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

Wenqiang Zhao\textsuperscript{a,c}, Sharon L. Walker\textsuperscript{b}, Qiaoyun Huang\textsuperscript{a}, Peng Cai\textsuperscript{a,\textsuperscript{⁎}}

\textsuperscript{a} State Key Laboratory of Agricultural Microbiology, College of Resources and Environment, Huazhong Agricultural University, Wuhan 430070, China
\textsuperscript{b} Department of Chemical and Environmental Engineering, University of California, Riverside, CA 92521, USA
\textsuperscript{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

A R T I C L E  I N F O

Article history:
Received 27 December 2014
Received in revised form 10 June 2015
Accepted 11 June 2015
Available online 14 June 2015

Editor: J. Fein

Keywords:
Bacterial pathogen
EPS
Attachment
Soil
Zeta potential
ATR-FTIR

A B S T R A C T

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\textsuperscript{−1} and 800 cm\textsuperscript{−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.

© 2015 Elsevier B.V. All rights reserved.

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

http://dx.doi.org/10.1016/j.chemgeo.2015.06.013
0009-2541/© 2015 Elsevier B.V. All rights reserved.
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, amine, 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-removed cell attachment. Hong et al. (2013) found that via the treatment with cation exchange resin (CER), the reduction in EPS had no apparent effect on bacterial attachment due to steric interactions which are not incorporated in DLVO theory (Chen and Walker, 2007; Liu et al., 2010).

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 (ddH2O) to remove all traces of the growth medium (Kim et al., 2010). Washed cells were subsequently resuspended in ddH2O at a concentration of 6.0 × 10⁹ 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 (10⁻⁶ 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 KNO₃ 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 ddH2O 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).
2.3. Potentiometric titration

Potentiometric titration was performed by an automatic potentiometric titrator (Metrohm titrator 836, Metrohm, Switzerland) to analyze the acid-base properties of full EPS- and partial EPS-pathogen surfaces (Fein et al., 2005; Ueshima et al., 2008; Kenny and Fein, 2011). Bacterial suspensions with concentrations of 6.0 × 10^9 cells per mL in 0.01 M KCl were titrated using 0.09 M HCl and 0.09 M KOH solutions under a N₂ atmosphere at 25 °C. Each suspension was continuously stirred with a small magnetic stir bar during the titration. A known amount of HCl was added at the beginning of the experiment to lower the pH to 2.5. The cell suspension was equilibrated for 40 min and titrated to pH 10.0 with KOH (Fang et al., 2010). The surface charge density was calculated from the amount of base consumed by suspended cells during the titration between pH 2.5 and pH 10.0, accounting for the cell surface area (Chen and Walker, 2007). At each titration step, a pH electrode stability of 0.1 mV s⁻¹ was attained before the addition of the next aliquot of titrant. Blank titration in the same electrolyte solution (0.01 M KCl) was performed and each experiment was conducted in triplicate. The bacterial surface site types were acidic and discrete. The deprotonation process can be generally expressed by the following reaction (Borrok and Fein, 2004):

\[ R-\text{AH}^+ \rightarrow R-\text{A}^- + H^+ \]  

(1)

where R is the bacterium to which the site type A is attached. The equilibrium constant \( K_a \) for reaction (1) can be given by:

\[ K_a = \frac{[R-A^-] \cdot \alpha_H}{[R-\text{AH}^+]} \]  

(2)

where \([R-A^-]\) and \([R-\text{AH}^+]\) stand for the concentrations of deprotonated and protonated sites, respectively, and \( \alpha_H \) represents the activity of protons in the bulk solution. Titration data were modeled using the software PrototFit to obtain dissociation constants (p\( K_a \)) and their corresponding site concentrations for proton-active chemical moieties on bacterial surfaces (Turner and Fein, 2006).

2.4. Zeta potential and hydrophobicity of bacteria and soil

Zeta potential and hydrophobicity of bacteria and soil across a range of IS (1–100 mM KCl) were measured and calculated according to the protocols described in the Supplementary data. The hydrodynamic diameters of bacteria were determined by dynamic light scattering in a Zetasizer (Nano ZS90, Malvern Instruments Ltd., UK). The specific surface areas (SSA) of bacteria were measured via methylene blue adsorption method (He and Tebo, 1998). Total EPS, sugar, and protein contents were assayed by ethanol precipitation, phenol-sulfuric acid (Biosynthesis Co., Ltd., Beijing) method, respectively (Gee and Bauder, 1986; Evangelou and Blevins, 1988). Zetasizer (Nano ZS90, Malvern Instruments Ltd., UK) was used to determine the surface charge density. The peak at 1740 cm⁻¹, for the stretching of C=O stretching associated with lipids and fatty acids; 1645, 1539, and 1460 cm⁻¹, for the C=O stretching of amide I, N−H bending/C−N stretching of amide II, and −CH₂/−CH₃ bending of proteins, respectively (Chen and Walker, 2007; Cao et al., 2011). The above cell properties are listed in Table S1.

Basic soil properties, including soil pH (5.8), texture (clay 14.2%; silt 64.1%; sand 21.7%), SSA (26.8 m² g⁻¹), organic matter content (14.7 g kg⁻¹) and cation exchange capacity (10.0 cmol kg⁻¹), were obtained by a pH meter (FE20, Mettler Toledo, Switzerland), pipette method, nitrogen adsorption measurement (Autosorb-1, Quantachrome Instruments, USA), K₂Cr₂O₇ digestion and NH₄Cl-H₂O₂ displacement, respectively (Gee and Bauder, 1986; Evangelou and Blevins, 1988).

2.5. Bacterial attachment to soil particles

Bacterial suspensions (contained 1.05 × 10¹⁰ cells) were mixed with 200 mg of soil particles in 20-mL centrifuge tubes. The bacteria-to-soil surface area ratios were 1:1.5 for S. suis and 1:3.8 for E. coli, respectively. KCl solution and ddH₂O were added to reach a final volume of 15 mL (1, 5, 10, 20, 40, 60, 80 and 100 mM KCl). Cell and soil mixtures were stirred on a rotary shaker for 1 h at 150 rev min⁻¹ and 8 °C to minimize microbial growth and maintain bacterial viability (Gantzer et al., 2001; Guber et al., 2007). Initial experiments showed that 1 h was sufficient for the cell–soil interaction to reach a plateau. The mixtures were centrifuged at 100 × g for 15 min, and the soil particles with any attached bacteria settled to the bottom of the tubes (Guber et al., 2007; Pachepsky et al., 2008). The calibration curves and the amount of un-attached cells in the supernatant were measured by UV–visible spectrophotometer (UV1102, Shanghai Tianmei Scientific Instrument Co., China) at a wavelength of 600 nm. Control tubes with only bacterial suspensions (or only soil suspensions) were processed in the same manner as the experimental tubes to correct for bacterial attachment to tube walls (or optical density of the soil supernatant) (Dai and Boll, 2003). The amount of attached bacteria was calculated from the difference between the amount applied and that recovered in the supernatant (Guber et al., 2005, 2007).

2.6. Application of DLVO theory

DLVO theory was applied to calculate the interaction energy profiles existing between the pathogen and soil particles. Total interaction energies were quantified as the sum of van der Waals and electrostatic interactions, which were calculated by considering the system as a sphere-plate model (Haznedaroglu et al., 2009; Kim et al., 2010; Cai et al., 2013). Calculation details are given in the Supplementary data.

2.7. Statistical analysis

All the potentiometric titration, zeta potential, hydrophobicity and attachment experiments were performed in triplicate. The data are presented as the mean ± standard deviation of the mean (x ± SDM). Statistical differences between mean values were analyzed using a Student’s t test. Linear regression analysis (Origin 8.0, OriginLab Co., Northampton, USA) were applied to evaluate the statistical correlations among bacterial surface property, DLVO energy barrier and attachment data. P values greater than or equal to 0.05 suggest differences are statistically insignificant within a 95% confidence interval.

3. Results and discussion

Fig. 1a displays the infrared spectra of full EPS- and partial EPS-S. suis from 1800 cm⁻¹ to 800 cm⁻¹. Based on previously reported spectral features for bacteria (Jiang et al., 2004; Yee et al., 2004; Parikh and Chorover, 2006; Ojeda et al., 2008; Ueshima et al., 2008; Gao and Chorover, 2009), the absorption bands of S. suis are summarized in Table S2. The peak at 1740 cm⁻¹ corresponds to C=O stretching associated with lipids and fatty acids; 1645, 1539, and 1460 cm⁻¹, for the C=O stretching of amide I, N−H bending/C−N stretching of amide II, and −CH₂/−CH₃ bending of proteins, respectively; 1397 cm⁻¹, for the symmetric stretching C=O; 1234 cm⁻¹, for the stretching of PO₂⁻ in phosphodiesters and phosphates, or vibrations of −COOH/C−O−C in esters; 1067 cm⁻¹, for the C−OH/C−O−C/C−C vibrations in polysaccharides, or P=O stretching of phosphodiester moieties; 887/843 cm⁻¹, for the C−O−P/P−O−P ring vibrations in polysaccharides, or O−P−O vibration. The infrared bands of full EPS-S. suis supported previous work that bacterial surface macromolecules were composed of a combination of protein, lipid, and carbohydrate (Eboigbodin and Biggs, 2008). After the partial removal of the EPS, some absorption peaks (1740 and 843 cm⁻¹) disappeared. The band at 1645 cm⁻¹ shifted to 1640 cm⁻¹. The disappearance and shift of some bands implied that some EPS constituents were removed, or the residual EPS molecules exposed after the cleaving of EPS. Ha et al. (2010) also reported that the intensities of peaks were significantly lower in the spectrum of Shewanella oneidensis without EPS.
3.2. Potentiometric titration

The titration curves of the model pathogens before and after CER treatment are presented in Fig. 2. The amount of OH⁻ consumed during the titration from pH 2.5 to pH 10.0 reflects the bacterial buffering capacity originated from the protons dissociated from surface acidic chemical moieties. The inflection points on the titration curves indicate the deprotonation of these different site types (Ams et al., 2004; Fang et al., 2010). Table S4 shows that the overall OH⁻ amount consumed by E. coli was larger than S. suis regardless of the EPS presence. The buffering capacities of full EPS-E. coli were higher as compared to those of partial EPS-E. coli, which suggested that the extraction of EPS removed some of the macromolecules and their dissociable chemical moieties. Dissimilarly, no obvious difference was found between the buffering capacities of full EPS-S. suis and partial EPS-S. suis. The relative order of surface charge densities as calculated from the titration curves (Chen and Walker, 2007) was full EPS-E. coli (541.1 μC cm⁻²) > partial EPS-E. coli (447.5 μC cm⁻²) > full EPS-S. suis (213.0 μC cm⁻²) > partial EPS-S. suis (198.9 μC cm⁻²). After EPS removal, the surface charge density on E. coli surface decreased by 17%, which was greater than that on S. suis (7%).

The potentiometric titration data were further evaluated by fitting by the non-electrostatic surface complexation model to quantify site concentrations and acid dissociation constants (pKₐ) for bacteria (Turner and Fein, 2006). The four-site model yielded the best fit to the titration curves (R² > 0.99), which implied the presence of four types of chemical moieties on the cell surfaces. As shown in Table 1, the pKₐ values of the four site types were 11.75, 9.06, 7.40, and 4.72 for E. coli and 12.22, 9.08, 7.61, and 4.21 for S. suis. The pKₐ values of the four site types were higher as compared to those of full EPS-S. suis and partial EPS-S. suis, which suggested that the extraction of EPS removed some of the macromolecules and their dissociable chemical moieties. The pKₐ values of the four site types were higher as compared to those of full EPS-S. suis and partial EPS-S. suis, which suggested that the extraction of EPS removed some of the macromolecules and their dissociable chemical moieties.
values for full EPS- and partial EPS-cells were determined to be 1.6–3.3 (pK_{A1}, 4.9–5.6 (pK_{A2}, 6.4–7.3 (pK_{A3}) and 9.4–10.1 (pK_{A4}). According to the typical deprotonation constants for short-chain organic sites (Fein et al., 1997), the proton-active sites on bacterial surfaces likely corresponded to the phosphodiester (pK_{A} < 4), carboxylic (4 < pK_{A} < 6), phosphoric (pK_{A} ≈ 7), and hydroxyl/amine (9 < pK_{A} < 11) moieties. ATR-FTIR data showed PO_2/OC=O–P–O stretching and N–H bending (Tables S2 and S3), further confirming the presence of these chemical moieties on bacteria. Previous studies also found four-site/three-site models with pK_{A} values of 3.2–4.2 (pK_{A1}, 4.2–5.9 (pK_{A2}, 6.4–7.7 (pK_{A3}) and 9.0–10.6 (pK_{A4}) for E. coli, S. oneidensis, P. putida, Rhizobium tropici, and Agrobacterium sp. with or without EPS (Castro and Tufenkji, 2007; Ueshima et al., 2008; Ha et al., 2010; Kenny and Fein, 2011). The deprotonation constants (pK_{A2}/pK_{A3}/pK_{A4}) in this study compared well to the above values in the literatures. However, the pK_{A}1 values (1.6–3.3) of E. coli and S. suis were obviously lower than those reported on other bacteria (3.2–4.2). It indicated that the bacterial strains used in this work possessed smaller proton binding capacities of phosphodiester moieties. Furthermore, the pK_{A} values significantly decreased or increased (P < 0.05) after EPS removal (e.g., pK_{A3} for S. suis and pK_{A4} for E. coli), suggesting that the presence of EPS molecules could alter the protonation behaviors of the biomass. The total site concentrations on full EPS-S. suis and full EPS-E. coli surfaces were 4.96 × 10^{-16} and 6.93 × 10^{-16} mol cell^{-1}, respectively; while those of partial EPS-S. suis (4.81 × 10^{-16} mol cell^{-1}) and partial EPS-E. coli (6.44 × 10^{-16} mol cell^{-1}) were very close to the full EPS-bacteria. No significant differences were found between the total site concentrations on full EPS-cell and on partial EPS-cell surfaces (P > 0.05). The two strains used in this study had more surface site concentrations than another pathogenic E. coli O157:H7 (2.74 × 10^{-16} mol cell^{-1}) reported by Kim et al. (2009b).

For each surface site, the decreasing trend for site concentrations was not always observed for the four site types after EPS removal (Table 1). It is interesting to note that the site concentration of phosphodiester moiety for partial EPS-E. coli (1.91 × 10^{-16} mol cell^{-1}) was larger than that for full EPS-S. suis (0.29 × 10^{-16} mol cell^{-1}). Partial EPS-E. coli also had more phosphoric moieties (increased by 0.18 × 10^{-16} mol cell^{-1}) than full EPS-E. coli. This phenomenon revealed that more phosphodiester and phosphoric moieties on the outer cell wall may be exposed with the removal of EPS, although the overall site concentrations decreased. It has been reported that EPS had different effects on cell surface site concentrations. For example, EPS removal increased the concentrations of all four site types on B. subtilis (Hong et al., 2013); whereas this treatment did not significantly alter the site concentrations of carboxyl/phosphoryl or reduced the phosphodiester/amine/hydroxyl moieties on B. licheniformis S-86 (Tourney et al., 2008). Such deviation among various studies likely resulted from the differences in cell strains and growth phase, which lead to the differential expression of EPS molecules on bacterial surfaces (Eboigbodin et al., 2007).

### 3.3. Charge properties of bacteria and soil

Zeta potential is defined as the potential at the shear plane of electrical double layer, which reflects the net surface charge of bacteria or soil particle (Kirby and Hasselbrink, 2004). The zeta potentials determined under varied IS conditions are shown in Fig. 3. Within the IS range tested, the natural soil sample and pathogens were all negatively charged. Full EPS-S. suis was the least negatively charged with its zeta potential increasing slightly from −11.9 mV to −6.5 mV with the increase of IS from 1 mM to 100 mM. The zeta potentials of partial EPS-S. suis (from −33.0 mV to −9.4 mV), full EPS-E. coli (from −37.7 mV to −13.4 mV) and partial EPS-E. coli (from −35.1 mV to −11.9 mV) also became less negative with increasing IS from 1 to 60 mM. This reduction of zeta potential was attributed to the double layer compression and charge screening by counter ions outside the cell surfaces (Walker et al., 2004; Shephard et al., 2010). Above 60 mM, the zeta potentials of partial EPS-S. suis/E. coli and full EPS-S. suis remained constant (P > 0.05), while only the full EPS-E. coli increased slightly by 3.2 mV. This result suggests that the compression of the double layer is effectively achieved around 60 mM (Mills et al., 1994; Zhao et al., 2012). Similar results were also observed in literature that the zeta potentials of E. coli O157:H7 and Salmonella pullorum SA1685 were unchanged (P > 0.05) at high IS conditions (Castro and Tufenkji, 2007; Haznedaroglu et al., 2009). The surface charges of the model soil showed less variation than the bacteria, with the zeta potentials changing only from −21.7 mV to −15.1 mV (1–100 mM).

Over the IS range examined, the relative zeta potentials of the cell types were as follows (Fig. 3): full EPS-E. coli > partial EPS-E. coli > partial EPS-S. suis > full EPS-S. suis. The charges of bacterial cells are related to the nature of macromolecules (e.g., EPS and lipids) in the outer cell wall (Wilson et al., 2001; Hong and Brown, 2006). Ionizable sites (e.g., phosphodiester, carboxylic, phosphoric, and amine/hydroxyl moieties) that make up these macromolecules are responsible for the surface charge exhibited under different IS (Castro and Tufenkji, 2007). Hence, the different site concentrations of cell walls for the four bacteria could explain the observed variability in the zeta potentials. At the tested pH range (5.6–5.8), the deprotonation of phosphodiester (pK_{A} < 4.0) and carboxylic moieties (pK_{A} < 5.6) primarily contributed to the overall net negative charges of the cells (Kim et al., 2009b). Titration results in Table 1 indicated that the sum of phosphodiester and carboxylic site

| Pathogen type          | pK_{A} | Site concentration (10^{-16} mol cell^{-1}) | Total site concentration (10^{-16} mol cell^{-1}) |
|------------------------|--------|---------------------------------------------|--------------------------------------------------|
| Full EPS-S. suis       | 1.64 ± 0.17 | 0.29 ± 0.12 | 4.96 ± 0.13 |
|                        | 5.55 ± 0.16 | 2.11 ± 0.14 |                                  |
|                        | 7.33 ± 0.34 | 0.89 ± 0.07 |                                  |
|                        | 9.56 ± 0.17 | 1.67 ± 0.06 |                                  |
| Partial EPS-S. suis    | 2.28 ± 0.16 | 1.91 ± 0.33 | 4.81 ± 0.69 |
|                        | 5.08 ± 0.25 | 1.50 ± 0.21 |                                  |
|                        | 6.38 ± 0.17 | 0.48 ± 0.10 |                                  |
|                        | 9.39 ± 0.45 | 0.92 ± 0.25 |                                  |
| Full EPS-E. coli       | 3.21 ± 0.30 | 1.27 ± 0.33 | 6.93 ± 0.89 |
|                        | 5.26 ± 0.53 | 0.98 ± 0.09 |                                  |
|                        | 6.82 ± 0.15 | 1.45 ± 0.51 |                                  |
|                        | 9.46 ± 0.07 | 3.09 ± 0.39 |                                  |
| Partial EPS-E. coli    | 2.44 ± 0.30 | 0.92 ± 0.16 | 6.44 ± 0.55 |
|                        | 4.88 ± 0.41 | 0.78 ± 0.20 |                                  |
|                        | 7.13 ± 0.38 | 1.67 ± 0.15 |                                  |
|                        | 10.09 ± 0.50 | 3.07 ± 0.37 |                                  |
concentrations for partial EPS-S. suis increased by 1.01 × 10^{-16} mol cell^{-1} compared with full EPS-S. suis, resulting in more negative charges on partial EPS-S. suis. In addition, the negative surface charges of partial EPS-S. suis (i.e., absolute zeta potential values) declined rapidly by 71.5% from 1 mM to 100 mM, while the surface charge trend of full EPS-S. suis was less sensitive to IS and decreased only by 45.4%. It suggests that EPS components on S. suis are able to reduce the effect of solution IS on the variation of S. suis surface charges. In contrast, the observed reduction in the total concentrations of these two chemical moieties (decreased from 2.35 × 10^{-15} mol cell^{-1} to 1.70 × 10^{-16} mol cell^{-1}) on E. coli after CER treatment may explain the reduced amounts of surface negative charges. Furthermore, both E. coli types exhibited the similar trends in zeta potential, which suggests that EPS presence did not change the general trends of E. coli surface charges at various IS. It is interesting to mention here that EPS removal also did not affect E. coli surface site types and the band positions from 1800 cm^{-1} that EPS removal also did not affect of E. coli. Furthermore, both treatment may explain the reduced amounts of surface negative charges.

Notably, the overall hydrophobicity values of the four cell types fall in the following order: partial EPS-S. suis ≥ full EPS-S. suis ≥ full EPS-E. coli ≥ partial EPS-E. coli. The only exception was full EPS-E. coli at 100 mM, which may be due to the conformational changes of hydrophobic moieties on EPS molecules at high IS condition (Shephard et al., 2010). The hydrophobicities of S. suis were higher than E. coli, indicating more nonpolar sites on S. suis surface regardless of EPS presence. Probably, a greater amount of acidic and polar sites are exposed on the surface of E. coli (Table 1, 6.4/6.93 × 10^{-16} mol cell^{-1}), resulting in a lower percentage of cells partitioning into the hydrocarbon (Walker et al., 2005b).

A greater hydrophobicity value was found for partial EPS-S. suis (30.6%–42.5%) than full EPS-S. suis (21.4%–34.0%). This difference may be ascribed to the removal of large amount of proteins (28.70 µg/10^8 cells) relative to sugar content (12.78 µg/10^8 cells) in S. suis EPS (Table S1). The outer proteins of bacteria that existed in EPS are mostly hydrophilic, which decreases cell surface hydrophobicity (Nikaido, 2003; Walker et al., 2005b). Further evidence of the sensitivity of bacterial hydrophobicity to proteins in extracellular polymers can be found in previous studies (Bruinsma et al., 2001; Chavant et al., 2002). The FTIR spectra in Fig. 1a also confirmed that the absorption peak for protein at 1645 cm^{-1} (amide I) shifted after treatment with CER. Interestingly, the average hydrophobicity values of partial EPS-E. coli decreased by 11% as compared with those of full EPS-E. coli at IS 1–100 mM. E. coli EPS had higher sugar content (11.73 µg/10^8 cells) than protein (4.81 µg/10^8 cells). Thus, the major parts of the molecular composition removed by CER were likely to be the uncharged hydrocarbon compounds C—(CH) in polysaccharides, which contained a relatively larger number of nonpolar sites than proteins (Kim et al., 2009b). It has been reported that the C—(CH) constituents could contribute to the increase in hydrophobicity (Hamadi et al., 2008). Therefore, a reduction of E. coli hydrophobicity after CER treatment can be attributed to the removal of C—(CH) constituents (polysaccharides) in EPS.

3.4. Surface hydrophobicity of bacteria and soil

Hydrophobicity values were quantified based on the percentage of bacterial or soil partitioning in a hydrocarbon phase (n-dodecane) and the results are presented in Fig. 4. Across the IS range tested, the hydrophobicities of S. suis and E. coli ranged from 21.4% to 42.5% and from 3.4% to 32.4%, respectively. These hydrophobicity values were comparable to those of other bacterial strains (S. pullorum SAI185 – 26.5%; E. coli O2:H7 – 2.8%; E. coli O157:H7 – 25.8%; E. coli O157:H16 – 33.1%) previously reported (Li and McLandsborough, 1999; Haznedaroglu et al., 2009; Kim et al., 2010). In the presence of 1–10 mM KCl, the hydrophobicities of both cell types with full EPS were approximately 30%, whereas the values gradually decreased to 21.4% and 8.9% at 100 mM for E. coli and S. suis, respectively.

A decreasing trend for E. coli O157:H7 hydrophobicity as IS increased (26.7%–16.4% across range of IS from 1 mM to 100 mM) was also reported in the literature (Haznedaroglu et al., 2009). Bacterial surface polymers (e.g., EPS) are compressed at high IS (Abu-Lail and Camesano, 2003), and probably led to the decrease in the exposure of hydrophobic sites outside the cell surface (Alizadeh-Pasdar and Li-Chan, 2000). For cells with partial EPS, their hydrophobicities remained variable and were around 35% for S. suis and 10% for E. coli, respectively, exhibiting irregular trends. No significant differences (P > 0.05) in hydrophobicity were observed for soil particle (70.0% ± 1.6%) over the entire IS range.

Notably, the overall hydrophobicity values of the four cell types fall in the following order: partial EPS-S. suis ≥ full EPS-S. suis ≥ full EPS-E. coli ≥ partial EPS-E. coli. The only exception was full EPS-E. coli at 100 mM, which may be due to the conformational changes of hydrophobic moieties on EPS molecules at high IS condition (Shephard et al., 2010). The hydrophobicities of S. suis were higher than E. coli, indicating more nonpolar sites on S. suis surface regardless of EPS presence. Probably, a greater amount of acidic and polar sites are exposed on the surface of E. coli (Table 1, 6.4/6.93 × 10^{-16} mol cell^{-1}), resulting in a lower percentage of cells partitioning into the hydrocarbon (Walker et al., 2005b).

A greater hydrophobicity value was found for partial EPS-S. suis (30.6%–42.5%) than full EPS-S. suis (21.4%–34.0%). This difference may be ascribed to the removal of large amount of proteins (28.70 µg/10^8 cells) relative to sugar content (12.78 µg/10^8 cells) in S. suis EPS (Table S1). The outer proteins of bacteria that existed in EPS are mostly hydrophilic, which decreases cell surface hydrophobicity (Nikaido, 2003; Walker et al., 2005b). Further evidence of the sensitivity of bacterial hydrophobicity to proteins in extracellular polymers can be found in previous studies (Bruinsma et al., 2001; Chavant et al., 2002). The FTIR spectra in Fig. 1a also confirmed that the absorption peak for protein at 1645 cm^{-1} (amide I) shifted after treatment with CER. Interestingly, the average hydrophobicity values of partial EPS-E. coli decreased by 11% as compared with those of full EPS-E. coli at IS 1–100 mM. E. coli EPS had higher sugar content (11.73 µg/10^8 cells) than protein (4.81 µg/10^8 cells). Thus, the major parts of the molecular composition removed by CER were likely to be the uncharged hydrocarbon compounds C—(CH) in polysaccharides, which contained a relatively larger number of nonpolar sites than proteins (Kim et al., 2009b). It has been reported that the C—(CH) constituents could contribute to the increase in hydrophobicity (Hamadi et al., 2008). Therefore, a reduction of E. coli hydrophobicity after CER treatment can be attributed to the removal of C—(CH) constituents (polysaccharides) in EPS.

3.5. Influence of ionic strength on cell attachment

Fig. 5 shows the attachment of bacteria to soil particles as a function of IS. The cell attachment percentages followed the sequence of full EPS-S. suis (51.8%–95.6%) > partial EPS-S. suis (21.5%–77.6%) > partial EPS-E. coli (19.9%–55.6%) > full EPS-E. coli (10.4%–37.6%). These obtained different attachment trends suggested that the pathogen type and EPS

**Fig. 4.** Hydrophobicities (%) of full EPS, partial EPS-pathogens and Yellow-Brown soil as a function of IS (1–100 mM). The hydrophobicity was quantified as the percentage of cells or soil particles partitioned into hydrocarbon (n-dodecane) phase. Error bars are the standard deviation of three replicates.

**Fig. 5.** Effect of IS on the attachment percentages of pathogens to Yellow-Brown soil (unadjusted pH 5.6–5.8, IS 1–100 mM). Error bars are the standard deviation of three replicates.
content had great influences on subsequent cell attachment to soil at varied IS.

Under the experimental conditions of pH 5.6–5.8, the pathogens and soil particle were all negatively charged (Fig. 3), resulting in the electrostatic repulsive forces existing between cells and soil (Haznedarglu et al., 2008). Full EPS-S. suis was the least negatively charged (from −11.9 mV to −6.5 mV), which yielded the weakest repulsive forces between full EPS-S. suis and soil, and hence the greatest attachment to soil was observed. Similarly, the greatest absolute zeta potentials of full EPS-E. coli (from −37.7 mV to −13.4 mV) generated the largest repulsive force, and subsequently the least attachment was found.

The attachment behavior could be quantitatively explained by the zeta potential values. Moreover, the sphere-plate DLVO theory was applied to gain insight into the relative interaction energies between the model bacteria and the soil as a function of separation distance (Hogg et al., 1966; Gregory, 1981; Torkzaban et al., 2007). A total positive interaction energy represents a repulsive force, whereas, a total negative interaction energy indicates an attraction (Vigeant and Ford, 1997). Fig. S1 illustrates the repulsive interactions existed at separation distances between bacteria and soil up to approximately 50 nm (1–60 mM), while some favorable conditions for cell attachment were predicted across the range of 60–100 mM, due to the total attraction regardless of separation distance (Haznedarglu et al., 2009; Zhao et al., 2014). Energy barriers that inhibited pathogen interaction in the primary minima were found to exist at separation distances of >3 nm. As shown in Table 2, the repulsive energy barriers of cell–soil interactions were in the order of full EPS-E. coli (0–377.1 kT) > partial EPS-E. coli (0–362.1 kT) > partial EPS-S. suis (0–268.5 kT) > full EPS-S. suis (0–762.2 kT). These heights of energy barriers were inversely related with the sequence of cell attachment percentages. It indicated that the sphere-plate DLVO model could quantitatively elucidate the different interaction energy trends and attachment behaviors of full EPS/partial EPS-cells.

The attachment percentages of full EPS-S. suis and partial EPS-S. suis remarkably increased by 85.2%–158.4% with IS increasing from 1 mM to 60 mM (P < 0.05). This attachment trend was in agreement with the increasing zeta potentials displayed in Fig. 3, which caused a reduction of electrostatic repulsion with increasing IS. DLVO prediction in Table 2 also revealed that the energy barriers of both S. suis types decreased (268.5–1.9 kT) with increasing IS, and the interactions became chemically favorable at 60 mM (i.e., no energy barrier) (Haznedarglu et al., 2009). While IS was above 60 mM, the zeta potentials of full EPS-S. suis (Fig. 3) were statistically unchanged (P > 0.05), and the trends of calculated DLVO energy curves at 60–100 mM got very close to each other (Fig. S1a). Thus, the extent of electrostatic repulsions were similar and the percentages of attached full EPS-S. suis was expected to reach a plateau. However, a decreasing trend was observed for full EPS-S. suis with increasing IS from 60 mM to 100 mM, which deviated from the prediction of DLVO theory. It suggested that additional mechanisms may play a role in the full EPS-S. suis attachment to soil at 80 and 100 mM, which are not incorporated in the traditional DLVO theory. The possible mechanism involved will be discussed in the next section.

The attachment of full EPS/partial EPS-E. coli to soil particles also showed increasing trends in the range of 1–100 mM (Fig. 5). This was an expected phenomenon due to double-layer compression occurring with increasing IS. Zeta potentials of E. coli cells confirmed this with decreased absolute values at higher IS (Fig. 3). As also indicated in Table 2, E. coli experienced smaller energy barriers (377.1–13.6 kT) at higher IS, and achieved its largest attachment capacity at 100 mM (37.6% and 55.6% for full EPS-E. coli and partial EPS-E. coli, respectively) where no barrier existed. Additionally, the comparable curves of full EPS-E. coli and partial EPS-E. coli at varied IS implied that EPS did not affect the attachment tendency of E. coli to soil particles. This result is generally in agreement with the zeta potential trends of full EPS/partial EPS-E. coli over the IS range, which may be attributed to the similar surface site types between full EPS-E. coli and partial EPS-E. coli (Fig. 1b).

Cell attachment data at varied IS were plotted against their surface properties to statistically investigate the main factors influencing the overall attachment phenomena. As seen in Fig. S2, a significant correlation (Y = −0.12 × X + 56.9, R² = 0.602, P < 0.01) was observed between bacterial attachment percentage (Y) and corresponding energy barrier (X) at 1–60 mM. This linear regression could be used to predict the extent of pathogen attachment in soil on the basis of existing energy barriers. Moreover, in the range of 60–100 mM where no repulsive energies appeared, the total interaction energies of partial EPS-S. suis and full EPS/partial EPS-E. coli at short distances of 0–5 nm became more attractive with increased IS (Figs. S1 b–d). The result verified that the attachment behaviors of the three bacterial types (partial EPS-S. suis and full EPS/partial EPS-E. coli) to natural soil particle at high IS were also in accordance with the sphere-plate DLVO calculations.

3.6. Other proposed mechanisms of EPS-induced cell attachment

In the current study, non-DLVO type forces may contribute to the attachment behaviors. The first possibility is hydrophobic effect. Previous studies showed that larger cell surface hydrophobicity was found to enhance its attachment affinity for soil minerals (Stenström, 1989; Kristian Stevik et al., 2004; Meyer et al., 2006). Attractive hydrophobic interaction can occur at separation distance of >10 nm and decay exponentially with distance (Israelachvili and Pashley, 1984; Rochex et al., 2004; Meyer et al., 2006). However, since DLVO calculations (Fig. S1) suggest that electrostatic repulsion dominates interactions as far as 50 nm, the cells will not approach the soil closely enough (in the order of 10 nm) to feel the hydrophobic force. This is further supported by the lack of correlation between attachment and hydrophobicity values. As shown in Fig. 6, the decrease in bacterial attachment to soil was generally observed with the increase of hydrophobicity. Although EPS had obvious impacts on cell hydrophobicities (Fig. 4), no significant positive correlation (P > 0.05) could be established (Fig. 6), indicating that the hydrophobic interaction had negligible effect on cell attachment.

Since the attractive secondary energy minima of cell–soil interactions were very small (around −1 kT, calculated by DLVO theory) in this study, it should not be sufficient to retain large amounts of bacteria on the soil surfaces. The average thermal energy of a bacterium is reported to be 0.5–1 kT (Hahn and O’Melia, 2004; Tufenkji and Elimelech, 2004). Some weakly attached cells may be knocked out of the secondary minima if only van der Waals and electrostatic forces were involved. Under this condition, other attractive forces possibly contribute to the bacterial attachment (e.g., surface roughness, charge heterogeneity and hydrodynamic force) (Li and Logan, 2004; Walker et al., 2005b; Chen et al., 2010).

The inability of DLVO theory to capture the interaction behavior entirely is partially due to the fact that the soil used in this study was not as homogeneous and smooth as DLVO theory assumes. Soil particles were irregularly shaped, and consisted of different particle sizes (clay 14.2%)

### Table 2

| IS (mM) | Energy barrier of pathogen–soil particle (kT) |
|---------|-----------------------------------------------|
|         | Full EPS-S. suis | Partial EPS-S. suis | Full EPS-E. coli | Partial EPS-E. coli |
| 1       | 76.2            | 268.5               | 377.1            | 362.1              |
| 5       | 49.2            | 159.7               | 284.4            | 230.8              |
| 10      | 16.3            | 101.4               | 206.0            | 137.3              |
| 20      | 1.9             | 58.7                | 132.7            | 111.6              |
| 40      | NB              | 15.7                | 53.3             | 52.8               |
| 60      | NB              | NB                  | 13.6             | NB                 |
| 80      | NB              | NB                  | NB               | NB                 |
| 100     | NB              | NB                  | NB               | NB                 |

Note: NB denotes no energy barrier.
silt 64.1%; sand 21.7%). The rough soil surfaces were likely to promote pathogen attachment. It was reported that roughness can lower surface energy, so that the electrostatic repulsive interactions are considerably less between the cell and rough surfaces than they are for more idealized smooth surfaces (Holm et al., 1992; Shellenberger and Logan, 2002; Li and Logan, 2004).

The next possible mechanism involved is surface charge heterogeneity. In this work, uniform distribution of surface charge is assumed to calculate electrostatic repulsion energy (Hogg et al., 1986; Ohshima et al., 1983; Walker et al., 2005b). This assumption, however, is not valid since real soil and cell surfaces are heterogeneous in nature (Song et al., 1994). It is well known that soil particles consist of multiple mineral and organic matter types (Brady and Weil, 1999). Soil grains have a tendency to aggregate and form heterogeneous surfaces exhibiting different charge distribution (Kemper and Rosenau, 1986; Tombácz and Szekeres, 2006). Local attachment of bacteria may occur to various mineral faces where the electrostatic repulsions between cells and some of these sites may have regions of lesser or different charge such that greater interaction is feasible (Song et al., 1994; Cai et al., 2013). The charge heterogeneity on cell surface could also result in the reduction of electrostatic energy barrier and more stable cell attachment (Redman et al., 2004).

It has been reported that a variety of charge heterogeneities can be found on the top of the peptidoglycan layer in Gram-positive (S. suis) or on the outer membrane in Gram-negative bacteria (E. coli), which arise from the differences in the arrangement of charged functional groups (Poortinga et al., 2002; Walker et al., 2005a). Hence, the local surface charge heterogeneity on soil/cell surfaces are important factors strengthening attachment.

Another possible interaction mechanism involved is hydrodynamic force. The cells and soil particles in attachment assay were suspended and mixed at 150 rev min⁻¹. It is possible that some level of shear force caused by this mixing may provide additional kinetic energy to help cells attach to the solid surfaces (Mohamed et al., 2000; Thomas et al., 2002; Chen et al., 2010; Hong et al., 2012).

There is also a possibility of polymer bridging as facilitated by EPS. It is well accepted that cell surface polymers may form covalent or hydrogen bonds that contribute to the attachment if the polymers are long enough to extend across the separation distance (Grasso et al., 2002; Chen and Walker, 2007). In this study, polymer bridging was found to play negligible roles in pathogen attachment. This is supported by data wherein the attachment of partial EPS-E. coli (19.9%–55.6%) was significantly greater (P < 0.05) than those of full EPS-E. coli (10.4%–37.6%). EPS on E. coli surface did not have substantial polymer bridging forces to improve its attachment. Besides, the attachment percentages (Y) of full EPS/partial EPS-pathogens were significantly governed by zeta potentials (X) of bacteria (Y = 2.1 X + 86.1, R² = 0.768, P < 0.01), rather than EPS content. Data and the above analysis suggest that EPS influenced cell attachment via electrostatic interactions stemming from the charged sites on the exposed surface polymers (full or partial EPS). Any contribution of polymer bridging or hydrophobicity was insignificant.

Finally, the short-range steric effect was likely to be responsible for the abnormal decreasing attachment trend of full EPS-S. suis between 60 and 100 mM (Kuznar and Elimelech, 2006). Table 2 illustrates that the energy barriers of full EPS-S. suis did not exist at this particular IS (60–100 mM). Total interaction energies were all attractive and S. suis cell was tightly attached at smaller separation distances. Under high IS conditions, the variation of electrostatic force was reduced. The strong steric repulsion could overcome the electrostatic force and contribute to the attachment at short distances (Abu-Lail and Camesano, 2003). It has been documented that the presence of excess ions suspended among the polymers may increase the intramolecular electrostatic interactions between individual polymer units, which lead to the polymers being more rigid. The rigid polymers restrain themselves from altering steric conformation, and interact directly with the soil surface to produce a steric repulsion (Chen and Walker, 2007; Kim et al., 2010). This hypothesis was corroborated by comparing with the attachment behavior of partial EPS-S. suis. Specifically, the attachment of partial EPS-S. suis increased with IS from 60 mM to 100 mM (i.e., attachment percentages of 55.7%, 67.0% and 77.3% at 60, 80 and 100 mM, respectively), verifying that the steric hindrance directly originated from the EPS components on S. suis.

Such phenomenon has also been reported by previous studies. For instance, Kim et al. (2009b) found the adhesion of E. coli O157:H7 to quartz sand decreased at IS (KCl) ≥ 10 mM. Chen and Walker (2007) reported that the steric force was repulsive when the IS (CaCl₂) was greater than 300 mM, as confirmed by the attachment efficiency of Halomonas pacifica to quartz decreasing at high IS. Otto et al. (1999) observed the number of attached E. coli MS7 to quartz crystal microbalance surfaces increased up to an IS (phosphate-buffered saline) of 122.5 mM or 185 mM, above which adhesion either leveled off or decreased. It should be mentioned that the solution IS value at which steric repulsion appeared in this study (≥80 mM) was distinctly different from the above investigations. This difference was possibly ascribed to the different natures of EPS on cell surfaces and dissimilar solution chemistry (Rijnaarts et al., 1999). On the contrary, E. coli had lower total EPS content than S. suis (Table S1). The small amount of EPS on E. coli (26.25 µg/10⁸ cells) was probably less rigid so as not to be sensitive to steric repulsion.

4. Conclusions

This work directly demonstrates that the EPS constituents could play contrasting roles in bacterial attachment to natural soil particles, as a function of cell type. Detailed insights into the mechanisms regarding the variation of EPS-induced pathogen surface properties were also provided. The obtained results showed that bacterial EPS had distinct influences on the surface charges and hydrophobicities of S. suis and E. coli, due to their different acidic site concentrations and EPS components. EPS removal impacted pathogen attachment to soil via the modification of cell surface charges. Steric repulsion from the EPS on S. suis surface was possibly involved in its attachment under high IS conditions. In contrast to previous reports using low-heterogeneous mineral surfaces (Li and Logan, 2004; Vadillo-Rodríguez et al., 2005; Chen and Walker, 2007), the effects of “EPS bridging” or hydrophobic interaction on bacterial attachment to soil particles were insignificant. Other physical (e.g., soil surface roughness and hydrodynamic shear force) and chemical factors (e.g., charge heterogeneity) may play more important roles and contribute to the attachment processes of full EPS/partial EPS-bacteria. Results of cell attachment to pure mineral experiments may
have limited implications for predicting the real functions of EPS in bacterial attachment phenomena in natural soil. Classic DLVO theory, including electrostatic and van der Waals forces, was able to explain the attachment trends of pathogens regardless of EPS content under low soil IS conditions. Findings from the current study highlight the importance of understanding the diverse functions of EPS that exist in bacteria–soil interactions, which is vital to better assess the fates of different pathogens in soil–aquatic environments.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (41171196), the National Basic Research Program of China (2015CB150504), Foundation for the Author of National Excellent Doctoral Dissertation of China (201066), and the Fundamental Research Funds for the Central Universities (2011PY005). The authors would like to thank Professor Junlong Zhao from the State Key Laboratory of Agricultural Microbiology for providing the S. suis SC05 and E. coli WH09 isolates.

Appendix A. Supplementary material

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.chemgeo.2015.06.013.

References

Abit, S.M., Bolster, C.H., Cai, P., Walker, S.L., 2012. Influence of feedstock and pyrolysis temperature of biochar amendments on transport of Escherichia coli in saturated and unsaturated soil. Environ. Sci. Technol. 46, 8097–8105.
Abu-Lail, N.I., Camesano, T.A., 2003. Role of ionic strength on the relationship of biopolymer substances from extreme acidic microbial biofilms. Geomicrobiol. J. 21, 511–519.
Aguilera, A., Souza-Egipsy, V., Martín-Úriz, P.S., Amils, R., 2008. Extraction of extracellular soil IS conditions. Findings from the current study highlight the importance of understanding the diverse functions of EPS that exist in bacteria–soil interactions, which is vital to better assess the fates of different pathogens in soil–aquatic environments.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (41171196), the National Basic Research Program of China (2015CB150504), Foundation for the Author of National Excellent Doctoral Dissertation of China (201066), and the Fundamental Research Funds for the Central Universities (2011PY005). The authors would like to thank Professor Junlong Zhao from the State Key Laboratory of Agricultural Microbiology for providing the S. suis SC05 and E. coli WH09 isolates.

Appendix A. Supplementary material

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.chemgeo.2015.06.013.

References

Abit, S.M., Bolster, C.H., Cai, P., Walker, S.L., 2012. Influence of feedstock and pyrolysis temperature of biochar amendments on transport of Escherichia coli in saturated and unsaturated soil. Environ. Sci. Technol. 46, 8097–8105.
Abu-Lail, N.I., Camesano, T.A., 2003. Role of ionic strength on the relationship of biopolymer formation during Brownian particle transport in soil. Langmuir 23, 6691–6697.
Aguilera, A., Souza-Egipsy, V., Martín-Úriz, P.S., Amils, R., 2008. Extraction of extracellular soil IS conditions. Findings from the current study highlight the importance of understanding the diverse functions of EPS that exist in bacteria–soil interactions, which is vital to better assess the fates of different pathogens in soil–aquatic environments.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (41171196), the National Basic Research Program of China (2015CB150504), Foundation for the Author of National Excellent Doctoral Dissertation of China (201066), and the Fundamental Research Funds for the Central Universities (2011PY005). The authors would like to thank Professor Junlong Zhao from the State Key Laboratory of Agricultural Microbiology for providing the S. suis SC05 and E. coli WH09 isolates.

Appendix A. Supplementary material

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.chemgeo.2015.06.013.

References

Abit, S.M., Bolster, C.H., Cai, P., Walker, S.L., 2012. Influence of feedstock and pyrolysis temperature of biochar amendments on transport of Escherichia coli in saturated and unsaturated soil. Environ. Sci. Technol. 46, 8097–8105.
Abu-Lail, N.I., Camesano, T.A., 2003. Role of ionic strength on the relationship of biopolymer formation during Brownian particle transport in soil. Langmuir 23, 6691–6697.
Aguilera, A., Souza-Egipsy, V., Martín-Úriz, P.S., Amils, R., 2008. Extraction of extracellular soil IS conditions. Findings from the current study highlight the importance of understanding the diverse functions of EPS that exist in bacteria–soil interactions, which is vital to better assess the fates of different pathogens in soil–aquatic environments.
Nikaido, H., 2003. Molecular basis of bacterial outer membrane permeability revisited.

Redman, J.A., Walker, S.L., Elimelech, M., 2004. Bacterial adhesion and transport in porous media. Appl. Clay Sci. 23, 19–30.

Pachepsky, Y.A., Yu, O., Karns, J.S., Shelton, D.R., Guber, A.K., van Kessel, J.S., 2008. Strain-dependent variations in attachment of Escherichia coli and enterococci to particles in runoff. J. Environ. Qual. 39, 1019–1027.

Stenström, T., 1989. Bacterial hydrophobicity, an overall parameter for the measurement of adhesion potential to soil particles. Appl. Environ. Microbiol. 55, 142–147.

Torkzaban, S., Bradford, S.A., Walker, S.L., 2007. Resolving the coupled effects of hydrodynamic and DLVO forces on colloid attachment in porous media. Langmuir 23, 9525–9530.

Tong, J., Nguyen, B.T., Mosselmans, J., Tietj, L., Cowie, G.L., 2008. The effect of extracellular polymeric substances (EPS) on the proton adsorption characteristics of the thermophile Bacillus licheniformis S-86. Chem. Geol. 247, 1–15.

Tsuneda, S., Aiakwa, H., Hayashi, H., Yuasa, A., Hirata, A., 2003. Extracellular polymeric substances responsible for bacterial adhesion onto solid surface. FEMS Microbiol. Lett. 223, 287–292.

Tyler, A.A., Chowdhury, I., Gong, A.S., Clevner, D.M., Walker, S.L., 2014. Deposition and disinfection of Escherichia coli O157:H7 on naturally occurring photovoltaic materials in a parallel plate chamber. Environ. Sci.: Processes Impacts 16, 194–202.

Thomas, W.E., Trinchita, E., Forero, M., Vogel, Y., Sukremenov, E.V., 2002. Bacterial adhesion to target cells enhanced by shear force. Cell 105, 913–923.

Tombáč, E., Szecheres, M., 2006. Surface charge heterogeneity of kaolinite in aqueous suspension in comparison with montmorillonite. Appl. Clay Sci. 34, 105–124.

Tong, M., Long, G., Jiang, X., Kim, H.N., 2010. Contribution of extracellular polymeric substances on representative gram negative and gram positive bacterial deposition in porous media. Environ. Sci. Technol. 44, 1837–1842.

Torkzaban, S., Bradford, S.A., Walker, S.L., 2007. Resolving the coupled effects of hydrodynamic and DLVO forces on colloid attachment in porous media. Langmuir 23, 9525–9530.

Toth, J.D., Aceto, H.W., Rankin, S.C., Dou, Z. 2013. Short communication: survey of animal-borne pathogens in the farm environment of 13 dairy operations. J. Dairy Sci. 96, 5756–5761.

Toory, J., Ngewya, B.T., Mosselmans, J., Tietj, L., Cowie, G.L., 2008. The effect of extracellular polymeric substances (EPS) on the proton adsorption characteristics of the thermophile Bacillus licheniformis S-86. Chem. Geol. 247, 1–15.

Tsuneda, S., Aiakwa, H., Hayashi, H., Yuasa, A., Hirata, A., 2003. Extracellular polymeric substances responsible for bacterial adhesion onto solid surface. FEMS Microbiol. Lett. 223, 287–292.

Uchima, M., Cinn, B.R., Haack, E.A., Szymansowski, J.E., Fein, J.B., 2008. Cd adsorption on Pseudomonas putida in the presence and absence of extracellular polymeric substances. Geochim. Cosmochim. Acta 72, 5885–5895.

Unc, A., Goss, M.J., 2004. Transport of bacteria from manure and protection of water resources. Appl. Soil Ecol. 25, 1–18.

Vadillo-Rodriguez, V., Busscher, H.J., van der Mei, H.C., de Vries, J., Norde, W., 2005. Role of lactobacillus cell surface hydrophobicity as probed by AFM in adhesion to surfaces at low and high ionic strength. Colloids Surf. B: Biointerfaces 41, 33–41.

Venglovsky, J., Sasakova, N., Placha, I., 2009. Pathogens and antibiotic residues in animal products from livestock production in comparison with montmorillonite. Appl. Clay Sci. 43, 4355–4360.

Walker, S.L., Hill, J.E., Redman, J.A., Elimelech, M., 2005a. Influence of extracellular polymeric substances on representative gram negative and gram positive bacterial deposition in porous media. Environ. Sci. Technol. 39, 2937–2943.

Walker, S.L., Redman, J.A., Elimelech, M., 2004. Role of cell surface lipopolysaccharides in bacterial adhesion under the inactivation of double layer: II. Curvature correction to the formula for the interaction of spheres. J. Colloid Interface Sci. 40, 239–242.

Ojeda, J., Romero-Gonzalez, M., Pouran, H., Banwart, S., 2008. In situ monitoring of the formation of Pseudomonas putida on hematite using flow-cell ATR-FTIR spectroscopy to investigate the formation of inner-sphere bonds between the bacteria and the mineral. Miner. Mag. 72, 101–106.

Otto, K., Elwing, H., Hermansson, M., 1999. Effect of ionic strength on initial interactions of Escherichia coli with surfaces, studied on-line by a novel quartz crystal microbalance technique. J. Bacteriol. 181, 5210–5218.

Pachepsky, Y.A., Yu, O., Karms, J.S., Shelton, D.R., Guber, A.K., van Kessel, J.S., 2008. Strain-dependent variations in attachment of Escherichia coli to soil particles of different sizes. Int. Agrophysics 22, 61–66.

Parikh, S.J., Chorover, J., 2006. ATR-FTIR spectroscopy reveals bond formation during bacterial adhesion to iron oxide. Langmuir 22, 4802–4805.

Poortinga, A.T., Bos, R., Norde, W., Busscher, H.J., 2002. Electric double layer interactions in bacterial adhesion to surfaces. Surf. Sci. Rep. 47, 1–32.

Redman, J.A., Walker, S.L., Elimelech, M., 2004. Bacterial adhesion and transport in porous media: role of the surface energy minimum. Environ. Sci. Technol. 38, 1777–1785.

Rijnja, H.H., Norde, W., Lyklema, J., Zehnder, A.J., 1999. DLVO and steric contributions to bacterial deposition in media of different ionic strengths. Colloids Surf. B: Biointerfaces 14, 179–195.

Rochex, A., Lecouturier, D., Pezon, I., Lebeault, J.M., 2004. Adhesion of a Pseudomonas putida strain isolated from a paper machine to cellulose fibres. Appl. Microbiol. Biotechnol. 65, 727–733.