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A Review on Microbial Exopolysaccharide

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ABSTRACT: Slight-, moderate-, and extreme halophiles are microorganisms that live in athalassohaline (saline aquatic environment with ionic proportions quite different from the dissolved salts in water) settings such as marine estuaries, salty and soda lakes, inland sun salterns, and acidic ecosystems according to their sodium chloride (NaCl) requirements. Taxonomic research including culturable diversity. The analysis of halophiles indicated that they belong to Archaea and Bacteria, respectively, representing the Archaea and Bacteria phyla. Halobacteriaceae, Methanosarcinaceae, and Gammaproteobacteria. The majority of halophiles synthesis as an adaptation strategy against strong salt stressors. Extracellular polysaccharides (EPS) are a type of extracellular polysaccharide that has a wide range of properties as a result of their physical, chemical, and material characteristics. A number of potent halophilic strains have been discovered to produce abundant amounts of EPS through process optimization and metabolic management, showing commercial feasibility. Furthermore, the importance of halophilic EPS production, physico-chemical, and biological features, as well as the potential uses of halophilic EPS in industry and biotechnology, have been highlighted.

KEYWORD: Exopolysaccharide, Screening, Carbon sources, Antibiotic resistance

I. INTRODUCTION

Extracellular polymeric substances are natural polymers of high molecular weight secreted by microorganisms into their environment. EPS establishes the functional and structural integrity of biofilms, and is considered the fundamental component that determines the physicochemical properties of a biofilm.

The polysaccharide are therapeutic molecules as chemical and structural diversity in microbial ecology, medicine, dairy industry, biofilms, agricultural industry, cosmetics, nutraceuticals (is a product and other nutrition also used as medicine) etc [1]. The microbial exopolysaccharide due to their health benefits and they are being treated as highly potential molecule [2]. In the realm of biotechnology, EPSs are preferred over natural or synthetic polymers due to their efficiency, adaptability, and biodegradability [3,4,5]. EPSs that are stable under these circumstances. In high salt concentrations, an extraordinarily broad range of organisms can be found. It is possible to find halophilic bacterial communities. These halophiles, which are taxonomically varied, can have a variety of functional roles, including as sulphur oxidation, denitrification, and phosphate transport. Antimicrobial, antiviral, and antimicrobial, antiviral, and antimicrobial, antiviral, and antimicrobial, antiviral, and antiviral, antimicrobial, antiviral, and Its anti-inflammatory qualities [6]. Such halophilic EPS can function as a salt-tolerant surfactant and find widespread use in high salt oil deposits due to its highly viscous and pseudoplastic nature [6,2]. They are frequently utilised as jellifying agents, viscosifiers, and/or both as metal binders and emulsifying agents [3,6,2,4].

Microbial polysaccharide are generally classified in three classes :

1. Capsular polysaccharide such as K30 O Antigen
2. Intracellular storage polysaccharide such as glycogen
3. Exopolysaccharide such as dextran, xanthan, cellulose etc [7].

Prokaryotes and eukaryotes can produce exopolysaccharide [8]. Bacterial exopolysaccharide include dextran, alginate, xanthan, curdlan, cellulose, succinoglycan, glucuronan, colanic acid etc. bacterial producing exopolysaccharide are *Acetobacter* spp., *Rhizobium meliloti*, *Pseudomonas auroginosa*, *Lactobacillus helveticus*, *Lactobacillus rhamnosus*, *Xanthomonas* spp., *Shigella* spp., *Escherichia coli*, *Salmonella* spp., *Enterococcus* spp., etc [9].

The bacterial EPSs are identified by their molecular structure, chemical composition, average molecular weight, confirmation of single molecule and their assemblies in case of aggregation process and gel systems [10]. Concentrated solution, viscosity and viscoelasticity are determined by rheometry [11,12].

EPSs have large molecular weights ranging from 10 to 1000 KDa, and their composition can be homopolymeric or heteropolymeric¹. Carbohydrates and proteins make up the majority of EPSs, nucleotide, humic compounds and lipid also present[13,14].

II. SOURCE OF EPS

EPSs producing microbes are present in ecological niches. Effluents from the sugar, paper and food sectors as well as sewage treatment plant, include these microorganisms[15]. They can be found in hot springs on land and in the sea. EPSs are produced by a variety of bacteria as solitary slime in the environment or as connected capsular material[16].

III. MICRO-ORGANISM PRODUCING EPS

Microorganisms that produce EPSs include a variety of algae, fungus and bacteria that can be thermophilic, mesophilic or halophilic. Famous mesophilic bacteria include Lactic Acid Bacteria (LAB). Other mesophilic bacteria are *Escherichia spp.*, *Bacillus spp.*, *Pseudomonas spp.*, *Acetobacter spp.*, *Streptococcus spp.*, *Aureobasidium spp.*, *Escherichia spp.*, and *Lactobacillus spp.* *Sulfolobus*, *Thermococcus*, *archaeoglobus fulgidus* are the thermophilic archaeobacteria[17,18]. *Co-cultures of methanococcus jannaschii*, *Thermotoga maritima* and *Geobacillus tepidamans* V264 produce lots of EPSs[19] many halophilic Archaea produce EPSs, including *haloferax*, *Halococcus*, *Natronococcus*, *Haloarcula* and *Halobacterium*[17].

IV. CLASSIFICATION OF EPS

The microbial exopolysaccharide from lactic acid bacteria can be divided into two groups
I. HOMO POLYSACCHARIDE

Homo exopolysaccharides are chemical compounds that are composed of a single type of monosaccharide. They are composed of the same repeating unit and a single type of monosaccharide is involved in the formation. They have a single structure compared to hetero exopolysaccharide.

V. HETERO POLYSACCHARIDE

Hetero polysaccharides are polysaccharides made out of two or more different monosaccharides. They are composed of different repeating units and different types of monosaccharides are involved in the formation. They have a complex structure.

Hetero- and certain homopolysaccharides are produced in microbial cells and secreted into the extracellular environment. Although synthesis of homopolysaccharide occurs outside the cells after specific enzymes are secreted[20]. Homo- exopolysaccharides consist of a single type of monosaccharide such as α -D-glucans, β -D-glucans, fructans, and polygalactans (fig : 1), whereas, hetero exopolysaccharides are formed of different types of monosaccharides including D-glucose, D-galactose, L-rhamnose, and their derivatives[21]. Classification of bacterial exopolysaccharide (fig : 1)[22]. Homo exopolysaccharides are the chemical structures of a single type of monosaccharide, whereas, hetero-exopolysaccharide.

Homo exopolysaccharides are the chemical structures of a single type of monosaccharide, whereas, hetero-exopolysaccharides contain regular 3-8 different carbohydrate moieties synthesized from intracellular sugar nucleotide precursors[23]. Both homo and hetero-homo-exopolysaccharide have different production processes. The enzymatic interaction of glucanase or levanase with sucrose are used to synthesize homo-exopolysaccharide[24], hetero-exopolysaccharide synthesis completes in four major steps involving sugar transportation, sugar nucleotide synthesis, repeating unit synthesis, and polymerization of the repeating units[25,2]. Dextran and mutan, for example, are glucan generated by *L. mesenteroides* and *S. Cerevisiae*, are the most important family of lactic acid bacteria-derived exopolysaccharides. Mutan, respectively, but *s. cerevisiae* mutation fructans like levan[26,27].

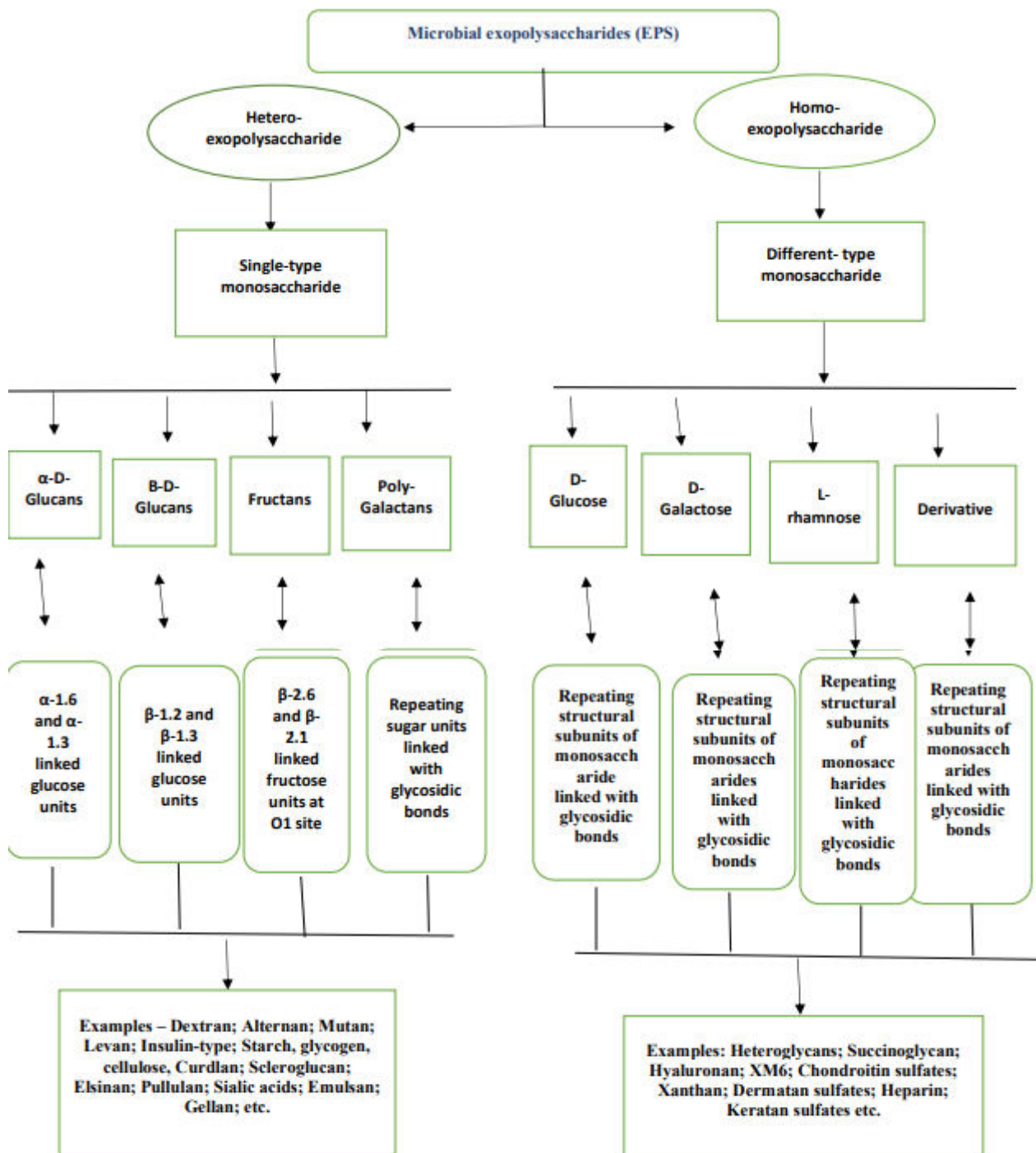


Figure : 1 classification of bacterial exopolysaccharide

V.CHEMICAL COMPOSITION AND CHEMICAL STRUCTURE

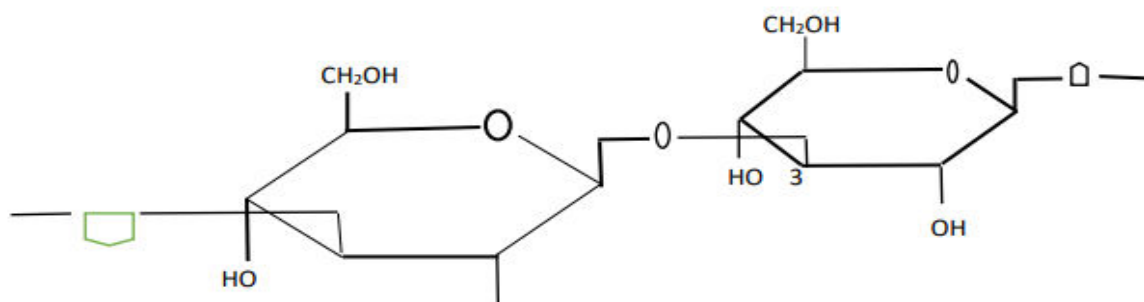


Figure : 2 structure of Curdlan

EPSs consist of carbohydrates and proteins but also contain DNA, lipids, humic substances, non-carbohydrate substituents, amino sugars such as N-acetyl amino sugars, neutral sugars and some uronic acids. Sugar residues, chain group elements such as phosphate and acetyl group, sugar based Repeating units are all detected by EPS chemical composition. Currently, Anion-exchange chromatography at high pressure utilized, together with electrochemical or Amperometric detection[28]. Capillary electrophoresis can also be used to characterize single carbs[29]. Curdlan is a linear beta-1,3-glucan with a high molecular weight that is insoluble in water. When heated in an aqueous suspension, curdlan, which is made of -(1,3)-linked glucose residues, transforms into elastic gels. *Alcaligenes faecalis* var was said to manufacture it.

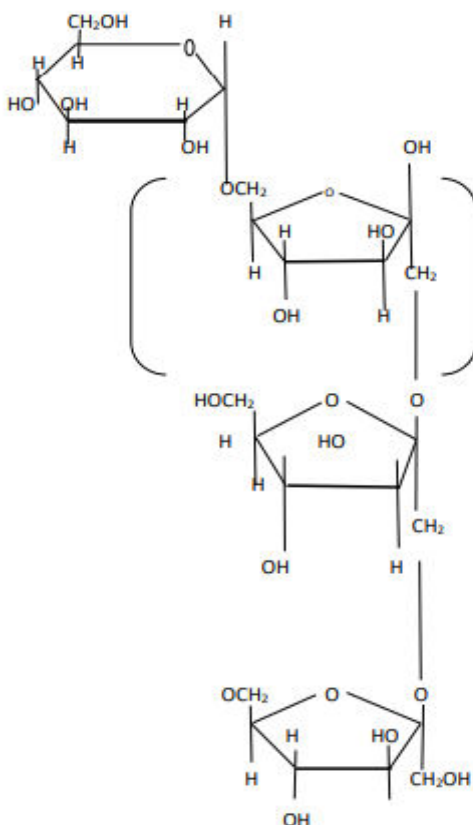


Figure : 3 structure of inulin

EPSs have intricate chemical structures ranging from linear to highly branched heteropolysaccharides because made up of variety of sugar-based monomeric components. monomeric bonding pattern this is accomplished by converting EPSs into incompletely methylated aldito acetates, which may be studied using GC-MS (Gas chromatography - mass spectrometry) Low molecular weight fragments are detected by GC-MS (Gas chromatography –mass spectrometry) and NMR (Nuclear magnetic resonance) spectroscopy . MALDI-TOF-MS(Matrixassisted laser desorption/ionization-time of flight mass spectrometry) is another option[30]. Figure 2 shows the structure of Curdlan, while Figure 3 shows the structure of inulin.

VI. MECHANISMS OF BIOSYNTHESIS OF EPS IN BACTERIA

VI.I. THE SYNTHESIS DEPENDENT PATHWAY

In this mechanism, complete polymer strand is secreted transversely to the cell membrane and the cell wall. Synthesizing proteins transport EPSs outside of the cell after polymerization. It's utilized in the manufacture of homopolymers.

VI.II THE EXTRACELLULAR PATHWAY

This process is governed by glycosyltransferases, covalently attached to the surface of the cell and EPSs are secreted outside the cell. EPS, such as dextran or levan is synthesized.

VI.III WZX/WZY-DEPENDENT PATHWAY

In repeating units are bonded to an undecaprenol diphosphate anchor (C55), organized by several glycosyltransferases and translocate across the cell membrane by a Wzx protein in the route at the inner membrane (flippase). Flippase responsible for polymerization in the periplasmic region[31,32].

VII. BIOLOGICAL ACTIVITIES OF EPS

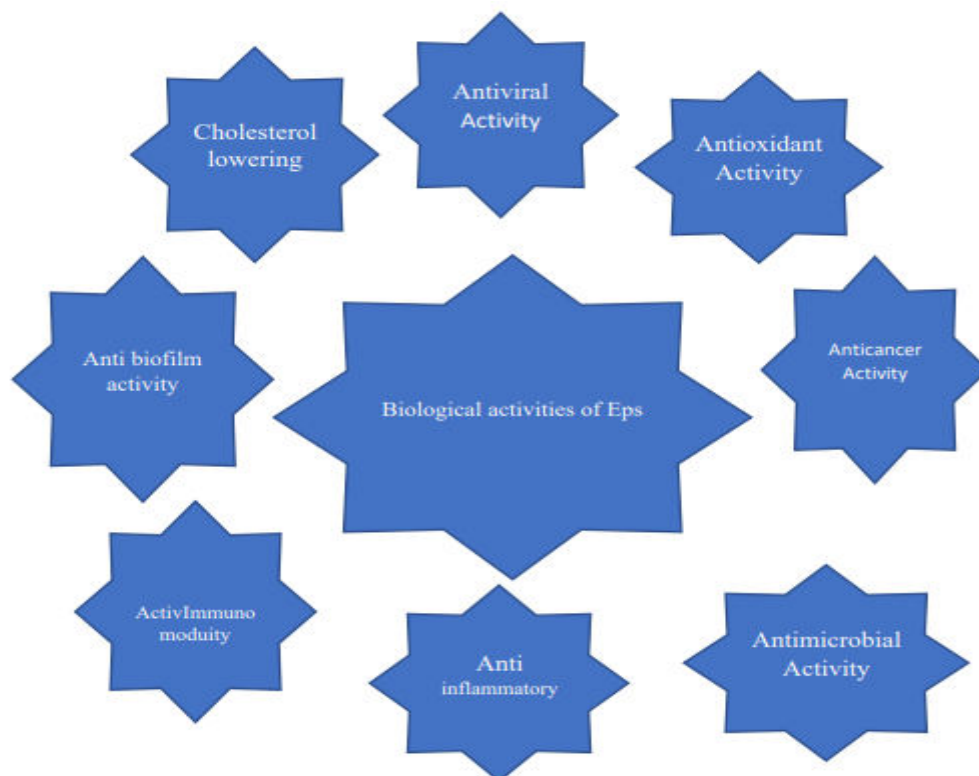


Figure: 4 biological activities of EPS

EPSs have been shown to significant health benefits, such as antioxidant properties[33] reducing cholesterol[34], antitumor[35], actions antiviral and immunomodulatory[36]. Various major disorders, such as Parkinson's disease atherosclerosis, cancer, and rheumatoid arthritis, are caused by reactive oxygen species(ROS), such as hydroxyl (OH), superoxide(O₂), and nitric oxide (NO). Parkinson's disease, atherosclerosis, cancer, and rheumatoid arthritis are few of the major disorders that can result. Antioxidant and free radical scavenging activities of EPSs have been demonstrated. The ability of EPSs to scavenging free radical can also be exploit to vegetables oil are protected against oxidation. show the exopolysaccharides (RPS) derived from rhizobium meliloti had antioxidant and free radical scavenging capabilities, as well as the potential of (RPS) to suppress lipid oxidation in sunflower oil emulsion during a 50-hour holding period at 60C. The EPSs of prebiotic Microorganisms have articular characteristics. The anti-ulcer effects of bifidobacteria, lactobacilli and Streptococci were investigated in rats using acetic acid-induced stomach ulcer and ethanol induced erosion models[37]. YIT4014 and 4043 bifidobacterium breve and Oral administration of bifidobacterium bifidum YIT4007 was found to have anti-ulcer properties for not just these bacteria but also other. But also the polysaccharide component of their bodies (PSFs). Rhamnose was the Main component of these anti-ulcer polysaccharides. Polysaccharides with higher Rhamnose Content, in particular, were more efficient in healing stomach ulcer. EPSs have been discovered to boost immune system and have anticancer properties. Polysaccharides from bifidobacterium adolescentis M101-4 was found to be beneficial in increasing the proliferation of mouse splenocytes by[35]. [38]extracted and assessed EPS from Lactobacillus rhamnosus RW-9595M in vitro. On mouse splenocytes from the C57BI/6 and BALB/c strains, as well as the murine RAW 264.7 cell line, putative modulating properties. EPSs stimulating macrophage-like cell lines and human peripheral blood mononuclear cell (PBMC), according to researchers. TNF, IL-6 and IL-12 can all be made from the cells mentioned above.

VIII. APPLICATION OF EPS

EPSs are necessary for biofilm development and cell adhesion to surface. EPSs makes up 50- 90 percent of a biofilm's total organic matter[13,14]. Biofilms are sometimes referred as microbial cities[39]. EPSs non-specific and specific host during the infection. they create antimicrobial resistance, provide production phagocytic protozoa, and cyanobacterial nitrogenase from the harmful effects of oxygen[1].A Polymer group of sulfated polysaccharides has been found to have a significant biological and functional characteristic[40]. These sulfated polysaccharide have been discovered to be strong antiviral drugs because they mimic pattern of sulfate substitution on glycosaminoglycan observe in cell membrane. Generally sulfated polysaccharides represent a complex polymer form of heterogeneous composition, Even in crude forms, a number of these Macromolecule have proved to have a powerful antiviral properties[40].

IX. BIOFILM FORMATION BY EPS

Bacteria residue in nature as colonies that form poly-bacterial aggregates such as mats, flocs, sludge, or biofilm, rather as planktonic scattered single cell in laboratory pure cultures[13,14]. Other microorganisms, in addition to bacteria, are involved in this quest. Our focus, however, is on bacteria and how their exopolysaccharides play a critical role. Furthermore, for clarity, we will refer to microbial aggregates that forms at the solid-liquid interface and are closed in matrix of highly hydrated extracellular biopolymers as biofilm. Despite the fact that this description does not include grouping of free people, flocs (floating microbial clumps) [1]. Biofilms have been dubbed“city of microorganisms”[39]and extracellular biopolymers, in which exopolysaccharides predominates, the “home of biofilm cells”[41]. The bacteria Pseudomonas auroginosa has been termed the “Escherichia Coli” of biofilm research since it has been studied the most. Microorganismplay an essential role in biofilm research[13,14].



IX.I.DISEASE ASSOCIATED WITH BACTERIALBIOFILM[1].

Table 1: Some human disease associated with bacteria biofilm

Human disease	Biofilm bacteria
Cystic fibrosis pneumonia	<i>P. aeruginosa and Burkholderia cepacia</i>
Otitis media	<i>Haemophilus influenzae (Non-typable strains)</i>
Periodontitis	<i>Gram negative anaerobic oral bacteria</i>
Dental caries	<i>Streptococcus spp. and other acidogenic Gram</i>
Musculoskeletal infections	<i>Staphylococci and other Gram-positive cocci</i>
Necrotizing fasciitis	<i>Group A streptococci</i>
Bacterial prostatitis	<i>E. coli and other Gram-negative bacteria</i>
Urinary catheter cystitis	<i>E. coli and other Gram-negative rods</i>
Biliary tract infection	<i>E. coli and other enteric bacteria</i>
Meloidosis	<i>Pseudomonas pseudomallei</i>

IX.II HEALTHBENEFITS OF EPS BIOFILM

Antimicrobial, immunomodulatory, anti-inflammatory, antioxidant, anti-viral, anti- tumour, Anti- diabetic , Anti- ulcer and cholesterol lowering effects have all been linked to EPS produced by probiotic bacteria.[42,43].

IX.III ANTIMICROBIAL ACTIVITY

according to several studies, LAB can create bacteriocins, diacetyl, organic acid, carbon dioxide, and other low molecular weights molecules like reuterin, reutericyclin, and antifungal peptides, among others[44,45]. Lactobacillus rhamnosus, an EPS-producing bacteria isolated from human. Under In-vitro testing, breast milk showed strong antibacterial activity against pathogenic E.coli and salmonella typhimurium[46].

IX.IV IMMUNOMODULATORYACTIVITY

various LAB and their extracellular polysaccharides have been discovered to modulate the host immune system by increasing T/B lymphocytes proliferation, natural killer (NK) cell tumoricidal activity, mononuclear cell phagocytic capacity, mitogenic activity, including cytokines and increasing the host immune defense against pathogen bacterium[47].

IX.V ANTI-INFLAMMATORY ACTIVITY:

Microorganisms causes inflammation by secreting the endotoxin lipopolysaccharide (LPS), which binds to toll-like receptor-4 (TLR-4) promotes the production of inflammatory genes[48], LPS is source of the production of mediators and cytokines including like IL-1 and IL-6, as well as tumour growth. TNF and anti-inflammatory cytokines like IL-10 are both suppressed when TNF- is present[49].

IX.VI ANTIOXIDANT ACTIVITY

Reactive oxygen species such as superoxide, hydroxyl radical, and hydrogen peroxide are formed during normal cellular metabolism and are linked serious health problems in human, including cancer , atherosclerosis, rheumatoid arthritis, and neurological illnesses[44,45]. Exopolysaccharides were isolated from a variety of probiotics. By analyzing their ability to degrade superoxide anion and hydrogen peroxide , they were assessed for their antioxidant potential. It is hoped to use them as a food supplement or in direct drug delivery because of their ability to reduce ROS and metal chelating activity[50].



IX.VII ANTICANCER ACTIVITY

The following are some of the proposed pathway by which EPS Exerts anti-cancer Activity: (1) carcinogenesis prevention, (2) cancer cell apoptosis induction, and (immunity improvement in the therapy of cancer, apoptosis, or programmed cell death , is required. Caspases dependent intrinsic and extrinsic mechanisms characterize it. The Inherent The extrinsic pathway is identified by the expression of caspase-3, caspase-9, BCL-2 and BAX, where intrinsic pathway is marked by the expression of caspase-3, caspase-9, BCL-2 and BAX. Caspase-8 and caspase-10 expression caspase caspase-8, caspase-9 and caspase-10 are caspase initiators. Caspase-3 is an effector caspase that is finally triggered by the Active caspase via attaching to particular proteins. Photolytic cleavage of inhibitors caspases[51].

IX.VIII HYPOCHOLESTROLEMICACTIVITY

The mechanisms behind cholesterol reduction by probiotic bacteria are follows: (i) cholesterol assimilation by growing bacterial cells, (ii) bile salt hydrolase deconjugation (iii) cholesterol precipitation with de-conjugation and (iv) cholesterol binding with cells[52,53]. Lactobacillus EPS is a type of EPS that comes from the bacteria Lactobacillus. RJF4 from plantarum was found to lower cholesterol by 42.24 percent[54].

IX.IX ANTI-VIRAL ACTIVITY

LAB has antiviral properties through a variety of pathway, including direct contact with viruses, the generation of viral inhibitory compound, and immune activation . The most crucial aspect is that even in crude from, sulfated polysaccharides are responsible for the antiviral Activity[55].

Table no: 2 Review of literature

Sr.no	AUTHOR	TITTLE	SUMMARY
1.	I.G.Nwoso, et al.,2019	Isolation, screening and characterization of Exopolysaccharide producing bacteria.	A study on the isolation and screening of exopoly saccharide frome soil and extreme environment.
2	Mu minah, et al., 2015	Isolation, screening and characterization of Exopolysaccharide producing bacteria from potato rhizosphere for soil Aggregation.	Explicated on the isolation of EPS producing isolates from various altitudes of different land slope and their production ability.
3	Muthuswamy Govarthanam, et al., 2015	Isolation exopolysaccharide producing heavy metal resistant Halomonas sp. MG	An article on isolation of Halomonas from ore taminated soil. Various technique employes such as X-ray diffractograms. SEM,FTIR for structural analysis of EPS.
4	Vivek k. Bajpai, et al	Exopolysaccharide and lactic acid bacteria: perception, functionality and prospects	The study on the EPS production abilities of lactic acid bacteria. It provides review about the properties, characterization and biological tailoring of EPS production.

5	K.V, Madhuri , K. vidya Prabhakar	Microbial exopolysaccharide biosynthesis and potential applications.	A review on various therapeutic applications of EPS producing lactic acid bacteria and microbial mechanisms for synthesis of EPS.
6	Madhuri K. V, Prabhakar K.V,	Recent trends in the characterization of microbial polysaccharides.	The study on detecting of biochemical properties of EPS and also the monomeric composition
7	Pawar,S.T, et al., 2014	Isolation,Screening,Optimization of exopolysaccharide's producing bacterium from saline soil	An article on isolation of EPS producing bacterium from saline soil,yield determination along with preferred carbon source.
8	Tejpreet Chadha 2014	Bacterial Biofilms: Survival Mechanisms and Antibiotic Resistance	An article on understanding intercellular communications within biofilms and the mechanisms of their formation.
9	Tiehua Zhang, et al., 2011	Growth and Exopolysaccharide production by Streptococcus thermophilus in skim milk.	Demonstrated the production potential of EPS from Streptococcus thermophilus with skim milk under varying concentrations, pH, carbon source.
10	J.Cerning, et al. 1994	Carbon source requirement for exopolysaccharide production by Lactobacillus casei CG11 and partial structure analysis of polymer	The work mainly focused on the EPS production from Lactobacillus casei using various carbon sources in the basal medium.

X. SYNTHESIS OF EXOPOLYSACCHARIDEPRODUCTION

X.I BIOSYNTHESIS OF EPS

The biosynthesis of EPSs occurs at many stages development ,depending on the environmental conditions and the organism produces them[26,27]. Extracellular enzyme produced by bacteria or bacteria themselves produce HoPSs. HePSs production is complicated and it takes places on the cell surface[34]. They're made from intracellular precursors that develop at the cytoplasmic membrane. The synthesis of EPSs involves a larger number of genes coding for enzymes and regulatory proteins which are not unique to EPSs production and secretion. Precursors for EPS biosynthesis are nucleotide diphosphate, which are intermediates in the central carbon metabolism begin the uptake of sugar from the surrounding medium[56]. In gram negative bacteria, undecaprenylphosphate serves as a lipid carrierfor EPS assembly. The cytoplasmic membrane is where the fundamental repeating units is assembled, and it involves[5]. the lipid-linked intermediates are usually translocated across the membrane and polymerized outside of the cell. Then, the EPS may be covalently linked to the cell surface to form a capsule, or released into the medium as slime. Undecaprenyl phosphate also involved in the manufacture of gram-positive bacteria. Four distinct reaction sequences

make up the biosynthesis pathway. These include sugar transport into cytoplasm, sugar – 1- phosphate production, sugar activation and coupling, the mechanisms involved in EPS export[2].

- The EPS producing bacteria were isolated from the soil sample following the serial dilution technique[57].
- The flask were inoculated for 3 days in rotary shaker at 120rpm at 30°C. Cell free supernatant (6000rpm, 10min) was precipitated overnight with the two volumes of cold ethanol at 4 °C[58].
- Precipitates of EPS were collected after centrifuging at 10,000 rpm for 20 minutes and later dried at 60⁰ C overnight in the hot air oven[63].
- Characterization of bacteria[26,27].
- MORPHOLOGICAL IDENTIFICATION :
- Gram Staining:
- The bacterial isolates were identified by the gram-staining method to determine the gram character according to the.
- Endospore Staining: Spore staining is carried out for the detection of endospores in the bacterial isolates according to[26,27].
- Biochemical test according to.
- Sugar Fermentation Tests: Sugar fermentation tests are used to discover bacterial isolated that fermenting sugar.
- Phenol-Sulphuric acid method used to determine the quantitative measurement of glucose concentration in the EPS precipitates[59].
- Various carbon source had different effects on bacterial isolates' EPS synthesis[26,27].
- The effect of different PH on the EPS production by bacterial isolates was detected[60].
- The effect of different temperature on the EPS production by bacterial isolates was detected[25].
- the multiple disc diffusion method was used to determine the in-vitro antibiotic susceptibility of EPS and Non-EPS isolates in vitro[61].

XI. CONCLUSION

EPS Producing bacteria isolated from the marine soil . Selected bacteria isolate screening for presence of EPS by alcohol precipitation and the dry weight determined. Bacteria isolate produce dry weight of EPS by quantitative method .to perform optimization Approach to produce carbohydrate content in the EPS Manufacture. The antibiotic susceptibility of EPS isolates and test clear zone , found to be resistance towards the antibiotics than the non- EPS isolates.

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