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The role of zeta potential in the adhesion of *E. coli* to suspended intertidal sediments

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ABSTRACT

The extent of pathogen transport to and within aquatic systems depends heavily on whether the bacterial cells are freely suspended or in association with suspended particles. The surface charge of both bacterial cells and suspended particles affects cell-particle adhesion and subsequent transport and exposure pathways through settling and resuspension cycles. This study investigated the adhesion of Faecal Indicator Organisms (FIOs) to natural suspended intertidal sediments over the salinity gradient encountered at the transition zone from freshwater to marine environments. Phenotypic characteristics of three *E. coli* strains, and the zeta potential (surface charge) of the *E. coli* strains and 3 physically different types of intertidal sediments was measured over a salinity gradient from 0 to 5 Practical Salinity Units (PSU). A batch adhesion microcosm experiment was constructed with each combination of *E. coli* strain, intertidal sediment and 0, 2, 3.5 and 5 PSU. The zeta potential profile of one *E. coli* strain had a low negative charge and did not change in response to an increase in salinity, and the remaining *E. coli* strains and the sediments exhibited a more negative charge that decreased with an increase in salinity. Strain type was the most important factor in explaining cell-particle adhesion, however adhesion was also dependant on sediment type and salinity (2, 3.5 PSU > 0, 5 PSU). Contrary to traditional colloidal (Derjaguin, Landau, Vervey, and Overbeek (DLVO)) theory, zeta potential of strain or sediment did not correlate with cell-particle adhesion. *E. coli* strain characteristics were the defining factor in cell-particle adhesion, implying that diverse strain-specific transport and exposure pathways may exist. Further research applying these findings on a catchment scale is necessary to elucidate these pathways in order to improve accuracy of FIO fate and transport models.

1. Introduction

In the early 2000s, it was predicted that local and foreign tourists spent two billion days each year at the coast worldwide (Shuval, 2003), and an estimated 20 million people used the coast and inland waters each year in the UK (Pond, 2005), with these numbers predicted to increase. Around this period, bathing at coastal sites caused an estimated 120 million cases of gastrointestinal illness worldwide (Shuval, 2003), and bathing at English and Welsh beaches and bathing waters caused an estimated 1.75 million cases of gastrointestinal disease annually (Georgiou and Langford, 2002). The most common disease associated with bathing in contaminated water is enteric illness with an associated risk of roughly 51/1000 bathers, and the risk of other respiratory, ear and eye disease between 20/1000 and 54/1000 bathers in water that contained <2000 faecal coliforms 100 ml^{-1} (Fleisher et al., 1998). The likelihood of gastrointestinal illness to sea-bathers compared to non-bathing beach goers increases 1.76 fold (Fleisher et al., 2010). However, risk is not solely associated with bathers, as an increase in enteric illnesses can be a direct result from increased contact with recreational beach sand (Heaney et al., 2012).

It is well established that survival of FIOs is greatly increased when in association with sediments compared to the overlying water in both freshwater and marine systems (Gerba and Mcleod, 1976; Moore et al., 2003; Pachepsky and Shelton, 2011). This is a

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result of many survival advantages including increased nutrient availability (Burton et al., 1987) and protection from UV (Fujioka and Yoneyama, 2002) and protozoan grazing (England et al., 1993). Faecal Indicator Organisms gain these survival advantages through adhering to particles in suspension, leading to incorporation of FIOs in sediments as the particle is deposited (Davies et al., 1995; Geldreich, 1970).

The transport and fate, and therefore spatial and temporal abundance, of faecal indicator organisms (FIOs) within aquatic systems is heavily dependent on whether cells are freely suspended, or associated with suspended particles (Bai and Lung, 2005; Jeng et al., 2005; Muirhead et al., 2006b). Particle association also governs FIO residence time through incorporation into the erosion, transport, deposition and consolidation (ETDC cycle) of particles (Whitehouse, 2000). The importance of differentiating between these phases has been realised in recent modelling approaches concerning FIO fate and transport on catchment scales (Cho et al., 2016b).

Free bacterial cells in the water column are maintained in suspension by Brownian motion but become susceptible to sedimentation when in association with particles because of the increased settling velocity. The mechanisms governing the adhesion of faecal bacteria to suspended particles are complex and may be determined by a range of physical, and biological factors (Oliver et al., 2007). Derjaguin, Landau, Vervey, and Overbeek (DLVO) theory is known to serve as a basic model for describing the initial adhesion of bacteria to suspended particles (Van Loosdrecht et al., 1990), and has been since been improved upon for the prediction of cell adhesion with the extended DLVO theory (xDLVO) (Perni et al., 2014).

Briefly, DLVO theory describes the interplay between electrostatic repulsion and the attraction of Van der Waals forces between colloidal particles. The strength of the electrostatic repulsion can be determined by measuring the particle charge, known as the zeta potential, of a colloidal suspension. As zeta potential becomes more positive or negative, the larger the electrostatic repulsion between particles, the less likely they are to flocculate (Van Loosdrecht et al., 1987).

In this study, phenotyping assays and zeta potential analyses were followed by a microcosm experiment using natural intertidal sediments and river and seawater in order to investigate the role of strain and sediment characteristics and particle charge in the adhesion of *E. coli* to suspended particles. It was hypothesised that the less negative zeta potentials of cell and/or sediments induced by higher salinity would correlate with increased cell-particle adhesion.

2. Materials and methods

2.1. *E. coli* strains

One of several wild-type *E. coli* strains isolated from intertidal sediment at the Ythan estuary, Scotland, UK (57°20′59.3” N 1°59′36.8” W) in May 2014 was selected for use and hereby referred to as Yth13 throughout. *E. coli* strains DSM 8698 and DSM 9034 were obtained from the Leibniz Institute DSMZ (Germany). Serological and isolation details are provided in Table 1. Unless otherwise stated, *E. coli* cultures were prepared from −80 °C stock cultures through overnight incubation in LB broth at 37 °C in a shaking incubator at 150 rpm. Cells were harvested by centrifugation at 3000 × g for 5 min, and washed three times in the appropriate dilution of seawater.

2.2. Bacterial strain characterisation

Swarm assays were performed using a similar method to that of Wolfe and Berg (1989). Cells from a single colony were stab-inoculated into the centre of a 0.4% LB agar plates (LB broth, Merck Millipore, Darmstadt, Germany). Three replicate plates for each strain at each temperature treatment (15 °C and 25 °C) were sealed with laboratory film to reduce moisture loss, inverted and incubated. The swarm diameter was measured to the nearest mm every 24 h.

Biofilm assays were performed using a similar method to that of Merritt et al. (2011). Briefly, *E. coli* cultures were grown overnight in 5 ml LB broth at 37 °C. Cultures were normalised to an OD600 of 0.5 using 70–850 μl disposable micro-cuvettes (Brand GMBH + CO KG, Germany) in a spectrophotometer (BioPhotometer 6131, Eppendorf). One μl of each normalised *E. coli* culture was inoculated into 4 replicate wells in a sterile 96 well cell culture plate (Costar, Corning, NY, USA) alongside 4 wells containing media only controls. One hundred μl of fresh sterile LB broth media was added to each well. The plate was covered with laboratory film to minimise evaporation and incubated at 25 °C for 48 h. After incubation, the plate was washed twice with DI H_20, and 125 μl of 0.1% (w/v) crystal violet in DI H_2O was added to each well and the plate incubated for 10 min at room temperature. The plate was rinsed as above and left to air dry before 200 μl of 80:20 ethanol: acetone solution (O'Toole et al., 1999) was added to each well and the plate incubated for 15 min at room temperature. The contents of each well were mixed via pipetting, and 125 μl transferred to a micro-cuvette with the OD600 of each biofilm elution measured against a blank of the ethanol: acetone solution.

Table 1
Summary information of the *E. coli* strains used in this study including clade (Clermont et al., 2013) and MLST Complex (Wirth et al., 2006). DSMZ refers to the Leibniz Institute Deutsche Sammlung von Mikroorganismen und Zellkulturen.

Strain	Serotype	Clade	MLST	Origin	Isolation	Details
Yth13	Unknown	B1	155	Ythan estuary, Scotland	2014	Isolated from a mixed mud sediment where salinity ~17 PSU.
DSM 8698	O111:H-	B1	20	DSMZ	1950	Enteropathogenic, isolated from human diarrhoea (Kauffmann and Dupont, 1950)
DSM 9034	O164:H-	–	–	DSMZ	1947	Enteroinvasive, isolated from human diarrhoea (Rowe et al., 1977)

Table 2
Summary of bulk sediment characteristics for the 3 sediment types used.

Sediment Type	UK Grid Reference (Lat., Lon.)	Bulk Density (g cm^{-3} ± SE)	Water Content (% core weight ± SE)	Organic Content (% dry weight ± SE)	Surface Area (sq.m g^{-1})
Organic Mud	57.359746, −2.017193	1.27 ± 0.01	65.42 ± 0.12	9.12 ± 0.12	7.101
Mud	57.334826, −2.004501	1.38 ± 0.07	62.82 ± 0.26	7.00 ± 0.12	6.014
Mixed Sand	57.313898, −1.993890	1.95 ± 0.03	23.66 ± 0.17	2.16 ± 0.02	1.136