



# Applications of Extracellular Polymeric Substances in Agriculture

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## Abstract

Extracellular Polymeric Substances, produced by the microbes are highly hydrated polymers of polysaccharides, proteins and nucleic acids which provide an ideal environment for biochemical reactions that can be beneficial for both soil and plants. Many bacterial strains are reported to produce different EPS which vary in composition and structures like gellan, alginate, emulsan, succinoglycan etc. which are produced by different species of bacteria as a result of their adaptive mechanisms to the vagaries of climate. These substances are either freely released into the rhizosphere or attached to the cell walls through complex structures like slimes, biofilms or capsules which are mostly derivatives of glycocalyx. Most of the EPS producing bacteria reside inside the biological soil crusts of arid regions, cow dung, natural botanicals, rhizosphere of xerophytes etc. Some of these strains (Plant Growth Promoting Rhizobacteria) are reported to produce Plant Growth Promoting Hormones like Indole Acetic Acid through Exopolysaccharides. Some Microbial strains that synthesize EPS collected from the rhizosphere of desert soils have the ability to hold moisture inside the soil and improve the sugar and protein content of the plants. Such microbes like *Pseudomonas*, *Bacillus*, *Rhizobium* etc. have the ability to impart resistance and protection to the plants from abiotic stress like changes in the pH, temperature fluctuations, drought and salinity. This review article provides an overview of the composition, types and functional role of EPS, and their applications especially in the field of Agriculture.

**Keywords:** Extracellular Polymeric Substances; Biofilms; Capsules; Glycocalyx; Soil Aggregation; Plant Growth and Agriculture

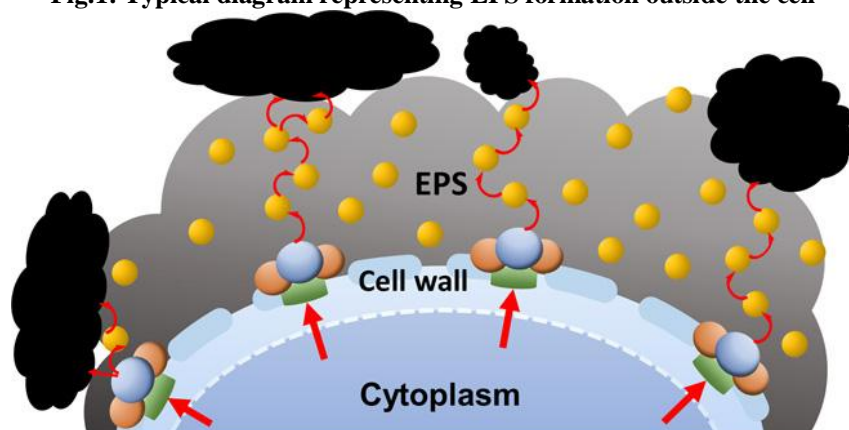
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## I. Introduction

EPS are the natural high molecular weight polymers of sugar residues secreted out by the microorganisms for their survival. These substances help the microbes to combat both abiotic and biotic stress. Extracellular polymeric substances may include exopolysaccharides and other macro-molecules such as DNA, lipids and proteins. In Fig. 1, a typical EPS formation from the cell has been represented. Due to their various compositions and functions, EPS are now used in different industrial sectors especially in Agriculture to impart resistance to plants against stress. The applications of EPS have been discussed in this paper.

Fig.1: Typical diagram representing EPS formation outside the cell



### Role of Microbial EPS

The microbial EPS are very much useful in cell defense (against phagocytosis, bacteriophage attack etc.). These substances also help in the microbial attachment to surfaces (E.g. *V. cholera* attaches intestines). EPS also helps in the gathering of nutrients (*S. mutans*). Other roles of EPS include cell movement, genetic exchange, biochemical signaling and communications, imparting insensitivity to abiotic and biotic stress, antigenicity, establishment of the structural and functional integrity of bacterial biofilms (cell aggregation), symbiosis with host plants, retention of water, sorption of exogenous organic compounds and inorganic ions.

### Different Structures of EPS

Microbial EPS generally exist in two forms viz. Cell bound and Released. Cell bound microbial EPS is mucoidal glycocalyx (capsules, sheaths, or slime layers) which closely adhere to the bacterial cell surface. Released type of EPS are usually released into the surrounding medium as free exopolysaccharide.

**Glycocalyx:** It is a gelatinous network of polysaccharides extending from the surface of bacteria. It is composed of polysaccharides, polypeptides, or both. It encompasses both capsules and slime layers.

Fig. 2: Structure of Glycocalyx; the complex chain of biomolecules (proteins and sugars).

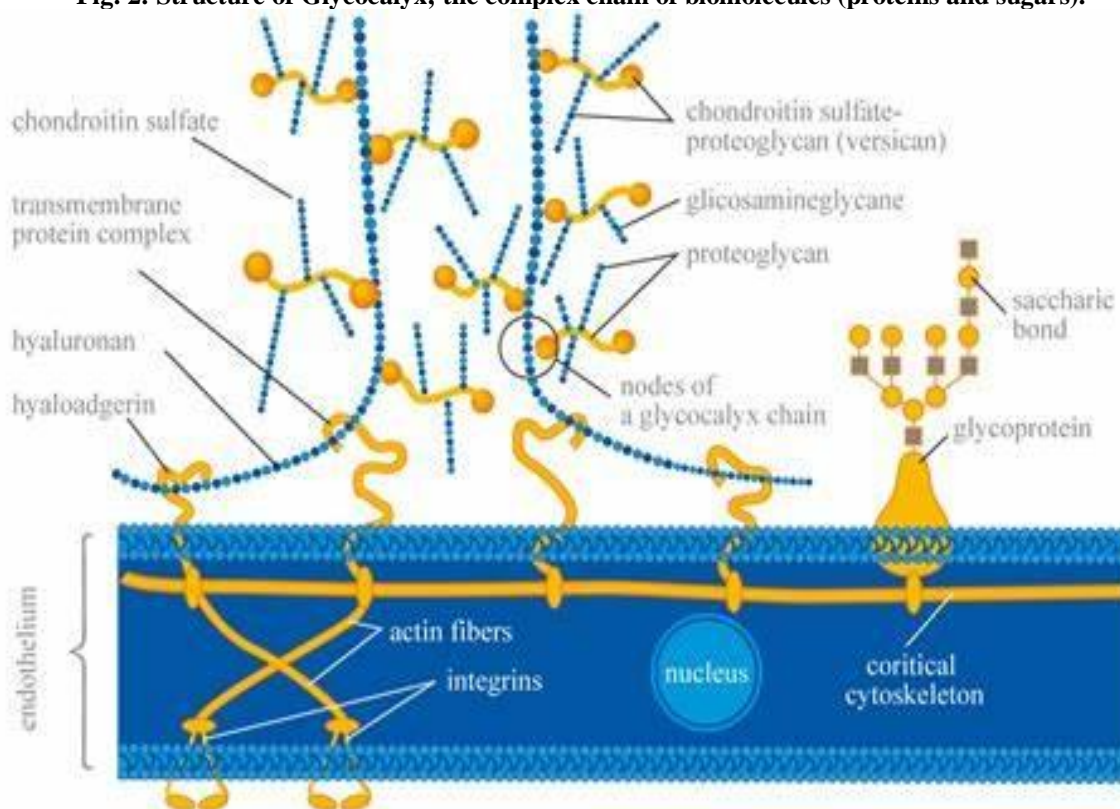
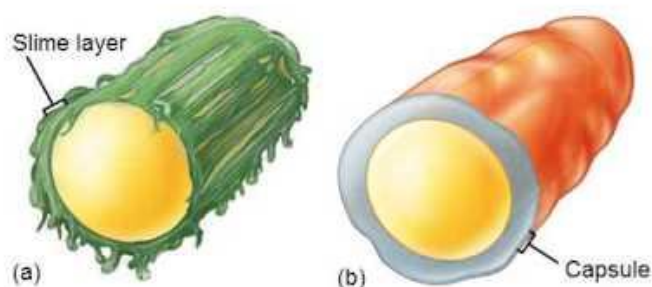


Fig. 3: The difference between Capsules and Slime Layers

## Glycocalyx

Coating of molecules external to the cell wall, made of sugars and/or proteins

1. **Slime layer** - loosely organized and attached
2. **Capsule** - highly organized, tightly attached



**Capsules:** These are well organized gelatinous layers. They contribute to cell adherence and protection. These cannot be easily washed off. The presence of capsules are determined by *negative staining*. Eg. *Bacillus anthracis* has a capsule of poly-D-glutamic acid.

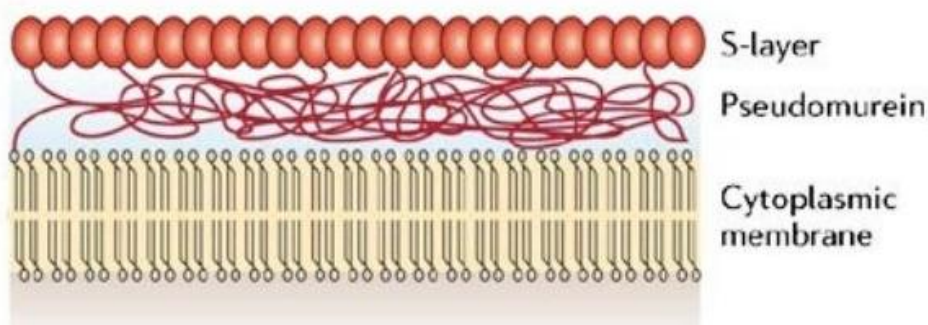
**Slime Layers:** These are unorganized glycocalyx that are loosely attached to the cell wall (i.e. easily removable). The difference between capsules and slime layers is shown in *Fig 3*.

**S-layer:** It is an evenly structured layer present on the surface of many gram-positive and gram-negative bacteria. It is very common among *Archeae*. It is mostly composed of proteins or glycoproteins.

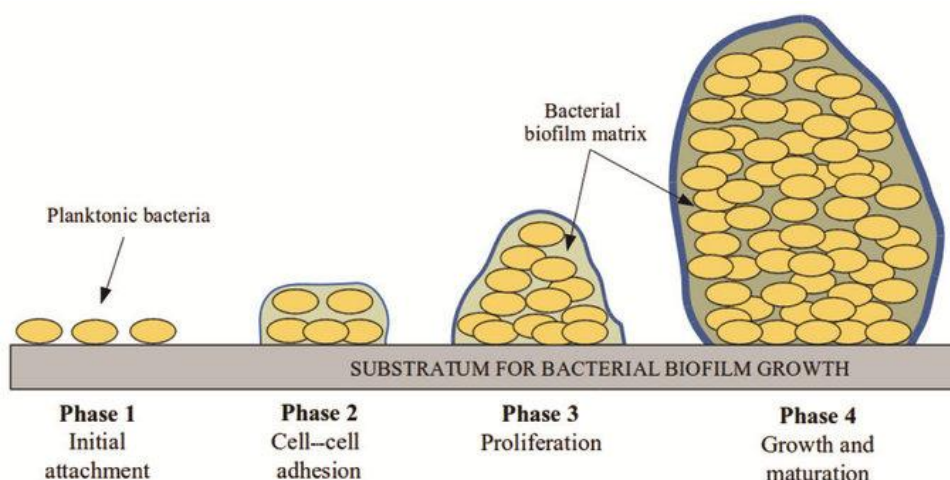
S-layers adhere directly to the surface of outer membrane (Gram-negative bacteria) and peptidoglycan (Gram-positive bacteria). S-layers protect cells against ions, pH, osmotic stress, enzymes, complement attack, phagocytosis and predacious bacterium *Bdellovibrio*. These layers also maintain cell shape and envelope rigidity. *Fig 4* depicts the structure of S-layer.

**Biofilms:** A biofilm is glycocalyx containing materials secreted by microbes in which the encased communities of the microbes survive. It is basically an exopolysaccharie atrix that adheres to an abiotic or biotic (plant roots) surface. E.g. *Bacillus species*. The structure of the Biofilm is represented in *Fig.5*.

Fig. 4: Structure of a typical S-layer



**Fig. 5: Biofilms formed by Bacteria**



**Important EPS**

There are many kinds of EPS found in nature formed by different types of *microflora and fauna*. Some of them are given in the Table 1.

**Table 1: Microbial Exopolymers and their Applications (Sutherland 1998; Kumar et al. 2007; Vu et al.2009)**

Biopolymers	Applications
Acetan	Viscosifier and gelling agent
Alginate	Immobilization and Microencapsulation
Cellulose	Temporary artificial skin, natural non-digestible fibers, hollow fibers or membranes, and acoustic membranes in audiovisual equipment
Curdlan	Gelling Agent
Cyclophorans	Encapsulation of drugs and food component
Dextran	Blood plasma extender or blood flow improving agent, cholesterol lowering agent, and microcarrier in tissue/cell culture
Emulsan	Emulsification and immobilization
Gellan	Solidification/gelling agent
Hyaluronic acid	Moisturization and synovial fluid replica
Kefiran	Gelatination and viscoelasticity
Levan and Alternan	Similar as dextran
Succinoglycan	Gelling agent and immobilization
Welan	Stabilizer and Viscosifier
Xanthan	Emulsification and gelatination

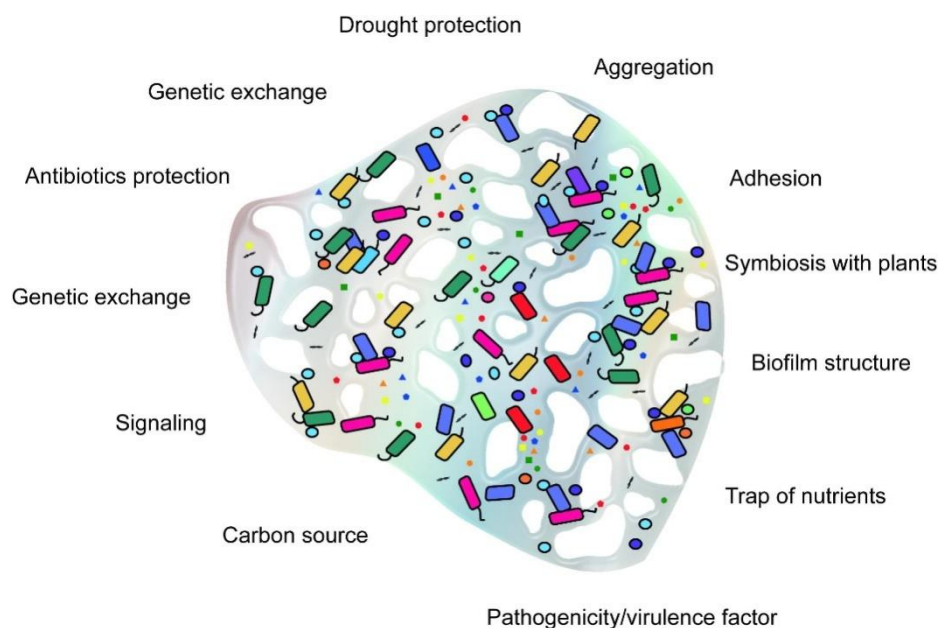
**EPS Biosynthesis**

The biosynthesis of EPS can be explained by three steps. In the first step, the microbes assimilate the Carbon through available substrates. Then polysaccharides are synthesized within the cells. Later the exudation of these polysaccharides out of the cells results in the formation of different types of EPS depending on the abiotic and biotic factors of the micro-climate where the microorganisms live.

**Application of EPS in Agriculture**

Microbial EPS can enhance the aggregation of soil particles and benefit plants by maintaining the moisture of the environment and trapping nutrients. The unique characteristics of EPS like gelling, biocompatibility and thickening capabilities can be used to protect plants against abiotic stresses like salinity and drought. EPS improve the soil aggregation and flocculation, important factors for soil structure, health and fertility. Some species forming EPS are also reported to decrease the oxidative stress of the plants. EPS have also the ability to resist the fluctuations in temperature and *pH*. It is also reported that EPS formation also influence the production of some plant hormones. The functions of EPS has been depicted in *Fig.6*.

**Fig. 6: Functions of EPS**



### Beneficial EPS forming Microbes for Agriculture

**Soil Aggregation:** Aggregates are the basic units of soil structures and are composed of pores and solid materials produced by the rearrangement of particles, flocculation, and cementation. These units influence the rheological properties of the soil like Water Holding Capacity, Porosity, Aeration etc. Soils with a balanced physical properties like these are ideal for plant growth. Some EPS forming bacteria are found to improve these qualities. For instance, *Lin et al. (2016)* reported that the electrostatic and adsorption capacity of the soils improved with EPS secreted by *Pseudomonas putida*. Other species like *Paenibacillus*, *Sphingobacterium*, *Bacillus*, *Pseudomonas* are also reported to increase the aggregation of soil. *Cyanobacteria* are also reported to improve the soil structures in arid of sandy soils zones (*Malam Issa et al., 1999*). Some EPS forming bacteria extracted from Biological Soil Crusts of Deserts also have the ability to improve the sand particle aggregation which improves the water holding capacity of the soils (*Nan Wu et al., 2014*).

**Plant Growth Promoters:** Plant Growth Hormones help the plants in its balanced growth and development. Sometimes plants themselves can't produce enough hormones for themselves. Therefore some Bacterial Strains residing near the rhizosphere of the host crops synthesize growth hormones such as Indole Acetic Acid. These bacteria are named Plant Growth Promoting Rhizobacteria (PGPR). It is reported that some strains of *Bacillus*, *Pseudomonas*, *Rhizobium*, and *Pantoea* form EPS which promote the synthesis of Plant growth hormones like IAA. This can enhance the crop yields (*Cipriano et al., 2016*). According to *Mu'minah et al., 2015*, there were 34 strains of Bacteria reported to produce IAA isolated from the rhizosphere of Potato grown in highlands.

**Salt Tolerance:** EPS are also involved in tolerance to salt stress. These molecules decrease Sodium uptake by the plants by trapping and decreasing the amount of ions in the soil (*Upadhyay et al., 2011*). EPS (formed by *S. meliloti*) prevents osmotic stress and nutrient imbalance which can benefit both microorganisms and plants. Species like *Pantoea agglomerans* play an important role in regulating the water content in wheat (*Amellal et al., 1998*).

**Drought Protection:** Drought stress has affected plants adversely. Plants which are xerophytic have the adaptive mechanisms to protect themselves from these climatic changes but most of the higher plants of mesophytic or hydrophytic habitat do not have any adaptive mechanisms to escape these sudden fluctuations in the atmosphere. Thus many crops are becoming more susceptible to drought. But some bacteria isolated from the arid regions are capable of secreting EPS to protect themselves and their host plants against environmental fluctuations like drought and salinity. For example, EPS produced by *Pseudomonas* showed higher Water retention. When such EPS producing bacterial strains were inoculated on the seeds of mesophytic plants like Maize, the plants showed higher water content, protein and sugars (*Hafsa et al., 2013*). It was also observed that the Water Holding Capacity of Sandy soils improved with the addition of EPS (*Roberson and Firestone, 1992*). Some bacterial EPS formed by *Proteus*, *Pseudomonas* etc. have the ability to promote plant growth and protect plants from drought (*Hafsa et al., 2013*). Rhizobacteriome which is an exclusive genetic material of bacteria resident to rhizosphere provides protection to the plants from abiotic stress like drought (*Kumar et al., 2020*). According to *Pollak et al., 2020*, Rhizobacteriome also has the capacity to protect the plants from pathogens like *Ralstonia species*.

**Symbiosis:** Symbiosis is a complicated process which involves signaling. Some biomolecules are also associated to form a symbiotic relationship between a plant and bacterial colony. One of these complex biomolecular structures is EPS. EPS also play an important role in the establishment of symbiosis between nitrogen-fixing rhizobia and plants that help in adaptation of both host and bacteria to changing environmental conditions. The composition and structure of EPS is different for different bacteria. For example, EPS formed by the *Rhizobium species* is mainly composed of xylose, mannose and glucose whereas the biofilm produced by *Bacillus* is composed of glycoproteins. The *Bacillus subtilis* species is also found to have association with the plant roots. It forms biofilm around the roots that protect the plants from infection (*Pascale B. Beauregard et al., 2013*). Other example include interaction between *Rhizobium leguminosarum* and *Pisum sativum* (*Chatli et al., 2014*).

**Sustainable Agriculture:** The N-P-K requirements of most of the crops are met by the chemical fertilizers which are toxic to environment. Thus, an organic fertilizing agent which is biodegradable in nature should be used to replace chemical fertilizers that are harmful for the environment. Bacterial Extramolecular Polysaccharides have the capacity to provide nutrients and protection from biotic and abiotic stress to the plants without causing pollution. These are also renewable in nature making them an ideal source of manuring agents for Sustainable Agriculture (*Saha et al., 2020*).

## II. Conclusion

Microorganisms have developed several approaches to survive environmental conditions, especially in soils. One among them is the formation of Extrapolymers. Being complex and diverse in forms, the knowledge on the applications of EPS is still limited. But efforts are being made to discover and characterize new polymers. EPS-based manures or solutions for crops can not only help in providing drought tolerance and salinity but also help in trapping the heavy metals causing soil degradation. Studies should focus on developing new techniques to understand the interactions of EPS with soil and plants, so that the knowledge can be applied to develop new EPS-based solutions in the field of Agriculture.

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