

BACTERIAL EXOPOLYSACCHARIDES FUNCTIONALITY AND ITS EMERGING ROLE: CURRENT AND FUTURE PERSPECTIVES. A REVIEW

TALIB HUSSAIN *

PhD Scholar, Institute of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan.
*Corresponding Author Email: talibhussain1822@gmail.com, ORCID ID: 0000-0003-3769-8943

NAGEEN HUSSAIN

Assistant Professor, Institute of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan. Email: nageen.mmg@pu.edu.pk

AQSA BASHIR

M.Phil Institute of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan.

FIZZAH MUQADDAS

M.Phil University of Engineering and Technology (UET), Lahore, Pakistan.

SIDRA GHAFOR

M.Phil Institute of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan.

RABIA IQBAL

M.Phil Institute of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan.

ABDULLAH MAQBOOL

MS Institute of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan.

Abstract

Bacterial exopolysaccharides (EPS) are the extracellular polymers of carbohydrates that forms either capsule or slime layer. EPS is secreted by many microbial genera and their production can be enhanced by using certain metabolic and genetic engineering strategies. Microbial EPS helps the bacterial cell in environmental adaptation, attachment, biofilm formation and stress tolerance. It shows wide range of applications in food and pharmaceutical industries. Additionally, they possess bioactive properties and performs anti-fungal, anti-viral, anti-microbial, antioxidant, antiaging, antiulcer, wound healing, anti-diabetic, anti-cancer, cholesterol lowering and immunomodulatory activities. Immense importance is given to bacterial EPS in human disease development and management. This review article casts a bird eye view on the types of EPS, their biochemical pathway of production, their involvement in causing and management of human diseases and their application in medical and industrial sectors. Collectively, this review article enlightens the comprehensive discussion on bacterial EPS.

Keywords: Exopolysaccharide; Biofilm; Polymers; Lactic Acid Bacteria; Bioactives; Physiochemical Properties.

INTRODUCTION

In the presence of unfavorable conditions in ecosystem, bacteria can colonize in various locations and form niches. Due to this, most of the bacteria has an outer cell envelope that not only perform functions of physical barrier but also help in the management of ecological stress[1, 2].

It also provides the opportunity of adaptation and communication with the members of its niche which they occupy. In case of most bacteria, the outer surface usually consists of exopolysaccharides (EPS). It is a polymeric system composed of various chains of carbohydrates.

Dextran was the first discovered EPS, and it was discovered by Louis Pasteur in 1861 in wine [1]. EPS performs various functions such as protection of bacteria from harsh environment, assistance and help in cell adhesion, antimicrobial activities, and desiccation etc.

EPS facilitates the formation of biofilms that helps bacteria in adaptation, survival, stress tolerance and persistent colonization [3]. As an integral part of cell wall, it also performs the protective functions and provide structural integrity.

As a part of capsule, it provides the surface attachment opportunity to bacteria that leads the formation of biofilm. In the near past, EPS has been isolated from thermophiles, extremophiles, psychrophiles, halophiles, alkaliphiles and acidophilic bacteria [4]. EPS has been isolated from different classes of bacteria and the major studied class is probiotic bacteria from class Lactic acid bacteria (LAB). EPS isolated from LAB has various functions in cosmetics, food, pharmaceuticals, petroleum, agriculture, and textile industries [5].

Bacterial EPS possess the excellent properties such as prebiotic effects, immune modulation, antioxidant, anticancer, antiulcer, antiviral, anti-gastritis, and antidiabetic properties. In the recent years, various biopolymers have been derived and characterized from bacterial origin [6]. EPS has shown excellent applications in medical field due to their close compatibility with the human metabolic activities and non-toxic nature [4]. **Figure 1** is illustrating a brief overview of source, applications, properties and biological activities of bacterial EPS.

Moreover, biofilm plays critical role in chronic infections. Due to this reason utmost efforts are being directed to understand the role of EPS and its utilization in therapeutic purposes. This review article will provide a critical analysis of EPS classification, their structural diversity, their importance in human disease management and industrial sector as well as pharmaceutical industries [7].

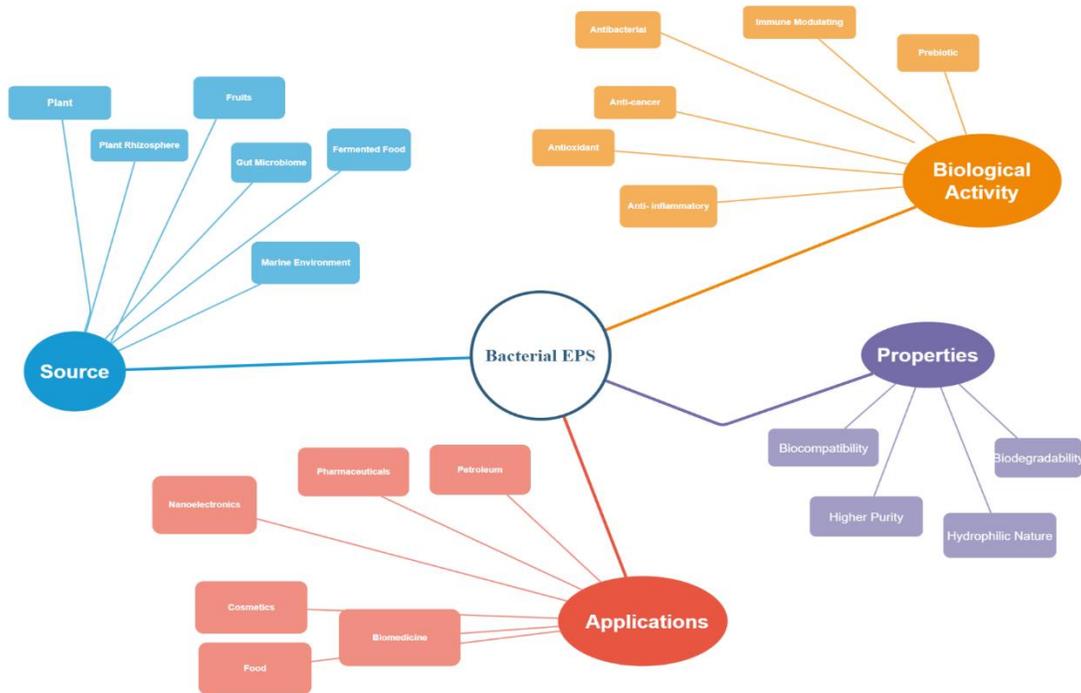


Figure 1: Bacterial EPS sources, properties, applications and biological activities

1.1. Fundamental of the bacterial EPS

The word “Exopolysaccharide” is combination of two words, ‘exo’ with the meaning of external and ‘polysaccharides’ that is a carbohydrate consisting of multiple monomers of sugar [8]. EPS are the natural polymers also known as biopolymers. They are usually found as an integral part of structure in various living organisms such as animals, plants, and microorganisms. Bacteria use simple to complex substrate to make a very long range of polymers and these biopolymers has a wide range of properties [9]. These biopolymers could be present inside or outside of the cell and are referred as intracellular and extracellular respectively. Intracellular has few properties but in contrast extracellular has a wide range of applications in multiple fields. Extracellular biopolymers are also known as extracellular polymeric substances and are divided into four major categories. These are polyesters, polysaccharides, polyamides and inorganic polyanhydrides [10]. Polysaccharides are the most abundant group. The molecular weight of EPS ranges from 10 to 1,000 kDa and are polymers of monosaccharides linked with the help of glycosidic bonds. EPS can be further divided into subclasses based on their location in the cell. These are EPS in bacterial cell wall, EPS in structures such as peptidoglycan in Gram-positive bacteria [11]. Glycocalyx is secreted by bacteria on their surface. Some microorganisms also make an outer layer known as capsule which helps them in attachment with surfaces [12]. Capsule is covalently attached to the body. They sometimes also develop some loosely attached layers known as slime layers. These are secreted from body to outer environment, but they can also develop it outside of the body and it is usually performed by some enzymes anchored with cell membrane [13].

Bacterial metabolism usually becomes most active during their exponential and stationary phases; hence generation of EPS takes place during these phases. In the abundant presence of sugar, more production of EPS takes place. It fulfills the need of energy and serves as carbon source [14]. The production of EPS is based on multiple factors, like nutrients availability and composition, temperature, pH, and nitrogen/carbon ratios. Different bacterial strains produce different type of EPS, so it is strain specific [15]. Some notable functions are cell aggregation, biofilm formation, cell to cell interaction and cell adhesion. Capsular polysaccharide protects the bacteria from phagocytosis, desiccation, bacteriophage attacks and osmotic stress [16].

2. CLASSIFICATION

EPS are classified on two bases i.e. chemical composition and functionality [17]. **Figure 2** detailed classification is explained.

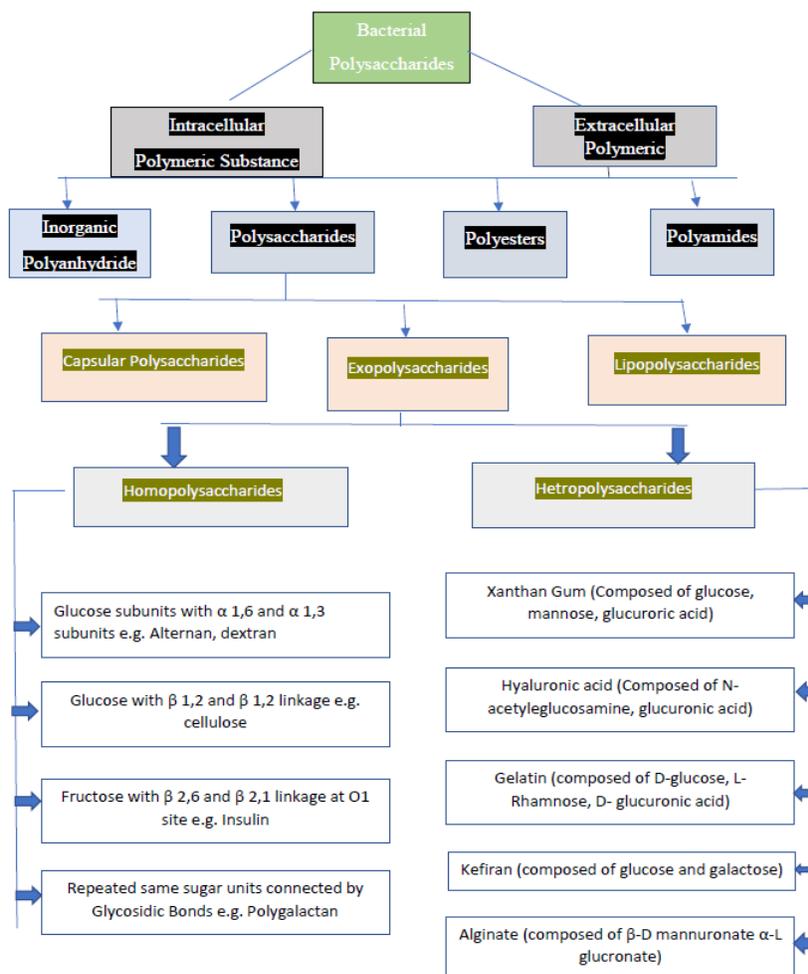


Figure 2: Bacterial Exopolysaccharides and its classification

2.1. Based on chemical composition

Same or different types of monomers combine to form the polysaccharides. On the base of chemical composition, EPS can be categorized into homopolysaccharides and heteropolysaccharides [18]. This division is usually based on the composition of polysaccharides, group substitutions and presence/absence of side chains. In case of homopolysaccharides only single type of monomers are used and are joined with the help of glycosidic bonds [19]. These are usually made up of fructose or glucose subunits. Based on the bonds of linkage, homopolysaccharides are divided into fructans, α -D glycans, β -D glycans and polygalactans [20]. Their structures can be branched or unbranched and depending upon the source of bacteria they have variable branches degree. As compared to the heteropolysaccharides, their quantity of production is very high. Their molecular weight is usually $>10^6$ Da. Glucans like mutan, reuteran, alteran and dextran are produced with the help of glucansucrase enzyme [17]. They had (a-1,3) (a-1,6 and a-1,3), (a-1,6 and a-1,4), (a-1,6 and a-1,3) and (a-1,6) glycosidic bonds respectively. The molecular weight of levan, reuteran and fructan is 2×10^6 Da, 2.8×10^7 Da and 10^7 Da respectively [21].

On the other hand, heteropolysaccharides are the important cocktails of various types of monosaccharide subunits. These are made up of repeating subunits of D-galactose and D-glucose [22]. In addition, some rare sugars such as arabinose, L-rhamnose, xylose, mannose, L-fucose, N-acetyl galactosamine and N-acetyl glucosamine were also found. Some other entities may also be there in rare cases [23]. These may include glycerate, acetate, pyruvate, amino acids, sulfate and phosphate etc. The secretion or synthesis of heteropolysaccharides depends upon the growth conditions of microbes. Under optimal conditions the yield is about 0.15 to 0.6 g/L [24]. One major difference between homopolysaccharides and heteropolysaccharides lies in the enzymes and site of their synthesis. Homopolysaccharides are produced outside of the cell and produced under the presence of specific substrate like sucrose [25]. Sucrose is converted into its polymers under the action of secreted enzymes. On the other hand, heteropolysaccharides are formed intracellularly and translocated outside of the membrane. This takes place with the help of isoprenoid glycosyl carrier lipids [26].

2.2. Classification based on functions

On the basis of their functions, EPS can be classified into seven categories. These categories are sorptive, constructive, redox-active, surface active, active, nutritive and informative. Architectural support for the formation of biofilm is provided by constructive or structural exopolysaccharides [21]. To manage the water retention and cell protection is their main goal. These are usually neutral in charge because of absence of any charged molecules. There are some charged particles present in case of sorptive exopolysaccharides [27]. These charged particles play important role in cell to surface interaction. Amphiphilic compounds usually combine to form the surface-active EPS. These have diverse surface and chemical structure properties [28]. They also possess antifungal and antibacterial activities. EPS with redox active properties can be formed by

some bacterial species like *Pseudomonas putida*, *Escherichia coli* and *Shewanella spp.* The redox sensitive pollutants or contaminants are migrated or bio-transformed with the help of these redox-active EPS. EPS produced by *Shewanella spp.* are useful in the biosorption, biodeposition and reduction of uranium [28]. The biotransformation of diphenozothiopene and arsenate reduction is facilitated the EPS produced by *P. putida*. Silver nanoparticles can be produced by positively charged silver ions with the help of EPS produced by *E. coli* [29].

3. BACTERIAL SOURCE OF EXOPOLYSACCHARIDE

Depending upon different types of available nutrients in the medium and other environmental conditions, EPS producing bacteria shows different type of characteristics and can be isolated from different environments [30]. Probiotic bacteria are the most common and important producers of bacteria. LAB are very important family in this regard and EPS isolated from LAB promises high therapeutic and health benefits for human beings [20]. Bacteria from different genera such as *Lactococcus*, *Streptococcus*, *Leuconostoc* and *Lactobacillus* are the most important EPS producing LAB. Most studied LAB in sense of EPS productions are *Lactobacillus casei*, *Lactococcus lactis*, *Lactobacillus reuteri*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus* and *Streptococcus thermophilus* [31].

3.1. Probiotic bacteria-derived exopolysaccharide

Dairy products such as milk and milk products, and non-dairy sources like vegetables, fruits and cereals are the most important source of probiotic strains of bacteria [20]. Another important source lies in animal products like meat and meat products [32]. Some important bacteria and their sources are described in **Table 1**. Some important sources are cabbage, Tibetan kefir, traditional Bulgarian yogurt, kimchi, pico cheese, Chinese paocai, home-made Sichuan pickle, curd, fermented dairy tofu, camel milk, pulp of durian fruit, Ngrai, gut of fish and native chicken [33]. Some of the important bacteria involved are *Lactobacillus bulgaricus subsp.*

Delbrueckii, *L. plantarum* 70810, *Enterococcus faecium* (BDU7), *Lactobacillus gasseri* FR4 and *L. plantarum* MTCC9510 etc. [33]. LAB EPS has a wide range of activities in biological world. One can found there applications as anti-cancer agent, antioxidant, immunomodulatory and role in gut microbiota regulation [34]. The complete explanation of LAB-EPS in different fields is elaborated in **Figure 3**.

Table 1: Important probiotic bacteria, and their biological properties

No.	Strain name	Source	Biological properties	Reference
1	<i>L. plantarum</i> H31	Pickled cabbage	Immune activity possession, helps during DNA damage	[18]
2	<i>Lactobacillus helveticus</i> LZ-R-5	Kefir	Immunomodulatory activity in in-vitro conditions	[36]
3	<i>Lactobacillus bulgaricus</i> subsp. <i>Delbrueckii</i>	Bulgarian yogurt	Activate NK cells, help in signaling driven by INF- γ , IL-18 and cytokines.	[37]
4	<i>L. plantarum</i> LRCC5310	Kimchi	It contains anti-viral activity especially against rota virus in in-vitro conditions.	[38]
5	<i>Leuconostoc citreum</i> L3C1E7	Cheese	Suppress the production of IgE and helps in allergy	[36]
6	<i>L. plantarum</i> 70810	Chinese Paocai	Suppress the proliferation of tumor cells especially HepG-02	[39]
7	<i>L. plantarum</i> HY	Sichuan pickle (home-made)	Possess anti-oxidant activity	[37]
8	<i>L. plantarum</i> MTCC9510	curd	Antimodulatory and antitumor activities	[18]
9	<i>L. plantarum</i> JLK0142	fermented dairy tofu	Impoves quantity of IgA and cytokines level in serum	[37]
10	<i>L. plantarum</i> C70	camel milk	Helps in colon cancer by showing antioxidant activities	[38]
11	<i>Lactobacillus</i> strains	pulp of the durian fruit	Reduces cholesterol level, antioxidant, antimicrobil	[40]
12	<i>Enterococcus faecium</i> (BDU7)	Ngari	Act as superoxide in scavenging radical compounds (in-vitro)	[37]
13	<i>E. faecium</i> MC13	gut of fish	Stops bio-film formation by <i>Listeria monocytogenes</i>	[33]
14	<i>Lactobacillus gasseri</i> FR4	chicken	Antioxidant and aantimicrobial activities against food born pathogens.	[18]

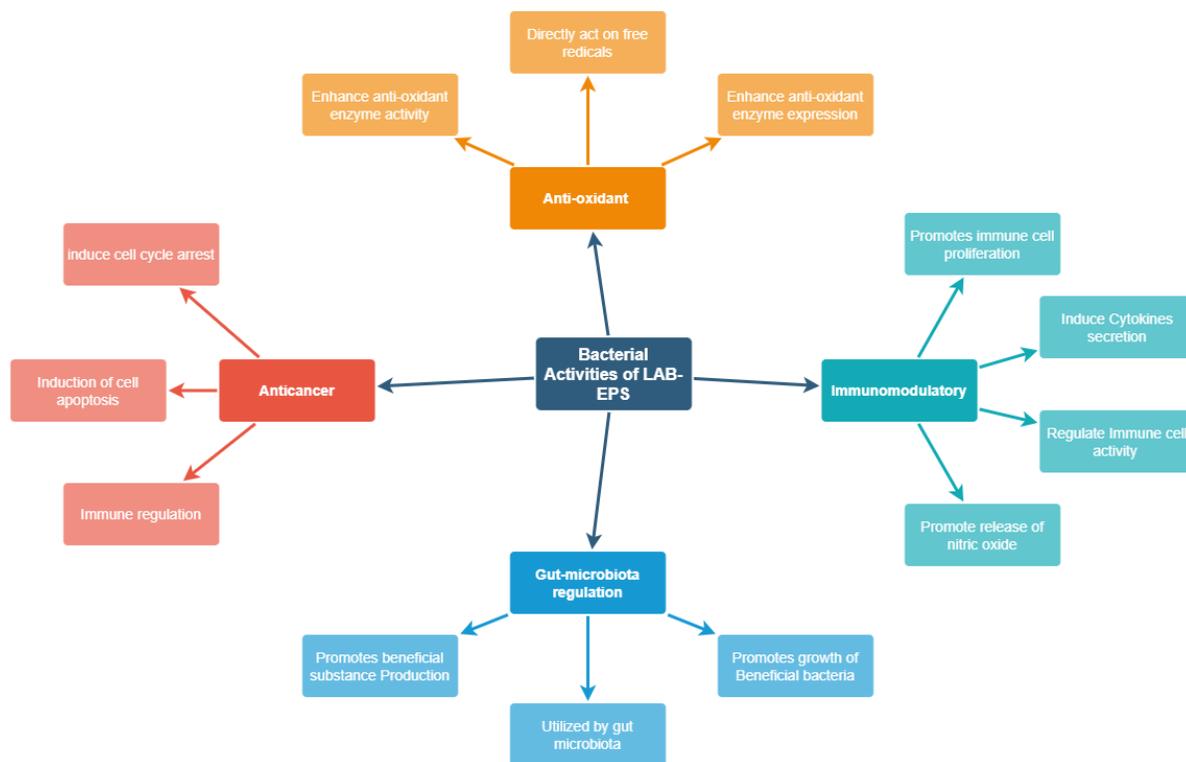


Figure 3: Biological applications of LAB EPS

3.2. Extremophile bacteria-derived exopolysaccharides

Extremophiles are those bacteria which can grow and survive in harsh environmental conditions like temperature, pressure, and salt concentration. The most important sources of these bacteria are hot springs, hydrothermal vents, salt lakes, arctic and Antarctic oceans [35]. The structural and functional properties of EPS derived from these bacteria are novel because of these harsh ecosystem and novel nutrients choice. Halophilic bacteria have an EPS capsule and can survive in extreme salt concentrations i.e. 13 to 20% [36]. The most important genera involved in EPS production are *Haloferax*, *Halococcus*, *Halomonas*, *Halobaculum*, *Halobacterium* and *Natronococcus* [37]. Some the important bacterial strains and their product properties are discussed in **Table 2**.

Table 2: Important bacterial strains and their EPS properties

No.	Strain name	Yield mg/kg	Source	EPS properties	References
1	<i>H. Maura</i>	34.4	Hot springs	antioxidant and anti-thrombogenic activities	[34]
2	<i>Bacillus thermoantarcticus</i>	67.2	Shallow hot marine vents	High amount of EPS	[43]
3	<i>Bacillus licheniformis</i>	88.1	Shallow hot marine vents	High amount of EPS	[39]
4	<i>Geobacillus thermodenitrificans</i>	43.3	Shallow hot marine vents	High amount of EPS	[40]
5	<i>G. tepidamans</i>	NR	Terrestrial hot springs	high thermostability	[44]
6	<i>Vibrio diabolicus</i>	73.4	hydrothermal vent	hyaluronic polysaccharides	[43]
7	<i>Xanthomonas spp.</i>	43.1	Hot water springs	Stability at high temperature	[45]
8	<i>Acetobacter spp</i>	39.1	Open environment	Can be used in food, biomedical and tissue engineering	[46]
9	<i>L. mesenteriodes</i>	45.2	Chines sauerkraut	Good stability, non-ionic	[7]
10	<i>P. aeruginosa and A. vinelandii</i>	49.0	Biofilm	Gelling capacity, film formation	[11]

Psychrophilic bacteria can survive in low temperature and most important EPS producing bacteria are *Pseudoalteromonas sp.*, *Colwellia psychrerythraea* and *Olleya marilimosa* [38]. Acidophiles can live in high acidic environment. They are usually involved in the process of bioleaching. Most important EPS producing bacteria are *Leptospirillum ferrooxidans*, *Acidithiobacillus ferrooxidans* and *A. thiooxidans*. Alkaliphilic bacteria can live in alkaline conditions and their optimal pH requirement is about 10. Genus *Bacillus* can produce EPS of diverse type [39].

4. BACTERIAL EPS BIOSYNTHESIS PATHWAYS

EPS are usually produced in the late period of stationary phase. Some influencing factors can affect the rate of EPS generation and that may include salt, nutritional imbalance, pH, and temperature etc. Residues of sugar are transported into cell boundary, their conversion into monomeric subunits takes place and they are polymerized [40]. Now they are ready to be linked with the biosynthetic machinery that is isoprenoid lipid carrier. After that, some important modifications are done in it and it is transported out of the cell to assemble them into polysaccharides [41]. It is very complicated process and various carriers; enzymes and transporter proteins are involved in it. The complete elaboration of this procedure is

given in **Figure 4**. There are three important pathways which are involved in the regulation of EPS synthesis, including Wzx/Wzy dependent pathway, ABC transporter dependent pathway, and synthase dependent pathway.

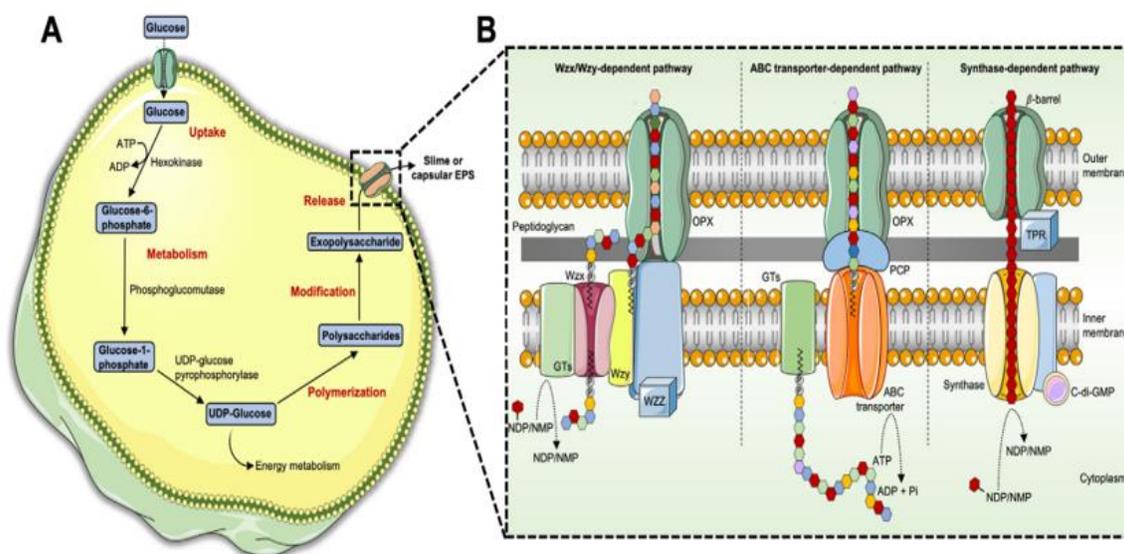


Figure 4: Biosynthetic Pathway of EPS formation. Inside and outside of the cell [39]

In case of gram-positive and gram-negative bacteria, Wzx/Wzy dependent pathway is used for the bulk production of polysaccharides [42]. The products may include O-antigen, exopolysaccharide, capsule, enterobacterial antigen, and spore coat etc. The cell to surface transport of poly and oligosaccharide is carried out by following the ABC transporter pathway. Actually, the polysaccharide chain is anchored on poly-kdo-linker, and it is transported out of the cell with the help of OPX and PCP proteins [43]. The biosynthesis of bacterial EPS is usually regulated by four enzymes: special pathway proteins such as permease, flippase, glycosyl transferases, glucose pyro-phosphorylase, and hexokinase. The EPS biosynthesis takes place in the strong control of Wzx/Wzy pathway. All the activities usually take place in the cytoplasm, one exception is the process of polymerization. Many enzymes take place in different reaction and in this process the core carbon metabolism intermediates serve as substrate for bacterial EPS production [44]. Some sugars like nucleoside diphosphate sugar acids serve as donors and precursors. Undecaprenyl di-phosphate anchor is present on inner part of plasma membrane and individual sugar nucleotides bound themselves to it and are transported outside of the cell with the help of flippase [41]. Isoprenoid alcohol serves as the pyrophosphate bridge with monosaccharide subunits and act as carrier lipid molecule. Certain enzymatic activities like sulphation, acetylation and methylation take place at this stage to form EPS. The formation of capsule or slime layer is done by the process permease, flippase or ABC transporters [45].

In Wzx/Wzy dependent pathway, individual repeating units are linked to “undecaprenol diphosphate anchor (C55) and it is present on the inner surface of membrane. Many types of glycosyltransferases (GT's) are involved in their assembly and Wzx protein is involved in the translocation across the membrane [46]. Here Wzy protein is involved, and polymerization takes place in the periplasmic space. Now transport of polymerized repeating units takes place from periplasm to the cell surface and many more proteins are involved in it [47]. These may include “outer membrane polysaccharide export” (OPX) families and “polysaccharide co-polymerase” (PCP) [43]. The polysaccharides that are assembled by using this pathway have diverse pattern of sugar. Commonly, four to five types of sugar are present in their assembly. This is important reason that they are classified as heteropolymers. One of the important example is xanthan. The operons of extracellular polysaccharides of all strains using this pathway possess polymerase (Wxy) and flippase (Wzx) [48].

The second important pathway involved in the production of EPS is “ABC transporter dependent pathway”. It is usually present in capsular polysaccharide (CPS) biosynthesis [49]. The polysaccharide formed by this process are usually not represent as EPS all they are linked to the cell surface so far. The GT's in this pathway are formed at the cytoplasmic side of inner membrane. If only one GT-containing operon is involved in the process, then homopolymers are formed and in case of multiple GT's heteropolymers are formed [50]. A complex is involved in their translocation from inner membrane to cell surface and it is formed of ABC transporter proteins, periplasmic proteins of OPX and PCP family. These proteins are usually closely related to that of proteins used in Wzx/Wzy pathway [51]. At the terminal end of EPS produced in this pathway contains conserved glycolipid that is composed of poly-2-keto-3-deoxyoctulosonic acid and phosphatidylglycerol linker. This is one of the major differences between products of both pathways [52].

Synthase dependent pathway is the third pathway of EPS production. This is involved in secretion of complete polymer strands across the cell wall and cell membrane. It is dependent on flippase for the translocation of repeating subunits [53]. Single synthase protein performs the process of not only translocation but also polymerization. This pathway is involved in the synthesis of homopolymers in case of requirement of single type of sugar precursor. One of the famous examples is of important example is curdlan where only β -(1-3) linked glucose is there [53]. Another important example is bacterial cellulose. It contains only the units of β -(1-4) linked glucose. The polymers in this pathway are formed as polymannuronic acid in the start of process. The processing of them takes place with the help of different epimerases. Some more enzymes like mannuronic/glucuronic acid are also involved for further modification. The ratio and sequence of these building blocks are different [54]. Both steps of polymerization and secretion takes place with the help of only enzyme (hyaluron synthase) in the synthesis of hyaluronic acid (HA). The assembly of polymeric disaccharide takes place with the help of two different precursors such as GlcNAc and glucuronic acid [55]. This shows that HA synthesis is different from all other formations which follow this pathway. But at protein

level, there is high level of similarities [48]. The complete elaboration of all these pathways is given in **Figure 5**.

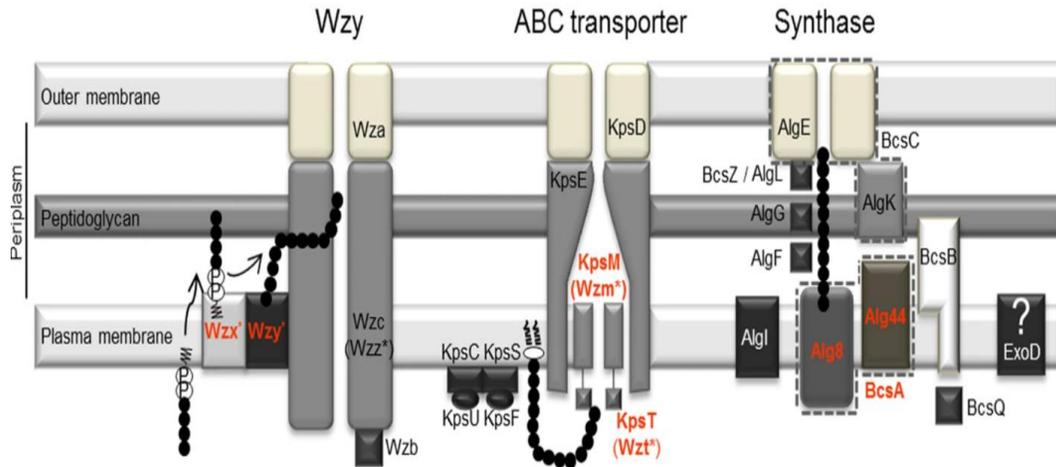


Figure 5: Pathways of EPS biosynthesis. [18]

5. ROLE OF EPS IN BIOFILM FORMATION AND QUORUM SENSING OF BACTERIA

Quorum sensing (QS) is a phenomenon used by the bacteria to adopt the harsh environmental conditions like temperature, osmotic pressure, and pH etc. It is cell to cell interaction of bacteria [56]. **Figure 5** is explaining the process involved in the formation of biofilm. Different processes of biofilm formation are identified so far. Some of the most important factors involved are peptides used as signal molecules in case of Gram-positive, LuxI/LuxR-type of QS in Gram-negative bacteria and LuxS-encoded auto-inducer-2 based QS in both Gram-positive and Gram-negative bacteria [57]. QS Molecules activate the genes that are involved in QS and the process of biofilm formation starts. The EPS production increased in case of increased QS process. In case of abundance of nutrients, bacteria use QS for colonization, spatial dominance, and survival [57].

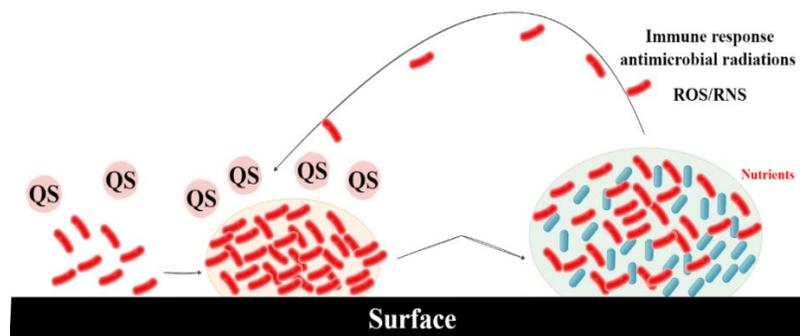


Figure 6: Bacterial biofilm formation

Quorum sensing autoinducer (QSAI) is involved in formation of EPS based stable biofilm in *P. aeruginosa*. It is estimated that about 2000 bacteria involved in the release of QSAI signal that initiate the of biofilm formation [58]. The major role of EPS in biofilm formation has been proved with the fact that QS inhibitors are designed to inhibit the production of EPS. For example, there is an important secondary metabolite of plant with the name of naringenin. It can reduce the production of AHL molecule and as a result, the expression level *lasI* is downregulated. As a result, the production of EPS also hampered [59]. Gut microbiota can form stabilized biofilm on surfaces of mucosa. The colonization is directly influenced by available nutrients and gut biogeography. Bacteroidetes and Firmicutes are important phyla of bacteria present in human intestine. It is reported that about 16.83% and 83.33% Bacteroidetes and Firmicutes respectively retain the orthologs of LuxS protein. This helps in the stable formation of biofilm [60]. Hence, biofilm formation is directly influenced by the formation of EPS and its quantity in the cell.

6. ROLE OF BACTERIAL EXOPOLYSACCHARIDES IN HUMAN DISEASE

The disease-causing property of EPS is directly associated with the formation of biofilm. Biofilm plays a major role in about 65% of bacterial diseases. A list is given in **Table 3** that explains different diseases associated with the biofilm formation [60].

Table 3: Role of biofilm in human diseases, tissue involved and pathogens.

Disease	Microbial biofilm roles	Tissue involved	Involved Pathogen	Reference
Infective endocarditis	Microbial colonization of endocardial surface of heart, it is present on leads of pacemaker most frequently, vascular grafts, and artificial valves heart	Heart	<i>S. mutans</i> , <i>S. aureus</i> , , <i>S. sanguis</i> , <i>S. bovis</i> , <i>S. mitis</i> , <i>S. salivarius</i> and <i>S. sanguinis</i>	[37,69]
Chronic rhinosinitis	Inflammation of tissues, obstruction of nasal tissues, involved in the prevention of antimicrobial agents action	Nasal tissues mucosa	<i>Streptococcus pneumoniae</i> , <i>S. aureus</i>	[18]
Atherosclerosis	The development of biofilms in severely damaged arteries may play a significant role in the chronic inflammatory pathways that lead to atherosclerosis.	Arteries	<i>Pseudomonas spp</i>	[69]
Wound infections	It generates the harmful poisons and enzymes that may encourage the persistent inflammation in wound and hinder wounds healing ability.	skin	<i>Bacteroides spp.</i> , <i>Peptostreptococcus spp.</i> , <i>S. epidermidis</i> , <i>Fusobacterium spp.</i> , <i>Clostridium spp.</i> <i>P. aeruginosa</i> and <i>S. aureus</i> ,	[36]
Tonsillitis	Antibiotics resistance, bypassing of immunity	Ear cavity	<i>S. pneumoniae</i> , <i>H. influenza</i> and <i>M. catarrhalis</i>	[37]

Cystic fibrosis	Antibiotic resistance, accumulation of mucus, Increased inflammatory processes, mutations,	Air ways/ lungs	<i>P. aeruginosa</i> , <i>S. aureus</i> and <i>Haemophilus influenzae</i>	[18]
Mastitis	It helps in antibiotic action prevention, promotion of damage of inflammatory tissue and increases adhesion of bacterial cells.	Breast	<i>S. aureus</i> , <i>Streptococcus dyslactiae</i> , <i>E. coli</i> , <i>Streptococcus agalactiae</i> and <i>Streptococcus uberis</i>	[36]
Chronic bacterial prostatitis	It Increases the bacterial colonization in prostate gland and it causes severe UTI and pelvic pain.	Prostate	<i>Klebsiella</i> , <i>Enterococcus faecalis</i> , <i>E. coli</i> , <i>Staphylococcus spp.</i> , <i>P. aeruginosa</i> , <i>Enterobacter</i> , <i>Serratia Proteus</i> ,	[36,69]
Bacterial vaginosis	It is involved in tissue damage, Inflammation, limiting of commensal growth and tissue invasion.	Vagina	<i>G. vaginalis</i> , <i>Mobiluncus</i> , <i>Atopobium vaginae</i> , <i>Prevotella</i> , <i>Bacteroides</i> ,	[11]
Pertussis	Colonization of pathogen increased due to the damage of tissue.	Respiratory tract	<i>Bordetella pertussis</i>	[2,11]
Melioidosis	It Induces the injury of inflammatory tissue, increased tissue colonization by stimulating cell lysis and autophagy.	Multiple organs	<i>Burkholderia pseudomallei</i>	[36,37]
Biliary tract infection	Act as nucleating factor for cholesterol and pigment gallstones	Gall bladder wall, biliary tract	<i>Salmonella enteritidis</i> , <i>E. coli</i> , <i>Acinetobacter baumannii</i>	[11,18]

EPS capsule could be a source of pathogenicity for many microorganisms. EPS acts as a barrier against the immune system in the biofilm system. They also provide resistance in case of phagocytic and anti-body mediated processes [61]. Biofilms are thought to be a major cause osteomyelitis, cystic fibrosis, wound infections and rhinosinusitis [62]. Biofilms are a cause of infection transfer from different medical appliances like 2% for joint prostheses; 2% for breast implants; 4% for mechanical heart valves; 4% for pacemakers and defibrillators; 10% for ventricular shunts and about 40% for ventricular-assisted devices [62]. A lot of attention is going to be put on treating the illnesses related to biofilm but there are a lot of challenges in it [20]. Various functional groups are present in EPS produced by bacteria. These groups may include phosphate, carbonyl and hydroxal groups. It is said that these functional groups are involved in the interaction of cell wall or cell membrane of pathogenic bacteria. Due to this, they may exert the antimicrobial activities. **Figure 7** is elaborating the biological and physiochemical properties of bacterial EPS.

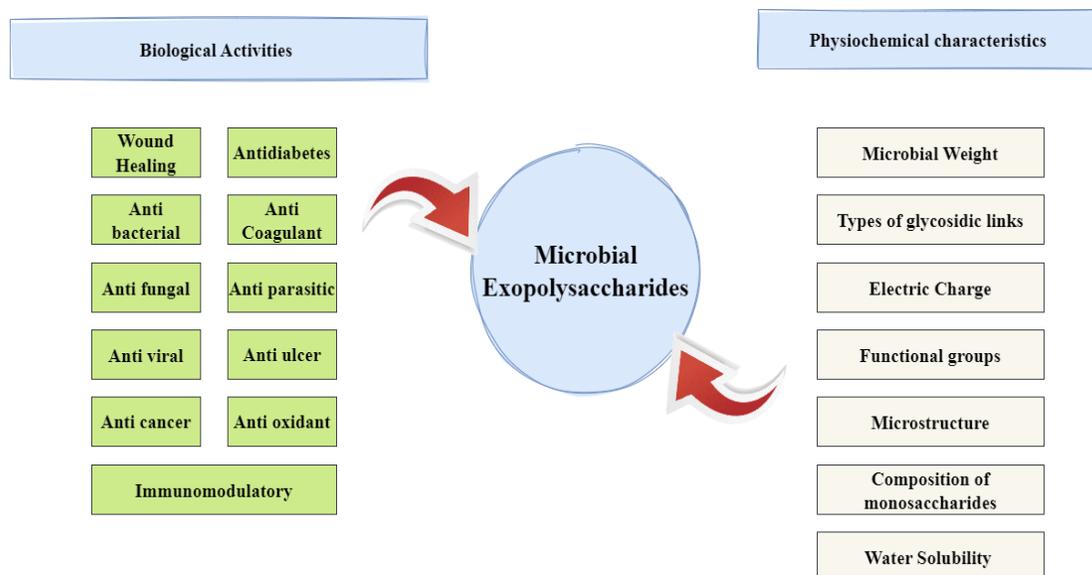


Figure 7: Physicochemical properties of microbial EPS and their biological activities

7. BIOACTIVITIES OF BACTERIAL EXOPOLYSACCHARIDES

Various bioactive properties have been shown by EPS in various institutes like immunomodulatory effect, probiotic effect, anti-cancer properties, cholesterol-lowering effects, anti-viral properties, anti-gastritis effects and anti-ulcer properties. Some of these properties are discussed below.

7.1. Prebiotic properties

Prebiotics are very important for human health as they promote the activity and growth of commensals in gut. Commensals benefit the human health. The energy for the growth and working of colon cells comes from the fermentation of carbohydrates and need is fulfilled by gut microbiota EPS prebiotics [63]. The most important strains of bacteria used as probiotics are *Lactobacillus* and *Bifidobacterium spp.* Many of these species produce the EPS. EPS produced by *Bifidobacteria* can be a substrate for the gut microbiota.

About 11 different types of EPS are produced by *Bifidobacterium* and it acts as the substrates able to be fermented. Dextran is produced by LAB and it acts as prebiotic [63]. Dextran can also be used by some important gut commensals. Levan is an indigestible EPS, and it serves as dietary fiber in the gut. Levan also has prebiotic properties. *Weissella cibaria* is able to make an EPS which is able to be fermented by gut microbes, hence act as prebiotics. In isolated conditions, this EPS showed low digestibility as compared to prebiotic insulin [64]. Hence, EPS produced by bacteria exhibit as good probiotic and prebiotic properties.

7.2. Anti-viral properties

The viral diseases are usually treated with chemoprevention, vaccination and chemotherapy. Now apart from these conservative methods, a new approach has been used. In this approach, the probiotic bacteria and their metabolically produced products are used as an anti-viral drug [44]. EPS shows different mechanisms like makes viral inhibitory substances, immune system stimulation and directly interact with different parts of virus and shows anti-viral properties. In their crude form sulfated polysaccharides are responsible to exhibit the anti-viral properties [65]. They stimulate innate and adaptive immune system and reduce the viral shedding, occurrence. They also shows the reduction in period of disease. Bacterial EPS enhance the virus specific antibodies to reduce the viral load and to control the disease. They use the pattern recognition receptors to help and enhance the communication between host and immunobiotics [66]. The studies have shown that the increased production of IFN- β (interferon beta), IFN- α (interferon alpha), RNase L (ribonuclease L) and other genes that play the role of first line of defense against virus. Some specific EPS has also shown antagonistic activity. *L. plantarum* produces EPS that shows very aggressive action against rotavirus [65]. The viral multiplication and few cytopathic changes were seen when MA104 cells were treated with EPS as compared to untreated ones. Some in-vivo studies on mice have shown reduced diarrheal period, less shedding of virus and less damaged epithelial layer in mice in rotavirus infection [67]. EPS can be good anti-viral options to control viral diseases, hence promises good health conditions in the world.

7.3. Antioxidant activities

The cellular metabolism of human beings produce the reactive oxygen species (ROS) in response to some important diseases such as cancer and rheumatoid arthritis. These ROS are responsible for the oxidation stress and as result, the damage of proteins and other harmful alterations can lead towards the death of cell. In chronic conditions, it can lead towards tissue damage as well[38]. Antioxidants are those substances that can hinder this process of oxidation. There are significant anti-oxidant activities if microbial EPS. The subunits known as monosaccharides has ketones and aldoses and acts as reducing sugars. There are various functional groups in bacterial EPS like carboxyl, hydroxyl, sulfhydryl, carbonyl, sulfate, acetyl, amide and thioether groups [68]. The anti-oxidant activity of EPS is usually due to these groups. These groups can release proton, donate an electron pair or can facilitate the process of binding in metals. Due to this character, conversion of free radicals into the stable substances takes place [69]. EPS hydrolysis can also be facilitated because these functional groups can facilitate the generation of acidic environment. Therefore, more antioxidant activity has been enhanced by the exposure of hemiacetal hydroxyl group [70]. This gives a sense about the production of antioxidants of high potency by chemically modifying them. Some influential and possible modifications in EPS may include sulfation, selenylation, phosphorylation, acetylation and carboxymethylation. The constituents of monosaccharides also effects the anti-oxidant properties of EPS [71]. It is observed that EPS shows more anti-oxidant

activities in the presence of naturally occurring monosaccharides like fucose, mannose, d-galactose, glucose, glucuronic acid and arabinose [72]. A detailed explanation of antioxidant activities of bacterial EPS is given in **Table 4**.

Table 4: Antioxidant activities of bacterial EPS

Bacterial strain producing EPS	Functional groups/ Constituent of monosaccharide	Concentration (mg/ml)	Molecular weight (kDa)	References
<i>Weissella cibaria</i> GA44	CH, OH, COOH and CO Glc and Rha	0.5-4	280	[94]
<i>Bacillus coagulans</i> RK-02	CH, OH, CO Glc, Man, Gal, glucosamine and fruc	50-500	30	[95]
<i>Lactobacillus gasseri</i> FR4	OH, CH ₂ , NH, C-O, C-O-C Man, Gal, Rha, Fruc	4	186	[96]
<i>Lactobacillus plantarum</i> BR2	OH, CH, CO Glc and Man	2-10	2380	[97]
<i>Lactobacillus plantarum</i> YML009	NR/NR	5-40	NR	[98]
<i>Lactobacillus plantarum</i> YW11	OH, CH, CO, NH, C-O-C Glc and Gal	High dose i.e. 50mg/Kg daily.	1.1x10 ⁵	[99]
<i>Lactobacillus helveticus</i> MB2-1 EPS-1, EPS-2 and EPS-3	OH, CH, CO, NH Gal, Glc, Man	0.125-4	2x10 ⁵	[100]
<i>Lactobacillus plantarum</i> YW32	OH, NH, CH, C-O-C Man, Fruc, Gal and Glc	5	1.03x10 ⁵	[99]
<i>Bacillus anthracis</i>	NH, CH, C-C, C-N, C-O-C/ Glc	0.2-5	1103	[101]
<i>Bifidobacterium animalis</i> RH	OH, CH, COOH Rha, Ara, Gal, Glc and Man	NR	21.3	[102]
<i>Lactobacillus plantarum</i> KX041	CH, NH, NH, COOH Ara, Glc, Gal, Man	0.2-5.6	38.67	[103]

NR: not reported.

7.4. Anti-microbial properties

Opportunistic microbes can grow and attack gut, but resistance is shown by gut commensals and one of the mechanisms of resistance is production of EPS. Many studies have shown resistance by using the mechanism of inhibition of bacteria and immunomodulatory activities [73]. All this is performed with the help of EPS production. EPS-Ca6 was isolated by LAB microbes, and it showed antibacterial resistance against important infection causing microbes such as *Salmonella enterica* and *Micrococcus luteus*. It created a zone of inhibition of 10 and 14mm respectively [74]. A heteropolysaccharide was isolated from *L. gasseri* and it showed antibacterial activity

against a wide range of pathogens. The EPS produced by *B. longum* can impair the cell division of microbes [75]. Some studies suggest that EPS can target the cell communication and can disrupt it by combining itself with receptor of glycocalyx and stops the production of biofilm. As a result, no biofilm formation takes place and infection can be controlled [8]. Microbes are responsible for a long list of human diseases. Anti-microbial of EPS properties can add positive contribution to control microbial diseases. Hence, health conditions could be improved.

7.5. Anti-cancer properties

Cancer is actually abnormal cells development and their uncontrolled division. This can results into the destruction of various tissues hence organs and can ultimately lead towards the death of the patient. One important type of all these cancer types is colon cancer, rate of death is increasing every year [76]. The radiation and chemo therapy has capability to kill cancer cells but along with cancer cells, they also kills the normal ones. EPS has considered better substitute of anti-cancer drugs due to their low cytotoxicity and less side effects. They perform some important mechanisms that make them able to fulfill the criteria to be called as anti-cancer.

- i. Prevention of tumorigenesis
- ii. Development of immunity
- iii. Induction of apoptosis of the cancerous cells. [77]

Anti-proliferating property in these polymers is due to the special characteristics in them like molecular weight and unique composition, and side chain linkages present in them. EPS G10 was isolated from *L. gasseri* and it inhibited the proliferation of HeLa cells [78]. A study found that human leukemia K562 proliferation was inhibited by an EPS produced by *Pseudoaltermonas* S-5. The EPS produced by *L. casei* was tested against HT-29 cell line and it showed the anti-proliferating results [79]. In the same way EPS produced from *L. plantarum* 70810 was tested against many cancerous cell lines and it showed anti-cancer activities in all lines. This is clearly suggesting the anti-cancer nature of EPS [79]. Cancer is a disease with high mortality rate. One of the reasons is high toxicity of anti-cancer drugs. EPS has low cytotoxicity and can be effective anti-cancer option in treatment of this lethal disorder.

8. APPLICATIONS OF EXOPOLYSACCHARIDES

Exopolysaccharides have a lot of physiochemical properties and possess economic nature in sense of mass production. Due to these important reasons, they have high demands in industrial zones.

8.1. Food industry

EPS has been used in food industry as emulsifiers, thickeners, and gelling agents for long time. Some other functions of these polymers include alteration in the rheology, moisture retention, biochemical food properties alteration for sensory properties, texture, odor,

mouthfeel and physical appearance, and induction of firmness [80]. LAB EPS has high demand due to their anti-microbial and antioxidant capabilities. Xanthan is used for oil recovery at large scale in early days, but since 1961, it has been used as food additive in USA after FOA approval [44]. Alginate has multiple functions such as gelling agent, thickener, emulsifier, and stabilizing agent. It also provides antimicrobial properties and long shelf-life after fruits and vegetable coating. It also has been used in packaging of coffee, tea and milk-based products [81]. Xanthan has been used in creams and suspensions to make them thicker and increasing their stability. In clinical studies, it has been proved as stable drug carrier and has promoted the controlled release of drug [82]. In pharmaceutical industries, it is being used as disintegrant agent in tablets and is being used as a part of microspheres, nanoparticles, liposomes and niosomes [83]. Some more important properties are being discussed in **Figure 8**.

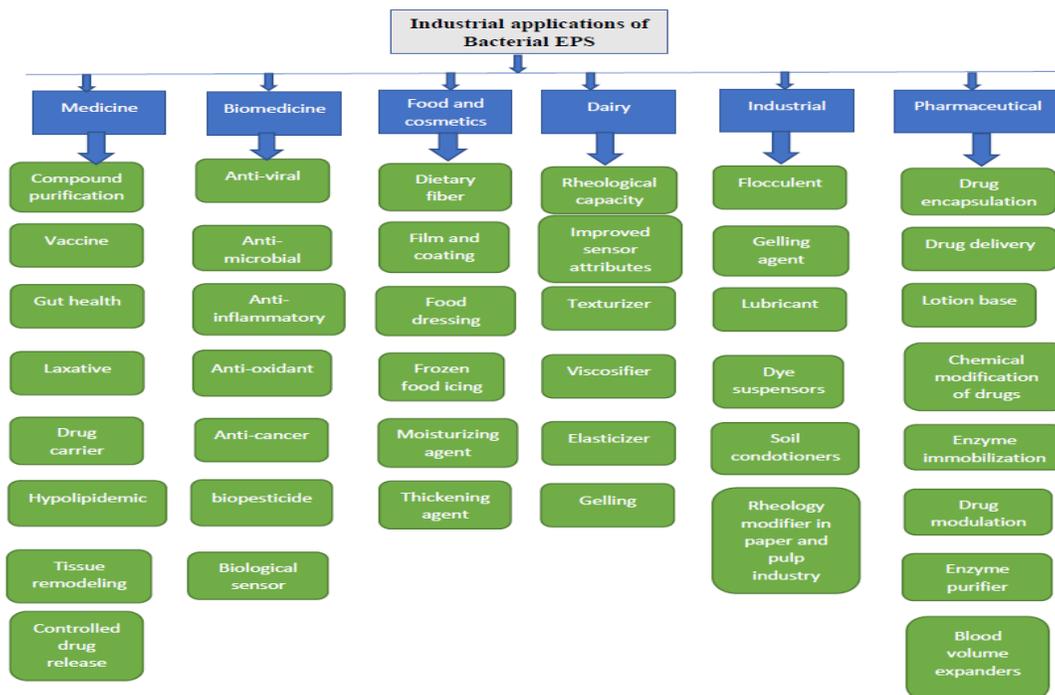


Figure 8: Applications of bacterial exopolysaccharides.

8.2. Pharmaceutical industry

Bacterial EPS has wide range of application in pharmaceutical industries. Xanthan is a polymer and has been used as a stabilizer and thickener agent in pharmaceutical industry like in suspensions and cream [83]. In some clinical studies, it has been proved as a stable drug realizing agent and drug stabilizer. The biofilms of alginate have been used as carrier agent in multi-vitamin preparations. Some other important applications include protein delivery system, hydrogels, wound dressing, oral dosage and many more [65].

Dextran is an integral part of plasma substitute; hence it has been used as boost in the patients of blood loss as it acts as enhancer of blood volume.

Calcium alginates convert exudates into gel. So, they are used in dressings of wound and provide moist environment of healing [31]. Gelatin is used in wound dressing, tablet and capsule formulation, substitute of blood volume and homeostatic sponges. Pullulan is important EPS polymer, and it is used in coating and granulation of tablets. It is specifically used in non-animal originating coating of capsule. Many more EPS has wide range of applications in pharmaceutical industry [32].

8.3. Agriculture industry

Empowerment of soil productivity and fertility are the major aspects of bacterial EPS application in agriculture. There are great applications of EPS in tissue culture in artificial production of seed [64]. They are good soil conditioners and bio-fertilizers. They protect seed from pathogens, desiccation, toxic metals, and anti-microbial agents. EPS exhibits cementing and water retention capabilities. It helps in flow of nutrients with help of water, biofilm formation, nutrients uptake and better aggregate formation of soil which help plant in growth enhancement [84]. EPS promotes the formation of nodules in roots of plant and help the total health of plant. They also help plant to bear the harsh environmental conditions in better way. Some studies have proved that EPS can act as bio-surfactants in the plant-soil environment. Xanthan gum can be used in drift control, soil improvement and encapsulation purpose. In aqueous system, it acts as rheological agent and act as stabilizing agent for the suspensions and emulsions. Xanthan has its application as fertilizer enhancer as well [85].

9. CONCLUSION

Due the large-scale functional diversity, bacterial EPS has enormous applications in medical and pharmaceutical industries. The major source of biologically active EPS is LAB and they have been used as probiotics for decades. The genomic study of various bacteria has been done so far and genetically modified strains of bacteria has been prepared with demand fulfilling ability to produce desired nature of EPS. But these bacteria are not enough for industrial purposes. Therefore, there is a need to produce more strains by using different metabolic and genetic engineering techniques. There is also demand of time to explore more information about the bioactivity of EPS by their application in in-vivo conditions. EPS can modulate the immune as well as mucosal immune responses. They are playing critical role in maintenance of gut barrier. Hence, the effects on intestinal mucosa need to be explored. As the involvement of EPS in biofilm forming, anti-biofilm strategies are going to be developed to stop bacterial EPS production. Gut health can be improved by utilizing EPS as probiotics. They are carbohydrate in nature and have high potential in improvement of human health. The chemical structure and composition of bacterial EPS is highly adjustable and which make them capable to be used in medical and pharmaceutical industries. Beyond the medical and pharmaceutical industry, EPS can be used in bio imaging and chemotherapy of

cancer. Usage of EPS can maintain the lost ability of normal flora in the body. On the other hand, encapsulations and nanoparticles of EPS are working as a practical tool for sustainable delivery of drug. Under the nutshell, these amazing polymers have potential for the future exploitation and commercial benefits of human beings. But adequate caution is necessary.

10. LIMITATIONS

This review article is focusing upon the classification of bacterial EPS, their production pathways and their various applications in food and pharmaceutical industries and agriculture. However, there are some limitations as well as the chemical compositions of bacterial EPS and their applications in some other fields have not been discussed. This review article will help the readers to enlighten more areas about bacterial EPS in their mind.

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