# Ecological functions and environmental fate of exopolymers of Acidobacteria

Costa, O.Y.A.

# Chapter 2

***Microbial extracellular polymeric substances: ecological function and impact on soil aggregation***

Ohana Y. A. Costa, Jos M. Raaijmakers, and Eiko E. Kuramae

Adapted from: Costa OYA, Raaijmakers JM, Kuramae EE (2018). Microbial Extracellular Polymeric Substances: Ecological Function and Impact on Soil Aggregation. **Frontiers in Microbiology**. 9:1636.

## Abstract

A wide range of microorganisms produce extracellular polymeric substances (EPS), highly hydrated polymers that are mainly composed of polysaccharides, proteins and DNA. EPS are fundamental for microbial life and provide an ideal substrate for chemical reactions, nutrient entrapment and protection against environmental stresses such as salinity and drought. Microbial EPS can enhance the aggregation of soil particles and benefit plants by maintaining the moisture of the environment and trapping nutrients. In addition, EPS have unique characteristics, such as biocompatibility, gelling and thickening capabilities, with industrial applications. However, despite decades of research on the industrial potential of EPS, only a few polymers are widely used in different areas, especially in agriculture. This review provides an overview of current knowledge on the ecological functions of microbial extracellular polymeric substances (EPS) and its application in agricultural soils to improve soil particle aggregation, an important factor for soil structure, health and fertility.

**Keywords:** EPS production, microorganisms, biosynthesis, ecological functions, soil aggregation.

## 1. Introduction

EPS are polymers biosynthesized by several strains of microorganisms. Composed mainly of polysaccharides, proteins and DNA, the production of these slimes is triggered primarily by environmental stresses. Since their biosynthesis is energetically expensive, they should generate some kind of advantage to the producer microorganism (Flemming & Wingender, 2010). Therefore, EPS production and functions have been studied for decades.

The polysaccharides are the most studied components of EPS. The investigation of EPS from numerous strains of microorganisms has demonstrated that the polysaccharides in these biopolymers vary immensely in composition and structure. They can be composed of one or many structural units, and the arrangement of these units is also exclusive for each different kind of EPS (Roca *et al.*, 2015). Aside from the carbohydrates, recently the interest in the structural proteins, enzymes and e-DNA has also been increasing. The analysis of e-DNA present in the EPS of a variety of strains has shown that the DNA is not innocuous, but can be a source of genetic exchange, signaling, attachment and moreover a very important structural component (Flemming & Wingender, 2010).

Besides the diversity of structures, EPS vary in their functions. A significant number of functions has been attributed to EPS, most of them related to protection. The matrix produced by EPS around microbial cells has the capability of shielding them against antimicrobial compounds and heavy metals; EPS matrix can also retain water, protecting microbes and the environment where it's contained against drought. In addition, other functions, such as adhesion, communication with other microbes and plants, antioxidant, aggregation, carbon storage, entrapment of nutrients have also been reported (Wingender *et al.*, 1999, Vardharajula & Ali, 2015, Wang *et al.*, 2015).

One of the roles of the EPS matrix that has been explored for decades is the capacity to aggregate soil particles, a function that is important for soil structure, health and fertility. Since EPS have a slimy texture and ionic charges, it can act like a glue, getting attached to clay and ions, holding solid particles together (Chenu, 1995). On the other hand, as stated before, EPS structures are variable, therefore their application efficiency in soils will vary accordingly. These polymers that are studied and produced in laboratorial conditions can be applied to soils for improvement of soil structure, fertility and quality. In this review we collate and synthesize the available information on EPS composition, biosynthesis, factors affecting EPS production, as well the ecological functions of microbial EPS and its application on soil particle aggregation.

## 2. EPS constituents

EPS are composed mainly of polysaccharides, proteins and DNA. However, the proportion of each component varies depending on the microbial strain and the method used to extract the EPS. Physical extraction methods, such as centrifugation, avoid the destruction of cells,

whereas aggressive methods, such as NaOH extraction, lyse cells, releasing their content into the EPS. Therefore, it is possible to find in the literature quantities of protein varying from 7.9 to 54.6 g per gram of EPS derived from the same sample using different extraction methods (Liu & Fang, 2002).

In general, polysaccharides are the main constituents of EPS and represent approximately 40% to 95% of the polymer (Flemming *et al.*, 2007). The polysaccharides can be classified as homopolysaccharides composed of a single type of monosaccharide or heteropolysaccharides composed of two or more types of monosaccharides (Sutherland, 2004). Common monosaccharides in EPS are D-glucose, D-galactose, D-mannose, L-fucose, L-rhamnose, D-arabinose, D-ribose and L-altrose. Less frequent are L-colitose, N-acetyl-L-fucosamine, N-acetyl-L-talosamine, L-iduronic acid, D-riburonic acid and 2-deoxy-D-arabino-hexuronic acid (Sutherland, 2004, Mishra & Jha, 2013). Polymers enriched with rare sugars are of potential interest because their unusual composition and structure may confer additional attributes, such as anti-inflammatory and antioxidant properties.

A significant number of proteins with different functions have been observed in EPS, including several trapped extracellular enzymes. The products of these enzymes consequently remain close to the cell, facilitating their uptake by the bacteria (Wingender *et al.*, 1999, Flemming & Wingender, 2010). Some EPS-modifying enzymes are capable of degrading the polymer during starvation. However, this process is slow, since no single enzyme is capable of degrading all of the polysaccharides present in the EPS matrix. In general, highly specific enzymes are required for this task (Sutherland, 2004, Flemming & Wingender, 2010). In addition, structural proteins are involved in the formation and stabilization of the polysaccharide chain and are responsible for the connection between the cell surface and the extracellular EPS (Flemming & Wingender, 2010).

Extracellular DNA (e-DNA) of different origins is an important EPS component in biofilms. Although the function and origin of e-DNA have not been completely elucidated, studies have shown that it is responsible for the structure of certain EPS and plays a role in adhesion to surfaces and signaling. E-DNA is likely an important structural component of *Staphylococcus aureus* biofilms but is not essential in biofilms produced by *Streptococcus epidermidis* (Flemming & Wingender, 2010). This conclusion is based on the fact that treatment with DNase I inhibits biofilm formation and detachment of preformed biofilms by *S. aureus* but not *S. epidermidis* (Izano *et al.*, 2007). In *Pseudomonas aeruginosa*, e-DNA is essential for biofilm formation, as DNase I inhibits this process (Whitchurch *et al.*, 2002). Moreover, *Bacillus cereus* mutants produce a weaker biofilm when lacking a purine biosynthesis gene involved in e-DNA production (Vilain *et al.*, 2009).

## 3. EPS biosynthesis

EPS production has been reported for bacteria and cyanobacteria as well as microalgae

(Parikh & Madamwar, 2006, Boonchai *et al.*, 2014), yeasts (Pavlova & Grigorova, 1999), basidiomycetes (Hwang *et al.*, 2004, Elisashvili *et al.*, 2009) and protists (Jain *et al.*, 2005). EPS are formed by the polymerization of repeating units of similar or identical monomers and are classified as loosely bound or tightly bound depending on their association with the cell (More *et al.*, 2014).

Initially, EPS was used as an abbreviation for "extracellular polysaccharides", "exopolymers" or "exopolysaccharides"; however, studies have shown that the matrix is much more complex and includes structural proteins, enzymes, nucleic acids, lipids and other compounds such as humic acids (Wingender *et al.*, 1999, Wingender *et al.*, 1999, Flemming & Wingender, 2010). The mucoid substances present in EPS are not only produced by the microorganism but are also derived from cellular lysis, hydrolysis of macromolecules and absorption from the environment. Each component contributes to the physicochemical characteristics of the matrix (Nielsen & Jahn, 1999). EPS physically involve microbial cells and mediate contact and exchange processes within microbial communities as well as with the environment. The EPS matrix provides a hydrated and buffered environment that facilitates chemical reactions (Wingender *et al.*, 1999).



Studies of genes involved in EPS production have focused on a few polymers, and the biosynthesis pathways of the polysaccharides composing EPS have been widely described. Four main biosynthesis pathways are known: (1) the Wzx-Wzy-dependent pathway; (2) the synthase-dependent pathway, (3) the ABC transporter-dependent pathway, and (4) extracellular synthesis by sucrase enzymes (Schmid *et al.*, 2015). In the Wzx-Wzy-dependent secretion pathway, the individual repeating units are assembled on an undecaprenyl-phosphate carrier located in the cytoplasmic portion of the inner membrane and then transported to the periplasm by a Wzx flippase (Whitney & Howell, 2013, Schmid *et al.*, 2015). Once in the periplasm, the putative polymerase Wzy assembles the polymer units, which are transported across the outer membrane by a complex formed by a polysaccharide copolymerase and an outer membrane polysaccharide exporter (Figure 1). The size of the chain is regulated by the polysaccharide copolymerase Wzz (Cuthbertson *et al.*, 2009). Xanthan produced by *Xanthomonas campestris* (Vorhölter *et al.*, 2008) and gellan produced by *Sphingomonas paucimobilis* (Wang *et al.*, 2006) are examples of EPS synthesized via this pathway.

In the ABC transporter-dependent pathway, the polymer is fully synthesized in the cytoplasm and then transported across the inner membrane by a dedicated ABC transporter. As in the Wzx-Wzy-dependent pathway, transport to the outside of the cell is accomplished by the polysaccharide copolymerase and polysaccharide exporter complex (Cuthbertson *et al.*, 2009, Whitney & Howell, 2013, Schmid *et al.*, 2015) (Figure 1). This pathway is involved in capsular polysaccharide production (Whitney & Howell, 2013).

For the synthase-dependent pathway, it has been proposed that biosynthesis and transport to the periplasmic space are accomplished by the same protein, a polymerizing

glycosyltransferase (synthase) (Hubbard *et al.*, 2012, Schmid *et al.*, 2015). Transport across the outer membrane is accomplished by a tetratricopeptide repeat (TRP) domain-containing protein and a β-barrel porin (Keiski *et al.*, 2010, Whitney & Howell, 2013) (Figure 1).

In general, this pathway is used for the production of EPS consisting of only one type of sugar precursor (Schmid *et al.*, 2015), such as the bacterial cellulose from *Komagataeibacter medellinensis* (Matsutani *et al.*, 2015) and curdlan from *Agrobacterium sp.* (Stasinopoulos *et al.*, 1999). Extracellularly synthesized EPS are assembled by glucansucrases, enzymes that are secreted and anchored to the cell wall. These enzymes catalyze the transfer of glucose from sucrose to the growing polysaccharide chain (Rehm, 2010, Schmid *et al.*, 2015). In some strains of microorganisms, such as the dextran producer *Leuconostoc mesenteroides*, the expression of glucansucrases is induced by sucrose (Kim & Robyt, 1994), whereas in some levan/inulin-producing *Lactobacillus strains*, the genes *levS* (levansucrase) and *inu* (inulosucrase) are expressed constitutively (Tieking *et al.*, 2004, Schwab *et al.*, 2007). The mechanism of sucrose induction, however, remains unknown. A summary of the genes, structure and producing microorganisms of industrially relevant EPS is provided in Table 1. For a review on the biosynthesis pathways of industrially important EPS, refer to Schmid et al. (2015).

EPS production and regulation have been studied for several decades because these polymers have biotechnological applications and are widely used in the pharmaceutical and food industries. For some polymers, such as xanthan, alginate and curdlan, the genes

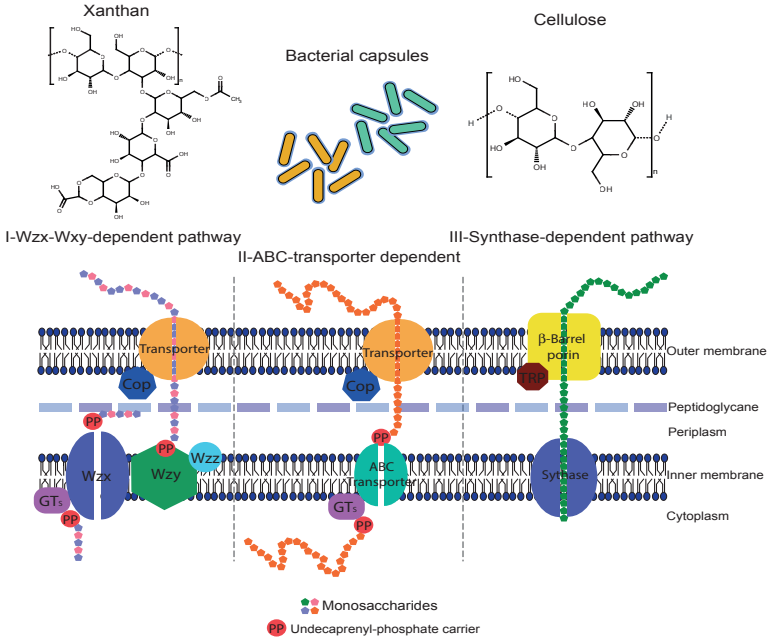


**Figure 1:** Simplified schematic representation of the three main intracellular biosynthetic pathways for microbial EPS production and example structures for each pathway. COP: copolymerase; GTs: glycosyl hydrolases; TRP: tetratricopeptide repeat protein. Figure modified according to Schmid *et al.* (2015).

responsible for production and structure have already been described and deeply explored. Current studies are focused on applying engineering strategies to improve the yields and characteristics of these polymers by targeting precursors and genes involved in their production or regulation. However, knowledge of the structures and functions of other types of polymers, especially EPS secreted by lesser-known strains, is relatively deficient.

## 4. Factors influencing EPS production



EPS production by microorganisms can be triggered by environmental and physiological conditions, such as carbon source, nitrogen starvation and ionic strength (Janczarek, 2011, Carzaniga *et al.*, 2012). Under stress conditions, EPS are synthesized to establish a physical barrier around the cell (Kehr & Dittmann, 2015). Microbial EPS production has mainly been observed in pure culture and varies according to environmental conditions (Chenu, 1995). In laboratory culture media, physiological conditions can be controlled to achieve high EPS yield and modify polymer characteristics, including relative molecular mass, polymer pattern, number of residues and degree of branching (Dumitriu, 2005). The composition of the polymer, however, is strain specific, and only some bacteria, such as *Enterobacter* strain A47 (Torres *et al.*, 2012), can be induced to change the polymer pattern (Roca *et al.*, 2015). Moreover, there are no standard conditions that promote high EPS production, since carbon and nitrogen sources, mineral requirements, optimal pH and temperature differ for each microorganism (Kumar *et al.*, 2007).

### 4.1. Carbon source

Carbon source is one of the main factors influencing EPS yield; therefore, many studies of EPS production have assessed the influence of a variety of carbon sources on EPS yield and the biomass of microorganisms. These carbon sources include glucose, fructose, lactose, maltose, mannitol, sorbitol, starch and sugar concentrates (Neosorb™, Cerelose™) (Kumar *et al.*, 2007). Glucose and fructose typically deliver the highest amount of EPS. Two strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* (Petry *et al.*, 2000), *Chryseobacterium indologenes* MUT.2 (Khani *et al.*, 2016), *Lactobacillus delbrueckii* subsp. *bulgaricus* strains B3 and G12 and *Streptococcus thermophilus* W22 (Yuksekdag & Aslim, 2008) produce EPS more efficiently with glucose as the carbon source in the culture medium. *Trametes versicolor*, however, produces EPS more efficiently when fructose is the carbon source (Bolla *et al.*, 2010). Other strains have different requirements, such as *Rhizobium leguminosarum* biovar *trifolii* TA-1, which has a preference for mannitol, and *Halomonas alkaliantarctica* strain CRSS, which requires acetate (Poli *et al.*, 2004) for high EPS production.

Table 1: Industrially relevant polymers: genes, structures and biosynthesis pathways.

| Polymer | Genes | Structure (example of producing microorganism) | Biosynthesis pathway | Reference |
|---|---|---|---|---|
| Alginate | *alg8, alg44, algD, algG, algX, algK, algE* | β-(1-4)-linked non-repeating heteropolymer (*Pseudomonas aeruginosa, Azotobacter vinelandii*) | Synthase-dependent | (Remminghorst & Rehm, 2006, Rehm, 2010) |
| Bacterial cellulose | *bcsA, bcsB, bcsC, bcsZ* | β-(1-4)-Glucan (*Acetobacter xylinum)* | Synthase-dependent | (Rehm, 2010) |
| Curdlan | *crdS, crdA, crdR, cdrC* and *crdB* | β-(1-3)-Glucan (*Agrobacterium* strains*)* | Synthase-dependent | (Rehm, 2010, Schmid *et al.*, 2015, Yu *et al.*, 2015) |
| Dextran* | *dsr* variations (*dsrF*, *dsrD*, *dsrX*, *dsrN*, *dsrR*) | α-(1-6)-glucose backbone with α-(1→2), α-(1→3), α-(1→4) branched linkages (*Leuconostoc mesenteroides*) | Extracellular (Dextransucrase) | (Kim & Robyt, 1994, Schmid *et al.*, 2015) |
| Gellan | pgmG, ugpG | β-(1,3)-linked repeating polymer composed by tetrasaccharide units (*Sphingomonas paucimobilis*) | Wzx/Wzy-dependent | (Sá-Correia *et al.*, 2002, Schmid *et al.*, 2015) |
| Inulin* | *inuJ*, *inuG*, *inuB*, *islA*, *ftf* | b(2-1) fructan (*Lactobacillus* and *Leuconostoc* strains) | Extracellular, (Inulosucrase) | (Anwar *et al.*, 2010, Schmid *et al.*, 2015) |
| Levan* | *levG*, *lsdA*, *levS*, *sacB*, *sacY* | β-(2,6) D-fructose backbone with β-(2,1) D-fructose and glucose branches (*Lactobacillus*, *Bacillus* and *Aerobacter* strains) | (Extracellular, Levansucrase) | (Srikanth *et al.*, 2015) |
| Succinoglycan | *exoC, exoB, exoN, exoY, exoF, exoA, exoL, exoM, exoO, exoU, exoW, exoP, exoT, exoQ, exoZ, exoH, exoV, exoK* | Octasaccharide repeating units with acetyl, pyruvyl, and succinyl substitutions (*Rhizobium*, *Agrobacterium*, *Alcaligenes* and *Pseudomonas* strains) | Wzx/Wzy-dependent | (Reuber & Walker, 1993, Schmid *et al.*, 2015) |
| Xanthan | *gumB, gumM, gumH, gumK, gumI, gumJ, gumE, gumC, gumB, gumD, gumL, gumF, gumG* | β-(1-4)-linked glucose backbone and a side chain composed of two mannose units and one glucuronic acid (*Xanthomonas campestris)* | Wzx/Wzy-dependent | (Becker *et al.*, 1998, Rehm, 2010) |

*The genes are not part of a cluster; each encodes a different glucansucrase in a different strain.

### 4.2. Nitrogen source

The main nitrogen sources employed in EPS production are ammonium sulfate, peptone, sodium nitrate, urea and yeast extract (Kumar *et al.*, 2007). However, the highest growth rates and EPS yields are reached when complex nitrogen sources are involved, probably due to the presence of growth factors (Farrés *et al.*, 1997), for which requirements vary among microorganisms. In addition, carbon found in the nitrogen source increases the carbon/nitrogen (C/N) ratio and thereby enhances EPS production (Kumar *et al.*, 2007).

Similar to carbon sources, several studies have compared different compounds to identify the best nitrogen supply for a variety of microbial strains reflecting the metabolic diversity among EPS producers. Among the nitrogen sources applied to optimize EPS production, the compounds that generally induce the highest yields are yeast extract and different types of peptones. However, inorganic nitrogen supplies can also induce high polymer production; ammonium sulfate is the best source for a high EPS yield from *Gluconacetobacter hansenii* LGM1524 (Valepyn *et al.*, 2012), whereas ammonium nitrate and sodium nitrate are optimal for *Bacillus megaterium* (Gandhi *et al.*, 1997) and some rhizobial strains (Kumar and Ram (2014).



### 4.3. Carbon/nitrogen ratio

The C/N ratio is as important as carbon type or nitrogen source and greatly affects microbial metabolism and, consequently, EPS production. Many studies have reported maximization of EPS production under nitrogen limitation and carbon excess. However, like other culture nutrient variables, there is no fixed ideal C/N ratio for all microorganisms (More *et al.*, 2014). EPS production by *Rhizobium tropici* reaches its maximum yield at a C/N of 20 (Staudt *et al.*, 2011), whereas *Rhodoblastus acidophilus* (formerly known as *Rhodopseudomonas acidophila*) requires a C/N ratio of 7.7 at low concentrations of carbon ($C_4H_4Na_2O_4$) and nitrogen sources ($(NH_4)_2SO_4$) (Sheng *et al.*, 2006). By contrast, for some strains of lactic acid bacteria (LAB), nitrogen limitation does not increase EPS yield. The production of EPS by *Streptococcus thermophilus* is dependent on high carbon and nitrogen concentrations (De Vuyst *et al.*, 1998). In addition, the effect of the C/N ratio may depend on the culture medium used for growth. Gonzalez Garcia et al. (2015) evaluated the effects of variable C/N ratios on EPS production by *Saccharophagus degradans* in basal culture medium (BM) and nutrient-limited medium (NL). In BM, variation of the C/N ratio did not affect EPS production, whereas an enhancement of EPS production was observed in NL, indicating a possible effect of the combination of the C/N ratio and nutrient limitation (N, P, K, Ca, Mg, and Fe).

### 4.4. Other nutrients and trace elements

In addition to carbon and nitrogen, nutrients such as Mn, Zn, Co, Mo, vitamins, P and $O_2$ are required for EPS synthesis and influence the conversion of precursors into polysaccharide (More *et al.*, 2014, González-García *et al.*, 2015). However, metal ion requirements differ among

microbial strains. $Mg^{2+}$ appears to enhance EPS production by *Lactobacillus rhamnosus* C83 (Gamar-Nourani *et al.*, 1998) and *Stemphylium sp*. (Banerjee *et al.*, 2009), whereas the addition of phosphate in the medium decreases the EPS yield of *Klebsiella* I-174 (Farrés *et al.*, 1997). The presence of $Na^{+}$ increases the EPS yields of *Rhodopseudomonas acidophila* (Sheng *et al.*, 2006) and *L. rhamnosus* (Gamar-Nourani *et al.*, 1998), suggesting a defensive response of the bacteria to salt stress. In addition to metal ions, compounds such as histidine, tyrosine, phenylalanine and xanthine are important for EPS production by *Stemphylium sp*. (Banerjee *et al.*, 2009).



### 4.5. Temperature and pH

Temperature and pH often influence EPS production (Kumar *et al.*, 2007, More *et al.*, 2014), but the optimal values of both parameters vary among microorganisms. Incubation at a temperature lower than the optimal temperature for bacterial growth typically enhances EPS biosynthesis because when cells grow slowly, the synthesis of the cell wall is slower, and more sugar precursors are available for EPS production (Sutherland, 2001). Most strains that produce EPS grow at a temperature range of 25-30 °C (More *et al.*, 2014). However, EPS production has been reported for psychrophilic microorganisms such as *Pseudoalteromonas* strain CAM025, which has an increased polymer yield between -2 °C and 10 °C (Nichols *et al.*, 2005), and *Colwellia psychrerythraea* strain 34H, which exhibits the highest yield at -8 °C (Marx *et al.*, 2009). EPS secretion has also been observed between 60 °C and 65 °C for the thermophilic bacteria *Bacillus thermodenitrificans* DSM 465 (Nicolaus *et al.*, 2000) and *Bacillus thermoantarcticus* (Manca *et al.*, 1996).

Many microbial strains grow and produce EPS in a neutral pH range, and EPS synthesis generally requires a stable pH for maximum production (Kumar *et al.*, 2007). Thus, extreme pH variation can decrease the polymer yield. Xanthan, curdlan and gellan, polymers used in industry, are produced at a pH range of 7.0–7.5 (Kalogiannis *et al.*, 2003, Nampoothiri *et al.*, 2003, Shih *et al.*, 2009). However, since the optimal pH varies among microorganisms, EPS formation has been observed at a wide range of pH. *Rhizobium tropici* (Staudt *et al.*, 2011) and *Rhizobium ciceri* (Küçük & Kivanç, 2009) produce EPS at neutral pH, with a drastic decrease in yield under acidic conditions. Synthesis of EPS by *Enterobacter* strain A47 decreases significantly with increasing pH of the medium (Torres *et al.*, 2012). By contrast, *Halomonas alkaliantarctica* strain CRSS can produce EPS at pH 8.0 and 9.0 (Poli *et al.*, 2004), and the optimum pH for highest EPS yield is 5.0 for *Antrodia camphorata* (Shu & Lung, 2004). These data illustrate the diversity of conditions for EPS production and the efforts of researchers to increase the yields of EPS secreted by different microorganisms. However, due to the high diversity of available EPS, many strains and polymers remain to be evaluated.

## 5. Ecological functions

EPS biosynthesis is an energy-demanding process. Therefore, its production requires selective advantages in the environment of the producing microorganism. In laboratory cultures, the production of EPS does not impact cell viability or growth and thus appears not to be essential for survival. However, in natural environments, most microorganisms live in aggregates, such as flocs and biofilms, for which EPS are structurally and functionally essential (Wingender *et al.*, 1999). Most of the functions attributed to EPS are related to protection of the producing microorganism. Diverse variations in abiotic conditions such as drought, temperature, pH and salinity can trigger the production of EPS as a response to environmental stresses (Wingender *et al.*, 1999, Kumar *et al.*, 2007, Vardharajula & Ali, 2015). The functions of EPS are summarized in Figure 2.



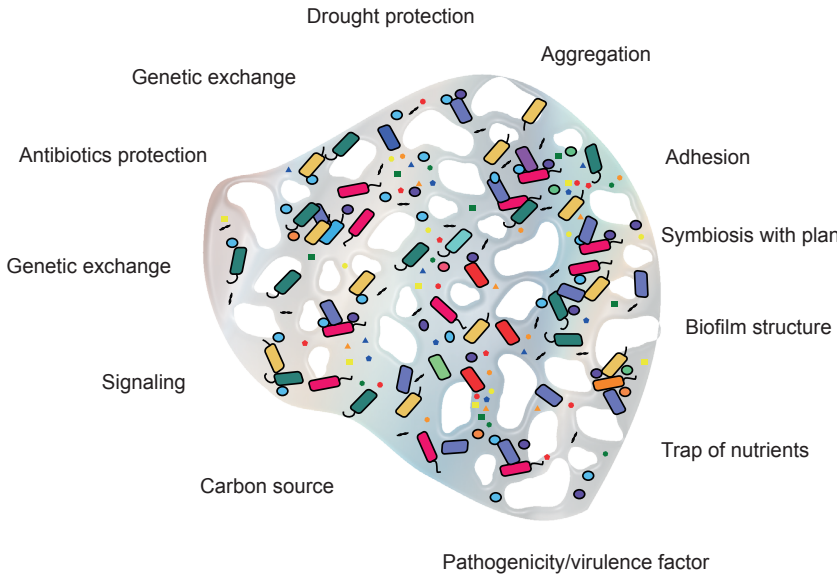


**Figure 2:** Conceptual framework of the functions of microbial extracellular polymeric substances (EPS) in soil.

### 5.1. Functions of EPS in interactions with the environment

#### 5.1.1. Adhesion/ Cohesion/ Genetic material transfer

EPS are responsible for the cohesion of microorganisms and adhesion of biofilms to surfaces, influencing spatial organization, allowing interactions among microorganisms, and acting as adhesives between cells (Wolfaardt *et al.*, 1999). These functions are important for the establishment and biological activities of biofilms and flocs. The polymers mechanically stabilize the microbial aggregates via several types of interactions between the macromolecules, including dispersion forces, electrostatic interactions and hydrogen

bonds. The resultant formation of a gel-like tridimensional structure around the cells allows the microorganisms to be retained near each other to establish stable consortia (Flemming *et al.*, 2000). For example, EPS of *Sphingomonas paucimobilis* have surface-active properties that promote and enhance attachment via the formation of polymeric bridges (Azeredo & Oliveira, 2000). The quantity of EPS can also influence cell adhesion, as demonstrated by Tsuneda *et al.* (2003). For the 27 bacterial strains evaluated, small quantities of EPS inhibited cell adhesion by electrostatic forces, whereas large amounts enhanced adhesion via interactions between functional groups in the EPS, such as uronic acids and acetyl groups. The nature of the interactions between the functional groups in EPS, however, is unknown. In addition, the matrix formed by EPS can facilitate chemical communication and even influence predator-prey interactions (Flemming *et al.*, 2007). Joubert *et al.* (2006) observed that ciliated protists preferred feeding on planktonic cells and the EPS matrix rather than on attached and biofilm-derived cells. In addition, the presence of protists appeared to enhance yeast metabolic activity in the biofilm.

Together with different protein adhesins, EPS are believed to be involved in the initial steps of microbial adhesion to surfaces. For instance, the polysaccharide produced by *Caulobacter crescentus*, called holdfast, is crucial for the initial surface attachment, together with other cellular structures (Entcheva-Dimitrov & Spormann, 2004, Wan *et al.*, 2013). However, the characteristics of each polymer are defined by their composition, as adhesiveness depends heavily on chain conformation, internal substituents and internal/external interactions (Berne *et al.*, 2015). Therefore, the extent to which the type of polymer contributes to the adhesive properties of bacterial cells remains to be determined.

In addition to polysaccharides, extracellular DNA (exDNA) seems also to be responsible for the adhesive properties of some EPS. Although the functions of exDNA have not been completely elucidated, studies have shown that it is responsible for the cohesion and structure of certain EPS and plays a role in adhesion to surfaces and signaling (Okshevsky & Meyer, 2013). Released by autolysis or active secretion by microorganisms, exDNA is likely an important structural component of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Ralstonia solanacearum* biofilms (Whitchurch *et al.*, 2002, Minh Tran *et al.*, 2016); however, it is not essential in biofilms produced by *Streptococcus epidermidis* (Flemming & Wingender, 2010). This conclusion is based on the fact that treatment with DNase I inhibits biofilm formation and detachment of preformed biofilms by *S. aureus* but not *S. epidermidis* (Izano *et al.*, 2007). Enhancement of genetic material transfer between microorganisms is another property of extracellular polymers. ExDNA of different origins is an important EPS component in biofilms, where microorganisms are surrounded by an EPS matrix. Although studies in this area are scarce, the rates of natural transformation and conjugation of bacteria appear to be higher within biofilms. Bae et al. (2014) demonstrated that *Campylobacter jejuni* transfers antibiotic resistance genes by natural transformation more frequently in biofilms than in planktonic cells. Other studies have shown that biofilm age and DNA concentration influence the frequency

of transformation events, whereas a high density of planktonic cells inhibits transformation in biofilms (Hendrickx *et al.*, 2003). Moreover, the number of events observed can depend greatly on the technique used to detect conjugative gene transfer in biofilms. For instance, Hausner and Wertz (1999) detected 1000-fold higher conjugation rates using confocal laser scanning microscopy than by classic plating techniques. It has been suggested that exDNA fractions can be used in environmental studies as an alternative method for microbial activity measurement. However, exDNA fraction separation and evaluation in complex samples, such as soils, has yet to be improved (Nagler *et al.*, 2018). Estimates of microbial community composition can be influenced by the presence of exDNA (Carini *et al.*, 2016).



### 5.1.2. Symbiosis

EPS play an important role in the establishment of symbiosis between nitrogen-fixing rhizobia and plants. Rhizobial surface polysaccharides are fundamental for nodule formation by some legumes, although the underlying mechanisms are not yet fully resolved. For example, to invade alfalfa nodules and establish successful symbiosis, *Sinorhizobium meliloti* Rm1021 must produce succinoglycan (Cheng & Walker, 1998). Mutants that do not synthesize succinoglycan produce modified polymers or overproduce EPS, reduce the capacity of *S. meliloti* Rm 1021 to infect and establish symbiosis. Although capable of producing nodules, *Rhizobium leguminosarum* biovar *viciae* glucomannan (*gmsA*) mutants are strongly outcompeted by wild-type bacteria in mixed inoculations of *Pisum sativum* (Williams *et al.*, 2008). The interaction between the EPS of *Mesorhizobium loti* strain R7A and *Lotus japonicus* was recently shown to be mediated by a receptor expressed by the plant. *Lotus japonicus* produces a receptor (EPR3) that binds to and permits infection by only bacteria that produce EPS with a specific structure; mutants with truncated EPS are less successful in infection (Kawaharada *et al.*, 2015). The expression of this receptor demonstrates that the plant is capable of recognizing the structure of EPS produced by rhizobia.

### 5.1.3. EPS as pathogenicity/virulence factors

For some bacteria, polymers function as pathogenicity and virulence factors. For example, the high virulence of *Erwinia amylovora* is a result of the production of amylovoran and levan. Both polymers contribute to the pathogenesis of the bacteria, and the absence of either amylovoran or levan dramatically decreases plant colonization (Koczan *et al.*, 2009). In addition, EPS can serve as a mechanical barrier between bacteria and plant defense compounds by decreasing the diffusion rates of these compounds. For example, the polymers of *Pseudomonas syringae* pv. *phaseolicola* and *Sinorhizobium meliloti* protect the bacteria against reactive oxygen species (ROS) produced by the plant host during infection, thereby decreasing oxidative stress (Király *et al.*, 1997, Lehman & Long, 2013). *Sinorhizobium meliloti* mutants overproducing EPS protect polymer-deficient mutants against $H_2O_2$ (Lehman & Long, 2013). Alginate, the EPS produced by *Pseudomonas aeruginosa*, a human

opportunistic pathogen, protects the bacteria against the inflammatory process of the host, avoiding free radicals, antibodies and phagocytosis and thereby aggravating the prognosis of patients infected by *P. aeruginosa* (Ryder *et al.*, 2007). Althought is known that EPS may act as an antioxidant, less in known the chemical mechanism of protection against ROS.

## 5.2. EPS and nutrition

### 5.2.1. Carbon reserves



EPS produced by microorganisms might act as carbon reserves, but few studies have investigated the role of EPS in nutrition or cross-feeding between organisms. Since EPS are generally complex molecules, their complete degradation would require a wide range of different enzymes (Flemming & Wingender, 2010). *Rhizobium* NZP 2037 can use its own poly-b-hydroxybutyrate (PHB) and EPS as sole sources of carbon for survival in carbon-restriction situations (Patel & Gerson, 1974). However, EPS is a higher potential carbon source than PHB. Stable isotope probing (SIP) is a powerful strategy for detecting microorganisms that can degrade polymers. Wang et al. (2015) labelled the EPS of *Beijerinckia indica* and observed that the polymer was assimilated by bacteria with low identities to known species, particularly members of the phylum *Planctomycetes*. In addition, the authors isolated bacteria that used the EPS as a sole carbon source, demonstrating the potential utility of these polymers for isolating new microbial species.

### 5.2.2. Nutrient trap

In addition to supplying carbon, EPS can accumulate other nutrients and molecules. The retention of extracellular enzymes in the EPS matrix promotes the formation of an extracellular digestion system that captures compounds from the water phase and permits their use as nutrient and energy sources (Flemming & Wingender, 2010). Many studies have investigated the adsorption of metal ions by EPS for heavy-metal remediation and recovery of polluted environments. The EPS of *Paenibacillus jamilae* adsorbs multiple heavy metals (Pb, Cd, Co, Ni, Zn and Cu) with strongler interaction with Pb, a maximum binding capacity of 303.03 mg/g, tenfold higher than the binding capacities for other metals (Morillo Pérez *et al.*, 2008). The polymers produced by *Anabaena variabilis* and *Nostoc muscorum* possess similar affinities for Cu, Cd, Co, Zn and Ni, with the highest affinity for Cu and the lowest for Ni. Both bacterial EPS are promising for the removal of toxic heavy metals from polluted water (El-Naggar *et al.*, 2008). The EPS of *Pseudomonas sp*. CU-1 has a high Cu-binding capacity and thus, protect bacterial cells against this metal ion (Lau *et al.*, 2005).

## 5.3. EPS in protection against abiotic and biotic stresses

### 5.3.1. Drought protection

EPS production can confer advantages to microorganisms in environments under drought

stress. A high water-holding capacity was observed for an EPS produced by a *Pseudomonas* strain isolated from soil; this EPS can hold several times its weight in water. When added to a sandy soil, the EPS altered its moisture by allowing the amended soil to hold more water than unamended soil (Roberson & Firestone, 1992). According to the authors, the EPS protected the bacteria against desiccation by acting like a protective sponge, thereby giving the bacteria time to make metabolic adjustments. This polymer exhibits significant structural modifications during desiccation and may be an important protection factor traps a reservoir of water and nutrients for bacterial survival (Roberson *et al.*, 1993). Cyanobacteria isolated from arid regions, such as *Nostoc calcicola* (2014) and *Phormidium* 94a (2004), are also capable of producing EPS, which may represent a strategy for water/nutrient retention and survival.



### 5.3.2. Salt tolerance

Some studies have revealed that microbial polymers are involved in tolerance to salt stress, not only for the producer microorganisms but also for the associated plants. The production of polymer by NaCl-tolerant isolates can decrease Na uptake by plants by trapping and decreasing the amount of ions available (Upadhyay *et al.*, 2011). Therefore, the polymer prevents nutrient imbalance and osmotic stress, which can promote survival of the microorganisms and benefit of the plant. *Sinorhizobium meliloti* strain EFBI cells severely reduce EPS production when inoculated in culture medium with low salt concentration. Since this strain was isolated from the nodules of a plant growing in a salt marsh with a salinity level of 0.3 M, a lower amount of salt can be considered a stressful condition. However, the relevance of this EPS for survival and symbiosis was not further studied (Lloret *et al.*, 1998).

### 5.3.3. Protection against low/high temperatures

The production of EPS at low temperatures is an important factor in the cryoprotection of sea-ice organisms as well as a natural adaptation to low temperatures and high salinities. High concentrations of EPS have been observed in samples collected from Arctic sea ice; the EPS shields diatoms against the severe environmental conditions during the winter season (Krembs *et al.*, 2002). In addition, EPS alter the microstructure and desalination of growing ice, consequently improving microbial habitability and survivability (Krembs *et al.*, 2011).
EPS can be a protection factor for thermophilic bacteria by shielding microorganisms from very high temperatures. The polymers produced by *Bacillus sp*. strain B3-72 and *Geobacillus tepidamans* V264 are not easily dissolved at high temperatures (Nicolaus *et al.*, 2000, Kambourova *et al.*, 2009). A few studies (Manca *et al.*, 1996, Nicolaus *et al.*, 2000, Nicolaus *et al.*, 2004) have evaluated EPS production by thermophilic bacteria and archaea for potential applications of these polymers in industry and the recovery of polluted environments. However, the structure and the ecological function of these slimes remain to be established.

#### 5.3.4. Protection against antimicrobials

The matrix that surrounds microorganisms in biofilms plays an important role in decreased susceptibility to antimicrobials. In general, biofilm matrices possess a negative charge and therefore bind positively charged compounds, protecting the innermost cells from contact. In addition, electrostatic repulsion can reduce the diffusion rates of negatively charged antimicrobials through the biofilm (Everett & Rumbaugh, 2015). Many studies have tested the inhibitory potential of bacterial EPS against antimicrobial compounds, particularly for clinically important bacterial strains. A few studies have demonstrated that the slime produced by *Staphylococcus* sp. is an effective antagonist to vancomycin, perfloxacin and teicoplanin, acting as a barrier to the compounds or even interfering with their action in the cell membrane (Farber *et al.*, 1990, Souli & Giamarellou, 1998). The EPS produced by *Acinetobacter baumannii* is also protective against tobramycin exposure and is effective regardless of the bacterial species exposed. By contrast, the polymer from *S. aureus* has no protective effect against tobramycin (Davenport *et al.*, 2014). EPS can also protect microorganisms against disinfection agents. Alginate produced by *Pseudomonas aeruginosa* enhances bacterial survival in chlorinated water, and removal of the slime eliminates bacterial chlorine resistance (Grobe *et al.*, 2001).

The few EPS isolated thus far have a wide range of functions, but a huge diversity of polymers produced by microorganisms with different functions awaits exploration and discovery. The different functions already discovered are consequences of the diverse EPS structures, and are connected to the benefits they can have when applied to soils. The production of EPS is not only and advantage to the microbes, but to the soil environment in general. The adhesiveness is important for gluing soil particles together; high water holding capacity protects microorganisms and plants against drought, as well as permits the diffusions of nutrients in the environment. EPS production also influences and is influenced by interactions between plants and microorganisms, increasing the availability of nutrients as a whole, promoting plant and microbial growth. In the next section, we summarize how the currently known EPS are applied to agricultural soils and their benefits for soil aggregation.

## 6. Application of EPS on soil aggregation

### 6.1. Soil aggregates and microbial communities

Aggregates are the basic units of soil structure and are composed of pores and solid material produced by rearrangement of particles, flocculation and cementation. These units define the physical and mechanical properties of soil, such as water retention, water movement, aeration and temperature, which in turn affect physical, chemical and biological processes (Alami *et al.*, 2000, Tang *et al.*, 2011). Aggregates are important for the improvement of soil fertility, porosity, erodibility and agronomic productivity by influencing plant germination and root growth (Dinel *et al.*, 1992, Bronick & Lal, 2005). Aggregate formation involves

numerous factors: vegetation, soil fauna, microorganisms, cations and interactions between clay particles and organic matter (Kumar *et al.*, 2013). The stability of aggregates depends on their internal cohesion, pore volume, connectivity, tortuosity and pore-wall hydrophobicity (Chenu & Cosentino, 2011). A good soil structure, dependent on aggregation, is fundamental for sustaining agricultural productivity and environmental quality, sustainable use of soil and agriculture (Amézketa, 1999).

The hierarchical model for classifying soil aggregates suggests that larger aggregates are composed of smaller units, which are formed from even smaller aggregates (Tisdall & Oades, 1982) (Figure 3). In persistent microaggregates (2-20 µm diameter), clay particles are united by inorganic amorphous binding agents such as aluminosilicates, oxides, humic substances and soil polysaccharides associated with metal ions. These persistent microaggregates are bound together into larger microaggregates (20-250 µm diameter) by plant roots, root hairs, and fungal hyphae. Microaggregates are glued to each other by transient binding agents such as polysaccharides and polyuronides to form macroaggregates (>250 µm diameter). Aggregation is influenced by the soil microbial community, mineral and organic compounds, plant community composition and past soil handling (Tisdall & Oades, 1982). For many decades, the microbial communities inside different classes of aggregates have been investigated using several techniques and experimental designs (Blaud et al., 2012, Zhang et al., 2018). Many studies determined the microbial community inside the different aggregate

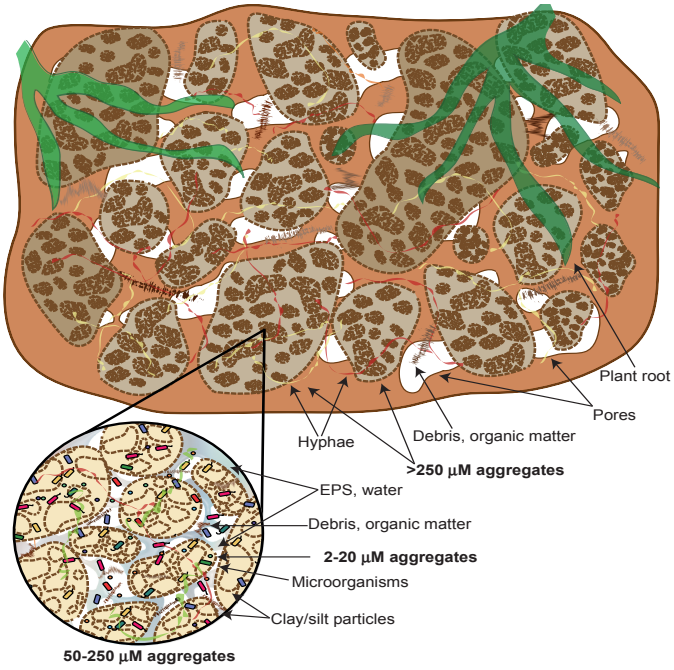


**Figure 3:** The hierarchical model of soil aggregate classification. Larger aggregates are composed of smaller units, which are formed from even smaller aggregates.

sizes in different agriculture management systems (Sessitsch *et al.*, 2001, Mummey & Stahl, 2004, Kravchenko *et al*., 2014), however, no studies evaluated the microbial community responsible for the aggregation. Studies on microbial effect on soil aggregations were limited to microbial isolated strains, albeit the role of microorganisms and their polysaccharides in soil aggregation have been studied for decades. Cesar Ton-That *et al*. (2007) used microaggregates (250 to 50 µm) from two agricultural ecosystems (40 years tillage and 9 years no tillage) to isolate bacteria and test their aggregation potential, as well as profiled both systems using Fatty Acid Methyl Ester (FAME). They observed that *Stenotrophomonas*, *Sphingobacterium*, *Bacillus* and *Pseudomonas* species could stabilize and increase aggregate strength in artificial aggregates, and that these species were mere frequent in partially undisturbed soils. In other study, Cesar Ton-That *et al*. (2014) investigated if soil aggregation and the culturable aggregating bacteria present in soils were influenced by different irrigation, tillage and cropping systems. In the irrigated no tillage and conservation areas, higher proportion of soil aggregating bacteria were isolated (81, compared to ~35). They were able to isolate 50 aggregating bacteria (from 1296 isolates), which were dominated again by *Pseudomonas* sp. and *Bacillus* sp. Interestingly, *Bacillus* and *Pseudomonas* are genus widely known to produce biofilms and EPS, which are involved in the stabilization of soil structure.



### 6.2. Inoculation of EPS producers in soils

The role of microorganisms and their polysaccharides in soil aggregation have been studied for decades. Microorganisms are fundamental for soil aggregation. Fungi and bacteria contribute to stabilization of soil structure by producing extracellular polymers and degrading aromatic humic materials that generate clay-metal-organic matter complexes (Umer & Rajab, 2012). Fungi also contribute by anchoring particles through hyphae, albeit with less persistence. However, the influence of microorganisms on soil structure stabilization varies and depends on the microbial species, available substrates and soil management (Beare *et al.*, 1994, Umer & Rajab, 2012). The aggregating potentials of numerous bacterial and fungal strains have been tested, demonstrating that the effect of microbial pure cultures on soil aggregation is dependent on the microbial species. Therefore, different microbial slimes and EPS have been explored as aggregation-capable components in different types of soils, for the recovery of soil quality and fertility.

Among the bacterial EPS producers that are the most investigated for soil aggregation potential are strains of *Pseudomonas*, *Bacillus* and *Paenibacillus* genera easily grown in laboratorial conditions, producing high amounts of EPS. However, other genera strains of *Pseudomonas*, *Streptomyces* and *Penicillium* had shown a significant positive effect on soil loss and erodibility, after rainfall simulation (Gasperi-Mago & Troeh, 1979). *Pseudomonas putida* strain GAP-P45 inoculation in soil increased aggregate stability in more than 50% in soils subjected to temperature, salt and drought stresses. Under stress conditions, the strain produced more EPS, protecting the bacteria against water stress and contributing to soil

structure (Vardharajula & Ali, 2015). An unidentified bacterium isolated from biological soil crusts from the Gurbantünggüt Desert stabilized sand surface, producing aggregation and slowing the soil water evaporation after only 8 days of inoculation. In addition, the EPS of the bacterium produced the conglutination of sand particles, as observed by scanning electron microscopy (HuiXia *et al.*, 2007). Another isolate from Gurbantünggüt Desert, *Paenibacillus* KLBB0001 – a strong EPS producer – was inoculated in the desertic soil to improve the recovery of biological soil crusts (BSC). After one year of field experiments, the strain stimulated the heterotrophic community in the soil and increased the numbers of bacteria, available nitrogen and phosphorus. Microscope images of the inoculation area revealed a glue-like polymer connecting sand grains, confirming the presence of EPS (Wu *et al.*, 2014). The studies showed the potential of the strains for the recovery of soil structure, especially under nutrient- and water-limited conditions.



Due to its high EPS production, *Bacillus amyloliquefaciens* strain HYD-B17, *B. licheniformis* strain HYTAPB18 and *B. subtilis* RMPB44 inoculation in soil improved aggregate stability in both the absence of stress and under drought stress conditions. For these strains, it was also observed a better aggregation effect with a larger bacterial population size, as well as an important role of larger incubation periods for EPS production and soil aggregation. All the strains produced more EPS under drought conditions, and strain HYD-B17 was the most efficient for aggregation among the strains studied. The differences in the performances of the strains could be explained by the different compositions of their EPSs. The performance of the strains demonstrate that they are also interesting for inoculation in situations of abiotic stresses (Vardharajula & Ali, 2014). Strains of *Pseudomonas* and *Bacillus* were also important for the stabilization of sand on the beach and at the edge of a dune in the study of Foster (Forster, 1979).

*Microbacterium arborescens*-AGSB is another example of an EPS-producer strain that can be used for the recovery of soils; its inoculation produces strong binding in sandy soil. In addition, the bacteria produced better aggregation in a sandy soil than in agricultural and mine reject soils, showing that the effect of microbial inoculation varies according to the soil type (Godinho & Bhosle, 2009).

In addition to other bacterial genera, the inoculation of soil with cyanobacteria has long been proved to be beneficial to soil structure and parameters. These bacteria were recognized as important in the stabilization of soil surfaces, primarily because of EPS production. In arid environments, cyanobacteria are major components of biological soil crusts (BSCs). BSCs are microbial assemblages developed on the top soil of drylands (Malam Issa *et al.*, 1999). They are integral components of arid and semi-arid ecosystems, which biological activities are important for soil fertility and reduction of erosion, influencing soil temperature, C and N content, hydrological dynamics and plant germination (Chamizo *et al.*, 2012, Rossi *et al.*, 2017, Velasco Ayuso *et al.*, 2017). Their main components are species of bacteria, microalgae, fungi, lichen and mosses, but their specific composition is variable (Wu *et al.*, 2014, Mugnai *et*

*al.*, 2017). The use of cyanobacteria for recovery of drylands and BSCs will not be discussed in this review since the focus is in agriculture soils.

Cyanobacterization improves soil structure, fertility, bioavalability of nutrients, benefits that are extended also to the subcrust. Recently, they have been investigated for improvement of quality of arable lands and treatment of degraded and desertified environments (Rossi & De Philippis, 2015). Characteristics such as stress tolerance drought resistance and oligotrophy make them optimal candidates, and their EPS improves soil stability and moisture content at the topsoil, stimulating soil biological activity (Guo *et al.*, 2007). Cyanobacteria exert a mechanical effect on soil particles, as they produce a gluing mesh, binding soil particles with their EPS. They promote the formation of hard entangled superficial structures that improve the stability of semi-arid soil surfaces, protecting them from erosion. In addition, they play a significant role in water storage, because of the hygroscopic properties of the EPS (Mugnai *et al.*, 2017). For instance, the inoculation of *Nostoc muscorum* improved the aggregate stability of a poorly structured silt loam soil in a greenhouse experiment. In this study, the authors investigated the effect of the inoculation of *N. muscorum* on the microbial population, soil nutrient status and fertility. The addition of the microorganism increased soil aggregation by an average of 18%, as well as increased soil total carbon by ~60% and total N by more than 100%; it also increased microbial population numbers and the emergence of lettuce seedlings in more than 52% (Rogers & Burns, 1994). Another strain of the genus *Nostoc* caused a positive impact in the physical characteristics of poorly aggregated soils from Guquka (Eastern Cape, South Africa). A dense superficial network of cyanobacterial EPS filaments covered soil surface after 4 and 6 weeks of incubation. The improvement appeared a short while after incubation, and increased with time and cyanobacteria growth (Malam Issa *et al.*, 2006). Other strains of cyanobacteria, such as *Oscillatoria*, *Lyngbya* and *Schizothrix delicatissima* AMPL0116 also showed positive effects in soil structure, by improving soil hydrological responses to rainfall, soil particle connections, soil permeability and water absorption (Mugnai *et al.*, 2017, Sadeghi *et al.*, 2017).

Inoculation of pure cultures of filamentous fungi is known to increase soil aggregation, however with different effectiveness than that of bacteria. Fungi not only can produce EPS that bind soil particles together but also produce hyphae that can enmesh aggregates (Baldock, 2002). The presence of *Strachybotrys atra* increased the aggregation of fumigated Peorian loess soil. However, the fungus was only able to produce this effect in a situation of reduced microbial community, demonstrating its establish as the dominant microorganism (McCalla *et al.*, 1958). In the study of Aspiras (1971), *Alternaria tenuis*, *Stachybotrys atra*, *Aspergillus niger*, *Mucor hiemalis* and the streptomycetes *Streptomyces purpurascens* and *S. coelicolor* promoted the stabilization of artificial soil particles from 3 different soils. The aggregation was a result of binding agents closely associated to the hyphae. Swaby et al (1949) tested the aggregation capacity of pure cultures of 101 bacteria, 5 yeasts and 50 filamentous fungi, finding that fungi had the best results. Among the best fungi there were species of *Absidia*,

*Mucor*, *Rhizopus*, *Chaetomium*, *Fusarium* and *Aspergillus*. For bacteria, *Achromobacter*, *Bacilllus* and unclassified *Actinomycetes* had the best aggregation potentials. A saprophytic lignin-decomposer evaluated by Caesar-TonThat & Cochran (2000) was able to aggregate and stabilize sandy soil, producing 90% of water stable aggregates. The fungus excreted insoluble extracellular compounds that acted as binding agents, forming a fibrillary network observed in soil micrographs. *Azotobacter chroococcum*, *Lipomyces starkey* and strains of *Pseudomonas* sp. and *Mucor hiemalis* were also able to promote soil stabilization (Lynch, 1981).

In addition of pure cultures, a combination of microorganisms can be an interesting option for soil inoculation. However, few studies investigate the addition of microbial consortia for improvement of soil aggregation. Nonetheless, when complex mixed cultures of microorganisms are inoculated (Swaby, 1949) in particles, aggregation is maximized as a result of interactions between different strains. Different species have different EPS properties; furthermore, EPS can have a complementary effect when associated with other EPS and other aggregating factors, such as EPS-coated fungal hyphae, resulting in greater adherence of soil particles compared to only physical involvement by the hyphae (Aspiras *et al.*, 1971). Moreover, the combination of organic fertilizers with microbial inoculants can strengthen microbial aggregation effects by enhancing EPS production, consequently improving soil structure, function, and quality (Rashid *et al.*, 2016).



### 6.3. Plant inoculation with EPS producers

Plant inoculation with Plant Growth Promoter Rhizobacteria (PGPR) and Arbuscular Mycorrhizal Fungi (AMF) is a very important agricultural practice. Microorganisms stablish interactions with plants, promoting plant growth, which stimulates the microbial community with the production of exudates. The organic carbon released by plant roots stimulates the growth of the microbial communities in the rhizosphere, which in turn, produce mucilaginous EPS, promoting soil aggregation and increasing Root Adhering Soil (RAS). RAS aggregation is important because it forms the immediate environment where plants take up water and nutrients for their development. The inoculation of plants with beneficial microbes can, in addition, increase the availability of nutrients, such as N, P, K and iron (Rashid *et al.*, 2016).

Among the best and most investigated bacterial candidates for plant inoculation are strains of *Bacillus*, *Pseudomonas*, *Rhizobium* and *Pantoea*, all known EPS producers and plant growth promoters. These strains can be inoculated directly in soil, or in seedlings, where they will also be beneficial for crop yield (Cipriano et al., 2016). The production of EPS in the rhizosphere of plants protects the environment against drying and fluctuations in the water potential, increasing nutrient uptake by plants and promoting plant growth. It protects seedlings from drought stress and stimulates root exudates. The improvement in aggregation and soil structure improves the growth of seedlings, because it promotes an efficient uptake of nutrients and water (Alami *et al.*, 2000, Bezzate *et al.*, 2000, Vardharajula *et al.*, 2009). Several studies have evaluate the effect of PGPR and AMF, however no focus on soil

aggregation, since the most are focused were on the plant growth.

*Rhizobium* strain KYGT207, which was isolated from an arid Algerian soil, is a wheat (*Triticum durum* L.) growth promoter and EPS-producing bacterium with significant soil structure-improving capacity. Inoculation of the strain on wheat increased the root-adhering soil dry mass/root dry mass ratio by 137% and enhanced the percentage of water-stable aggregates due to reduction of soil water stress by the EPS (Kaci *et al.*, 2005). Equally significant are the effects of *Pantoea agglomerans* NAS206 and its polymer on the rhizosphere of wheat and on soil aggregation. The strain can colonize the wheat rhizosphere, causing significant aggregation and stabilization of root-adhering soil. It also increased aggregate mean diameter weight, formation of water-stable aggregates (diameter > 0.2 mm) and RAS macroporosity. Thus, *Pantoea agglomerans* NAS206 is an interesting candidate for inoculation, since it can play an important role in regulating water content in the rhizosphere of wheat and improving soil aggregation (Amellal *et al.*, 1998, Amellal *et al.*, 1999).

The levan produced by *Paenibacillus polymyxa* CF43 also has notable effects on the aggregation of soil adhering to wheat roots (Bezzate *et al.*, 2000). The authors tested the role of levan in aggregation of soil adhering to wheat roots by producing a mutant strain. In comparison with the mutant, the wild type EPS producing strain increased the mass of RAS, demonstrating the influence of the EPS in aggregation and suggesting that the production of levan is the main mechanism involved in the improvement of the RAS structuration.

The role of EPS in soil aggregation has also been evaluated under the application of different environmental stresses. Inoculation of chickpea plants (*Cicer arietinum* var. CM-98) with the EPS-producer strains *Halomonas variabilis* HT1 and *Planococcus rifietoensis* RT4 protected the plants from salinity, promoted plant growth and improved soil aggregation in more than 75% under elevated salt stress. These results demonstrated that both bacteria can be applied to enhance plant growth and soil fertility under salinity (Qurashi & Sabri, 2012). In another study, the EPS-producer *Rhizobium* YAS-34 positively affected soil aggregation and water and nitrogen uptake by sunflower plants under normal and water stress conditions. It increased RAS in up to 100%. The strain acted as a plant growth promoter, increasing shoot and root biomass and also increased soil macropore volume. These effects were attributed to EPS production, which increased soil water hold capacity (WHC) and reduced water loss (Alami *et al.*, 2000). The strains of *Bacillus* and *Aeromonas* evaluated by Ashraf et al (2004) increased the aggregation around roots of wheat in a moderate saline soil, restricting $Na^+$ uptake by plants and promoting plant growth.

The effects of plant inoculation of several fungi have also been extensively evaluated, also with more focus on plant growth promotion than in rhizosphere soil aggregation. The mechanisms involved in the aggregate stabilization by fungi are entanglement of the soil particles by hyphae as well as the production of EPS. AMF also produce glomalin, a glycoprotein that acts as a glue (Kohler *et al.*, 2006). Forster and Nicholson (1981) examined the effects of the interactions among grass (*Agropyron junceiforme*) and microorganisms

(*Penicillium* sp and *Glomus fasciculatus*) in the aggregation of sand from an embryo dune. Experiments showed that the addition of selected microorganisms increased both plant growth and soil aggregation. Even though roots alone affected sand aggregation, the best results were due to the association of microorganism inoculation and plants.

The mycorrhizal inoculation of *Olea europaea* and *Rhamnus lycioides* with *Glomus intraradices* showed beneficial effects for rhizosphere aggregation. Together with the addition of composted residue, inoculation increased rhizosphere aggregation in comparison with non-rhizosphere soil by 1.8 fold (Caravaca *et al.*, 2002). The effects of the inoculation of *Glomus intraradices*, and *Pseudomonas mendocina* were evaluated by Kohler (2006) in lettuce. The inoculation of both strains increased the percentage of water soluble carbohydrates and stable aggregates. *P. mendocina* also had a positive effect on soil enzymatic activities, such as dehydrogenase and phosphatase. The combination of *P. mendocina* with inorganic fertilization increased stable aggregates in 84% compared to the control.

Inoculated microorganisms can have a significant effect on soil properties and quality by interacting with natural microorganisms in the rhizosphere, in addition to the improvement of plant productivity. Good soil structure and aggregate formation are important for controlling germination and root growth. Microbial inoculants have been studied for decades, but there is still a need for the enhancement of microbial growth conditions, for the production of high quality inoculants, with higher biomass and EPS production. Therefore, strains will be able to have an optimal performance in field conditions, with efficient colonization and dominance over the native microbial community.

### 6.4. Addition of pure EPS to soil

Several studies link microbial products to soil aggregate stability. It has been long know that polysaccharides, are involved in the maintenance of soil structure, even though they are not the primary aggregating agents. Other molecules, such as humic acids are also responsible for soil structure. The treatment of natural and synthetic soil aggregates with various chemical substances, such as periodate and tetraborate frequently does not result in a consistent pattern, demonstrating that polysaccharides are important, but more than one single substance are the main factors sustaining soil aggregates (Mehta *et al.*, 1960, Sparling & Cheshire, 1985). Angers and Mehuys (1989) observed that the correlation between aggregate mean weight and carbohydrate content suggested that at least part of the water-stable aggregation was related to carbohydrates in soils . Treatment of the soil with sodium periodate prior to wet sieving confirmed partial involvement of carbohydrates in the stabilization of aggregates by crops.

The resistance of the biopolymers to degradation may be related to its importance for the soil structure. The greater the resistance, the longer is its persistence in soils. The association of polymers with metal ions and colloids, such as clay may also influence the degradation rates of polymers, because of their influence in enzymatic activity. Since the addition of polymers

to soil started to be investigated, it has been demonstrated that the binding power of plant and microbial polysaccharides is variable. However, characteristics of the soil such as pH also influence the action of polysaccharides, because the charges of molecules are essential for binding particles (Martin, 1971). Some characteristics of polysaccharides that influence their binding activity are linear structure and length and flexible nature, that allow the formation of Van der Waals forces; large number of OH for hydrogen bonding and presence of acyl groups, allowing ionic binding to clays (Martin, 1971).

The effects of many different EPS produced by fungi and bacteria were already tested as soil aggregating agents. The direct application of polymers in soil can be an alternative to the inoculation of microorganism. The aggregating potential of the EPS of *Bacillus subtilis*, *Leuconostoc dextranicum* and *L. mesenteroides* were evaluated by Geoghegan and Brian (1948). The different EPS had a significant in soil aggregation tested by wet sieving, and even small amounts of levan (0.125 to 0.05%) were able to stabilize aggregates. The EPS of *Chromobacterium violaceum* had also an interesting effect in soil, being more resistant to degradation than a variety of plant polysaccharides. It exhibited the best binding performance among all polysaccharides tested, improving the hydraulic conductivity of a soil with neutral pH (Martin & Richards, 1963).

Some EPS molecules have a very high WHC. A xanthan tested by Chenu and Roberson (1996) demonstrated a WHC of 15 times its weight. The dextran tested in the same study had a lower WHC, due to differences in structure. For both EPS, diffusion of glucose was tested, and it was observed that diffusion rates were slower than in water. A high WHC of EPS can protects microorganisms, soil and plants against drought stress, promoting hydrating conditions and bridging among soil particles and clay. In addition, the nutrients are still able to diffuse until the microorganisms during low water potentials, maintain physiological functions even during dry periods. The EPS of a *Pseudomonas* strain isolated from soil can also hold several times its weight in water. When added to a sandy soil, the EPS altered its moisture by allowing the amended soil to hold more water than unamended soil. The addition of a small amount of EPS increased the amount of water held by the sand (Roberson & Firestone, 1992).

There are evidences that xanthan stabilizes soil against disruptive effect of wetting and drying cycles (Czarnes *et al.*, 2000). In comparison with control soil and dextran, soils amended with xanthan were less sensitive to this kind of stress. Differences in structure of both polysaccharides could explain their different behaviors. Rosenzweig et al. (2012) also tested the WHC of two sandy soils amended with xanthan, and observed that the addition of >1% xanthan increased dramatically the water holding capacity of the soil, as well as soil porosity.

Many of the studies that evaluate the application of microbial biopolymers in soil are in the engineering area. There are several studies that evaluate the application of microbial biopolymers and plant polymers, such as guar gum and cellulose for stabilization and soil binding for constructions. Such studies in the engineering area also confirm the usefulness of biopolymers application in soil, but with different purposes.

The strength of biopolymers can be observed by their application in the fields of construction and geotechnical engineering, as soil binders (Chang *et al.*, 2017). The commercial polymer from *Aureobasidium pullulans* was efficient in the treatment and stabilization of a residual Korean soil, increasing the compressive strength of soil more than 200% (Chang & Cho, 2012). It was considered an economically competitive and environmentally friendly alternative for soil binding. In another study, a very small amount of microbial EPS (such as xanthan and gellan gum 0.5%) mixed with soil resulted in a higher compression strength in comparison to the addition of a large amount of cement. Xanthan forms connection bridges between particles, enhancing particle alignment, improving strength. The effect is a result of the matrix strength and electrostatic bonds between xanthan and fine soil particles. These polymers can be naturally decomposed, not requiring construction demolition. They are promising for construction as building materials (Chang *et al.*, 2015). The application of xanthan gum can also be used to treatment of collapsible soil, reducing collapsible potential (Ayeldeen *et al.*, 2017).



In addition to the direct application of EPS to soil, there are evidences that EPS production in soil can be modulated by N management. Roberson et al. (1995) evaluated the effect of the N addition in EPS production and soil aggregation, by indirect measurements, carbohydrate content and monosaccharide composition. While intermediate and high amount of N fertilization gave similar crop yield, the soil properties had different results. Intermediate N fertilization induced better aggregation, saturated hydraulic conductivity and the monosaccharide composition was more related to microbial polysaccharide. Therefore, the addition of nutrients could also induce EPS production directly in soil, consequently improving soil aggregation.

Many studies have demonstrated that EPS production can increase soil aggregation, improve soil quality and contribute to soil fertility. Moreover, in addition to improving soil structure, the presence of EPS in soil and in plant roots can improve nutrient uptake and water availability for both plants and microorganisms, thus benefiting not only the producer but also the environment as a whole. Several works show that both bacteria and fungi are important for soil aggregation, Their EPS are capable of binding soil particles, and their interactions, as well as their interaction with plants, and the addition of organic fertilizers altogether are enhancers of soil structure and stability. Microorganisms have an enormous potential, which can be enhanced by the improvement of the knowledge of the structure of EPS, as well as the development of microbial consortia and large-scale EPS production.

## 7. Conclusion and perspectives

Microorganisms have developed several approaches to survive environmental conditions, especially in soils. EPS production is an important strategy for providing a moist environment, entrapping nutrients, facilitating chemical reactions, and protecting cells

against environmental conditions, antibiotics and attack by predators. Microbial extracellular polymers are highly diverse compounds with multiple functions that depend on their composition and structure.

EPS have long been of interest due to their biodegradability, biocompatibility, and thickening, gelling and emulsion capacities. The polymers and their production can be manipulated to achieve high yields, but such manipulations are dependent on the characterization and physiological study of EPS-producing microorganisms. Improving polymer production requires an understanding of the underlying mechanisms and regulatory pathways. In contrast to the intensive work focused on improving EPS yield and altering the characteristics of well-known polymers, novel EPS and polymers produced by less-studied microbial strains are still underexplored. The investigation of the genetic mechanisms involved in the biosynthesis of any type of molecule involves complex and time-consuming techniques, and thus the development of knowledge in this area may proceed slowly. Many microorganisms produce EPS, and because each polymer is different, many opportunities remain for investigation and discovery.



EPS are complex substances and our understanding of their composition, structures, functions and genetic regulation, although very broad, is far from complete. There is a need for a fundamental understanding of the genes and mechanisms involved in the biosynthesis and regulation of EPS. Furthermore, the discovery and characterization of new polymers could lead to interesting other applications, especially for the environment. EPS can be employed in wastewater treatment, recovery of polluted environments, and, potentially, in the recovery of soil aggregation and improvement of soil fertility. Advances in modern techniques and approaches, such as high-throughput sequencing, confocal laser scanning microscopy, nuclear magnetic resonance and scanning electronic microscopy, stable isotope probing in association with classic microbiology techniques will enhance efforts to discover and characterize new EPS and their functions in the soil ecosystem. The understanding of structure and properties of EPS is fundamental for understanding their interactions with soil. The combination of classic microbiology techniques with modern high-throughput methods and integration of different fields are fundamental for increasing knowledge on EPS composition, structure and function and applications.

## Acknowledgments

O.Y.A. Costa was supported by an SWB grant from CNPq [202496/2015-5] (Conselho Nacional de Desenvolvimento Científico e Tecnológico).