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Effects of extraction methods on the composition and molar mass distributions of exopolymeric substances of the bacterium *Sinorhizobium meliloti*

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ABSTRACT

The influence of the extraction methods on the composition, size diversity, molar mass and size distributions of exopolymeric substances (EPS) from the bacterium *Sinorhizobium meliloti* wild type (WT) and by the exoY strain deficient in exopolysaccharide production was investigated. EPS obtained by centrifugation, EDTA and formaldehyde/NaOH were compared. It was found that the extraction method influenced TOC, TN and total protein content in EPS from both strains. However, no difference between EDTA and formaldehyde/NaOH methods was observed for the exopolysaccharide components. Similar functional groups and fluorescence pattern were found in the EPS obtained by different methods; however their relative abundance was method dependent. The extraction method also affected the molar mass and size distribution, HP SEC diversity among different treatment and bacterial strains.

1. Introduction

Extracellular polymeric (or exopolymeric) substances (EPS) are composed of a wide range of organic polymers such as polysaccharides, proteins, nucleic acids and phospholipids, excreted by eukaryotic and prokaryotic organisms (Wingender et al., 1999). EPS are considered to play a key role in bacterial flocs and biofilms (Flemming and Wingender, 2010), to be central in toxic metal bioremediation (Pal and Paul, 2008) as well as to greatly influence the performance in waste water treatment systems (Sheng et al., 2010; Subramanian et al., 2010). Exopolymeric substances can be divided to: (i) soluble EPS in the extracellular environment, not covalently linked to the cell surface and (ii) bound EPS tightly linked via a covalent or non-covalent association to the cell wall (Wingender et al., 1999). Usually the soluble and bound EPS are separated by centrifugation and different procedures have been developed to extract bound EPS based on physical (e.g. centrifugation, ultrasonication, blending and heat), chemical treatments (e.g. extraction with ethylenediamine tetraacetic acid (EDTA), NaOH, NaCl or formaldehyde) or their combination (Donot et al., 2012; Pal and Paul, 2008; Sheng et al., 2010). The available methods were evaluated and compared with respect to their extraction efficiency, the chemical composition and fluorescence properties of the extracts (Comte et al., 2006a; Domínguez et al., 2010a; Donot et al., 2012; Ni et al., 2009; Sheng et al., 2010). It was shown that different extraction procedures influence the quantity and the composition of the extracted EPS (Comte et al., 2006a; Domínguez et al., 2010a; Donot et al., 2012; Ni et al., 2009; Sheng et al., 2010), the quantity and the composition of the mineral fraction present in the EPS extracts (Bourven et al., 2011), as well as EPS binding properties to protons and different metals (Comte et al., 2006b; d'Abzac et al., 2010; Kenney and Fein, 2011). EPS are also broadly distributed in size and molar mass, thus the extraction procedure could be expected to affect their molar mass and size distribution and thus their environmental reactivity. However, very few and often contradictory studies are available about the physicochemical characterization of EPS, their molar mass and size distributions and the effects of extraction treatments. Several studies employed high pressure size exclusion chromatography (HP-SEC) to elucidate the influence of the EPS extraction on their HP-SEC fingerprints and

Abbreviations: EPS, extracellular polymeric substances; WT1, soluble EPS isolated from wild type *Sinorhizobium meliloti*; WT2, EPS of wild type bacteria extracted by EDTA; WT3, EPS of wild type bacteria extracted by formaldehyde/NaOH; EXOy1, soluble EPS isolated from exoY mutant *S. meliloti*; EXOy2, EPS of exoY mutant extracted by EDTA; EXOy3, EPS of exoY mutant extracted by formaldehyde/NaOH; FT-IR, fourier transform-infrared spectroscopy; EEM, excitation emission matrix; AFlFFF-UV-DRI-MALS, asymmetrical flow field-flow fractionation hyphenated with UV, differential refractive index and multiangle laser light scattering detection; HP-SEC, high pressure size exclusion chromatography; FPLC, fast protein liquid chromatography; TN, total nitrogen; TOC, total organic carbon.

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