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Adhesion of bacterial pathogens to soil colloidal particles: Influences of cell type, natural organic matter, and solution chemistry

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ARTICLE INFO

Article history:
Received 3 September 2013
Received in revised form
20 November 2013
Accepted 5 January 2014
Available online 16 January 2014

Keywords:
Pathogen
Adhesion
Soil colloid
Solution chemistry
DLVO theory

ABSTRACT

Bacterial adhesion to granular soil particles is well studied; however, pathogen interactions with naturally occurring colloidal particles (<2 μm) in soil has not been investigated. This study was developed to identify the interaction mechanisms between model bacterial pathogens and soil colloids as a function of cell type, natural organic matter (NOM), and solution chemistry. Specifically, batch adhesion experiments were conducted using NOM-present, NOM-stripped soil colloids, *Streptococcus suis* SC05 and *Escherichia coli* WH09 over a wide range of solution pH (4.0–9.0) and ionic strength (IS, 1–100 mM KCl). Cell characterization techniques, Freundlich isotherm, and Derjaguin–Landau–Verwey–Overbeek (DLVO) theory (sphere–sphere model) were utilized to quantitatively determine the interactions between cells and colloids. The adhesion coefficients (K_f) of *S. suis* SC05 to NOM-present and NOM-stripped soil colloids were significantly higher than *E. coli* WH09, respectively. Similarly, K_f values of *S. suis* SC05 and *E. coli* WH09 adhesion to NOM-stripped soil colloids were greater than those colloids with NOM-present, respectively, suggesting NOM inhibits bacterial adhesion. Cell adhesion to soil colloids declined with increasing pH and enhanced with rising IS (1–50 mM). Interaction energy calculations indicate these adhesion trends can be explained by DLVO-type forces, with *S. suis* SC05 and *E. coli* WH09 being weakly adhered in shallow secondary energy minima via polymer bridging and charge heterogeneity. *S. suis* SC05 adhesion decreased at higher IS 100 mM, which is attributed to the change of hydrophobic effect and steric repulsion resulted from the greater presence of extracellular polymeric substances (EPS) on *S. suis* SC05 surface as compared to *E. coli* WH09. Hence, pathogen adhesion to the colloidal material is determined by a combination of DLVO, charge heterogeneity, hydrophobic and polymer interactions as a function of solution chemistry.

1. Introduction

The United States and China annually produce 317 × 10^6 and 300 × 10^7 tons of animal manure, respectively (Browner et al., 2001; Huang et al., 2008); whereas, the production is approximately 10^{10}–10^{11} tons worldwide (Pachepsky et al., 2006). Over the past decades, large amounts of fecal wastes have been applied to the soil as a source of plant nutrients. Meanwhile, these wastes may contain various pathogenic microorganisms

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0043-1354/$ – see front matter

http://dx.doi.org/10.1016/j.watres.2014.01.009

if not managed properly (Tyrrel and Quinton, 2003; Cotruvo et al., 2004). Application of solid wastes to soil introduces high concentrations of pathogens associated with human diseases (Santamaria and Toranzos, 2003; Haznedaroglu et al., 2009). For instance, *Streptococci* and fecal coliforms have been widely detected in animal manures and soil environments by genetic techniques (Unc and Goss, 2004). During the summer of 2005 in China, a large outbreak of acute diseases linked to *Streptococcus suis* appeared suddenly, which was ascribed to close contact with pig wastes or ingestion of contaminated food (Gottschalk et al., 2010). The Beijing Centers for Disease Control and Prevention reported that 205 people were infected, with a mortality rate of nearly 20%. A case of male farmer infection was also reported in the United States in 2006 (Gottschalk et al., 2007). Another bacterial pathogen of concern is *Escherichia coli* (referred to as an indicator of fecal contamination), from which outbreaks linked to contaminated vegetables, soil or groundwater have led to serious diseases (Cai et al., 2013). It was reported that pathogenic *E. coli* causes ~73,000 illnesses, ~2200 hospitalizations, and ~61 deaths annually in the United States (Wang et al., 2011). Pathogens presence, through their retention in the upper layers of soil or surface runoff, may pose a risk to human and livestock health (Dhand et al., 2009). Therefore, a thorough understanding of pathogen adhesion to soil components is of great importance for assessing the fate of pathogens in soil and aquatic environments.

Generally, a two-step mechanism mediates bacterial adhesion to solid surfaces (Zita and Hermansson, 1994). The first step is governed by long-range physiochemical interactions such as Lifshitz–van der Waals and electrostatic forces that described by the Derjaguin–Landau–Verwey–Overbeek (DLVO) theory (Bakker et al., 2004), as well as bridging effects among the organic matter or polymers that exist on collector surfaces (Parent and Velegol, 2004). These forces determine whether the cells are able to get close enough to solid surfaces such that adhesion can occur. The second step involves short-range irreversible adhesion by forming hydrophobic force, hydrogen bonding, or steric interaction (Gordesli and Abu-Lail, 2012). Both steps are affected by the surface properties of the interacting surface and electrolytic environment. The investigated factors include bacterial strain (Li and Logan, 2004; Morrow et al., 2005), mineral type (Salerno et al., 2004; Rong et al., 2008), solution chemistry (Yee et al., 2000; Farahat et al., 2010), cell surface features (e.g., proteins and polysaccharides) (Walker et al., 2005a; Shephard et al., 2010), and organic matter (Parent and Velegol, 2004; Foppen et al., 2008; Park and Kim, 2009). The organic matter (e.g., humic acid) used in previous cell adhesion studies was usually purchased from a company and had been modified (Parent and Velegol, 2004; Foppen et al., 2008; Park and Kim, 2009), which differs from the real heterogenous state of natural organic matter (NOM) in the soil. Additionally, most previous work examined the impacts of various physical, chemical, and biological factors on cell adhesion to granular and flat mineral surfaces, far less attention has been directed towards the natural colloidal soil particles. Extrapolating the adhesion mechanisms in pure minerals to those in soil particles is more complicated because of the higher complexity of the latter consisting of NOM and multiple mineral types (Brady, 1990). Moreover, it should be noted that pathogen adhesion to soil particle surfaces (soil colloids <2 μm in size for this study) has not been systematically explored across a wide range of ionic strength (IS) and solution pH.

After pathogens were introduced through the application of biosolids in soil, they can be trapped in soil pores or adhered by soil particles, fragments of vegetation, and manure particles (Tyrrel and Quinton, 2003; Pachepsky et al., 2006). Once suspended in water during the rainfall and irrigation events, bacteria may also be transported with surface or subsurface water flow as free or adhered cells via association with soil particles (Pachepsky et al., 2006; Guber et al., 2007). Recently, a small number of studies have shown that soil colloids played an important role in bacterial adhesion. For example, Oliver et al. (2007) found that most of the *E. coli* cells (65%) were associated with soil particulates <2 μm in diameter. For the 2–3 μm, 4–15 μm, 16–30 μm, and ≥ 31 μm size fractions, the percentages of adhered cells were 7, 14, 12, and 2% respectively. Guber et al. (2007) observed that in the absence of manure colloids, fecal coliforms adhesion to soil silt (2–50 μm) and clay particles (<2 μm) was much higher than those to sand particles (62.5–500 μm). Soupir et al. (2010) found that more than 60% of adhered *E. coli* and enterococci were associated with fine-size particles (8–62 μm). The lowest *E. coli* concentration in runoff occurred from the silty loam soils, which have higher clay and organic contents. Wu et al. (2011) reported that the maximum number of *Pseudomonas putida* cells that adhered to the clay fraction of Red soil (Ultisol) was 4 and 62 times as great as that by silt and sand fractions, respectively. Despite these initial efforts, few studies have given sufficient explanation with regard to bacterial adhesion to colloidal particles. Information on the comprehensive surface properties of pathogens and soil colloids under varying solution parameters is lacking. To the best of our knowledge, the interaction forces between pathogens and natural soil particles have never been investigated.

The objectives of the present work were to elucidate how pathogens (*S. suis* SC05 and *E. coli* WH09) adhesion to soil colloids respond to the changes in cell type, NOM, and solution chemistry. Surface physico-chemical properties of the bacteria and soil colloids were extensively characterized over a wide range of pH (4.0–9.0) and ionic strength (IS, 1–100 mM). These solution values encompass most soil environmental relevant conditions. Furthermore, the DLVO theory (sphere–sphere model) was firstly utilized to predict energy profiles and provide insight into the interaction mechanisms of pathogen-soil colloid systems.

2. Materials and methods

2.1. Bacterial growth and preparation

Gram-negative *E. coli* WH09 and Gram-positive *S. suis* SC05, obtained from the State Key Laboratory of Agricultural Microbiology, were isolated from soils around a pig farm in Wuhan, Hubei Province, China. Bacterial growth and preparation methods are provided in the Supplementary data.

2.2. Bacterial cell characterization

The zeta potential and hydrodynamic diameter of bacteria were determined by Zetasizer (Nano ZS90, Malvern