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Macromolecular and chemical features of the excreted extracellular polysaccharides in induced biological soil crusts of different ages

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ABSTRACT

The development of biological soil crusts (BSCs) is widely recognized as beneficial to soil fertility due to their contribution to the stabilization of soils and to the increase in their carbon and moisture content. An important role in these processes is played by the extracellular polysaccharidic (EPS) matrix embedding microbial cells and soil particles in BSCs. The present study was aimed at investigating the molecular and chemical features of the EPSs and the degradation processes of the polysaccharidic matrix (i.e. dehydrogenase and sucrase activities) in induced biological soil crusts (IBSCs) of different ages displayed within an investigation area in Hobq Desert (Dalatequi County, Inner Mongolia, China). Two operationally-defined EPS fractions, the colloidal (C-EPS) and the EDTA extractable (tightly bound, TB-EPS) fractions, were analyzed. In BSCs, C-EPSs are loosely bound to cells and sediments while TB-EPSs are tightly bound to the crustal biotic and abiotic constituents of the crusts. In this study, the C-EPS and TB-EPS fractions extracted from the IBSCs of different age (4-, 6- and 8-years old IBSCs) were found present in comparable amounts but showed marked differences in terms of their molecular size distribution and monosaccharidic composition. C-EPS showed to be mostly constituted by sugar fractions with molecular weight (MW) distributed in the range 2000–76 kDa and in the range 64–0.34 kDa. Conversely, the TB-EPSs showed to be prominently constituted by one fraction having a MW in the range 2000–76 kDa. While the chemical and macromolecular characteristics of TB-EPSs did not show significant changes with the age of the crusts, the older IBSCs showed a lower content of low MW C-EPSs, as well a higher number of different types of monosaccharides constituting the C-EPS. Moving from these results, it can be hypothesized that C-EPSs, which are dispersed in the soil and thus more accessible, have been rather easily degraded by the heterotrophic microorganisms dwelling in mature IBSCs and reduced to low MW carbohydrates that are easily metabolized by chemoheterotrophs. This hypothesis is supported by the higher activity observed in older IBSCs of the two enzymes associated with sugar degradation in the soil, dehydrogenases and sucrases, that is consistent with an increased release of low MW carbohydrates in the crusts.

The results obtained suggest that the colloidal fraction of the EPSs, which is more dispersed in the soil, is more easily degradable by the microflora, while the EPS fraction tightly bound to the soil particles, which is characterized by a high MW, plays a key role in giving a structural stability to the crusts and in affecting the hydrological behavior of the soil covered by IBSCs.

1. Introduction

Biological soil crusts (BSCs) are highly-specialized communities covering the first millimeters of topsoil in many ecosystems, notably arid and semi-arid environments. BSCs reportedly play an important role in major ecological processes in badlands systems, particularly those involving early stage soil succession on degraded

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soils (Belnap and Gillette, 1997; Harper and Belnap, 2001; Langhans et al., 2009). Within these biotic assemblages, cyanobacterial filaments, fungal hyphae and excreted extracellular polysaccharides (EPSs) enfold soil particles, creating an organo-mineral layer in the first soil millimeters (Belnap et al., 2001; Li et al., 2005).

Cyanobacteria are the first pioneers after disturbance events. They significantly contribute to the C and N input in the soil and are major EPSs producers (De Caire et al., 2000; Acea et al., 2003; Hawkes, 2003; Langhans et al., 2009). EPSs are molecules of varying MW, capable of constituting gels of varying hardness depending on their chemical nature and on environmental parameters (i.e. pH, available counter-ions, water availability etc.). While harder and more condensed fractions may constitute stable layers surrounding microbial cells (named sheaths or capsules), less condensed and loosely bound fractions, encompassed under the name of slime, may be massively released in the surrounding environment (De Philippis and Vincenzini, 1998; Nielsen and Jahn, 1999).

Beside their ecological role in BSCs, recently thoroughly documented by Mager and Thomas (2011), EPSs represent a huge carbon source which can be exploited by residing microorganisms and colonizing plants and give an important contribution to the hydrological behavior of the crusts (Rossi et al., 2012b; Colica et al., 2014). In some cases, the amount of these substances was reckoned to represent up to 500% of the cell biomass (Chenu, 1993). Complex high molecular weight (HMW) polymers are degraded by the soil biota to simple sugars such as glucose, galactose (Gross et al., 1998; Brüll et al., 2000; De Brouwer and Stal, 2001) and fructose, which can be readily utilized as carbon source or energy reserve (Bertocchi et al., 1990) by the crustal microbial community and/or by adjacent plants. Indeed, C from EPSs was demonstrated to be the primary substrate respired in the pulse of CO_2 typically observed in dry lands after rainfall events (Thomas et al., 2008). In other systems it is documented that, owing to microbial activity, EPSs are eventually reduced to smaller polymers with a broad range of molecular sizes (Arnosti, 1995, 1996; De Brouwer and Stal, 2001) and chain lengths (Bender et al., 1994), depending on the structure and composition of the microbial community.

Notwithstanding the key role of BSCs in starting and enhancing biogeochemical cycles on bare substrates and in increasing soil total organic carbon (TOC), a limited number of studies have been so far addressed to analyze enzymatic activity in crusted soils (Miralles et al., 2012, and references therein), especially enzymatic activity of BSCs at different developmental stages. In this connection, it is worth stressing that the activity of dehydrogenase in the soil can provide correlative information on microbial activity and microbial populations, and in many cases it is related to soil organic matter content (Dormaar et al., 1984). On the other hand, sucrase, which is an extracellular enzyme that catalyzes the hydrolysis of sucrose into glucose and fructose, may represent the hydrolytic activity on complex sugars carried out by the chemoheterotrophic microbial community.

The present study was aimed at investigating the macromolecular and chemical features of the EPSs and the degradation processes of the polysaccharidic matrix (i.e. dehydrogenase and sucrase activities) in induced biological soil crusts (IBSCs) displayed within an investigation area in Hobq Desert (Dalatequi County, Inner Mongolia, China). At the time of this study (2011), IBSCs of different ages were available due to previous massive inoculations of sandy dunes carried out in different years (2003, 2005 and 2007) with *ex-situ* cultivated cyanobacteria (Chen et al., 2006).

2. Materials and methods

2.1. Study area and cyanobacterial crust induction

Investigation area is located in Hobq Desert, Dalateqi County, Inner Mongolia, China (40°21′30″–22′30″N; 109°50′30″–51′50″E). Hobq Desert is a hyper-arid plateau at an altitude of 1040 m above sea level, characterized by a mass of sand dunes with average height of 5 m. The climate belongs to a typical continental monsoon pattern, with an average minimal annual temperature of −34.5 °C and an average annual maximum temperature of 40.2 °C. Windy days (wind velocity >5 m s^{-1}) occur on more than 180 d yr^{-1}. Mean annual precipitation and mean annual evaporation are 293 mm and 2448 mm respectively.

In a period spanning from 2003 to 2007, two cyanobacterial strains, *Microcoleus vaginatus* Gom. and *Scytonema javanicum* Born et Flah, were mass cultivated in raceway ponds located in a greenhouse and then mixed (*M. vaginatus*: *S. javanicum* = 10:1 w/w ratio) and inoculated onto the sandy soil in three different sites of the investigation area. The inoculation procedure used has been described in details by Chen et al. (2006) and Wang et al. (2009). Briefly, cultures were harvested by filtration through silk and inoculated directly onto unconsolidated sand with a sprayer, as homogenously as possible. Sites were inoculated at a concentration of biomass approximately corresponding to 6 μg chlorophyll *a*/cm^2 soil. Then, the sites were irrigated for 15–18 days, from 9:00 to 16:30, by automatic sprinkling micro-irrigation facilities, until crusts were visible on the soil. Site 1 (40°21′30″N, 109°50′30″E) was inoculated in 2003 (8 years before the sampling); site 2 (40°21′45″N, 109°50′42″E) was inoculated in 2005 (6 years before the sampling); site 3 (40°21′54″N, 109°50′51″E) was inoculated in 2007 (4 years before the sampling).

In all the experimental sites, the desert is characterized by a mass of dunes with an average height of 5 m above the ground and the soil is constituted by bare sand carried by the wind from the desert area between the towns of Urad Qianqi and Dengkou (Inner Mongolia, China) (Colica et al., 2014). In this case study, the physicochemical characteristics of the soils, classified as sandy soils by Lan et al. (2010), are the same in all the investigated sites.

2.2. Field sampling

Sampling was carried out on August 2011 in all the three inoculated sites. For each of the experimental sites, three dunes were randomly chosen ($N = 3$, statistical replicates) and for each of these three dunes, standing 100–200 m apart one from another, three crust samples (experimental replicates) were collected on the dune-top and three in the interspaces between adjoining dunes. At the time of sampling, IBSCs were separated by areas with shrubs and other small plants, strongly reducing the interferences between the sampling points. This allowed to have more robust replicates.

The collection was performed digging soil pits on well developed cyanobacterial crusts and picking up samples with a thickness ranging from 0.1 to 1 cm, depending on the structure of the crusts, using a sterilized spatula. In each sampling site, unconsolidated sand was also collected 20 cm under the crusted surface in the inoculated areas and was analyzed separately. In order to have a control (CKs), samples were also collected in three different points ($N = 3$) standing 100–150 m apart one from another, in a non-inoculated area located closely to the three sites (40°20′28″N, 109°51′20″E). Non-inoculated area did not show any biological soil crust and it appeared constituted by unconsolidated sand with the same texture and the same characteristics formerly showed by the sites before the inoculation.

Samples were stored in small plastic containers and carried to field laboratory.

2.3. Analysis of chlorophyll a content

Samples of IBSCs were manually ground to pieces of roughly 1 cm^3, air-dried and treated with 95% ethanol in the dark at 4 °C for