kill bacteria by secreting cytokines, regulate immunity, stimulate phagocytosis, and present bacterial antigens to helper T cells [79]. EPS produced by probiotic bacteria has been reported to control inflammatory mechanism of the immune cells [80]. In particular, more in-vitro studies on macrophage cell lines are performed by several researchers to evaluate the innate immune response induced by EPS. The purified novel EPS from *Lactobacillus pentosus* LZ-R-17 showed significant immunostimulatory activity by increasing the viability of RAW264.7 macrophage cells, enhancing phagocytosis, improving macrophage activation and promoting the secretion of NO, TNF-α, Il-1β, IL-6 and IL-10 [81]. EPS from *Lactobacillus sp.* isolated from healthy human vagina showed considerable anti-inflammatory activity with decrease and increase in the production of TNF-α and IL-10 respectively in HeLa cells [29]. The polymer extracted and purified from *Bifidobacterium longum* BCRC 14634 demonstrated mild immunomodulatory activities on J77A.1 macrophages with the increase in the secretion of IL-10 and reduction in the level of TNF-α [10]. In-vitro evaluation of EPS produced by the *Lactobacillus rhamnosus* KL37 stimulated the production of both pro-inflammatory (TNF-α, IL-6, IL-12) and anti-inflammatory (IL-10) cytokines in mouse peritoneal macrophages [42]. The probiotic strain *Lactobacillus paraplantarum* BGCG11 tested in the peritonitis rat model induced by carrageenan decreased the levels of pro-inflammatory mediators, IL-1β, TNF-α and iNOS, and enhanced the secretion of anti-inflammatory IL-10 [82]. The EPS from *Bacillus licheniformis* BioE-BL11 and *Lactobacillus mesenteroides* BioE-LMD18 (Korean Kimchi) showed inhibition in secretion of pro-inflammatory cytokine IL-6 in LPS stimulated RAW264.7 mouse macrophages and enhanced secretion of the anti-inflammatory cytokine IL-10 in dose dependent manner. This property encourages EPS to be employed in food, cosmetic and pharmaceutical industries [83]. The study conducted with EPS obtained from isogenic *Bifidobacterium animalis* subsp. *lactis* strains represents its capability to stimulate TLR-4 regardless of their molar mass in mouse colitis model [84]. The monosaccharide galactose present in EPS of *Lactobacillus reuteri* Mh-001 influenced anti-inflammatory activity on the macrophages [85]. Acidic EPS103 produced by *Lactobacillus plantarum* significantly reduced excessive release of IL-6, TNF-α, NO and Prostaglandin E2 (PGE2) through the suppression of NF-κB activation by inhibition of Iκ-Bα phosphorylation in RAW 264.7 macrophages activated by LPS [86]. The EPS extract from *Lactobacillus fermentum* Lf2 combined with yoghurt given to BALB/c mice resulted in the increased concentration of short chain fatty acids such as acetate and butyrate [87]. These fatty acids are volatiles produced by gut microbiota and have intestinal anti-inflammatory properties. It was observed that *Bifidobacteria* can protect the host against enteropathogenic infections through the production of acetate [88]. Butyrate participates in the mobility of the colon, reduces inflammation, increases visceral irrigation, induces apoptosis and inhibits the progression of tumor cells, contributes with the prevention of colorectal cancer [89]. Based on these recent research findings, diagrammatic representation of in-vitro anti-inflammatory activity of EPS on LPS stimulated RAW 264.7 macrophages is proposed in Fig. 5.

6.4. Antioxidant activity

Reactive oxygen species (ROS) such as superoxide, hydroxyl radical, and hydrogen peroxide are produced from normal cellular metabolism responsible for severe ill-effects in humans, including cancer, atherosclerosis, rheumatoid arthritis, and neurodegenerative diseases [65]. Superoxide free radicals considered the most harmful ROS, serves as a precursor for other ROS such hydroxyl radical, hydrogen peroxide and singlet oxygen. Oxidative stress created by these ROS results in damage of proteins, mutations in DNA, oxidation of membrane phospholipids, and modification in low-density lipoproteins which leads to tissue damage and death [90,91]. Antioxidants are substances that retard and hinder oxidation. Although synthetic antioxidants like butylated hydroxyl-anizole, butylated hydroxyl-toluene and n-propyl gallate are used to encounter these free radicals but fails in their complete elimination resulting in carcinogenesis and liver damage [92]. There are many reports on EPS from probiotic bacteria with potential antioxidant activity. In general, LAB stains confronts free radicals through inherent cellular antioxidant defense by secreting enzymes like superoxide dismutase and assists the production of the major non-enzymatic antioxidant and free radical scavenger glutathione (GSH). The exopolysaccharides extracted from various probiotics were evaluated for their ability to exhibit antioxidant potential by the degradation of superoxide anion and hydrogen peroxide, reduction of ROS and metal chelating activity and it is desired to employ them as food supplement or in direct drug delivery system [93]. The partially purified EPS of *Lactobacillusplantarum* YML009 has been proved to have antioxidant effects that may involve scavenging the ROS, up-regulation of enzymatic and non-enzymatic antioxidant activities. The same report admitted that probiotics and their secondary metabolites lowers the ROS accumulation as well as degrades superoxide anion and hydrogen peroxide [94]. The EPS from *Lactobacillusdelbrueckii* sp. *bulgaricus* SRFM-1 which was obtained from fermented milk provides satisfactory results on antioxidant activity [95]. The EPS extracted from *Lactobacillus plantarum* ZDY2013 has been reported to show increased antioxidant activity after sulfonation [96]. The DPPH scavenging activity of EPS from *Lactobacillus gasseri* FR4 isolated from native chicken at 4 mg/ml concentration was reported as 75.95% with ascorbic acid (92.94%) as standard [97]. Glucan (500 μg/ml) isolated from idli batter displayed significant antioxidant activity in DPPH (74%) and hydroxyl radical scavenging assays (97.8%). This ensures that the glucan has the promising future in food and

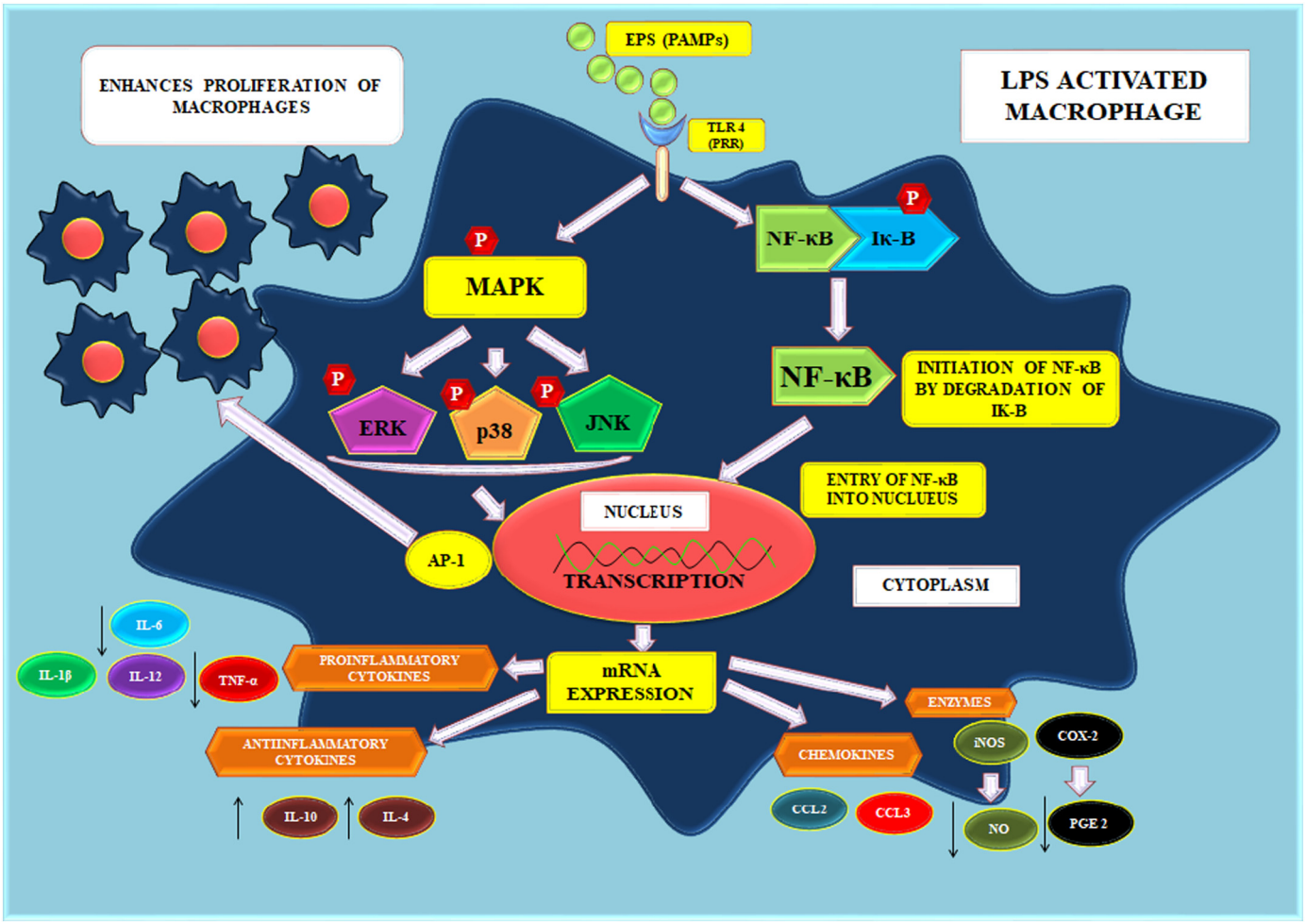

Fig. 5. In-vitro anti-inflammatory activity of EPS from LAB on LPS stimulated RAW 264.7 macrophages. EPS binds to TLR-4 on macrophage cell surface and activates the cell through NF-κB and MAPK pathways. It has been observed that EPS treatment down regulates the NF-κB activation by inhibiting the phosphorylation of NF-κB inhibitor (Iκ-B). Consequently the level of pro inflammatory mediators decreases and anti-inflammatory mediators increases in the cell. Activation of MAPK pathway results in enhanced proliferation of macrophages. The expression of relevant mRNAs and proteins were confirmed by qRT-PCR and western blotting.

pharmaceutical industry [98]. Two EPS fractions of acidic HePs rich in mannose and galactose produced by *Weissella cibaria* SJ14 showed strong antioxidant activity [99].

6.5. Anticancer activity

Cancer is an abnormal development of cells leading to destruction of various organs of the body resulting in death. Among different types of cancer, the prevalence of colon cancer has become common, increases death rate every year. The current chemo and radiation therapy given to cancer patients destroys tumor cells as well as normal cells. The current research aims to develop antitumor drugs with low side effects on the immune system than current synthetic drugs [100]. Anticancer potential by Lactobacilli involves apoptosis induction, differentiation of cancer cells, and binding of genotoxic carcinogens [101]. The possible mechanisms of anticancer activity exerted by EPS are follows: (1) prevention of tumorigenesis, (2) induction of cancer cell apoptosis, and (3) improvement of the immunity. Apoptosis or programmed cell death is necessary in the treatment of cancer. It is characterized by caspase-dependent intrinsic and extrinsic pathways. The intrinsic pathway is indicated by the expression of caspase-3, caspase-9, BCl-2 and BAX, while, the extrinsic pathway is marked by the expression of caspase-8 and caspase-10. Among caspases, the caspase-8, caspase-9 and caspase-10 are initiators that are activated via binding to specific proteins; while caspase-3 is an effector caspase that is eventually activated by the active initiator caspases through photolytic cleavage [102,103]. Activation of Caspase-3 is often the indicator to confirm that the cancer cells have been subjected to cell shrinkage, nuclear fragmentation and chromatin condensation without affecting surrounding healthy tissues [104]. EPS of probiotic origin displays anti-cancer activity with reduced side effects based on their chemical characteristics like molecular composition, chain linkage and molecular weight [105,106]. The diversity in sugar composition of EPS is responsible for anti-proliferative effects. A recent finding reported that the EPS G10 from *Lactobacillus gasseri* inhibits the proliferation of HeLa cells. At the concentration 400 μg/ml, G10 EPS showed better anti-proliferation activity because of its up-regulation of BAX in HeLa cells and an increase in Caspase 3 protein expression that activates apoptosis was also noted [29]. EPS-M41 from probiotic marine source *Pediococcus pentosaceus* M41 was reported to possess antitumor activity against Caco-2 and MCF-7 cells [107]. EPS from *Lactobacillus plantarum* NCU116 increased the expression of pro-apoptotic genes such as Fas, FasL and c-Jun, through toll-like receptor-2 (TLR-2) which mediates apoptosis in mouse intestinal epithelial cancer cells [108]. EPS produced by wild and mutant *Lactobacillus delbrueckii* has been studied to disclose their inhibitory role on tumor cell by the reduction in carcino-embryonic antigen level of tumor induced mice [109]. Silver nanoparticles synthesized using EPS from *Lactobacillus brevis* demonstrates various applications in the biomedical field especially as a powerful anti-proliferative agents against various human cancer cell lines [110]. In-vitro anti-cancer activity exhibited

by newly extracted MSR101 EPS from *Lactobacillus kefiri* on HT-29 cancerous cells showed satisfactory apoptotic mechanism through up-regulation of the expression of cytochrome-c, BAX, BAD, caspase-3, caspase-8 and caspase-9 [111]. Acidic EPS produced by *Lactobacillus strain* SB27 showed maximal increase of capsase-3 activation inducing apoptosis, G0/G1 cell cycle arrest and anti-proliferative activity on HT-29 cells [112].

6.6. Hypocholestrolemic and antidiabetic activity

The accumulation of cholesterol in the human leads to cardiovascular disorders resulting in life-threatening conditions [113]. The high serum cholesterol level can be considerably managed through cholesterol lowering drugs but ends with side effects. The mechanisms behind cholesterol reduction by probiotic bacteria are as follows: (i) assimilation of cholesterol by growing bacterial cells, (ii) de-conjugation of bile salts by bile salt hydrolase, (iii) precipitation of cholesterol with de-conjugated bile and (iv) binding of cholesterol to cells [114]. Very limited researches has been conducted on cholesterol lowering properties but in recent studies aiming at lowering blood cholesterol level through EPS of probiotic origin gained significant attention. The EPS produced by *Lactobacillus paracasei* M7 reported the reduction of cholesterol level (30 μg at 25 °C for 20 min) to 70.78% in-vitro with optimum dose of 0.1% EPS [115]. The EPS derived from *Lactobacillus plantarum* RJF4 was shown to reduce the cholesterol to 42.24% [1]. The EPS of a novel probiotic strain *Enterococcus faecium* F1 isolated from fermented milk product kalarei may be helpful in lowering raised cholesterol level if incorporated in functional foods [50]. Diabetes mellitus is a metabolic disorder characterized by increased blood glucose level due to abnormal glucose metabolism which results in multiple organ dysfunction [116]. The most essential biochemical reaction in the human body is carbohydrate metabolism since any alteration in carbohydrate metabolism will result in metabolic disorders such as Type-2 diabetes mellitus (T2DM). Anti-diabetic drugs such as acarbose, voglibose and miglitol causes abdominal distension, bowel disruption and diarrhea, unsuitable for patients with gastrointestinal disorders [117]. The only and most satisfied anti-diabetic activity exerted by EPS from LAB came in this current year 2020. The two common pathways examined were PI3k/AKT (Phosphatidylinositol 3-kinase/protein kinase B) and AMPK (AMP activated protein kinase) pathways which plays prominent role in the coordination of anabolic and catabolic processes, particularly in T2DM. The HePs obtained from *Lactobacillus plantarum* H31 decreased α-amylase and increased the gene expression of GLUT-4 (insulin-regulated glucose transporter type-4), AKT-2 and AMPK in insulin resistant HepG2 cell line in in-vitro condition [118]. These genes enhance glucose uptake in the insulin resistant cells through insulin signaling pathway. The action of insulin in glucose uptake from blood into cells (hepatocytes) through translocation of GLUT-4 vesicles to the plasma membrane is regulated by PI3K/AKT signaling pathway. With insulin resistance, the normal amount of insulin secreted is not sufficient to move glucose into the cells [119]. The main reason for T2DM is insulin resistance, which involves failure in the translocation of GLUT-4 vesicles to the plasma membrane, leading to inhibition of glucose consumption. In liver, AMPK is a key master switch in regulating glucose and lipid metabolism [120]. Therefore, medium dosage of EPS H31 treatment with insulin-resistant HepG2 cells increased expression of GLUT-4, AMPK and AKT-2 indicates that glucose consumption has been achieved. This research suggests that the EPS could be employed to treat hyperglycemia but requires in-vivo studies to evaluate reduction in blood glucose level [118].

6.7. Anti-biofilm activity

As an outcome to exogenous stress exerted on the microorganisms by the environmental conditions, group of microorganisms forms biofilm and gets attached to protect them from host antagonistic activity [121]. These biofilm producing pathogenic bacteria are responsible for antibiotic resistance, chronic and recurrent infections because of their capability to reside in medical surfaces and in dwelling devices [122]. These biofilms are a great threat to food safety and they are resistant to conventional therapy [123]. The potential of EPS extracted from LAB to lower the levels of biofilm could be used in the treatment and prevention of infectious diseases caused by biofilm producing pathogenic bacteria. The inhibition of initial auto-aggregation and cell attachment of bacterial cells occur either through weakening the cell surface modifications or by reducing cell to cell interactions [124]. The highest activity on disrupting the pre-formed biofilms and biofilm inhibition was detected in EPS-BMS produced by *Leuconostoc citreum* isolated from bovine meat sausages [47]. The cell free supernatant of *Lactobacillus acidophilus* A4 restrained the biofilm formation of entero-hemorrhagic *E. coli* O157:H7 [122]. EPS from *Lactobacillus fermentum* LB-69 isolated from children feces displayed highest biofilm inhibition on *Bacillus cereus* RSKK 863 [123]. The dextran produced by probiotic bacteria *Weissella confusa* isolated from Romanian yoghurt has shown 70% anti-biofilm activity on *Candida albicans* SC5314 strain [125].

6.8. Anti-viral activity

Generally viral diseases are treated with vaccination, chemoprevention and chemotherapy. Apart from these treatments, a new approach prevails in which probiotic microorganisms and their metabolic products provide favorable results in counteracting viruses. LAB exerts antiviral activities by various mechanisms like, direct interaction with viruses, production of viral inhibitory substances or by stimulation of immunity [126]. Most important aspect is that sulfated polysaccharides are responsible for the antiviral effect even in crude form [92]. Immunobiotics are supplement that combines both prebiotics and probiotics with immunoglobulin to boost immunity and promote intestinal health. It was proved that immunobiotics provides protection against viral infection by enhancing innate and adaptive antiviral immunity that leads to the reduction in the duration of the disease, the number of episodes and viral shedding. EPS of LAB permit the communication of immunobiotics with the host by interacting with pattern recognition receptors (PRRs) expressed in non-immune and immune cells. A research conducted on antiviral immune response used polyinosinic-polycytidylic acid (poly (I:C)), a synthetic analogue of viral ds RNA and this is considered as a common tool for scientific research on the immune system. EPS extracted from *Lactobacillus delbrueckii* OLL1073R-1 improved antiviral activity in poly (I:C) induced porcine intestinal epithelial cells which significantly increased expression of IFN-α, IFN-β, MxA and RNase L and other interferon stimulated genes [127]. The EPS of probiotic bacteria *Lactobacillus*, *Leuconostoc* and *Pediococcus* are indicated to show stronger antagonistic activity against human adenovirus type 5 [128]. *Lactobacillus plantarum* LRCC5310 EPS reduced the duration of diarrhea, limited the epithelial lesions, decreased the rotavirus replication in the intestine and shortened the time to recovery of suckling mice [129]. EPS obtained from *Lactobacillus plantarum* showed inhibitory effect on transmissible gastroenteritis corona virus proliferation in epithelial swine testicle cell line [130].

7. Conclusion

Exopolysaccharides produced by microorganisms has favorable advantages with regard to industrial as well as therapeutic applications when compared to other natural agents. EPS obtained from Lactic acid bacteria contains remarkable valuable properties which replaces polysaccharides of plant or animal origin. In recent years, non-dairy sources that possess probiotic bacteria are targeted to explore their health promoting and rheological properties. In this review, the studies conducted on EPS of probiotic bacteria during latest years along with their sources, chemical structure, biosynthesis, applications and biological potential are recorded. Some researchers have investigated the improved level

of therapeutic activities upon chemical modification in EPS structure. More in-vitro studies have been reported on antioxidant, antimicrobial, anticancer, anti-biofilm, immunomodulatory activities etc. Only limited researches were done on in-vivo models to evaluate antitumor and anti-inflammatory activities. Novel EPS are identified from different resources with outstanding benefits for commercial purposes. From this review, we conclude that more scientific investigations on increase in the yield of EPS and in-vivo studies on therapeutic properties are required to exploit EPS to its complete potential.

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