

Special Article: Phytoremediation

Pseudomonas Exopolysaccharides: A Game Changer for Attaining Environmental Sustainability

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Abstract

Exopolysaccharides (EPS) are the polymeric substance obtained from diverse species of the genera *Pseudomonas* for a variety of purposes. Recently, large focus have been given on isolation and characterization of EPS from diverse microorganisms employing various strategies, but very less attention have been given on the theme of harnessing EPS from the master microbe *Pseudomonas* and exploiting its multifaceted roles. Hence, the main essence of this review article is to emphasize protocols opted for EPS production, extraction and optimization, detailing its architecture, composition, mechanisms and genetic regulation. In the process it will also discuss various approaches used nowadays for novel usage of EPS bioformulations derived from *Pseudomonas* for plant growth promotion, stress amelioration, bioremediation and disease management in an eco-friendly and sustainable manner.

Keywords: Bioformulations; Biofilm; Biocontrol; Exopolysaccharides; Metabolites; *Pseudomonas*

Introduction

Polysaccharides commonly known as glycans are abundant in nature. These polysaccharides can be isolated from various living organisms including algae, plants, animals, fungi, protozoa and bacteria [75]. Nature of polysaccharide varies in terms of physical properties, chemical nature and biological attributes. Some bacterial cells are enclosed by a slimy polysaccharide layer, commonly known as the *glycocalyx*. Capsular polysaccharides are produced by those organisms in which glycocalyx are attached to the bacterial cell surface by covalent bonds. When polymer is very loosely bounded to the cell surface, it creates slime, and then it is referred as Exopolysaccharides (EPSs) [47,66]. The concept of EPS was first introduced by Sutherland in 2001. They are high-molecular-weight compounds, made from carbohydrate, proteins, DNA and some non-carbohydrate components including acetate, phosphate, pyruvate, succinate etc depending on the bacterial strains [23,101]. EPSs are broadly classified as homo-EPS and hetero-EPS. Homo-EPS are the one made up of only one mono-saccharide unit and hetero-EPS constitutes diverse mono-saccharides unit within it [114]. In nature, EPS is present in the form of capsular material in bacterial cell, and even obtained in the structure of slimes. Bacterial cells have multifaceted reasons for releasing EPS, some of them includes [1] maintaining cell growth under stress environment, [2] providing attachment during biofilm construction, [3] nutrient pool or carbon storage, [4] plant growth promotion and [5] biological control.

Hence, the main aim of this chapter is to highlight how this metabolite EPS can serve as a game changer in future for attaining environmental sustainability. The chapter will also focus on the multi-dynamic roles of EPS with reference to the genera *Pseudomonas*. In the process it will also discuss various strategies; protocols opted for EPS extraction, detailing its architecture, composition and genetic regulation. Novel usage of EPS bioformulations derived from *Pseudomonas* for plant growth stimulation and disease management has been also included (Figure 1). This chapter will especially help those researchers who are exploring the areas of EPS in conjugation with the genera *Pseudomonas* for developing multifaceted bioformulations in order to attain environmental sustainability.

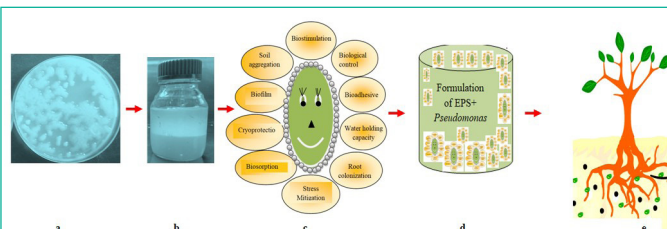


Figure 1: Plant growth stimulation and aggressive root colonization after applying EPS supplemented bioformulation.

a) Thick, slimy, or mucoid colonies of EPS producing bacteria on the growth medium; b) Extraction of EPS from bacteria, c) Diverse function performed by EPS; d) bioformulation developed from multifaceted EPS; e) Plant growth stimulation and aggressive root colonization after EPS amended bioformulation under stressed conditions.

Table 1: Diverse mechanisms adopted by EPS producing *Pseudomonas* strains for plant growth stimulation, biocontrol and bioremediation using different medium and culture conditions.

EPS producing <i>Pseudomonas</i>	Media for EPS extraction	Dry weight of EPS	Mechanism of EPS for plant growth /biocontrol/ bioremediation	References
<i>Pseudomonas</i> sp.	Mineral salt medium at 0.2-mPa	-	EPS creates a microenvironment around the bacterial cells, hold high amount of water, suggesting its moisture holding capacity and maintain bacterial survivability under high desiccation state.	[89]
<i>Pseudomonas aeruginosa</i> RB 28	Minimal medium supplemented with 2% crude oil	2.7 g/l after 60 h incubation	High emulsification activity of the extracted EPS shows the presence of high glycolipid and rhamnolipid. These biosurfactants increased cell surface hydrophobicity that helped in biodegradation of crude oil.	[97]
<i>Pseudomonas putida</i> GAP-P45,	Trypticase soya broth supplemented with 25% polyethylene glycol	4.06 mg/ mg protein under minimum water potential (−0.73 Mpa)	Strain displayed high drought tolerance by raising its cell number, increasing RAS/RT ratio, strengthening root colonization and soil aggregation. GAP-P45 produced biofilm like communities, where bacteria were connected together by an EPS matrix with micro colony formation under water stress condition and enhanced sunflower growth	[94]
<i>P.aeruginosa</i> PF23	Davis Minimal Medium supplemented with 2000 mM NaCl	1.323 g/l at 2000 mM NaCl	Saline tolerant EPS producing strain PF23 displayed multifaceted role of plant growth promotion, disease management and stress amelioration under non saline and saline conditions with sunflower crop	[103]
<i>P.aeruginosa</i> PF07	Davis minimal medium supplemented with 0-1600 mM NaCl	1.298 g/l EPS observed at 1600 mM NaCl	Bioformulation enhanced early seed germination, plant growth parameters, raising seed weight and ameliorating stress in saline affected regions by embracing RAS/RT, enhancing porosity, boosting nutrients uptake in sunflower crop	[104]
<i>Pseudomonas</i>	Mineral salt medium	-	EPS improved soil moisture contents, plant biomass, root and shoot length, and leaf area of maize Under drought stress, the inoculated plants showed increase in relative water content, protein, and sugar.	[79]
<i>Pseudomonas</i> sp. AK-1	Nutrient Broth supplemented with NaCl	5.3 g/l at 500 mM NaCl	Strain AK-1 was able to bind free Na ⁺ from the soil, making Na ⁺ unavailable to soybean roots under saline (200 mM NaCl) conditions. EPS binds with Na ⁺ and reduces salinity stress in the soil and reduced electrical conductivity of soil from 1.1 to 0.9dS/m on soybean crop	[55]
<i>Pseudomonas</i> psd	Gluconate minimal medium with and without ZnSO ₄ supplementation.	0.6 mg/mL of alginates after 5-days of incubation without Zn ²⁺ , In the presence of Zn ²⁺ , up to 3-fold increase was recorded in the amount of alginate produced	Zinc supplementation enhanced EPS production, siderophore production, phenazine-1-carboxylic acid synthesis, phenazine production displaying biofilm formation and aggressive root colonization that helped in the mungbean plant for growth stimulation	[108]
<i>Pseudomonas fluorescens</i> DR7+ <i>P.fluorescens</i> DR11+ <i>Enterobacter hormaechei</i> DR16+ <i>Pseudomonas migu-lae</i> DR35	RCV-sucrose medium supplemented with 40g/l sucrose	<i>P.fluorescens</i> DR7 produced 11.63 mg/ mg protein, DR16 displayed 5.44 mg/ mg protein, DR35 showed 3.33 mg/mg protein (−1.03 MPa)	Multiple PGP traits (ACC, EPS, IAA, Phosphate solubilization) displayed by the organisms DR7, DR11, DR16, and DR35 increased the percentage of foxtail millet seeds germinating under drought stress conditions between −0.30 MPa and −1.03 MPa Such traits improved plant growth promoting activity, possibly by improving soil structure, soil texture and raising the number of root colonizing microbes around the root surface under drought stress conditions in Fox tail millet	[81]
<i>P.fluorescence</i> PF17 EPS+salicylic acid	Davis minimal medium supplemented with 600 mM NaCl	0.9 g/l at 500 mM NaCl	Combinational effect of EPS+SA helped in stress amelioration, plant stimulation and biocontrol, as stimulation of EPS enhanced SA production, augmenting seed germination thereby proving them to be biopriming agents on sunflower seeds	[105]
<i>Pseudomonas</i> sp. PFAB4	King's medium B base	2.63 g/l	Thermotolerant EPS was mixed with AgNO ₃ in 1:1 ratio and this combination displayed effective killing against target bacterial and fungal pathogen The non-porous, compact nature of the EPS biopolymer is useful for several industrial applications; most likely of which is bioplastic production.	[10]
<i>Pseudomonas entomophila</i> PE3	Nutrient broth medium supplemented with NaCl (0-8% NaCl)	EPS production of 12.5 g/l was observed at 2% NaCl	Enhanced EPS production during salinity stress (2% NaCl) resulted in increased antioxidant potential, hydroxyl scavenging activity, antioxidant activity and osmolyte accumulation. Excessive root colonization raised sunflower growth	[31]
<i>P.aeruginosa</i> MTCC 1688	Nutrient broth	26 mg/50 ml after 96h of incubation at pH 6 and 32°C temperature	EPS helped in sequestration of heavy metals. The extracted EPS has been applied for removal of Ni (II) and Cr (VI) ions from aqueous system by biosorption.	[22]

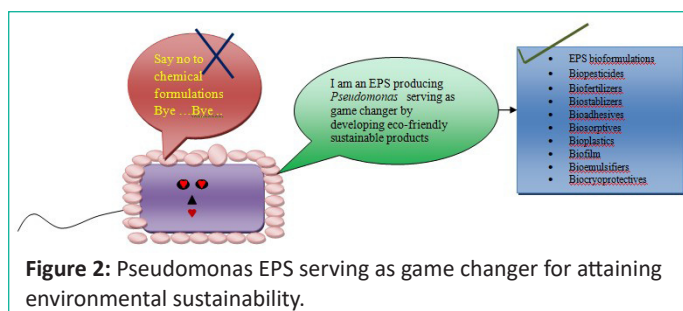


Figure 2: *Pseudomonas* EPS serving as game changer for attaining environmental sustainability.

Pseudomonas: Versatile Bacteria

The genus *Pseudomonas* includes the most ingenious and ecologically remarkable group of bacteria on the planet earth. *Pseudomonas*, belongs to the class gamma (γ) Proteobacteria. At present, there are more than ten genera of Pseudomonadales that includes, *Burkholderia*, *Caulobacter*, *Ralstonia*, *Sphingomonas*, *Stenotrophomonas*, and *Xanthomonas* [21]. The family Pseudomonadaceae, contain the foremost genera *Pseudomonas*, that are principally gram-negative, rod-shaped, straight and slightly curved shaped bacterial cells with polar flagella [21]. They are generally aerobes or facultative anaerobes, positive for catalase and oxidase test, negative for vogues proskauer, indole and methyl red tests. Advance methods for identifying the genus *Pseudomonas* have been done on the basis of 16S rRNA sequencing technique, with universal primers like, forward primer Ps-for (20-mer [5'-GGTCTGAGAGGATGATCAGT-3']) and reverse primer Ps-rev (18-mer [5'-TTAGCTCCACCTCGCGGC-3']) (Widmer 1998). Diverse members of the genera *Pseudomonas*, are established in majority of the natural environments including freshwater, marine, terrestrial, extreme habitats etc. Some of the *Pseudomonas* species can form close intimate relations with plants and animals [91]. Diverse *Pseudomonas* species including *Pseudomonas alcaligenes*, *Pseudomonas chlororopsis*, *Pseudomonas dimunita*, *Pseudomonas elongata* *Pseudomonas fluorescense*, *Pseudomonas putida*, *Pseudomonas syringae* etc are comprehensively used in the area of agricultural and environmental microbiology for its various applications.

A number of major *Pseudomonas* applications includes: plant growth elongation, biological control, biofilm formation, antimicrobial metabolite production, quorum sensing, plant microbe synergistic interaction, chemotactic interaction, uptake, and catabolism of various plant secretions [92]. There are many reviews and book chapters available on the subjected cited above [41], but the role of *Pseudomonas* in EPS production and exploiting its novel applications is reported rarely. Generally various species of Rhizobia are regarded as EPS producers and much work has been cited on this topic [105,106]. But, throwing light on the role of EPS with reference to *Pseudomonas* is a new thought that needs to be explored.

EPS Production in Pseudomonas

Bacteria that produce EPS are usually characterized by thick, slimy, or mucoid colonies on the growth medium (Figure 1a). The concentration, amount and composition of EPS may vary within bacterial genera, species and strain [100]. Different *Pseudomonas* isolates obtained from the potato rhizosphere showed 1.75 to 2.24 mg/ml of EPS in the medium [100]. For producing large amount of EPS, bacteria desperately require energy from miscellaneous carbon sources. Hence, the type of carbon source utilized in the medium is an essential element that determines the quantity, composition and extent of EPS production. The EPS production is not solely dependent on carbon sources, but other factors including carbon/nitrogen ratio, genotype of mi-

crobe, phase of the microbial growth, salts used (NaCl , CaCl_2), culture conditions (pH, temperature), and incubation time also impacts synthesis and production of EPS [95]. Composition of EPS varies in terms of carbohydrates, lipids, proteins, nucleic acids, humic substances etc depending on the strains. Different functional groups are embellished in the matrix of EPS such as amino group, amides, carboxylic group, hydroxyl group, phosphate etc that constitutes an essential component of EPS [22]. Bouchotroch et al. (2000) isolated 32 unusual *Halomonas* isolates from the salinized environment; authors also stated that all the diverse isolates showed variation in EPS productivity, chemical composition and physical properties, even under same environmental conditions.

In laboratory, EPS production is recorded maximum during the stationary phase [27,69], but there are few species of *Pseudomonas*, where the high EPS rate is observed during exponential phase. Bacterial genera including *Alteromonas* obtained from deep-sea hydrothermal vent showed high EPS production under nitrogen limited stationary phase [32]. The study was conducted by Nevot et al. (2006), where the worker isolated *Pseudoalteromonas antarctica* glacial marine sludge of South Shetland Islands. *P. antarctica* displayed, high EPS production during exponential phase. When *Pseudomonas aeruginosa* MTCC 1688 was inoculated in nutrient broth (pH 6) at 32°C for 4 days, maximum EPS production was monitored of about 26 mg/50 ml after 96 h of incubation [22]. Lower incubation temperature assist in manufacturing EPS by dipping *P. aeruginosa* growth and raising the accessibility of precursor for EPS synthesis. Production of EPS in case of *P. aeruginosa* was recorded significantly higher, when, there is an increase in incubation of bacterial cells under starved conditions. Under nutrient limitation, glucose is dominant in the EPS and glucosyl units are detected in the EPS matrix of *P. aeruginosa* cells [76]. Table 1 elucidate diverse culture conditions, growth medium, required by the bacteria for enhancing EPS production in *Pseudomonas* sp.

Diverse Methods for Extracting Pseudomonas Exopolysaccharids

There are different methods and protocols employed for extracting and quantifying EPS form various species of *Pseudomonas*. Protocol for isolating EPS in *P. fluorescens* Biovar II, (ATCC 55421) was given by Hung et al. (2005). Total carbohydrates in the EPS of *P. fluorescens* Biovar II, was analyzed as per the modified protocol of Hung and Santschi (2001). Different sugars present in EPS consisted of arabinose, fucose, galactose, glucose, mannose, rhamnose, ribose, and xylose [49].

Titus et al. (1995) isolated EPS from marine bacterium *P. alcaligenes* using basal salt medium, EPS of diverse quantity was examined under different phases of growth medium. Slight variation in the concentration of C and N sources brought major change in amount and composition of EPS. Copious amount of EPS production was observed on active surfaces including copper, rather than on glass and titanium. EPS which promotes bacterial adhesion on immersed surfaces contained 19.8%, 16.8%, 3.5% and 0.7% of carbohydrates, proteins, uronic acids and sulphate respectively [107]. Amount of pyruvate was analyzed in traces and 52.5% of inorganic matter was also recorded in the EPS of *P. alcaligenes*.

Forde and Fitzgerald (1999) described the method for isolating EPS from *P. aeruginosa* ATCC 10145, in which amount of EPS production varied as per incubation time and nutrient availability. After 96 h and 120 h of incubation, the amount of

EPS was recorded to be $5 \mu\text{g}/10^9 \text{ CFU}$ and $18 \mu\text{g}/10^9 \text{ CFU}$ respectively. Quantification of total EPS from *Paeruginosa* ATCC 10145 was done by acid hydrolysis as per the protocol of Parkar et al. (2001). The quantified data was analyzed and it revealed different monomeric constituents within it including arabinose, fructose, glucose and galactose. The amount of these sugars showed variation in their values at 96 h and 120 h of incubation ranging from 0.17 to 6.60 % [76]. The mutational studies was conducted by Tewari and Arora (2014) on saline tolerant *Paeruginosa* PF23, that displayed high EPS production under salinity stress conditions using using 5 bromouracil. It was observed that wild strain (PF23 ^{EPS+}) was EPS producer, whereas, mutant strain (PF23 ^{EPS-}) was defective in EPS production. Tewari and Arora (2014) conducted study on Davis minimal medium, and it was observed strain PF23 ^{EPS+} showed high salinity tolerance upto 2000 mM NaCl concentration with 1.323 g/l of EPS production. With progressive increase in salinity from 100 to 2000 mM NaCl, there was significant enhancement in EPS production. Nearly, 66% increase in EPS was observed at 2000 mM NaCl concentration in comparison to non-saline conditions (0 mM salt). EPS defective mutant (PF23 ^{EPS-}) showed nearly, 87% reduction in EPS in comparison to wild strain (PF23 ^{EPS+}). Composition of EPS also showed variation in terms of raising salt concentration, for example at 0 mM NaCl, only glucose was present, whereas, progressive raise in salinity resulted in glucose, galactose and trehalose as its sugar components [103]. Sandhya et al. (2009) also reported the isolation and quantification of EPS from *P. putida* GAP-P45 by changing the amount of poly ethylene glycol in the trypticase soy broth medium. Authors reported high amount of EPS of about 4.06 mg mg⁻¹ protein at water stress conditions (-0.73 Mpa), and this EPS producing strain was effectively used under drought stress for raising sunflower productivity. Characterization of EPS by TLC displayed presence of glucose, mannose, and rhamnose as its constituents.

Kasotia et al. (2016) performed EPS extraction using *Pseudomonas* sp AK-1 taking nutrient broth medium supplemented with 0 to 500 mM NaCl concentration. In absence of salt, strain AK-1 displayed 0.32 g/100 ml dry wt of EPS, that raised to 0.53g/100 ml at 500 mM NaCl. While performing experiments on two different species of *Pseudomonas* viz; *P. aeruginosa* G1 and *P. putida* G12, Celik et al. (2008) observed 3% and 2% xylose respectively as preferential carbon source for obtaining high EPS production of about 368 mg l⁻¹ and 262 mg l⁻¹ respectively. *P. aeruginosa* resistant to chromium was found to produce 863 mg l⁻¹ of EPS [57]. Study was conducted by Upadhyay et al. (2017), in which workers have isolated EPS from Fluorescent *Pseudomonas* strain Psd, on Gluconate Minimal Medium (GMM) in two sets with and without ZnSO₄.7H₂O supplementation. EPS was extracted from strain Psd, using the protocol of Bitton and Freihofer (1977). High EPS production was obtained in zinc supplemented medium rather than other one. Freitas (2009) used glycerol rich products and pure glycerol as carbon source for the EPS production in *P. oleovorans* NRRL B-14682. Amount of EPS, obtained was approximately 11.82 g l⁻¹ and 12.18 g l⁻¹, when pure glycerol and glycerol rich products respectively, were used as carbon source, in the medium at 30°C and pH 6.8. *P. oleovorans* displayed heteropolysaccharide EPS, consisting of 37-80% galactose, 2-30% glucose, 0.5-25% mannose, 0.5-20% rhamnose and acyl group substituent's like acetyl, succinyl and pyruvil. As per the findings of Dimitrijević (2011), EPS extracted from extremophilic *P. aeruginosa*, documented alginate type EPS production of 36.5 mg l⁻¹ in LB broth after supplementation

of sunflower oil and tween 80. Several workers reported that mixing sunflower oil and detergents into the medium raised EPS production in *Pseudomonas* [62]. There is a proposed mechanism by which surfactants improve the EPS production. It may be due to the fact that surfactant molecules cooperate with the cell membrane in such a way that it would augment the polymerization process and thereby assist in the discharge of the polymer from the membrane [7]. Ali et al. (2020) documented elevated EPS from a psychotropic bacterium *Pseudomonas* sp. BGI-2 purified from the Batura glacier of Pakistan. EPS assembly was enhanced by changing the amount of different nutritional and physiological conditions. Glucose, galactose, glycerol, mannose and mannitol, were the preferable carbon sources that were used by this bacteria. Molasses (waste of sugar industry), was used as a major substrate for the bacterial growth and EPS production. Strain displayed high EPS production of 2.01g l⁻¹, when following conditions were maintained in growth medium, viz: glucose (100g l⁻¹), yeast extract (10g l⁻¹), NaCl (10g l⁻¹) and C/N ratio (10/1), pH 6 and temperature 15°C. Diverse sugar components present in EPS were glucose, galactose, and glucosamine that were detected using high-performance anion-exchange chromatography with pulsed amperometric detection technique.

Extracting EPS from Waste

It could be seen from various examples cited above that to obtain good quantity of EPS majorly good quality of carbon sources are required, that make the process of EPS extraction a bit expensive. Hence, it is utmost important to optimize the culture conditions for extracting high amount of EPS, using cheap nutrient sources or agrowaste materials. Different agro food industries are excreting hazardous waste materials that are toxic to the environment; hence, proper strategies should be opted for disposing agrowaste (Kanimozhi et al. 2018). Naturally these agro-wastes are allowed to decay in the fields. These waste are too polymeric and fibrous, to be digested by monogastric animals, so they are not suitable as animal food and fodder. Agro wastes are sparingly soluble in water containing upto 70% carbohydrates that can be effortlessly used by microbes as their energy source [112] (Sadh et al 2018). Agro waste material display dual role, firstly, they can be used as carbon source by the microbes to derive energy and secondly, they can be used to obtain EPS as by-products. As per the findings of Li P et al. (2016) *Xanthomonas campestris* can convert kitchen waste into xanthan gum. Similarly, agricultural waste could be converted to pullulan that is produced from *Aureobasidium pullulans* [96]. Kumar et al. (2022) observed that the *Pseudomonas* strain BGI-2 used crude glycerol (waste product of biodiesel industry) as a carbon source. To cut down the cost of EPS production, several workers examined the usage of citrus fruits, mango peel, milk whey, pineapple waste, cabbage, spoiled guava, beet molasses, olive mill wastewater, sweet lime, potato starch waste, date syrup, rotting tropical fruits and mixed fruit waste for EPS production [109,121]. Though, these substrates have been tried and tested to extract EPS from diverse bacterial genera, more focus should be given in using them with particular reference to the genera *Pseudomonas* for EPS extraction.

Genetic Regulation of EPS

Basic requirement for EPS synthesis is monosaccharide units that are obtained from catabolized sugars including nucleoside diphosphate sugars, Uridine Diphosphate (UDP)-N-acetylglucosamine and Guanosine Diphosphate (GDP)-mannuronic acid [9]. Plasmid or chromosomal DNA is the sites that target EPS

gene synthesis in the bacterium. These genes are organized in the form of gene clusters. It has been observed that more than one gene cluster is present in a bacteria, that participates in EPS synthesis [59]. The gene cluster involved in EPS synthesis contain numerous functional genes, with desired properties such as gene assembly, determining chain length, gene exportation, gene polymerization, and gene regulation (De Vuyst et al. 1999). On the basis of detailed analysis of EPS biosynthetic and exportation mechanisms, three main pathways have been elucidated. First pathway is involved, in catalyzing both polymerization and exportation [115]. In the second pathway, ATP-binding cassette transporter plays an important role in exporting repeating biosynthesized unit. In the third pathway, Wzx-Wzy proteins are involved [117]. Wzy protein helps in polymerization and assist in elongating growing EPS chain whereas, final translocation is done by Wza [87,115]. Repeating unit is catalyzed by glycosyltransferase, and exported outside the cell across the inner membrane by Wzx protein [87,115]. Bacterial tyrosine kinases are coupled with EPS biosynthesis in bacteria [30]. Genes encoding bacterial tyrosine kinases are often associated with genes involved in EPS production. The kinases are known to regulate EPS production by phosphorylation and thereby, activating a biosynthetic enzyme in the pathway of EPS production [71]. Several workers reported that three major types of EPS are secreted by *Pseudomonas* sp. They are alg, Psl and Pel [36,43,67]. Alg for alginate synthesis, Psl constitutes galactose and mannose-rich polysaccharide that helps in the primary attachment, biofilm and micro colonies formation [67]. Pel, is a glucose rich cellulosic polymer necessary for pellicle development at air-liquid interface [39]. Divyashree et al. (2022) mentioned prevalence of top 5 gene for EPS synthesis including gene pslB (71.21%), gene pslA (57.57%), gene algD (43.93%), gene pelA (45.45%), and pelD (27.27%), respectively, in *P. aeruginosa*. Kamali et al. (2020) reported some interesting results in which 85% of *P.aeruginosa* isolates displayed algD, pslD, and pelF genes. The genes related with biofilm formation pslA (83.7%) and pelA (45.2%) respectively, were also reported by Ghadaksaz et al. (2015) in *Pseudomonas. P. putida* KT2440, contain four important genes, they are alg (alginate), bcs (cellulose), peb (biofilm stabilization), pea (*putida* EPS A), important for forming biofilm, carbohydrate processing, polysaccharide synthesis and transport respectively. *Pseudomonas entomophila* L48 exhibited the presence of pea and alg locus and alginate biosynthesis loci [113].

Alginates are linear EPS consisting of β -1,4-linked-D-mannuronic acid and α -L-glucuronic acid [44]. Alginate is a form of EPS secreted by various types of *Pseudomonas* sp. The three main gene clusters responsible for producing alginate are PA1381-1393, PA2231-2245, and PA3552-3558 [70]. In *P. aeruginosa* PAO1, gene cluster PA2231-2245 (pslA to -O) plays an imperative function in biofilm formation. 18-gene cluster are required for synthesizing EPS 273 in marine bacterium *P. stutzeri*, if any of the gene is deleted from this gene cluster it may affect EPS synthesis. Divyashree et al. (2022) observed 66 different isolates of *P. aeruginosa*, obtained from hospital waste.

Franklin et al. (1994) reported that in *P.aeruginosa* 13 proteins are directly linked with alginate synthesis (except AlgC), and encoded by the *alg* operon. 3 main enzymes are involved in synthesizing alginate precursors including guanosine 5'-diphospho-D-mannose pyrophosphorylase (Alg A), phosphomannomutase (Alg C) and GDP-mannose dehydrogenase (Alg D). These enzymes catalyze the conversion of fructose-6-phosphate to GDP-mannuronic acid via four steps: primarily, alginate is syn-

thesized as a linear homopolymer from the GDP-mannuronic acid to polymannuronic acid by catalytic subunit alpha-1,3-glucosyltransferase (Alg8). Later, it interacts with Alg44 polymerase positioned at the cytoplasmic membrane, Thirdly, enzymes allow the movement of the alginate precursor across the inner membrane for polymerization, lastly, Alg44 also demonstrated the capability of binding the second messenger cyclic dimeric guanosine monophosphate (c-di-GMP) synthesized by MucR, a membrane-anchored protein, for synthesizing alginate [45,68].

The cellulose EPS has been described as a key component in the development of *P. syringae* pv. *syringae* UMAF0158 biofilm [46]. Strain UMAF0158 contains two additional genomic regions that putatively encode for alginate and a Psl-like polysaccharide. The Psl-like polysaccharide of UMAF0158 play an important role in virulence similar to that has been described for cellulose, also they are involved in surface colonization in case of *P. aeruginosa*. The significant role of Psl EPS in biofilm formation, plant colonization, plant microbe interaction and virulence, is well documented in case of the *P. syringae*. Since, the genomic region that encodes Psl is very well conserved. The strong relation is observed in between the production of Psl EPS and swarming motility. Direct relationship in between the two suggests the connection between the expression of psl gene and rhamnolipid genes (Heredia-Ponce et al. 2022).

EPS: A Game Changer for Environmental Sustainability

EPS are natural, non-toxic bioproducts with diverse biological activities. These natural biopolymers are serving as game changers for attaining environmental sustainability. As these polymers are pure, green and eco-friendly, so they can serve as a chemical free source for designing bioformulations including biopesticides, biofertilizers, biostabilizers, bioadhesives, biosorptives and bioplastics (Figure 2). Various industries produces non-degradable plastics, that are extreme threat to the natural resources, mostly affecting the aquatic water bodies [77]. Usage of microbial EPS based bioplastics can serve as a next generation eco-friendly, and sustainable approach. Keshavarz and Roy (2010) reported the superiority of biological polymers in comparison to petro chemically derived polymers, in terms of biocompatibility, biodegradability, environmental and human compatibility. Recently the findings of Tewari and Sharma (2020) documented the role of microbial derived EPS as biostimulant, thereby serving as an alternative to replace the usage of hazardous agrochemicals, and safeguarding the survival and productivity of very important pulse crop *Arhar* in marginalized saline lands of India. Similar biostimulatory efficiency of *Pseudomonas* derived EPS was even proved by Fatimah and Arora (2021) and, its efficacy was tested on oilseed crop sunflower. Supplementation of pure bacterial EPS or in combination with *Pseudomonas* cells can provide a sustainable solution for bioremediation of salinized soil, augmenting plant productivity and disease management [103,104]. Authors also reported the usage of combinational bioformulation developed from *Pseudomonas* + EPS can work effectively as a potent biological control against *Macrophomina phaseolina* and a potent stress ameliorator protecting sunflower plants against salinity stress conditions [103-105]. Nutrient imbalance under salinity stress could be mitigated, by utilizing *Pseudomonas* EPS, as it promotes massive root colonization and assist in augmenting plant growth and development under osmotic stress conditions [104]. EPS producing *Pseudomonas* are the potent biofilm producers, and, helps in naturally creating slimy microbial aggregation rather than chemical films [43]. Nowadays, there is a

massive interest in the usage of microbial derived EPS, at industrial level. EPS from psychrophilic bacteria *Pseudoalteromonas* sp KCTC 12867BP showed, excellent cryoprotective, membrane stabilizing and non cytotoxic nature (Roca et al. 2016). Hence, this EPS can be used as a substitute towards conventional cryoprotective agents that are cytotoxic and hazardous at high concentration to humans and animals. Certain bacterial species have been used commercially for exploiting various EPS such as alginate, xanthan, gellan, and dextran due to its gluing and bio-adhesive nature. These microbial products could be used in future as a potent substitute over hazardous chemical adhesives [38]. Bioemulsifiers developed from *Pseudomonas* are more ecofriendly over synthetic emulsifiers (heptadecane) as they are biodegradable and non- carcinogens (Caruso et al. 2018). Another important feature of *Pseudomonas* EPS is its biosorption capacity that could be used to clean up the toxic heavy metal and salts from the environment. Microbe mediated biosorption is a green approach for sustainable future in comparison to chemical sorption [22]. A number of *Pseudomonas* species have been reported to sequester toxic contaminants including zinc, mercury, lead, chromium etc from waste water [22,108]. All these multifunctional attributes of *Pseudomonas* EPS, makes it a potent candidate for designing next generation bioformulations. Such EPS based biological products can serve as a game changer and set up a new horizon in attaining environmental sustainability in an eco-friendly manner.

Plant Growth Promoting Roles of *Pseudomonas* EPS

Structure, synthesis and genes responsible for EPS production are highly diverse. Besides this diversity, EPS also vary in terms of its functionality. Diverse functions have been carried out by EPS, like EPS matrix offer protection to bacterial cells against environmental stressors. It also covers bacterial cells and shields them from antimicrobial compound and toxic heavy metals. Water Holding Capacity (WHC) of EPS protects bacteria against excessive drought (Costa et al. 2018). Apart from this it also help in cell to cell communication, adhesion, aggregation, carbon storage, and a nutrient reservoir (Vardharajula and Ali 2015; Wang et al. 2015). In this section detailed diversity of EPS has been highlighted in terms of its functionality.

EPS offers tremendous protection to the microbial cells under stress conditions. Diverse abiotic conditions including drought, temperature, pH, and salinity can elicit the formation of EPS as a response to environmental stresses [110,119]. Roberson and Firstone (1992) observed that under drought stress conditions, EPS produced by *Pseudomonas* strain isolated from soil displayed high survivability in comparison to non-EPS producers. It was observed that under high desiccation state, EPS act as a protective covering, that help in trapping water and nutrients for bacterial survival, thereby allowing the bacteria to make metabolic as well as structural modifications [88]. Thus, it may act as a strategy for retaining water/nutrient and maintaining bacterial survival. Role of EPS in mitigating salinity stress has been elucidated by Tewari and Arora (2014) in *P. aeruginosa* PF23 under soil stress conditions while working on sunflower crop. The synthesis of EPS by salt tolerant isolates PF23 decreased Na⁺ uptake in plants by trapping and declining the amount of salt ions available (Upadhyay et al. 2011). EPS prevents nutrient imbalance under osmotic stress, and promote the growth of root colonizing *Pseudomonas*, thereby, benefitting the plant growth and development [104]. Kasotia et al. (2016) observed that EPS producing *Pseudomonas* sp. AK-1 was able to bind free Na⁺ from the soil, making Na⁺ un-

available to soybean roots and maintain normal plant growth up to 200 mM NaCl. Application of AK-1 EPS on soybean plant helped in minimizing soil electrical conductivity from 1.1 to 0.9 dS/m and protects bacteria from salinity stress. Seed pelleting with EPS of *P. putida* AKMP7 on wheat, under heat stress conditions enhanced the amount of cellular metabolites including proline, reduced membrane damage and the activity of several antioxidant enzymes such as super oxide dismutase, ascorbate peroxidase and catalase [44]. Treatment of plants with EPS-producing bacteria showed higher accumulation of proline, sugars, and free amino acids under water deficit stress conditions. Co-inoculation of PGPR with EPS producing bacteria is a promising measure to combat drought/salinity stress and, increasing global food security [78]. Importance of EPS has been also elucidated by Nunkaew et al. (2015) while performing experiments on rice crop using EPS producing bacteria *Rhodopseudomonas palustris* (strains TN114 and PP803).

Combinational effect of EPS producing *P. fluorescens* DR7+*P. fluorescens* DR11+*Enterobacter hormaechei* DR16+*P. migulae* DR35 was observed on foxtail millet (*Setaria italica*), it enhanced seed germination and profuse plant growth under drought stress conditions (-0.30 MPa and -1.03 MPa). Multiple PGP traits displayed by the microbes including ACC deaminase activity+EPS+ IAA+ phosphate solubilization, could be one of the reason for plant growth stimulation. EPS producing *P. fluorescens* DR7 showed the highest level of ACC deaminase activity, DR7 could efficiently colonize the root adhering soil, increased soil moisture, and enhance the root adhering soil/root tissue ratio (RAS/RT). Bacteria having dual activity of EPS +ACC deaminase, may appears to improve the plant growth stimulation of desired crop plant, possibly by improving soil structure, soil texture and raising the number of root colonizing microbes around the root surface. Such EPS producing bioinoculants can be effective in long run for maintaining sustainable agricultural in drought effected arid habitat (Niu et al. 2018).

EPS producing strain of *P. entomophila* PE3 act as biostimulants for raising sunflower growth, yield and productivity under salinity stress. Talc based EPS bioformulation was prepared taking various concentration of purified EPS ranging from (0.1%, 1%, 2% and 5% EPS) and combination of EPS+PE3. Combinational set (EPS+PE3) and pure EPS (2%) set displayed significant enhancement in growth stimulation of sunflower crop in comparison to untreated sets receiving only bacterial treatment [31]. Potency of pure EPS bioformulation and combinational EPS bioformulation in growth promotion and biological control of pigeonpea crop was proven by Tewari and Sharma (2020) on Rhizobia. Naseem and Bano (2014) showed augmented growth and productivity of maize crop when combination of EPS+*Pseudomonas* was added in the saline soil. Tewari and Arora (2014) also developed talc based EPS bioformulation from saline tolerant *P. aeruginosa* PF07 and monitored its potency on sunflower crop under saline conditions in pot trails (125 mM NaCl concentration). Author's suggested the benefits of employing EPS formulation in enhancing early seed germination, enhancing plant growth parameters, raising seed weight and ameliorating stress in saline affected regions. EPS bioformulation also enhanced soil texture by embracing RAS/RT, enhancing porosity, boosting nutrients uptake, and thereby considering it as a commercially important bioformulation for restoration of stressed sites. In 2018, same workers performed another experiment in which they have used the combination of two powerful metabolites EPS+Salicylic Acid (SA) for monitoring dual effect on sunflower seeds. The results highlighted

that that combination of these two metabolites EPS+SA helped in enhancing plant growth of sunflower crop under saline conditions and combated the growth of dreadful phytopathogen *M.phaseolina* [103].

Certain *Pseudomonas* species produce high amount of EPS, that serves as potent cryoprotective agent, and help the microorganism against frost injuries. Mishra et al. (2011) documented, temperature ranging from 0-15°C display significant increase in EPS production in comparison to optimum conditions (20-30°C). Psychrotolerant *Pseudomonas* harvested from the north western Himalayan regions were able to produce higher amounts of EPS under low temperature conditions in comparison to ambient temperature (Mishra et al. 2011). The reason why EPS producing bacteria can help plants to thrive best under cold conditions may be due to the fact that the bacterial EPS act as an excellent chelator and, it chelates Na⁺ ions, restricting sodium uptake by roots, thereby, defending the plants against cold-mediated dehydration [74]. Ali et al. (2020) performed study on cold loving bacteria *Pseudomonas* sp. BGI-2, that displayed copious amount of EPS production, guarding bacterial cells against harsh environmental conditions including freeze thawing, chemical lysis or detergent treatment. It was amazing to observe that EPS extracted from strain BGI-2 safeguarded mesophilic *Escherichia coli* k12, suggesting its cryoprotective nature. BGI-2 also provided noteworthy stability to the RBC membranes by detergent treatment. Increased inhibition of lipid peroxidation by BGI-2 EPS, further authenticates its role in cell membrane protection. High colonization of EPS producing *Pseudomonas* on glacier ice was observed by [17]. Authors stated, the inhibitory effect of EPS from psychrophiles on lipid oxidation is very critical as oxidative stress increases at low temperature. Carrión (2015) isolated cryoprotective EPS from Antarctic *Pseudomonas* sp. ID1. Arcarons (2019) reported the cryoprotective role of EPS by monitoring its efficacy on adult cow oocytes. Application of 10 µg ml⁻¹ EPS obtained from *Pseudomonas* sp. ID1 was added to vitrification and warming media, it protected bovine oocytes against cryodamage. EPS derived from *Pseudoalteromonas* sp. strain CY01 (KCTC 12867BP), is a novel strain isolated from the polar ice caps. It showed excellent ability to cryoprotect cells, and display no cytotoxicity. Thus, this EPS can be used as an alternative towards conventional cryoprotective agents that show cytotoxic attributes when used at high concentrations. This work paved new avenues for the usage of EPS as a cryoprotective and cell membrane stabilizing agents. With all these facts it could be speculated that, the usage of EPS as cryoprotective agent at industrial level will be a noteworthy. Currently, chemical based cryoprotective agents like dimethyl sulfoxide and methanol have taken a hold in the industries, but there usage will be toxic to cells at higher concentrations. Hence, microbial derived EPS can be used as a non-toxic, pure and biological approach towards the conventional method.

High-quality soil structure depends on its aggregation. Soil aggregation is important for agricultural sustainability [5]. Rearrangement of soil particles by flocculation, and cementation results in soil aggregation. Aggregated soil particles usually define the physical and mechanical properties of soil, including WHC, water upsurge and aeration which in turn affect chemical and biological processes [2,102]. Aggregates are important for the improving soil fertility, porosity and agronomic productivity as it influences plant germination and root growth [13]. The stability of aggregates depends on their internal cohesion, pore volume, and pore-wall hydrophobicity [20,64]. demonstrated

that electrostatic interactions are important in between the EPS of *P. putida* X4 and mineral kaolinite, montmorillonite, and goethite. It was observed that *Stenotrophomonas*, *Sphingobacterium*, *Bacillus*, and *Pseudomonas* species could stabilize and increase aggregate strength. Interestingly, *Bacillus* and *Pseudomonas* are genus widely known to produce biofilms and EPS, which are involved in the stabilization of soil structure [93]. EPS obtained from *P. aeruginosa*, *Erwinia*, *Ralstonia*, and *Azotobacter vinelandii* helps in soil aggregation as it holds high adhesive properties. These adhesive natures help in gluing the bacteria to the soil particles and enhance root colonization. Mu'minah et al. (2015) reported high amount of EPS producing strains from the potato rhizosphere that were also potent IAA producers which in synergy assisted in plant growth development. There are several documented proofs that EPS producing *Pseudomonas* can enhance the aggregation of soil particles, this binding helps in maintaining soil moisture and nutrient entrapment. Within this process plants are benefitted and their productivity enhances. In addition to this gummy nature of EPS, it also provides unique traits including biocompatibility, gelling, stabilizing, and thickening capabilities, with enormous industrial applications. As per the reports of Roberson and Firestone (1992) EPS obtained from *Pseudomonas* can hold high amount of water, suggesting its moisture holding capacity. Addition of EPS in sandy soil, alters its moisture by allowing the EPS amended soil to hold more water than unamended soil. Endophytic bacterium strain PsJN (*Pseudomonas* sp. PsJN) was isolated from onion root that displayed root colonization on *A. thaliana* [40]. EPS are responsible for maintaining cohesive and adhesive interactions amongst microorganisms and biofilms respectively to the attaching surface. It influences the spatial association, allowing microbial interactions, and acting as cementing material between the cells [120]. These functions are imperative in establishing the biological activities of flocs and biofilms. EPS, provides mechanically support as it stabilizes the microbial cluster *via* various macromolecular interactions involving hydrogen bonds, dispersion forces and electrostatic interactions. These interactions leads to the formation of tridimensional gel like structure around the cells and help them in maintaining stable microbial consortia [33]. Exopolymers from *Pseudomonas* benefits the survival of other marine organisms by facilitating attachment and adherence to surfaces [48]. Chen and Stewart (2002) concluded that EPS is responsible for both adhesion and cohesion interactions and play a crucial role in maintaining structural integrity of *P. aeruginosa* biofilms. EPS act as a preconditioner, making the adhesion process more favorable. In some cases, EPS matrix also enables the marine bacteria to capture nutrients. In certain bacterial genera, exDNA is accountable for the cementing nature of EPS. exDNA is an autolytic secretion released by *P. aeruginosa* biofilms [72,116]. The exact function of exDNA has not been completely elucidated, but few studies demonstrated that may be related to the cohesion/ adhesion to surfaces and signaling [83].

Bioemulsifiers obtained from *Pseudomonas* have significant advantages over synthetic emulsifiers, as they are biodegradable and environmental friendly (Caruso et al. 2018). There are large number of *Pseudomonas* species that have been reported to form polymers with emulsifying property [24]. Microorganisms that produces bioemulsifiers can be categorized into three major groups: The first one, are those microbes that use only alkanes as carbon source. Second one, includes those organisms that uses only water soluble substrates as the carbon source and third one, involves those organisms which includes both

alkanes and water soluble substrates as carbon source [112]. *Pseudomonades* are the main example included in the third category. There are certain species of fluorescent pseudomonads, where, the emulsification activity of bacterial EPS was almost found to be similar to that obtained from any hydrocarbon. The *P. fluorescens* EPS exhibited highest emulsification in comparison to controls including diesel and olive oil. Sifour et al. (2007) have reported high emulsification activity of EPS producing *P. aeruginosa* when sunflower oil, heptadecane and paraffins were taken as control. Abouseoud et al. (2007) reported the usage of diesel and kerosene oil as the substrate for enhancing the EPS production in *P. fluorescens* and it may further raise the emulsification activity. There is a direct correlation observed in between the emulsification activity and EPS production [112].

EPS producing strains play a significant role in biofilm formation and quorum sensing. Various studies have shown that Acylhomoserinelactone (AHL), (3OC6-HSL, 3OC8-HSL and C6-HSL) mediated quorum sensing is an important factor for biofilm enlargement, and in some species this is mediated through regulation of EPS production [25,61]. Genes required for EPS production are responsible for producing biofilm in bacterial cells. AHLs, are involved in the regulation of EPS production. *P. aeruginosa* has been regarded as model organism for biofilm formation. *P. aeruginosa* contain three types of diverse EPS synthesis viz alginate, Psl, and Pel. These EPS contribute to the formation of biofilm in this microorganism. Pel is a polymer of cellulose and highly rich in glucose. Increase in the amount of Pel production is related with the appearance of wrinkled colony and it is helpful in cell to cell communication that assists in the process of biofilm formation [43]. Polysaccharide containing Psl is highly rich in galactose and mannose. It is involved in initial adhesion and biofilm maturation during planktonic stage. In mature colonies Psl is linked with the caps of mushroom-like microcolonies. *P. aeruginosa* also produces alginates, a polyanionic EPS composed of uronic acids [39]. Excessive production of alginate results in forming large finger-like microcolonies. Alginate has been shown to contribute to decreased susceptibility of biofilms to antibiotic treatment and human antibacterial defense mechanisms [90].

Various industries releases huge amount of waste effluent containing heavy metal contamination that may cause serious threats to animals and other water bodies. Entrapment of this toxic heavy metal from such source using microbial assisted biosorption could be a clean and green approach for sustainable future. Detailed structure of EPS analyzed by electron microscopy shows many grooves within it, in which multiple heavy metals can bind. It was observed that when *P. aeruginosa* is exposed to 10 mg l⁻¹ of chromium dosage, there was significant increase in rhamnolipid concentration [85]. It indicates the involvement of rhamnolipids, that assist in chromium removal from the natural system like soil and water [85]. Taking into these considerations, EPS producing different species of *Pseudomonas*, have been used to sequester heavy metals such as cadmium, copper, chromium, nickel etc from aqueous solution [22]. EPS, obtained from *P. aeruginosa* MTCC 1688, has the potential to remove 26% Cr and 9% Ni respectively from the water waste system [22]. Fluorescent *Pseudomonas* strain Psd is a soil dweller, possessing zinc biosorption capacity. As per the findings of Upadhyay et al. (2017) it was documented that EPS play significant role in zinc biosorption. The reason for this was the production of alginates by *Pseudomonas*, that are involved in producing alg 8 gene. Alginate plays significant role in the biosorption of zinc. If alg8 gene was mutated, it may lead to the significant reduction in

EPS production and compromised biofilm production. Authors also demonstrated that when *Pseudomonas* cells are exposed to zinc, it raises the concentration of alginate production, and further improved biosorption formation in psd strain.

Antagonistic role or biocontrol activity of EPS have been elucidated by various workers in details [10,103,106]. Role of EPS in antagonizing dreadful pathogen is new and very less literature is available on this subject. Aullybux et al. (2019) isolated EPS from bacteria residing in the marine water habitat, that displayed wide range of antibacterial activity against *Acinetobacter*, *Bacillus*, *Campylobacter*, *Enterobacter*, *Enterococcus*, *Escherichia*, *Proteus*, *Salmonella*, *Streptococcus* and *Staphylococcus*. Banerjee et al. (2020) isolated EPS producing novel thermotolerant and haloalkalophilic strain of *Pseudomonas* sp. PFAB4 from hot spring. Strain was able to produce 2.63 g l⁻¹ dry weight of EPS on Kings B medium. EPS obtained from this strain was monitored for antagonistic activity against gram negative bacteria by agar cup assay. Equal ratio of AgNO₃: EPS (1:1) demonstrated effective killing against gram negative bacteria *E. coli*. The EPS coated nanoparticles exhibited high antimicrobial property against target fungi and bacteria. Such green and eco-friendly measures could be used over conventional chemicals [10]. Tewari and Arora (2014) reported antifungal activity of *P. aeruginosa* PF23 EPS, against phytopathogen *M. phaseolina* that caused charcoal rot disease in sunflower crop under salinity stress conditions. Antimicrobial activity of EPS producing strains of *P. aeruginosa* B1 and B2 were monitored against gram positive and negative bacteria Onbasli and Aslim 2008).

Mixed Mechanisms Working With EPS

Multiple traits of EPS displayed by *Pseudomonas* are interwoven and knitted with each other. *Pseudomonas* strain psd is a soil bacteria present in the rhizosphere of mung bean plant. It has strong PGP and biocontrol potential. Psd is a potent EPS producer, displaying biofilm formation and aggressive root colonization. The stimulatory effect of Fluorescent *Pseudomonas* strain Psd on root growth has been studied by Sirohi et al. (2015). Biofilm producing *Pseudomonas* resulted in improved plant growth by facilitating dense bacterial population to produce various phytohormones, antibiotics, exoenzymes and secondary metabolites [60,98]. The secondary metabolites and antibiotics like Phenazine-1-Carboxylic Acid, (PCA) produced by *Pseudomonas* sp. contribute to the biocontrol properties. Supplementation of Zn²⁺ resulted in the stimulation of PCA production in *P. fluorescens*, which further strengthen the biocontrol potential of the strain (Slininger and Jackson 1992). Zn²⁺ absorption in plants is mediated by alginates in strain Psd that may also serve as important determinants in plant stimulation. Strain Psd showed an increase in the production of siderophores and phenazine in the presence of added Zn²⁺ in the medium, suggesting that Zn²⁺ modulates the production of these crucial secondary metabolites. The production of EPS is not only an advantage to the microbes but also to the soil environment in general. The adhesiveness is important for gluing soil particles together; high water locking capacity protects microorganisms and plants against drought, as well as permits the diffusions of nutrients in the environment. EPS production is also influenced by the interactions between plants and microorganisms, as it increases the availability of nutrients as a whole, promoting plant and microbial growth [23].

EPS can serve as a mechanical barrier between bacteria and plant defense compounds. EPS of *Pseudomonas syringae* pv. *phaseolicola* and *S. meliloti* protect the bacteria against Reactive

Oxygen Species (ROS) produced by the plant host during infection, thereby decreasing oxidative stress [58,63]. Alginate, the EPS produced by *P. aeruginosa*, a human opportunistic pathogen, protects the bacteria against the inflammatory process of the host, by avoiding free radicals formation [90]. EPS can act as an antioxidant, but very less is known about the chemical mechanism of protection against ROS. Alginates produced by *P. aeruginosa* enhances bacterial survival in chlorinated water, and removal of the slime eliminates bacterial chlorine resistance (Grobe et al. 2001).

Conclusion

In the coming years, there has been a budding interest in the isolation and identification of new strains of *Pseudomonas* and exploiting them for harnessing EPS. Diverse species of *Pseudomonas* produce different exopolymers with exploitable properties. As the property of one polymer varies from other, it may provide opportunities to the researchers for further isolation, characterization and examination of many new EPS from novel strains. Till now isolation of *Pseudomonas* has been done from common environmental habitats including soil and water habitat, from which different *Pseudomonas* species and strains are isolated for routine laboratory experiments. In order to exploit novel EPS, more focus should be given on isolating new strains of *Pseudomonas* from atypical environment like extremophilic habitats (hydrothermal vents, hot sulphur springs) that are still untouched and unexploited. Deep-sea hydrothermal vents, marshy areas, hot deserts, salty lakes, ice frost glaciers etc may be recommended as a new source for isolating novel *Pseudomonas* species that could be purposefully used for isolating different exopolymers with exploitable properties. EPS from such thermophiles, alkalophiles and halophiles could be employed for remediating stressed sites, it may also serve as a game changer for designing pure green and ecofriendly emulsifiers, viscosifiers, stabilizers, gelling agents, plant growth promoters, root colonizers, pathogen controller, texture enhancers and bioformulation developers. More focus should be given in exploiting the strategies for isolating diverse EPS and, harnessing them at significantly higher rates by manipulating the current procedures using economical substrates. Hence, deeper insight is required, so as to underline the mechanisms, pathways and genetic regulation of *Pseudomonas* EPS. While studying novel EPS of biotechnological interest, working on various model organisms will help in investigating how different biomolecules present in the EPS are stabilized when subjected to extremities.

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