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Adsorption mechanism of extracellular polymeric substances from two bacteria on Ultisol and Alfisol☆

Li-ying Ren [a, b], Zhi-neng Hong [a], Wei Qian [a], Jiu-yu Li [a], Ren-kou Xu [a, *]

[a] State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China
[b] College of Resources and Environment, Linyi University, Linyi 276000, China

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ABSTRACT

The primary objective of this study was to identify the capacity and mechanism of extracellular polymeric substance (EPS) adsorption on soil colloids of Alfisol and Ultisol at different pH and ionic strengths. Two kinds of EPS were extracted from *Bacillus subtilis* and *Pseudomonas fluorescens* by centrifugation, and their adsorption on Ultisol and Alfisol was investigated using a batch adsorption experiment and attenuated total reflectance–Fourier transform infrared spectroscopy (ATR-FTIR). The average diameter of EPS from *B. subtilis* and *P. fluorescens* was 1825 and 1288 nm, respectively, and both the EPS were negatively charged. The zeta potentials of the two EPS became more negative with increasing solution pH from 3 to 8 and less negative with increasing ionic strength from 0 to 80 mM. The maximum adsorption capacity of EPS-C and EPS-N on Alfisol was higher than that on Ultisol, whereas the maximum adsorption capacity of EPS-P on Alfisol was lower than that on Ultisol. The adsorption of EPS-C, EPS-N, and EPS-P of both the EPS on Ultisol and Alfisol decreased with increasing solution pH from 3 to 8. Adsorption of EPS-C, EPS-N, and EPS-P of both the EPS on Alfisol significantly increased with increasing ionic strength from 0 to 10 mM, whereas it remained constant, slightly increased, or reduced, when the ionic strength was increased from 10 to 80 mM. The adsorption of EPS-C, EPS-N, and EPS-P on Ultisol slightly increased with increasing ionic strength from 0 to 80 mM. Saturation coverage determined by ATR-FTIR showed that adsorption of whole EPS on Ultisol was higher than that on Alfisol at pH 6 after 60 min. Thus, electrostatic force between EPS and soil colloids played an important role in EPS adsorption. Besides, proteins and phosphate groups in EPS also contributed to EPS adsorption on soil colloids.

1. Introduction

In soil environments, extracellular polymeric substances (EPSm) are produced during the growth and metabolism of heterotrophic biomass. The main components of EPS are carbohydrates, proteins, humic substances, and nucleic acids, with carbohydrates and proteins accounting for 75%–90% of EPS (Long et al., 2009; Cao et al., 2011). Most of the EPS molecules are negatively charged owing to the presence of anionic functional groups, such as carboxyl, phosphoryl, hydroxyl, and sulfhydryl groups (Ha et al., 2010). These acidic functional groups get ionized in response to solution chemistry changes and affect cell attachment to solid substrates (Gong et al., 2009; Karunakaran and Biggs, 2011; Mukherjee et al., 2012). When released into soil, EPS may adsorb onto mineral surfaces and affect the hydrophilicity and charge of the mineral surfaces (Cao et al., 2011; Mikutta et al., 2011), biomineralization (Bontognali et al., 2008), bioleaching (Sand and Gehrke, 2006), and binding of organic and inorganic compounds (More et al., 2014) in soils. Therefore, knowledge of EPS adsorption on soil surface could help in understanding the function of EPS on biochemical changes in soils.

EPS adsorption on minerals is influenced by solution pH and ionic strength. It has been reported that the adsorption of EPS from *Bacillus subtilis* on goethite decreased with increasing solution pH from 3 to 9 and increasing NaCl concentration from 1 to 100 mM (Omoike and Chorover, 2006). In another study, quartz crystal microbalance with dissipation (DCM-D) analysis showed that the deposition efficiency for EPS from *Escherichia coli, Pseudomonas* sp. QG6, *Rhodococcus* sp. QL2, and *B. subtilis* on silica surface increased with increasing ionic strength in both $CaCl_2$ and NaCl solutions (Zhu et al., 2009). Furthermore, attenuated total

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* Corresponding author.
E-mail address: rkxu@issas.ac.cn (R.-k. Xu).

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reflectance–Fourier transform infrared spectroscopy (ATR-FTIR) revealed that EPS from *B. subtilis* and *Pseudomonas aeruginosa* were bound to goethite via inner-sphere complexation (Omoike et al., 2004). Amide groups in proteins and phosphate groups in phosphodiester bridges of nucleic acids have been reported to play an important role in *Pseudomonas putida* EPS adsorption on goethite (Fang et al., 2012). Confocal laser scanning microscopy revealed that proteins were predominantly distributed on the surface of montmorillonite and kaolinite, while nucleic acids were mainly adsorbed on the goethite surface (Lin et al., 2016). The adsorption mechanism of bacterial EPS on mineral surface includes electrostatic, hydrophobic, covalent, and polymer-polymer interactions (Tsuneda et al., 2003). Moreover, interaction of EPS with soil inorganic colloids influences the adhesion and transport of bacteria, dissolution of minerals, and accumulation of metals in soil (Banfield et al., 1999; Kim et al., 2009; Fang et al., 2010). Although some studies have elucidated the adsorption mechanism of EPS on homogeneous mineral surfaces, there is only limited information about EPS adsorption on heterogeneous natural soil particles.

In the present study, colloids (<2 μm) of Ultisol and Alfisol were used to investigate the adsorption of EPS extracted from *B. subtilis* (Gram-positive bacterium) and *Pseudomonas fluorescens* (Gram-negative bacterium). The purposes of this study were (1) to compare the adsorption of EPS from the two bacteria on the colloids of the two soils at different solution concentrations; (2) to investigate the effects of pH, ionic strength, and reaction time on EPS adsorption on soil colloids using batch adsorption experiment and ATR-FTIR; and (2) to identify the mechanisms underlying EPS adsorption onto soil colloids.

2. Materials and methods

2.1. Preparation of soil colloids

Two soils were used in this study. Ultisol was collected from Jiangxi Province, China (28°23′ N, 116°17′ E), and was derived from Quaternary red earth. Alfisol was collected from Jiangsu Province, China (31°9′ N, 118°9′ E), and was derived from loess deposit. The samples of both soils were collected from sub-layer and thus contain low content of organic matter (Table S1). Sedimentation was used to separate soil colloids of less than 2 μm from bulk soils, as previously described (Liu et al., 2015a). The collected soil colloids were purified by electrodialysis at a potential gradient of 15 V cm^{-1} until a constant specific electric conductance was achieved. After drying at 60 °C, the colloids were ground and passed through a 60-mesh sieve. The particle size of soil colloids was measured with dynamic light scattering technique using a 90Plus/BI-MAS Zeta potential instrument (Brookhaven Instruments Corp., Holtsville, NY, USA), and the average diameter of Ultisol and Alfisol colloids was 527.13 and 560.97 nm, respectively. The selected properties of soil colloids are shown in Table S1. The clay mineral composition of the soil colloids was determined using X-ray diffraction (XRD) (Ultima IV, Rigaku, Tokyo, Japan) (Table S2).

2.2. Bacterial EPS extraction and purification

Bacillus subtilis (CGMCC 1.88) and *P. fluorescens* (CGMCC 1.1802) were obtained from the China General Microbiological Culture Collection Center (Beijing, China), and EPS were extracted from these bacteria by centrifugation (Omoike and Chorover, 2006). In brief, the bacteria were grown in beef extract peptone medium at 28 °C and 180 rpm for 24 h until the stationary growth phase: 0.67 mg mL^{-1} by wet weight and 1.153×10^8 CFU mL^{-1} for *B. subtilis*; 0.73 mg mL^{-1} by wet weight and 1.362×10^8 CFU mL^{-1} for *P. fluorescens*. Subsequently, the culture medium was centrifuged at 5000 × g for 15 min, and the supernatant was collected and centrifuged at 12,000 × g for 30 min to remove residual cells. The EPS was precipitated from the supernatant by adding ethanol at 3:1 vol ratio and stored at 4 °C for 48 h. Then, the EPS precipitate was separated from ethanol by centrifugation, dissolved in deionized water, purified using cellulose membranes (3500MWCO from Spectrum, Viskase, USA), and freeze-dried.

2.3. Particle size determination

The size distribution of EPS was measured with dynamic light scattering technique using a 90Plus/BI-MAS Zeta potential instrument (Brookhaven Instruments Corp., Holtsville, NY, USA). The average diameter of intensity weighted size was used in the interaction energy calculation.

2.4. Zeta potential measurement

The zeta potentials of soil colloids and bacterial EPS were determined using a 90Plus/BI-MAS Zeta potential instrument (Brookhaven Instruments Corp.). The concentration of the soil colloids and bacterial EPS was 250 mg L^{-1}, respectively. For determining the influence of pH, 1 mM NaCl was used as the background electrolyte, and the pH of the suspension was adjusted to 3–8 using 0.1 M HCl or NaOH. For ascertaining the effect of ionic strength, the concentration of NaCl was set in the range of 0–80 mM and the solution pH was adjusted to 6. All the experiments were repeated thrice, and the data are shown as mean ± standard deviation.

2.5. Isothermal adsorption experiment

A series of 50-mL plastic centrifuge tubes were prepared, and 100 mg of soil colloids were added to each tube. Then, various amounts of 2 mg mL^{-1} EPS solution were added to maintain the EPS concentrations in the range from 0 to 1 mg mL^{-1} in a total suspension volume of 20 mL, and 1 mM NaCl was used as the background electrolyte. The pH of the suspensions was adjusted and maintained at 6 with 0.1 M HCl or NaOH, and the mixtures were agitated at 25 °C for 2 h. Then, the mixtures were centrifuged at 10,000 × g for 30 min, and the total organic C and total N contents in the EPS in the supernatant were determined by Jena Multi N/C 3100 Analysis Instrument (Analytik Jena AG, Germany). Furthermore, the EPS was digested with potassium peroxydisulfate ($K_2S_2O_8$) and the total P content in the EPS was determined by molybdenum blue colorimetric method (GB/T 11893–1989). The adsorbed EPS-C, EPS-N, and EPS-P were calculated as the difference between the amounts of these elements in added EPS and the EPS remaining in the supernatant. The EPS-C, EPS-N, and EPS-P contents were 539.25, 206.51, and 45.92 mg g^{-1} in the EPS from *B. subtilis*, and 554.86, 189.83, and 47.41 mg g^{-1} in the EPS from *P. fluorescens*, respectively.

2.6. Influence of pH and ionic strength on EPS adsorption

To investigate the effect of pH on EPS adsorption, 100 mg of soil colloids and 5 mL of EPS solution (2 mg mL^{-1}) were added to the tube, and the suspension pH was adjusted to various values ranging from 3 to 8. As the background electrolyte, 1 mM NaCl was used, and the total volume of the suspension was 20 mL. To investigate the influence of ionic strength on EPS adsorption, 100 mg of soil colloids and 5 mL of EPS solution (2 mg mL^{-1}) were added to the tube, and NaCl was added to adjust the final ionic strength ranging from 0 to 80 mM. The suspension pH was adjusted to 6, and the total volume of the suspension was 20 mL. Subsequently, the same procedures as those employed in the isothermal adsorption