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Contrasting effects of extracellular polymeric substances on the surface characteristics of bacterial pathogens and cell attachment to soil particles

Wenqiang Zhao [a,c], Sharon L. Walker [b], Qiaoyun Huang [a], Peng Cai [a,*]

[a] State Key Laboratory of Agricultural Microbiology, College of Resources and Environment, Huazhong Agricultural University, Wuhan 430070, China
[b] Department of Chemical and Environmental Engineering, University of California, Riverside, CA 92521, USA
[c] Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization & Ecological Restoration Biodiversity Conservation Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China

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ABSTRACT

Extracellular polymeric substances (EPSs) have been confirmed to affect bacterial surface properties and cell attachment to minerals. However, no systematic work has been done to clarify the contrasting roles of EPS in cell attachment to natural soil between different pathogenic strains. This study compared the different surface properties and attachment behaviors of two bacterial pathogens (with full or partial EPS) using attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy, potentiometric titration, zeta potential, hydrophobicity analysis, DLVO theory, and attachment tests. Cation exchange resin (CER) was employed to remove the EPS on *Streptococcus suis* and *Escherichia coli* such that the contribution of EPS to cell attachment to soil could be determined. ATR-FTIR confirmed the binding sites differed between *S. suis* and *E. coli* EPS. Notably, after partial EPS removal the absorption bands of *S. suis* between 1800 cm^{-1} and 800 cm^{-1} shifted or disappeared, whereas the lack of EPS did not affect the infrared absorption peaks for *E. coli*. This result suggests the overall surface site types within the *E. coli* EPS were similar to the residual EPS fractions or cell wall. The partial removal of EPS also changed the proton-active site concentrations of both cell types, and reduced the bacterial surface charge densities by 7%–17%. The negative charges on bacterial surfaces followed the order of full EPS-*S. suis* < partial EPS-*S. suis* < partial EPS-*E. coli* < full EPS-*E. coli* (ionic strength 1–100 mM; pH 5.6–5.8). With the removal of EPS, the average hydrophobicities of *S. suis* increased by 5% while those of *E. coli* decreased by 11%. EPS removal inhibited the attachment of *S. suis* to soil particles (<2 mm) but enhanced *E. coli* attachment across the IS range of 1–100 mM, which was attributed to the alteration in electrostatic repulsion. At IS 60–100 mM, a sudden reduction in the attachment was observed only for full EPS-*S. suis*, which could be ascribed to the steric hindrance derived from EPS. However, full EPS-*E. coli* and partial EPS-*E. coli* showed similar increasing attachment trends at IS 1–100 mM. This study clearly showed the distinct contribution of EPS to pathogen attachment to soil as a function of cell type and EPS present.

1. Introduction

Livestock manure and wastes from animal feeding operations are increasingly applied to lands as agricultural fertilizers for silage, grazing, or crop production (Guber et al., 2007). These biosolids may serve as a source of pathogens that contaminate soil, fresh products, surface/ground water and water supply systems (Venglovsky et al., 2009). Pathogens can survive for extended periods after they are spread to agricultural land with manure (Nyberg et al., 2010; Toth et al., 2013). The retention of pathogens in upper soil layers could influence their metabolic activities (Rong et al., 2007), survival time (Franz et al., 2008), as well as transport process in surface runoff or saturated soil (Keller and Auset, 2007). A wide variety of bacterial pathogen groups have been found in animal feces, such as fecal coliforms, streptococci, *Salmonella* spp., *Campylobacter* spp. and *Listeria monocytogenes* (Unc and Goss, 2004). Over the past decades, the emergence of serious disease outbreaks has been associated with consumption of fecal contaminated food or water, and in many cases animal manures that released into soil environments were identified as the likely source of these outbreaks (Gerba and Smith, 2005; Abit et al., 2012). Therefore, an understanding of the fate of bacterial pathogens in soils – notably the degree to which they attach to the soil – is needed to assess their availability and potential risk to public health.

A number of physical and chemical factors have been proposed to govern bacteria–solid surface interactions (Stevik et al., 2004), including particle size (Soupir et al., 2010), surface coating (Li and Logan, 2004), charge property (Jacobs et al., 2007), hydrophobic effect (Shephard et al., 2010), and electrolyte composition (Hong and Brown, 2008). Additionally, biological factors such as bacterial cell

* Corresponding author.
E-mail address: cp@mail.hzau.edu.cn (P. Cai).

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type (Foppen et al., 2010), extracellular polymeric substances (EPSs) (Kim et al., 2009b), and lipopolysaccharides (Walker et al., 2004) have also been shown to have considerable influence on bacterial attachment. Among the various components of bacterial cell surfaces, EPS is the major heterogeneous component composed dominantly of polysaccharides and proteins, with nucleic acids and lipids as minor constituents (Eboigbodin and Biggs, 2008; Long et al., 2009; Cao et al., 2011). EPS contains various acidic functionalities (carboxyl, phosphoryl, amide, amino, hydroxyl) that ionize in response to changes in solution chemistry, and is of particular importance which affects cell surface characteristics and attachment to solid substrates (Gong et al., 2009; Karunakaran and Biggs, 2011; Mukherjee et al., 2012). Interactions between bacteria and solid surfaces are typically considered as an abiotic physicochemical process that can be approximated by the balance of electrostatic, van der Waals and hydrophobic forces (Hermansson, 1999). It has been shown that bacteria–mineral interactions were well predicted by the classic Derjaguin–Landau–Verwey–Overbeek (DLVO) theory (Hori and Matsumoto, 2010; Zhao et al., 2014). In some cases, the DLVO theory is not always valid to predict cell-surface interactions due to the presence of complex polymer layers extending into the liquid medium (Tsuneda et al., 2003; Kim et al., 2009a). Depending on solution ionic strength, the presence of EPS may alter the extent of microbial attachment due to steric interactions which are not incorporated in DLVO theory (Chen and Walker, 2007; Liu et al., 2010).

The influence of EPS on cell interactions with solid surfaces has been drawing increasing attention (Tong et al., 2010). Several studies have investigated the attachment of cells with and without EPS coating to well-characterized minerals under controlled conditions via batch attachment tests, parallel plate flow chamber, radial stagnation point flow (RSPF) system, and packed-bed columns. For instance, by comparing the attachment efficiencies of untreated and proteinase K treated *Escherichia coli* O157:H7 on quartz sand in a batch system, Kim et al. (2009b) reported greater cell attachment occurred for cells with EPS-partially removed when IS ≥ 10 mM, suggesting the presence of EPS hindered cell attachment. Hong et al. (2013) found that via the treatment of cation exchange resin (CER), the reduction in EPS had no apparent effects on *Bacillus subtilis* adhesion on montmorillonite, kaolinite and goethite when the wet bacteria/mineral mass ratio was less than 0.4. However, at higher cell concentrations, the removal of EPS reduced bacterial adhesion to kaolinite and montmorillonite, and enhanced its adhesion to goethite. Taylor et al. (2014) examined the deposition of *E. coli* O157:H7 (full or partial coating EPS) on bare and hematite coated quartz in a parallel plate chamber. Their results indicated the ability of EPS to facilitate interactions between cells and surfaces. Kuznar and Elimelech (2006) investigated the attachment kinetics of untreated and digestive enzyme-treated *Cryptosporidium parvum* oocyst to quartz surfaces using a RSPF system. It showed that the removal of surface macromolecules increased attachment efficiencies. Gargiulo et al. (2007) observed that the proteolitic enzymatic treatment of *Rhodococcus rhodochrous* significantly decreased the amount of attached cells in the silica sand column, while straining behavior for treated and untreated bacteria was quite similar.

Despite the efforts discussed above, it should be noted that the literature mainly focused on homogeneous mineral surfaces; whereas the contribution of EPS in bacterial attachment to more heterogeneous natural soil particle has not been investigated. Furthermore, the majority of studies focused on a single microbe. Overall, very little work has been conducted to comprehensively interpret the EPS-induced dissimilar attachment phenomena that may exist in different pathogens. Hence, this study was developed to address this gap in knowledge.

The objective of this paper is to explore the role of EPS in surface characteristics of different pathogens and their subsequent attachment to natural, heterogeneous soil. Two model bacterial pathogens – *Streptococcus suis* (Gram-positive) and *E. coli* (Gram-negative) – were selected based on their differing outer membrane properties. The CER extraction method was employed to remove EPS associated with the outer surfaces of cells such that the EPS-extracted cells could be compared with those with full EPS. Surface properties of full EPS- and partial EPS-bacteria were extensively characterized via attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy, potentiometric titration, zeta potential, and hydrophobicity analysis. In addition to attachment tests of full EPS/partial EPS-pathogens, DLVO theory was applied to interpret results and evaluate the contribution of EPS to cell attachment across the ionic strength (IS) range tested (1–100 mM).

2. Materials and methods

2.1. Bacterial pathogens and soil particles

The two model organisms used in this study were Gram-positive pathogenic bacteria *S. suis* SC05 and Gram-negative *E. coli* WH09, both obtained from the State Key Laboratory of Agricultural Microbiology. These cell types were isolated from soils around a pig farm in Wuhan, Hubei Province, China (Zhao et al., 2014). The bacteria were grown and harvested according to the protocols described in the Supplementary data. After harvest, the cells were washed two additional times with sterilized distilled-deionized water (ddH_2O) to remove all traces of the growth medium (Kim et al., 2010). Washed cells were subsequently re-suspended in ddH_2O at a concentration of 6.0×10^9 cells per mL and then divided into two portions. One portion of cell suspension was used as untreated bacteria (full EPS). The other portion of cell suspension was used to prepare partial EPS-bacteria via the employment of cation exchange resin (CER) technique (Long et al., 2009; Tong et al., 2010). The detailed CER treatment procedure is presented in the Supplementary data. This method has been reported as the most effective way to remove EPS from cell surfaces and most of the cells were intact (Aguilera et al., 2008; Tourney et al., 2008). Preliminary experiments were also conducted to test the viability of cells. Bacterial suspension ($\sim 10^8$ cells mL^{-1}) was stained with a dye solution consisting of 40 μL Live/Dead BacLight stain (L-7012, Molecular Probes, Eugene, OR) in 2 mL of 1–100 mM KNO_3 for 15 min. Most of the cells (94.8%–98.0%) were confirmed to be viable (green color) by using fluorescence microscopy (IX-70, Olympus).

A Yellow-Brown soil was collected from the top 20 cm of farmland in Wuhan, Hubei Province, China. After removing the organic residues and stones, the soil was air-dried, sieved to pass through 2-mm sieves, and autoclaved at 121 °C and 0.105 MPa for 30 min (repeated for 3 times) (Guber et al., 2005). No microorganisms were detected in the sterilized soil through plate counting procedures on tryptone soy agar (TSA). The soil particles were then oven-dried at 60 °C prior to the attachment experiments.

2.2. ATR-FTIR spectroscopy

To compare the functional groups on full EPS- and partial EPS-cell surfaces and ensure the effectiveness of the EPS removal, infrared spectra of bacteria were determined by ATR-FTIR spectroscopy. This technique permits in situ investigation of functional groups on bacterial surfaces in water (Parikh and Chorover, 2006; Ojeda et al., 2008). The washed bacterial suspensions in ddH_2O were centrifuged at 8000 ×*g* for 10 min, and the cell pellets were spread on the ZnSe internal reflecting element (IRE) crystal to obtain their spectra. The ATR-FTIR spectra for the full EPS/partial EPS-bacteria were collected over the scan range of 3500 cm^{-1} to 800 cm^{-1} (Vertex 70, Bruker Optics, Germany) (Parikh and Chorover, 2006). Each sample was scanned 256 times with a resolution of 4 cm^{-1}, and the obtained spectra were baseline corrected. The FTIR spectra of both full EPS- and partial EPS-bacteria were normalized to the height of the peak at 1539 cm^{-1} for *S. suis* and 1545 cm^{-1} for *E. coli* (amide II), respectively. Following the same protocol, the supernatants of bacterial suspensions were also scanned as the background spectra (Ueshima et al., 2008).