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Study on Rhizospheric Bacterial Exopolysaccharide and Its Application in Plant Growth Promoters and Antimicrobial Activity

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Abstract
In rhizhosheric soil contains high number of organism produce high molecular weight substances having polysaccharide but also non sugar components like protein, nucleic acid and lipid. Exopolysaccharide (EPS) are the produced microbial cell and secret outside. EPS play important roles in constructing materials of bacterial settlement, remain attached to outer surface of cell. EPS found various applications in agriculture field such as improvement of soil properties, disease control and plant growth. In the present review article highlighted on EPS production in rhizhosheric soil and application of plant growth promoters and antimicrobial activity. Plant growth promoting rhizobacteria (PGPR) can promote plant growth and directly effect on plant metabolism. PGPR play in evacuate abiotic stress conditions. Microorganisms produce antimicrobial compounds as secondary metabolites. It used as antibiotics to control various infections pathogens. Rhizosphere soil is best to study PGPR and antimicrobial activity .
Keywords *– Exopolysacharride , Rhizosphere, PGPR plant growth promoting rhizobacteria, antimicrobials.*

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I. INTRODUCTION

In the rhizospheric region large amount of organic material are found it support variety of beneficial organism to produce exopolysacharide. (Hafsa Naseem et al 2018). Those help to stimulate microbial activity and biomass which helps to produce exopolysaccharide.(Philippe Hinsinger 2009). Microbial polysaccharide have wide range industrial and agricultural application. EPS structures are simple, lack of complexity without extensive branches; so it is great interest to do work on EPS production. Exopolysaccharide have wide range applications in commercial market of food and healthcare industry (Misu Moscovici 2014). Different microorganisms such as bacteria, fungi, actinomycetes produce exopolysaccharide during their life cycle. Polysaccharide play important role in protection of bacterial cell from desiccation, invade of toxic metals, antibiotic, phygocytosis and phage attack. (Uchechukwu U. Nwodo 2012) .Now days bacterial polysaccharide used as immunostimulatory, immunomodulatory, antitumor, antiviral, anti-inflammatory and antioxidants agents in various medical and pharmaceutical industries. Microbial exopolysaccharide such as dextron, xanthon, gellan, pullulan, yeast, glucans and bacteria alginates are used in many industries as food additives (Prasad Andhare,Kishor Chauhan 2014). They are used in a wide variety of industrial applications, including food, textile dye removal from water, pharmaceutical, emulsifiers, beverages, adhesives.

EPS gives numerous benefits like resist in abiotic conditions (Rossi et al2012) biofilms formation, nutrient and water reservoirs (Castro et al 2014) . The important feature of EPS is biodegradable nature; it can release under extreme environmental conditions like temperature and pH (kodali et al;2009) . Microbial EPS enhance soil aggregation, these benefits to plant by maintaining moisture content and trapping nutrients. Bicompatibilty, gelling and thickening capabilities of soil are happened due to EPS . Only few polymer are widely used different areas such as agriculture, due to the EPS production helps to improve soil structure, health and fertility (Ohana Y. A. Costa 2014). The matrix produced by EPS around microbial cell helps to shielding cell against antimicrobial compounds and heavy metals (Bhavana V. Mohite 2017). EPS matrix also retain water, protecting microbes and environment against drought. Because of their special components, EPS matrices show adsorption abilities, biodegradability, and hydrophilicity or hydrophobicity (H. Koo, 2013) .Also an EPS play an important role in mass transfer via biofilm, in adsorption of different metals and organic/inorganic compounds and most importantly in providing structural support to the biofilm . EPS of bacteria are hydrated compounds with water polymer matrix which protection against desiccation.(Wngender et al.,1999; Hunter et al., 2005; Bhasker and Bhosle, 2006). Bacteria produce EPS in two forms slime EPS and capsular EPS. The important roles exhibited by EPS are protective, surface attachment, biofilm formation, microbial aggregation, plant–microbe interaction, and bioremediation.(Ohana Y. A. Costa 2014).

Biosynthesis

Enzyme and regulatory proteins are involved in the biosynthesis process of microbial exopolysaccharide (Schimid, Sieber and Rehm,2015) Exopolysaccharide microorganisms use sugar as their energy source, ammonium salt and amino acids as a source of nitrogen. (L. Zhang 2011) The production of exopolysaccharide by microorganisms is largely dependent on the presence of carbon and nitrogen availability in the surrounding areas. Although low nitrogen concentration leads to high production of exopolysaccharide. to introduce four common methods known for the production of exopolysaccharid from bacteria: (i) the Wzx / Wzy-based method; (ii) the ATP (ABP) supplier router; (iii) a method dependent on synthase and (iv) the extraction of cells using a single sucrase protein. In Wzx / Wzy the method relies on individual recurring units, linked to the undecaprenol diphosphate anchor.(C55) in the inner membrane, bound to several glycosyltransferases and transferred to the cytoplasmic membrane with the Wzx protein. In the next step its polymerization process takes place in the periplasmic space by the Wzy protein before it is transferred to the cell site (Cuthbertson et al., 2009; Morona et al., 2009; Islam and Lam, 2014). Repeated transport of polymerized units from periplasm to cell component has been shown to depend on additional proteins (s) supplied by polysaccharide co-polymerase (PCP) and outer polysaccharide membrane (OPX; formerly OMA families) (Cuthbertson et al. 2009; Morona et al., 2009)

All polysaccharides composed of the Wzx / Wzy line have a very different sugar pattern and are therefore classified as heteropolymers. All components using this method carry the genes of flippase (Wzx) and polymerase (Wzy) within their operations outside of polysaccharide cells. (Jochen Schmid 2015)

Application of EPS

1. Industry

EPS producing cultures are widely used in dairy foods to provide viscosity, stability and water-binding functions to dairy products therefore it gives mouth-feel, texture and taste perception of fermented dairy products .One of the most important commercial application of EPS is in yogurt manufacture, and it is helpful for production of biscuits because it increases water absorption, dough development time and dough stability due to this ability shelf life increases. Pullulans are used in replacement of starch in baked food. It is also used in beverages and sauces, in frozen food EPS used as binder and stabilizer. It can be used as protecting glaze on the food. EPS is used as thickener, stabilizer, emulsifier or binding agent like in icings, jams, jelly, pastries, cheese, sauces, pet food, salad dressing, etc. (Torres et al., 2010)

2. Personal care products

In cosmetics, EPS are widely used in creams and lotions as moisturizer . EPS from mushroom Grifola frondosa is used in sun screen protection creams. pullulan has used in cosmetics creams, lotions, and shampoos. Xanthan have high cream stability so it used in toothbrush technology. EPS used in hydrogel in skin care fabrics, where they are implanted in fabricss. These hydrogels are used in moisturizing, whitening, brightening, and anti-ageing effects on human skins (Hilor, 2012.)

3. **Textile industry**

In textile industries EPS are used as a binding agent with color dyes. EPS is also used in smart fabric. It is used as aroma for finishing fabric. Hydrogel helps property such as viscosity, stabilizer and its cross linking ability with fabrics. EPS used to produce photochemical textiles and also in sizing and printing.

3.Bioremediation:

Bacterial EPS have ability to bind heavy metals. EPS contains negatively charged functional groups and strong binding capabilities to heavy metal ions and organic pollutants. EPS producing bacteria like *Bacillus cereus* possess the ability of bio corrosion of stainless steel thus are used in bioremediation to remove excessive steel compounds in stainless steel industries.

EPSs are also mentioned for the prevention of tumor cell development, formation of white blood cells and in the treatment of the rheumatoid arthritis (Vanhooren and Vandamme 2000).

PGPR

Bacteria that live in the rhizosphere and promote plant growth and tolerance are known as plant growth which promotes the rhizobacteria (PGPR) properties of PGPR that they have the ability to bind to the root zone .They can survive, multiply and compete with other microorganisms. Extracellular PGPR directly increase plant growth by increasing phytohormone, siderophore, biofilm, and exopolysaccharide production. and by improving the availability of nutrients in the rhizosphere or indirectly, by protecting plants from bacterial infections. PGPR therefore acts as a biofertilizer. (Kloepper et al., 1999) For example, bacteria have been described as altering the structure of their membranes or combining movement to increase their availability during low groundwater. Polysaccharides are hygro-scopic and, therefore, can keep water much higher in a small colonial area than in large soils as rainfall decreases (Roberson EB, Firestone MK. 1992) and planting dry content compared to irrigated and pressed control plants. Increased plant growth observed by bacteria producing moderate levels of indole acetic acid IAA include *Azospirillum sp., Alcaligenes faecalis, Klebsiella sp., Enterobacter cloacae, Acetobacter diazotrophicus, Rhizobium* (Costacurta et al, 1995). The microbial colonizing rhizosphere includes bacteria, fungi, actinomycetes, protozoa, and algae. However, bacteria are the most numerous insects present in the rhizosphere (Saharan, 2011). Kloepper and Schroth (Kloepper, 1978) paved the way for greater access to PGPR. Effective methods of PGPR can also be categorized into direct and indirect. PGPR operating systems can also be categorized directly and indirectly. Specific methods are biofertilization, promoting root growth, rhizoremediation, and control of plant pressure. On the other hand, the way in which biological control rhizobacteria is involved in promoting indirect plant growth is to reduce the impact of diseases, including antibiotics, systemic resistance, and competition for nutrients and niches. (García-Fraile, 2015). PGPR that promotes plant growth in tolerance to abiotic stress on plants, nutrient adjustment for plant extraction, plant growth regulators; production of siderophores; production of organic compounds; and the production of a protective enzyme (.Choudhary, D.K. 2011 and García-Fraile, 2015). PGPR has the potential to increase the concentration of nutrient in the rhizosphere by modifying the body's genes, thereby preventing them from excreting. A free example - a nitrogen-fixing organism is *Azospirillum*, which is often associated with grains in cooler climates and is reported to be able to improve rice yields. (Lavakush et al. 2014) studied the effect of PGPR on nutrient uptake by rice. They use PGPR pressures such as *Pseudomonas fluorescens, Pseudomonas putida, and Pseudomonas fluorescens*. Iron is usually present in the environment in the form of Fe3 +, which is not very soluble; To solve this problem, PGPR hides siderophores. Siderophores are low protein-binding proteins involved in the process of chewing ferric iron (Fe (iii)) from the environment. Organic chemicals (VOCs) produced by plant growth-promoting rhizobacteria (PGPR) play a key role in improving plant growth and making systematic resistance (ISR) to pathogens. Several species of bacteria, from a variety of sources including *Bacillus, Pseudomonas, Serratia, Arthrobacter, and Stenotrophomonas* produce VOCs that contribute to direct and / or indirect plant growth in plant biomass growth, disease resistance, and tolerance to abiotic stress (Effmert, U.12). Biofertilizer is becoming increasingly important for organic farming and a major player in the global economy and agricultural production. Biofertilizer products are usually derived from plant microorganisms (PGPM). *Pseodomonas sp.* PGPR two of the most reported active bio-control agents: *Bacillus subtilis, Basillus amyloliquefacients, and Bacillus cereus* are the most effective strains of plant disease.

Application of Rhizosoheric bacterial exopolysachride on different plants in drought stress

Drought stress can make physico-chemical and biological properties of soil unsuitable for soil microbial activity and crop yield. Water availability controls the production and consumption of protein and polysaccharides by the bacteria (Roberson and Firestone 1992) The current climate change scenario has resulted in global warming combined with intermittent precipitation, resulting in an increase in irrigation demand. Rainfed soils are unable to move water through capillary action from deeper layers of soil. These soils have a unique composition. Whereas, the plants have a weak structure and have a lower water holding capacity, as well as fewer nutrients; however, the plants have a better structure and have a higher water holding and fertility status (Khan N, Bano A 2019) A PGPR- induced increase in the development and yield of crops has been demonstrated in both greenhouse and field trials (Sharafzadeh S 2012)

Salicylic acid is known for its defensive role when present in plants under appropriate concentrations; it also plays a key role in the plant development process by modulating plant responses to abiotic (Khan and Bano, 2019). Requirement of water for maize (135 mm/month) at the time of seedlings is 4.5mm/day which increase up to 195mm/month that is 6.5mm/day during hot and windy conditions. The maize inoculated with *Pseudomonas sp.* strains has increased plant biomass, relative water contents of leaves, leaf water potential, root adhering soil/root tissues ratio, stability of soil aggregates and decreased the leaf water loss. The EPS-producing *Pseudomonas* strain GAP-P45 act as a plant growth promoting rhizobacteria and can alleviate the effect of drought stress in sunflower plants possibly through improved soil structure and plant growth promoting substances(V. Sandhya & Ali SK. Z. & Minakshi Grover & Gopal Reddy & B. Venkateswarlu 2009). The Okra plant growth was evaluated under different treatment of EPS producing bacteria namely *Pseudomonas aeruginosa* and *Bacillus coagulans*. Different growth parameters like seed germination, plant height, number of leaves, leaf area were evaluated in the field under water stress condition (Shyam Nath Yadav, Ajay Kumar Singh1, Jyotsna Kiran Peter, Harison Masih, Jane C. Benjamin, Deepak Kumar Singh, Siddhant Chaudhary, P.W. Ramteke and Surendra Kumar Ojha2018).

Under drought stress conditions following Pgpr are useful (Anirban Basu 2020)

Table no 1-

PGPR	Crops	Reference
Pseudomonas fluorescens DR11, Enterobacterharmaech ei DR16, Pseudomonas migulae DR35, Bacillus subtilis, Archromobacter piechaudii ARV8, Paenibacillus polymyxa, Rhizobium tropici, Azospirillum brasilence	Foxtail millet (*Setriaitalica* L),Maize (*Zea mays* L.), Bean (*Phaseolus vulgaris* L.) *Arabidospsis thaliana*, Tomato (*Lycopersicumesculentum* Mill cv. F144), Pepper (*Capsicum annuum* L. cv .maor), Wheat (*Triticum aestivum* L.)	Timmusk, S.;2014Niu, X.; Song 2018 Ilyas , N 2020

Antimicrobial activity and applications-

EPS are reported to possess unique physiological activities including anti-microbial, anti-tumor, and anti inflammatory (Kruti K Pate 2010) wheat, rice, maize, and potato are major crops grown round the world and are encountered by several phytopathogens causing significant economic losses and food shortage at an area, national and global scale. If the case isn't controlled, the globe will see high yield losses shortly resulting in a severe shortage of food especially in food-deficit regions with a fast growing population. The matrix that surrounds microorganisms in biofilms plays a crucial role in decreased susceptibility to antimicrobials. In general, biofilm matrices possess a electric charge and so bind charged compounds, protecting the innermost cells from contact. additionally, electrostatic repulsion can reduce the diffusion rates of charged antimicrobials through the biofilm (Everett and Rumbaugh, 2015). EPS may also protect microorganisms against disinfection agents. Alginate produced by *P. aeruginosa* enhances bacterial survival in chlorinated water, and removal of the slime eliminates bacterial chlorine resistance (Grobe et al., 2001). The few EPS isolated to this point have a large range of functions, but a large diversity of polymers produced by microorganisms with different functions awaits exploration and discovery. The production of EPS isn't only a bonus to the microbes but also to the soil environment normally. The isolation of antibiotics from microorganisms is comparatively easy as compared to chemical synthesis of antimicrobial agents. The isolation of antibiotics from microorganisms improved the invention of novel antibiotics that would act as better chemotherapeutic agents. The use of antimicrobial drugs for prophylactic or therapeutic purposes regularly in human, veterinary and agricultural purposes is favoring the survival and spread of resistant organisms. (Geetanjali and Pranay Jain 2016). The screening of microbial natural products continues to represent a vital route to the invention of valuable chemicals, for the development of latest therapeutic agents and for evaluates of the potential of recent microbial taxa. Soil is rich in microorganisms capable of antibiotic synthesis, but quantity and quality of nutrients as nutrients don't seem to be dispersed uniformly throughout soil, but rather, are localized within the rhizosphere of plants and therefore the ability to compete successfully for them are major determinants of microbial population size and their metabolic activity which regulates antibiotic synthesis. The rhizosphere may be a densely geographic region during which the microbes, including bacteria, fungi and insects feeding compete for space, water, and mineral nutrients. So there's a population of microbes which kill other microbes for survival and exhibit antimicrobial property. Most of the antibiotics in current use for the treatment of varied infectious diseases are microbial products. There is an emerging menace of drug resistance among microorganisms because of inappropriate use of antibiotics by general health practitioners worldwide. This example has become an alarming condition to drug manufacturers and public health practitioners. Rhizosphere bacteria are known to confer resistance/protection against many plant pathogens (Compant et al., 2010). Only a few studies have reported the biocontrol agents having antagonistic potential against multiple fungal pathogens along with plant growth promotion ability. Soil microorganisms perform many vital processes and participate within the maintenance of soil 328 health and quality. They play an important role in organic matter turnover, nutrients release, stabilization of the soil structure and ensure its soil fertility. Moreover, many microorganisms of them act as biological control agents by inhibiting the expansion of pathogens (Varma and Buscot, 2005). Nitrification and/or denitrification rates were also influenced by antibiotic exposure, and the consequences were strongly passionate about the kind of antibiotic and also the length of exposure. For example, sulfadimethoxine inhibited soil nitrification; however, this effect was only observed on some sampling days and just for a high sulfadimethoxine treatment. Nitrification and/or denitrification rates were also influenced by antibiotic exposure, and the effects were strongly addicted to the kind of antibiotic and also the length of exposure. Antibiotics may additionally change the ratio of iron in soil enzyme activity indicates the potential of microbial communities to hold out the biochemical processes that are essential to keep up soil quality. Any application of a toxicant that may affect the expansion of soil microorganisms can induce alterations within the general activity of enzymes, like dehydrogenases (DHAs), phosphatases (PHOSs) and urease (URE) (Gil-Sotres et al., 2005; Hammesfahr et al., 2011; Cycoń et al., 2016)The manure microbiome can influence the soil microbiome through direct competition and transfer of antibiotic resistance genes (ARGs)(Chee-Sanford 2008)The soil microbiome has been linked to overall soil quality because it's involved in nutrient cycling, helps maintain soil water content, and influences soil acidity (Trivedi P,2016)The recent study showed that the bacteria isolated from sugar cane field soil sample are found to be good EPS producers. In addition, MIC values of the EPSs suggested that they

were effective in inhibiting the growth of test organisms at lower concentrations as well. The antimicrobial activities of EPSs could be used as a potential source for the development of antimicrobial drugs (Muhammad et al., 2015). Citrus, for example, has high water content and nutrients, making it very susceptible,to infection by microbi *m chrysogenum ,streptomyces griseus,Cephalosporium acremonium ,Bacillus subtilis, Streptomyces erythreus ,Streptomyces fradiae, Streptomyces rimosus ,Streptomyces orientalis, Streptomyces kanamyceticus, Streptomyces nodosus, Streptomyces hachijoensis ,Bacillus polymyxa, Bacillus brevis, Bacillus cereus ,Acremonium fusidioides, Chaetomium cochlioides* . In a pathogens like Peni subtilis strains and their metabolites are claimed to have strong antimicrobial activities towards green mold pathogen (P. digitatum) of the citrus fruit *Cillium digitatum* (*P. digitatum*) during the period between harvest and consumption(Talibi, 2014). There are twenty strains of *Bacillus* species isolated from soil showing antagonistic activities in vitro towards the *P. digitatum* pathogen. Volatile compounds produced by *B. subtilis* strains can exhibit 30- 70% inhibition of fungal growth (Leelasuphakul, W 2008). Thus, *B. subtilis* 155 is considered to be potent biological control agents to suppress the growth of *P. digitatum* in postharvest protection of citrus fruits.(Talibi, I 2014)
EPS producing bacteria showing antimicrobial activity against plant pathogen.

Table No 2

PGPR	Antibiotic	Target phytopathogen	Reference
Pseudomonas sp.	2,4Diacetylphloroglucinol	*Gaeumannomyces graminis var.tritici*	de Souza
P.fluorescens	Phenazine-1-carboxylic acid (PCA)	Pathogenic fungi	Weller (2007)
Bacillus amyloliquefaciens	Lipopeptide and polyketide	Soilbourne pathogens	Sacherer Et al. (1994)
Pseungomonad sp.	Phenazine	*F.oxysporum and Gaeumannomyces graminis*	Chin-A- Woeng et al. (2003)
P. chlororaphis PCL1391	Phena-zine-1-carboxamide	Pathogenic fungi	Hernandez Et al. (2004), Hass and Defago (2005)
Bacillus ssp.	Polymyxin, circulin and colistin	Pathogenic fungi	Makismov Et al. (2011)
1. P. cepacia *2. P.fluorescens pf5*	Pyrrolnitrin	*1. Bipolaris maydis* *2. Sclerotina homoecarpa*	Sayyed et al. (2013)
1. P.fluorescens 2-79 *2. P.aureofaciens 30-84* *3. P.aureofaciens PGS12*	Phenazines	1. Various sp. Of bacteria and fungi 2. G.*graminis tritici* 3. G.*graminis tritici*	Sayyed et al. (2013)
B. subtilis st.	Iturin and fengycin	*Podosphaera fusca*	Romero Et al . (2007)
Bacillus, Streptomyces, and Stenotrophomonas sp.	Oligomycin A , Kanosamine, zwittermicin A , and xanthobaccin	Prevents the proliferation of plant pathogens (generally fungi)	Compant Et al. (2005a,b)

II. CONCLUSION

Exopolysaccharide is environment friendly product has great interest in commercial application like PGPR and antimicrobial activity. Plant growth-promoting rhizobacteria (PGPR) are enhancing plant growth. In different environmental conditions such as drought and we can develop sustainable crop management in abiotic environment. This should help to improve organic crop farming. It offers huge potential to global agriculture sustainability and climate change scenario. EPS producing organism show antimicrobial activity it gives various industrial use. Most Microbial products are used as antibiotics in pharma and health care industry. It is also useful to fight against plant pathogens, most of plant and animal antibiotics made of these EPS producing organisms. Nowadays numbers of resistance pathogens are increased it will be more dangerous in our future generations. This situation is alarming to our healthy environment so we continue to work more on this field.

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