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REGULAR ARTICLE

Complex role of the polymeric matrix in biological soil crusts

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Abstract
Background Extracellular polymeric matrix (EPM) is a complex component of the organo-mineral assemblages created by biological soil crusts (BSCs). Mainly of polysaccharidic origin, it embeds soil and sediments and provides key benefits to the crust community. Services provided include: sediment cohesion and resistance to erosion, moisture provision, protection from external harmful factors, as well as support to plant establishment and growth. EPM is the product of BSC microbial community, and it is constituted by exopolysaccharides (EPS) associated to other substances, organized in a three-dimensional structure having different levels of gelation, and degrees of condensation.
Scope This review aims at focusing scientific attention, for the first time, on the characteristics and the roles of three operationally defined EPM fractions, one water soluble, one more adherent to cells and sediments, and one firmly attached to microbial cells. The latest results obtained by analyzing EPM of natural and induced (i.e, the result of cyanobacteria inoculation) BSCs are outlined, and the optimized extraction methodology is described in details.
Conclusions The review underlines the complexity of investigating the characteristics and the role of microbial EPS, and its supra-structure (EPM), in natural conditions (as opposed to cultures in laboratory conditions), where the matrix is subjected to continuous microbial rearrangement due to biosynthetic, self- and cross-feeding processes, and where microbial activity affected by environmental parameters.

Keywords Biological soil crusts (BSCs) · Cyanobacteria · Microalgae · Microfungi · Exopolysaccharides (EPS) · EPS extraction · Extracellular polymeric matrix (EPM)

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Introduction

Biological soil crusts (BSCs) are highly specialized complex microbial communities which are an integral component of arid and semiarid ecosystems. By colonizing the uppermost soil layers, they control the exchange of gases and nutrients (Pointing and Belnap 2012; Weber et al. 2015), play important ecological functions (Bowker et al. 2011; Maestre et al. 2011) and provide soil stability and N-enrichment, two key factors supporting vascular plants establishment (Bowker 2007; Miralles et al. 2012). They are composed of bacteria, microalgae, microfungi, green algae, lichens and mosses (Wu et al. 2013), although some of these organisms may be lacking, depending on climate and terrain age (Belnap and Lange 2001).

Microbial-produced exopolysaccharides (EPS) accumulate as a heterogeneous extracellular polymeric matrix (EPM) that is strictly associated to organisms and sediments in BSCs. Other than for exopolysaccharides, the term EPS has been used as acronym for extracellular polymeric substances to account for a number of other components that, along with the dominant polysaccharidic fraction, constitute the EPM (see section 3). EPM constitute a supra-structure of EPS, having a three-dimensional organization and varying degrees of condensation, from mucilaginous to solid gel (Fig. 1). The concept of EPM is present on a wide array of studies on microbial biofilms, although not thoroughly stressed, and addressed with slightly different terms, including extracellular polymeric substance matrix (Battiston et al. 2015; Fish et al. 2016; Gu et al. 2017) and biofilm matrix (Limoli et al. 2015).

EPM provides a wide array of services to the crust community, from conferring physical integrity and stability, to providing an optimal microenvironment with increased moisture, nutrients, and protection from harmful biological and physical agents.

Although many BSC organisms produce EPS, cyanobacteria and microalgae are known prominent contributors (Belnap and Lange 2001). Other less-acknowledged producers of EPS are microfungi (Selbmann et al. 2003), and members of proteobacteria and actinobacteria (Martínez-Cánovas et al. 2004; Suela Silva et al. 2013) that are present in crust communities (Nagy et al. 2005; Kuske et al. 2012; Rossi et al. 2012c). The excretion of EPS is an important physiological process from the first stage of BSC development, when bare soil is colonized by sheathed filamentous cyanobacteria (Belnap and Eldridge 2001). Incipient cyanobacterial crusts, representing an early stage of BSCs (Lan et al. 2013), are a nutrient-rich substrate easily colonizable by other phototrophic species and heterothrophic bacteria. Although several authors recognize the importance of EPS in BSC - water relations and survival (Mazor et al. 1996; Mager and Thomas 2011), and consider their amount an index of soil stability (Hoppert et al. 2004) and of the metabolic capacity of the community (Bu et al. 2014), there is still a lack of information concerning their contribute in increasing the resilience of BSCs to environmental constraints and in nutrient and water diffusion. In addition, information concerning their physiochemical properties and their modifications in space and time is still limited.

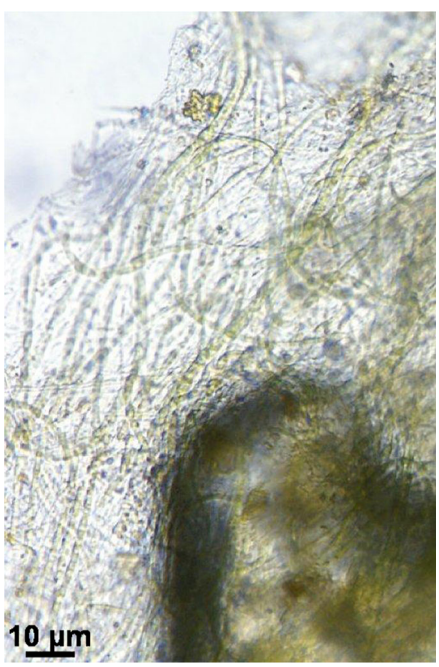

Fig. 1 Microscopical image showing the EPM of a cyanobacterial crust of S*chizothrix* cf. *delicatissima*. Picture by GM

While the roles of cyanobacterial and microalgal EPS have been pointed out by several researchers (Mazor et al. 1996; De Philippis and Vincenzini 1998; Mager and Thomas 2010; Rossi and De Philippis 2015a), the majority of these scientific advances were attained under lab conditions, and employing strains growing in liquid culture. Studies under natural conditions (e.g., field colonies) are limited to a few cases. Some of them point out that cyanobacteria may produce compositionally different EPS depending on whether they face nutrient limitations and constraints, or optimal growth conditions (Huang et al. 1998; Brüll et al. 2000). For microalgae and cyanobacteria, several factors were demonstrated to influence EPS productivity and, in some cases, EPS characteristics. Those include light intensity and temperature; availability of carbon, nitrogen, phosphorous an sulphur; moisture level and salinity (Rossi and De Philippis 2015b).

EPM characteristics in natural microbial associations are strongly dependent on the dynamics and the activities of the microbial community (Flemming and Wingender 2010). Thus, an increasing level of complexity is expectable in moving from studies performed on laboratory isolates, to studies conducted in situ on composite microbial aggregates.

This work aims at giving an overview of the characteristics and the roles of EPS in BSCs, with a particular attention to their organization in definite stable three-

dimensional structures, an aspect which has not been studied so far. In addition, we propose a clear terminology consistent with previous studies conducted on EPM in other complex microbial associations.

Cyanobacterial, microalgal, and fungal EPS: Morphological and chemical characteristics and roles

The synthesis of EPS, which is an energy-consuming process, has important ecological implications for its producers (Li et al. 2001). Several studies led to believe that EPS production is a physiological mechanism increasing organism survival, and tolerance to environmental harsh conditions. Several known roles of EPS are reported in Table 1.

It is generally believed that EPS production does not confer concrete advantages in laboratory cultures, in contrast to natural conditions, under which cells experience competitive multispecies environments and multiple stresses (Costerton et al. 1987). Cyanobacteria and microalgae have been generally considered as initiators of BSC development on bare oligothrophic soils. Being proficient EPS producers, they strongly promote the first crucial soil stabilization. Although other nonphotosynthetic bacteria were demonstrated to be significant EPS producers, and to have a role in crust formation (Wu et al. 2010), EPS produced by cyanobacteria, microalgae and filamentous fungi bear generally a higher complexity in terms of monosaccharidic composition (Osińska-Jaroszuk et al. 2015; Pereira et al. 2009). In particular, Wu et al. (2010) underlined how nonphotosynthetic bacteria in BSCs contribute compositionally simple polysaccharides, with mannose, galactose, glucose, and a glucose isomer accounting for more than 98%. Conversely, cyanobacterial and microalgal EPS may also contain high relative amounts of non neutral sugars (see below).

Cyanobacteria and microalgae produce EPS excreted as sheaths and capsules, or unevenly dispersed as mucilage (Rossi and De Philippis 2015b) depending on their chemical features, and on abiotic factors (e.g., available ions, pH). These outermost structures have also been described for other prokaryotes (e.g., Decho and Lopez 1993; Vincent et al. 1994), sometimes termed "glycocalyx" (Wingender et al. 1999). Bacterial capsules and sheaths can be attached to cells through non-covalent interactions, but also covalently to phospholipids and lipid-A molecules at the cell surface (Roberts 1996). Concerning cyanobacteria and green microalgae, the nature of these outer investments has been described in details in past publications (De Philippis and Vincenzini 1998; De Philippis et al. 2001; Pereira et al. 2009; de Paniagua-Michel et al. 2014, Rossi and De Philippis 2015b). On the other hand, regarding fungi there is a more limited amount of information, especially regarding the biosynthetic processes. In laboratory cultures, some species encompassing lower filamentous fungi and yeasts from different ecological niches produce EPS (Mahapatra and Banerjee 2013). Black yeasts such as *Exophiala crusticola* and *Rhodotorula*, often detected in BSC communities, were indicated as highly probable contributors of soil stability in desert systems owing to their EPS productivity (Bates et al. 2006). EPS excretion in fungi has been correlated with the production of sclerotia, presence of conidia, and to the phytopathogenic behavior (Selbmann et al. 2003). As for cyanobacteria and microalgae, fungal EPS production is affected by several parameters, including temperature, oxygen concentration, pH, and N source (Mahapatra and Banerjee 2013; Seviour et al. 1992).

EPS produced by cyanobacteria and microalgae can be compositionally complex. In cyanobacteria, they can contain up to 15 sugar moieties (Pereira et al. 2009), organized in complex repeating units and often having a molecular weight (MW) up to 1–2 MDa. Non saccharidic components like peptides, lipids and nucleic acids are also present. Glucose, galactose, arabinose, xylose and uronic acids have been frequently detected in major amounts. Methyl, pyruvyl, succinyl and sulphate groups were also detected in some cases. The presence of hydrophilic moieties on one side (sulphated sugars, uronic acids and ketal-linked pyruvyl groups, among others), and hydrophobic on the other (acetyl groups, dehoxysugars and peptides) confers an amphiphilic character to the macromolecules and hence provide greater plasticity in organisms' response to surrounding environment (Rossi and De Philippis 2015b). While hydrophobic EPS fractions are more involved in the adhesion to solid surfaces, hydrophilic fractions are more involved in binding minerals, nutrients and water molecules (Rossi et al. 2012a). Some cyanobacteria are also reported to excrete cellulose (de Winder et al. 1990), which is often localized in the sheath (Stuart et al. 2016).

Table 1 Known major roles of microbial EPS

Role	Details	References
Cell adhesion and cohesion	Enhancement of capability of cell to bind to solid substrates, and enhancement of bounds between cells. Promotion of the formation of biomineral layers and influence on the physico-chemical properties of cell aggregates (charge, viscosity, flocculation).	(De Philippis and Vincenzini 1998; Rossi et al. 2012a; Xiao and Zheng 2016)
Tolerance against desiccation and freezing	Constitution of a hydrated surrounding of the cells that control the uptake and the release of moisture. Prevention of drought-impairment of O_2 evolution. Improvement of the resilience to freezing and thawing.	(Tamaru et al. 2005; Pereira et al. 2009; Varin et al. 2012)
Protection from external specific and non-specific threats	Protection against protozoan predation, antibiotics, host defenses, lysis from other bacteria and viruses. Capsulated cells are less efficiently digested than noncapsulated. Slime EPS is more easily digested, possibly due to the less abundant proteic portions included.	(Decho and Lopez 1993; De Philippis and Vincenzini 1998; Li et al. 2001; Pereira et al. 2009)
Protection from UV-radiation	The UV-screening pigments scytonemin and mycosporine aminoacid-like substances (MAAs) are contained in the sheath of several cyanobacterial species. In addition, the thickness of the EPS casing is a barrier hindering the radiation from reaching the cells.	(Garcia Pichel and Castenholz 1991, 1993; Rossi and De Philippis 2015a)
Cell gliding	Some cyanobacteria are motile by gliding. The junctional pore complex system (JPC), observed on the cell wall of *Phormidium uncinatum* and *Anabaena variabilis*, is a structure constituted by a proteic scaffolding and fibrils, and operates thanks to EPS extrusion that provides the thrust. JPC was demonstrated to be involved in cell propulsion.	(Hoiczyk 1998; Hoiczyk and Baumeister 1998)
Nutrient and mineral accumulation	EPS secretions, due to their ionic nature, help the accumulation by ionic interactions, of minerals and nutrients.	(Sutherland 1994; Welch and Vandevivere 1994; de Alexandre et al. 2013; Chen et al. 2015)
Support to photosynthetic systems	Assistance to the re-establishment of damaged photosynthetic apparatus after the state of dormancy.	(Harel 2004)
Protection of nitrogenase against the harmful effects of oxygen	Creation of an effective barrier for oxygen transfer to the cells, protecting nitrogenase from inhibition.	(Sabra et al. 2000)

Fungal EPS can consist of polymers with a high MW (around 2 MDa), and be organized in very diverse repeating units with a complexity similar to that observed for EPS produced by cyanobacteria, although not always displaying a similar high compositional complexity (Seviour et al. 1992). In some cases, glucose, mannose and galactose are the only components, whereas in other cases uronic acids, aminosugars and rhamnose may be present (Mahapatra and Banerjee 2013).

The EPM of complex natural microbial communities

EPM of BSCs is similar to those already described for other typologies of bacterial aggregates (Wingender et al. 1999). It is an ordered, hydrated, semi-solid state structure organized in a polymeric three-dimensional network embedding organisms and soil sediments. Its physical state is governed, to a large extent, by environmental parameters. It provides structural and functional integrity to BSCs.

Being the result of microbial synthesis and demolition, EPM of natural communities is in continuous modification and rearrangement, with its composition and distribution varying spatially and temporary according to the prevailing activities of the biofilm (Wingender et al. 1999). The presence of a so structured matrix allows consortial activities, needed by microorganisms to maximize their fitness through cooperative interactions, while synergistic activities enhance the resilience

to stress factors and oligotrophic conditions (Wingender et al. 1999). Within the EPM, the spatial arrangement creates gradients of oxygen (determining aerobic and anaerobic habitats), other electron acceptors, as well as organic substrates (e.g., proteins, peptides, aminoacids and colloids), and pH value (Costerton et al. 1987; Kepkay 1994; Mayer et al. 1995; Wingender et al. 1999). Some processes such as the accumulation of nutrients and other substances from the bulk soil water, gene exchange, and quorum sensing are favored.

Beside exopolysaccharides, EPM is constituted by other components that are the result of secretion processes or are released after cell lysis (Gu et al. 2017; Wingender et al. 1999), although the polysaccharidic component is often strongly dominant (Al-Thani 2015). Proteins, nucleic acids, and amphiphilic substances as (phospho)-lipids can be often detected in varying amounts as part of the matrix in the extracellular space. Extracellular proteins may establish hydrogen bonds within the EPM structure (Dignac et al. 1998); some can be glycosylated to create glycoproteins, or substituted with fatty acids to form lipoproteins. One main function of extracellular proteins is to act as enzymes for the digestion of exogenous macromolecules (Wingender et al. 1999).

Several authors have attempted to define the different EPM fractions observed in complex biofilms. Some used the term "slime" to indicate EPM fractions that are loosely bound to cells (and soil sediments) and less condensed, although not dissolved. Dissolved EPM fractions are referred to as "colloidal" (Nielsen and Jahn 1999). A generic distinction used by some authors is between "bound" EPS (sheaths, capsules, condensed gels, loosely bound polymers, attached organic material) and "soluble" EPS (soluble polymers, colloids, slimes) (Nielsen et al. 1997). Operationally, the more easily recoverable fraction is that which is less condensed, and weakly attached to cells and sediments (loosely bound EPS, LB-EPS) (Fig. 2). A second fraction consists in molecules with a higher level of gelification and thus thickened, having strong bonds with cells and sediments (tightly bound EPS, TB-EPS). This fraction may include more sub-levels of gelification and the extent of recovery is strictly determined by the extraction methodology (see section 4). In addition to LB-EPS and TB-EPS fractions, we identify a third "glycocalix" fraction (G-EPS) (Wingender et al. 1999), which is firmly attached to the cells (capsules or sheaths). G-EPS may be either containing filaments or hollow, following filament migration.

Extraction and analysis of EPS from complex microbial associations: The case of BSCs

In most studies present in the literature, the selected extraction procedure defines the typology of recovered fractions (Nielsen and Jahn 1999). Most of the extraction procedures rely on the fact that EPS fractions have varying levels of solubility. The immediately soluble fractions can generally be removed with a washing with H_2O, whereas more hydrophobic fractions are not expected to be recovered in this way. Less soluble fractions can be recovered by applying proper extraction methods. For the majority of studies on complex microbial biofilms, extraction methodologies can be encompassed in the following procedural scheme (Table 2).

Any extraction method must be developed and optimized according to the type and structure the biofilm and the type of environment (soil or aquatic environment) which it belongs to.

There is not an universally followed extraction method to recover EPS from BSCs. The existing extraction procedures that appeared recently in literature were adapted from methods previously applied to other types of biofilms, or to cyanobacterial strains grown in liquid cultures.

It has to be stressed that if the analysis is aimed only at the EPS, the maintenance of cell integrity is a prerequisite. In this case, the extraction procedure must be selected in a way to not cause cell leakage, or the EPS will be contaminated with intracellular material. When the extraction causes cell lysis, the subsequent analysis, whether simple quantification or macromolecular characterization, is performed on "total polysaccharides" or "total carbohydrates" and not on EPS. The determination of cell lysis after extraction is not straightforward, since some intracellular substances, e.g. proteins and nucleic acids, may be naturally present in the extracellular environment (see section 2). The use of truly intracellular compounds as markers for cell lysis is suggested. One compound is ATP, although in past studies some concerns regarded the level of accuracy in measuring this molecule (Grotenhuis et al. 1991). Another suggested marker is the enzyme glucose-6-phosphate dehydrogenase (G6PDH) (Platt et al. 1985). Stuart et al. (2016) used this enzyme to evaluate the extent of cell lysis after EPS extraction from cyanobacterial mats. Another parameter supporting the loss/maintenance of cell integrity is the content of chlorophyll in the extracts, although the reliability is related

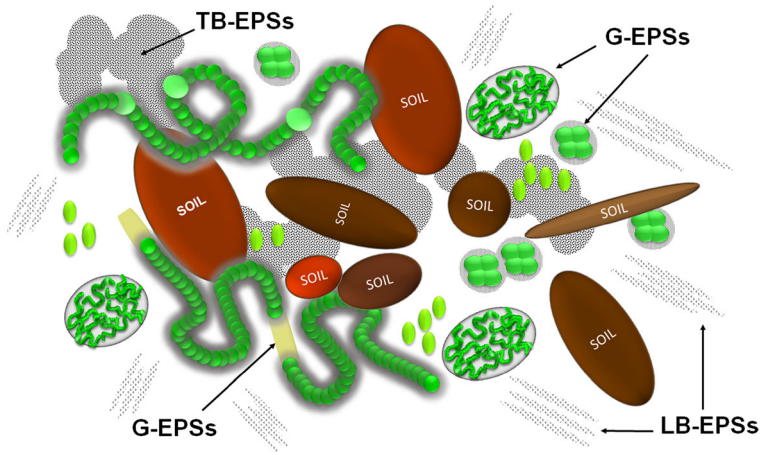

Fig. 2 Representation of the three main operationally-defined fractions of EPM in a phototrophic biofilm: loosely bound EPS (LB-EPS), tightly bound EPS (TB-EPS) and glycocalyx EPS (G-EPS), the latter encompassing bacterial capsules and sheaths

to the abundance of the phototrophic fraction of the community.

Extraction procedure is generally a combination of chemical and physical approaches, based on considering the major type of interactions that keep EPS together in EPM, namely van der Waals forces, electrostatic interactions, hydrogen bonds, hydrophobic interactions and covalent bonds (Christensen 1999). The application of only physical methods (addition of water followed by centrifugation, mixing, shaking or sonication) gives lower EPS yields than combining with chemical methods (Nielsen and Jahn 1999). Physical methods alone result in a minimal, if not null, release of bound EPS.

Table 2 Theoretical procedural workflow for extracting EPS from organo-mineral microbial aggregates

Operation	Details
1) Sampling	Collection of BSCs from natural setting or from laboratory setting (e.g., microcosms).
2) Preparation of samples for extraction procedure	Preparation procedures may include washing or homogenization without disruption of the cells. Homogenization is aimed at dispersing soil aggregates and allows to eventually perform normalization on a dry soil basis.
3) Extraction procedure	Selection of the most suitable procedure to recover EPS from the organo-mineral layer.
4) Purification of EPS	After extraction, EPS can be purified to remove non-carbohydrate components or salts. Treatment generally include dialysis to remove salts, and use of proteases to remove peptides.
5) Analysis of EPS	This phase include the preparation of the samples for the different possible analytical and instrumental procedures (e.g., hydrolyisis, removal of coarse particulate).

Chemical methods include the use of a wide array of substances that are meant to facilitate the release of TB-EPS. Extractants include pyridine acetate, used for *Escherichia coli* (Pelkonen et al. 1988), NaOH (Sato and Ose 1980) and NaCl used for *Pseudomonas aeruginosa* (May and Chakrabarty 1994). The use of alkali (e.g., NaOH) leads to the ionization of charged groups in EPS, due to their isoelectric point that is generally below pH 4–6. The result is a strong repulsion within the EPM and the increase of water solubility of more condensed fractions, although this process seldom leads to the removal of G-EPS. The structural order of EPM can be shifted to disorder on heating or removal of ions (Sutherland 1999). For example, the removal of cations such as Ca^{2+} and Mg^{2+} using complexant agents such as ethylenediaminetetraacetic acid (EDTA) and ethylene glycol-bis(ß-aminoethyl-ether)-N, N, N′, N′ – tetraacetic acid (EGTA) strongly compromises the stability of the EPS strands, and EPM tends to fall apart. In a study dedicated to the comparison of several methods for the extraction of EPS from soil biofilms, Redmile-Gordon et al. (2014) suggested that the best method is based on the use of cation exchange resins as they are capable of maintaining the integrity of the cells thus preventing the contamination of the extracted EPS with humified soil organic matter.

The extraction procedure can be aimed at a general quantification of EPS in a BSC sample, or at recovering specific fractions for separate quantifications. A method to extract and quantify EPS from intertidal sediments proposed by Underwood et al. (1995) was recently successfully applied to quantify EPS in BSCs (Rossi et al. 2012c; Colica et al. 2014). It consists of extractions in 0.1 M Na_2EDTA of small amounts (~100 mg) of homogenized BSC for 15′ at room temperature. The extracts can be assayed for total extracellular carbohydrate amount by applying phenol-sulfuric acid assay (Dubois et al. 1956). To quantify actual EPS (the fraction with a MW ≥ 100 k Da), the quantification must be performed after treating the extract with ethanol (70% final concentration) (Decho and Lopez 1993). The extraction efficiency of the method in analysis preliminary to those published in Rossi et al. (2012c) was evaluated by extracting EPS from BSCs collected in North American deserts (*F. Rossi*, personal communication). The analysis was conducted employing two BSC typologies (described in Rossi et al. 2012c), one collected in the Chihuauan Desert, and one in the Mohave Desert, that were different for relative abundances of species (significant differences in cyanobacterial and proteobacterial relative abundances), and in the percents of sand and silt contents.

These results pointed out that repeated extractions may increase extraction yields. At least five extractions were needed to remove all the extracellular carbohydrates, although the carbohydrates removed with the first two extractions represented over 60% of the total amount recovered with the sum of all the extractions (Fig. 3) (*F. Rossi*, personal communication). Although the needed number of extractions may depend on the typology of crust and soil texture, the method is relatively rapid, and allows to process multiple samples simultaneously.

According to some authors, treatment with EDTA may cause cell wall destabilization due to divalent cations removal causing cell leakage (Nielsen and Jahn 1999). Nonetheless, Underwood et al. (1995) observed low, if not null, intracellular contamination extracting intertidal sediments. In using EDTA extraction on cyanobacterial biofilms, Stuart et al. (2016) ruled out cell lysis.

We recommend the procedure described in Fig. 4 to recover LB-EPS, TB-EPS and G-EPS from BSCs. Most of the hereby proposed methods have been published in several previous papers (Rossi et al. 2012c; Colica et al. 2014; Chen et al. 2014; Colica et al. 2015).

The procedure includes recovering LB-EPS by water extraction and then recovering TB-EPS by extracting with 0.1 M Na_2EDTA. These two treatments in sequence will leave a pellet of sediments, cells and G-EPS. Several methods have been proposed for the removal of sheaths and capsules for microalgae and cyanobacteria. Those include sucrose gradients, acidic treatment and heat treatment (Rossi and De Philippis 2015b). To remove G-EPS, we suggest treating the pellet resulting from the removal of LB- and TB-EPS with hot water (80 °C) for 1 h after washing with 1,5% NaCl (Mugnai et al. 2017).

Following the recovery of the three fractions, the phenol-sulfuric acid assay can be used to quantify them. It is possible to further purify the fractions, or treat them for further analytical analysis. The purification processes often require precipitation in alcohol/acetone (we recommend isopropyl alcohol or ethanol) of the extract. Although the process removes impurities, it also removes low MW polymers that will not be detected in further macromolecular analysis (e.g., gel permeation chromatography). The level of purification strictly depends on the aim(s) of the investigation and may involve further purification steps depending on the hydrophobicity/hydrophilicity of the macromolecules to be removed. When not of interest, proteins may be removed by using proteases, phenol extraction or gel filtration chromatography. DNA or RNA may be removed by using nucleases.

The EPM of BSCs: Morphological and chemical characteristics of EPS and their role

Morphology of EPM of BSCs

Mager and Thomas (2011) reported the presence of EPS-containing structures in BSCs from the Kalahari Desert, and they categorized them as “capsules”, “granules” and “slime”, basing on their morphology. To our knowledge, it was the first time that a tentative morphological description of EPM was attempted. Arid soil pioneers like the non-heterocystous cyanobacterium *Microcoleus vaginatus,* members of the genera *Schizothrix* or *Hydrocoleum* forms filaments constituted of rope-like bundles of trichomes encased in tubular

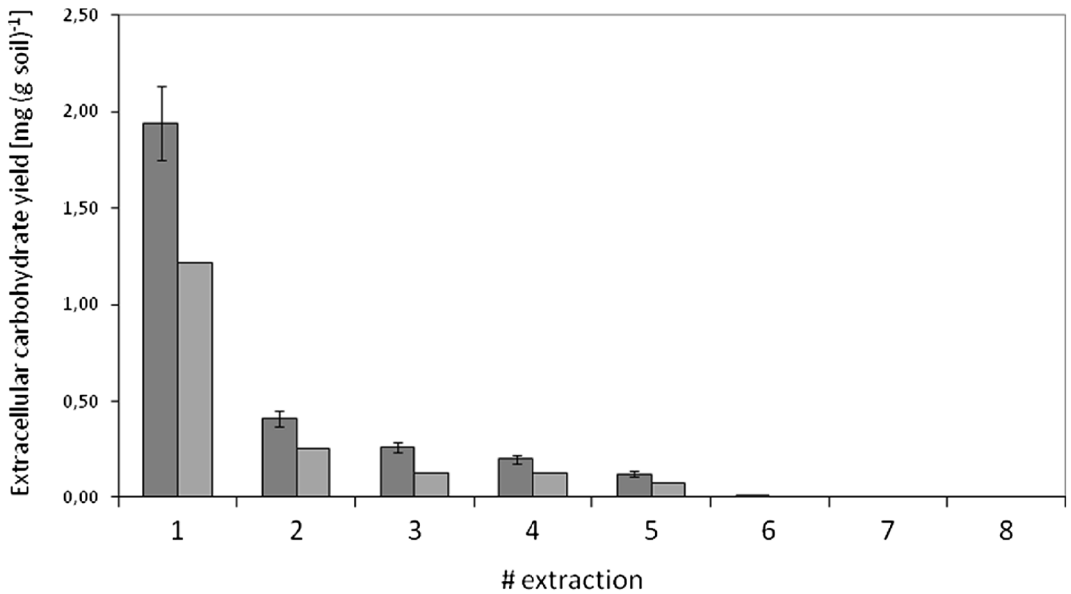

Fig. 3 Repeated extracellular carbohydrate extractions from two different typologies of BSCs from North American deserts, utilizing the method of Underwood et al. (1995) for the first time on BSCs. Dark brown bars, Chihuahuan Desert crust; light brown bars, Mohave Desert crust. Yields were expressed as mg of extracellular carbohydrates (g soil)$^{-1}$ for each extraction on each sample. Each sample type was extracted in triplicate and results were expressed as value ±SD (*F. Rossi*, personal communication)

exopolysaccharidic sheaths (Garcia Pichel and Wojciechowski 2009), resulting from one or more secretion events. This is recognized as the first step in BSC formation. Cyanobacterial sheaths can be considered a central element in the formation of EPM, as they constitute the first accumulation of EPS material on which early stages of BSCs are structured (Rajeev et al. 2013). Organized in "large bodies" (~100 μm), sheaths bind soil particles stabilizing soil against erosion by wind or water (Belnap and Büdel 2016; Belnap and Gardner 1993). In studying early-stage BSCs from Gurbantunggut Desert, China, Zhang (2005) observed that cyanobacterial sheaths either contained filaments, or were leftover material, after filament migration or death. Empty sheaths remain solidly attached to the organo-mineral material as a cement stabilizing the crust structure. Unless crashed by compressional disturbances, a primitive discernible organization of the EPM is visible from the first stages of development of BSCs by microscopical observations. Three month-old artificial cyanobacterial crusts obtained by inoculating the cyanobacterium *Schizothrix delicatissima* AMPL0116 on bare sand in microcosms were constituted by an EPM distinctly organized in a LB-EPS and a TB-EPS fraction (Fig. 5) (Mugnai et al. 2017).

Chemical and macromolecular characteristics of EPM in BSCs

The chemical and macromolecular characteristics of EPM of BSCs have been studied in only a limited number of cases. Recently, the monosaccharidic composition and MW distribution of EPS extracted from BSCs of known age, collected in the Hopq Desert, Inner Mongolia, China, was carried out. The area is hyper-arid, with a climate classified as semiarid, temperate and continental monsoon. These BSCs were the result of an inoculation-based treatment carried out in different years (Chen et al. 2006; Li et al. 2014; Wang et al. 2009). Notwithstanding the stressful environmental conditions which would suggest a compositional simplicity (Mager and Thomas 2010), the extracted EPS showed instead a certain complexity, unrelated to the age of the crusts. Up to 13 different types of sugars were identified. They included the hexoses galactose, fructose and glucose, which had the highest relative abundance, the dehoxy-sugars fucose and rhamnose, the amino-sugars galactosamine and glucosamine, and the pentose ribose. In addition, uronic acids, namely galacturonic and glucuronic acids, were also detected (Chen et al. 2014; Colica et al. 2015). Extracted EPS showed mainly MWs

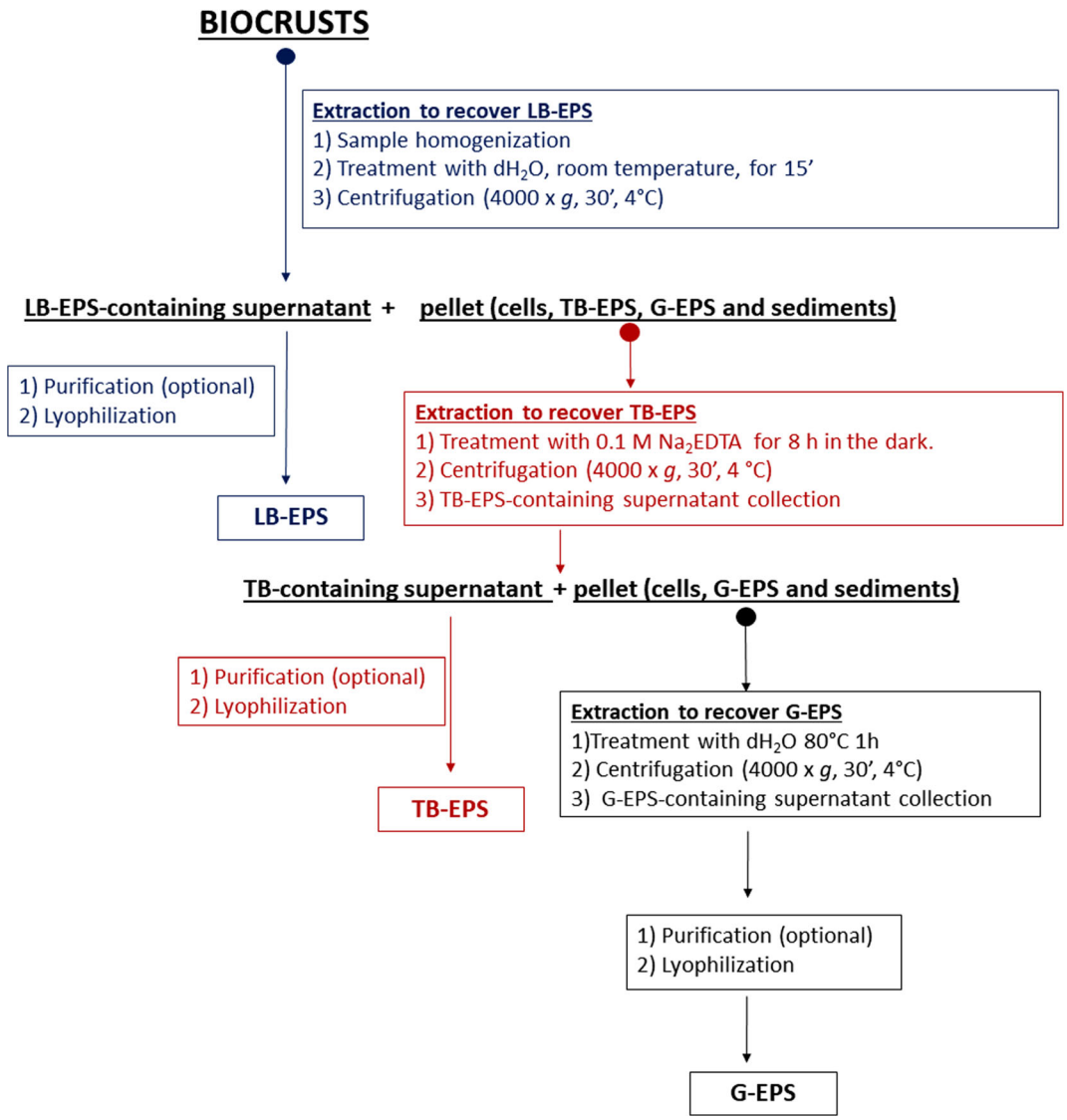

Fig. 4 Suggested procedure for the extraction of EPM fractions from BSCs

comprised between 2 M and 485 kDa (in the range characterizing EPS produced by cyanobacteria) and between 72.6 k Da and 0.34 k Da, comprised of small MW saccharides, dimers and monomers. In a further study, the composition and MW distribution of LB-EPS and TB-EPS were determined and compared. While the two fractions had a similar compositional pattern, they appeared to be different in MW distribution. The 90% of TB-EPS had a MW between 2 M and 0.76 M Da, while the less condensed fractions were polymers in the lower MW ranges (Chen et al. 2014). In Arctic BSCs, collected around Ny-Ålesund, Svalbard archipelago, the cyanobacterial relative abundance was very low in favor of the relative abundances of Proteobacteria, Actinobacteria and Acidobacteria (Mugnai et al. 2015; Rossi et al. 2012b). Notwithstanding this, and the obvious different environmental characteristics, monosaccharidic composition was of a similar complexity between the two types of crusts. On the contrary, the MW distribution profile was significantly different, with high relative percentages of small MW carbohydrates. The comparison of these first surveys suggests that MW distribution profile may be more distinctive among crusts with different characteristics, and among different environments.

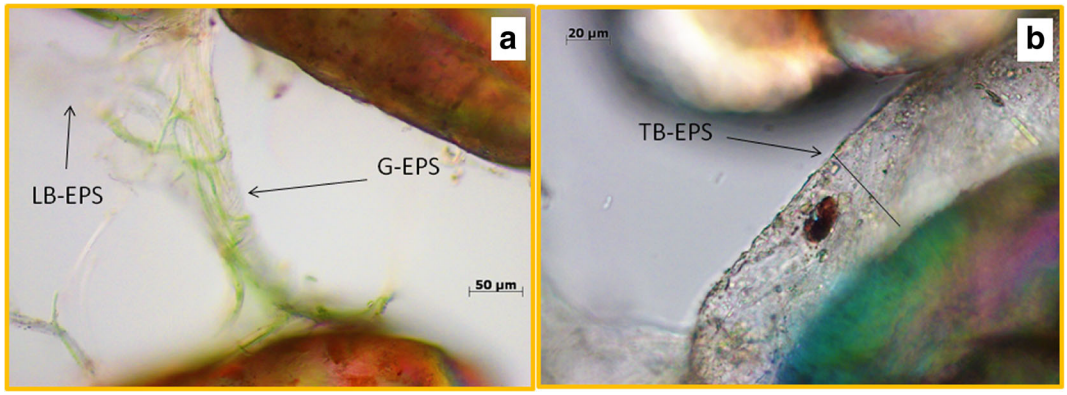

Fig. 5 Microscopical images of the operationally-defined EPM fractions in a 15 day-old induced cyanobacterial crusts obtained by inoculating the cyanobacterial strain *Schizothrix* cf. *delicatissima* AMPL0116 in microcosms. G-EPS, glycocalix EPS (encompassing sheaths and capsules); LB-EPS, loosely bound EPS; TB-EPS, tightly bound EPS. a) Filaments and sheaths of *S. delicatissima* gluing two adjacent sand particles. The picture underline the prominent role of EPS in sediment cohesion. b) TB-EPS covering a sand grain surface. Pictures by GM

Structural role of EPM in BSCs

EPM is essential for crust physical structure, prompting the cohesion between the biotic and the a-biotic crust components, and the adhesion to solid substrates (Rossi and De Philippis 2015a).

Due to the stability that they confer to the BSC structure, and their capability to counteract erodibility, EPS are considered by some authors as an index of soil aggregation and BSC development (Bowker et al. 2008).

The sediment-cementing nature of EPS has been often observed and acknowledged (Zhang 2005; Malam-Issa et al. 2007). This structuring role is important for the incipient cyanobacterial colonization of bare soils. Some BSC-dwelling cyanobacteria and microalgae, tested for soil stabilizing capability, provided evidence that EPS productivity is in agreement with their capability of stabilizing fine sand grains (Hu et al. 2003) and crust resistance to wind erosion (Hu et al. 2002). EPS strands bind fine soil particles (< 65 μm) enforcing the stabilizing action of bacterial filaments, mosses and lichens (Bowker et al. 2008). In addition, Malam Issa et al. (2009) provided evidence that EPS, due to their amphiphilic properties, confer to bacterial filaments the capability to drive the formation of additional soil pores ("microbial pores"), affecting their geometry, and determining soil spatial organization within the BSC thickness.

By studying the EPM of three to eight year-old induced BSCs in a semiarid environment, it was observed that TB-EPS is the fraction more involved in providing a structural role, appearing more "preserved" from enzymatic activity, and being more condensed than LB-EPS (Chen et al. 2014).

EPS are also involved in the formation of induced sedimentary structures, which include roll-ups, folds, desiccation polygons, especially on sandy soils (Garcia Pichel et al. 2016) and in hot arid conditions. The formation of these structures may have an ecological meaning for crust community. In natural dry hot environments the formation of cracks may provide open pathways to increase soil permeability and aeration under crust layers (Williams et al. 2012), leading to new colonizable niches with enhanced moisture regimes (Danin et al. 1998).

Role of the EPM in BSC-water relations

One of the first reported effects of the presence of EPS in BSCs was that their swelling, and pore-clogging effect upon re-wetting, leading to run-off during rain events (Kidron et al. 1999; Fischer et al. 2012). The role of EPM of BSCs in affecting soil infiltrability was investigated in two different studies (Rossi et al. 2012c; Colica et al. 2014), considering soil textures ranging from silt loam to sandy, according to the USDA classification (e.g., Groenendyk et al. 2015). Some differences related to soil texture appeared evident: loamy sand and silt loam soils showed a high positive correlation of sorptivity values with sand content and negative with silt and clay. At the same time, no correlation was found between sorptivity and EPS content (Rossi et al. 2012c). Nonetheless, the application of a simple nondestructive

extraction of EPS (detailed in Rossi et al. 2012c) resulted in a more compacted crust structure that lost the ability to absorb water. This strongly supported the idea of a contribution of the EPM in structuring BSC waterways. This aspect was further investigated by Felde et al. (2016) that examined the effects of this extraction procedure by using X-ray computerized tomography. This analysis ruled out any "artificial" modification of the crust structure, apart from EPS extraction, limiting the cause of sorptivity change only to their removal. Studies conducted with BSCs growing on sandy soils in the Hopq Desert, Inner Mongolia, China, where water infiltration velocity is high, depicted a different scenario. A significant correlation between EPS content and soil sorptivity was found. In this context, the presence of BSCs diminishes remarkably water infiltrability (up to 90% detected reduction). The correlation observed supports the notion that EPS swell following water contact, reducing the volume of soil pores (Fischer et al. 2010a), and promoting the maintenance of the moisture in the very first soil layers which are the most biologically active.

The presence of an EPM is very important for cells to counterbalance water-deficient conditions. Owing to their amphiphilic character, EPS accumulate water, and regulate water loss (Pereira et al. 2009), maintaining hydration (Mazor et al. 2009) and counteracting evapotraspiration. The presence of a moistened environment at the soil surface is very important for an optimal physiological activity of the crust community. In addition, EPS are involved in dew formation at the surface of BSCs (Fischer et al. 2012).

In one study conducted on BSCs grown on sandy soil in the same Hopq Desert, it was demonstrated that *i*) EPS are fundamental to retain humidity and *ii*) EPS are fundamental for water uptake from non-rainfall water sources (i.e., dew, fog and plant guttation) (Colica et al. 2014). The Hopq Desert site is characterized by an average yearly evapotranspiration that broadly exceeds that of precipitation. The capability of BSCs to retain water against evapotranspiration was correlated to the presence of EPS, especially those having a high MW. After removing 90% of EPS from BSC samples, utilizing the previously mentioned nondestructive method, water uptake capability decreased sensibly, to being not statistically different from that of bare sand. These studies expanded the knowledge on the important roles of EPM in: *i*) delaying water movement through the soil when sorptivity is high, and contributing to the creation of viable waterways when sorptivity is low, *ii*) regulating water uptake and water loss from the cells (Pereira et al. 2009), and *iii*) reducing evaporation loss and increasing soil water-holding capacity (Mager and Thomas 2011).

EPM in BSCs as a nutriment source and plant fertilizer

EPM is a medium constantly subjected to enzymatic and abiotic degradation, to modification and condensation processes. For example, humic substances are thought to have a chemical structure which is the result of partial enzymatic degradation and condensation (Hedges 1988). Enzymes mostly involved include hydrolases, (less frequently) lyases, invertases, and sucrases (Wingender et al. 1999; Miralles et al. 2012). The activity of hydrolases can provide correlative information on microbial activity and microbial population and in some cases it is related to soil organic content (Chen et al. 2014). The hydrolytic enzymatic action in BSCs increases according to the level of development (LOD), from cyanobacteria-dominated crusts to lichen-dominated crusts, primed by an increase in C and N (Miralles et al. 2012). In one study, it was observed that the activity of hydrolases and sucrases is correlated not only to LOD, but also to the age of BSCs (Chen et al. 2014).

EPM promotes the association in consortia that depolymerize complex compounds to simple molecules easily assimilable even by community members having a smaller genome, a less specialized lifestyle, and not possessing a full range of degradative enzymes (Mba Medie et al. 2012). Some microorganisms are able to degrade and feed on their own EPS. Although some authors deem it limited to a few cases, the "reuse" theory has been demonstrated for fungi and cyanobacteria. Fungi possess glucanases, which degrade (1–3)-ß- and (1–6)-ß-glucans with several mechanisms of action and functions (Seviour et al. 1992). Stuart et al. (2016) demonstrated that in induced cyanobacterial microbial mats, cyanobacteria can degrade oligosaccharides, proteins and nucleic acids. In addition, they found that cyanobacteria are able, in a short time, to feed on the C from their own EPS, both in the light and in the dark. This information may be important for fully describing C cycle within these communities.

Since arid and semiarid soils contain low amounts of C, the emission of EPS is of vital importance. According to Chenu (1993), EPS may represent up to 500% of cellular biomass, and were hypothesized to be the primary substrate respired after heavy rainfall events

(Thomas et al. 2008; Fischer 2009; Thomas and Hoon 2010). A study on the enzymatic activity in induced BSCs of different years, was coupled with a characterization of the EPM fractions (Chen et al. 2014). It was observed that the enzymatic activity was mostly directed towards the more soluble LB-EPS, which displayed substantial variation in MW distribution comparing BSCs of different ages. A study conducted by Decho and Lopez (1993) on the digestibility of EPS-producing bacterial cells, demonstrated an higher resilience to digestion of G-EPS, which has more stable, ordered and definite secondary structures, in comparison with less condensed EPS fractions. This supports the idea that slime diffusing in the soil is the principal more easily degradable EPS fraction, while TB-EPS and G-EPS, due to their lower degradability, constitute more a "structural skeleton" of BSCs.

EPM may also act as a nutrient accumulator, concentrating dissolved organic matter (DOM) with compounds containing C, N, P and trace metals which are essential for cell metabolism (Wolfaardt et al. 1999; Flemming and Wingender 2010). In this respect, such a nutrient source may be of importance for vegetation establishment, determining an increase of essential nutrients in plant tissues (Zhang et al. 2016).

Studies addressing the effects of BSCs in plant establishment are often contradictory (Zhang et al. 2016). It is nonetheless obvious that, since EPM conditions soil cohesion, soil properties, soil porosity and the formation of microbial induced sedimentary structures (e.g., roll-ups, folds, desiccation polygons) (Garcia Pichel et al. 2016) BSCs indirectly affects seeds entrapment and subsequent emerging. For example, BSCs enhance germination and emergence of seeds in cool desert (Belnap 2003a, b) where morphology is characterized as rolling or pinnacled (Belnap 2001).

Whether EPS are directly effective and benign to plant establishment and growth is still unknown. One study (Xu et al. 2012) seems to suggest so. The treatment of the seeds of *Caragana koshinskii*, a desert-dwelling shrub belonging to Leguminosae, with the solubilized EPS produced by *Phormidium tenue* (Oscillatoriales), a cyanobacterial species commonly found in BSC communities (e.g., Hu et al. 2002), promoted germination (germination index, and germination energy) as well as root growth, nutrient and water uptake, photosynthetic efficiency, and defense from oxidative damages. Although these data are the result of a single study, they point out the need of a more in-depth exploration the bioactive characteristics of EPS.

Conclusions

In spite of the unanimously recognized importance of the EPM in the formation and stabilization of BSCs, and compelling SEM imagery illustrating this importance (Belnap 1993; Zhang et al. 2006; Fischer et al. 2010b; Mager and Thomas 2011), an increasing number of reports on the characteristics of the most important constituents of EPM, namely EPS, has appeared only recently. The papers reviewed here clearly show the complexity of the functions of EPM. Indeed, one of the most striking recent results obtained is that EPS are constituted by more than one fraction, with each fraction having different chemical properties and possibly playing different roles in BSCs. This may contribute to the amazing capability of these complex structures to withstand very harsh environmental conditions. Moreover, the presence of such complex EPS structures points out the need of applying specifically developed methods for the extraction of the different fractions in order to obtain a sound characterization of their chemical and macromolecular features.

However, many important aspects still have to be investigated and clarified in order to fully understand the role of EPS in BSCs. In our opinion, future studies should be focused in particular on investigating:

i) the complex interplay between EPS producers and consumers in BSCs, defining the specific role of the microorganisms;
ii) how the EPS affect the flux of nutrients (C, N, P and inorganic ions) in the EPM;
iii) how the different EPS fractions interact with water molecules, affecting their movement within the crusts;
iv) if and how the characteristics and the role of the EPS are affected by the different typologies of soils where BSC developed.

Answering these questions will provide a fundamental contribution to the understanding of the role of the EPS in the dynamic and complex habitat constituted by BSC but we are sure that at the same time it will also open new questions to be addresses in further studies.

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