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Effects of Outer Membrane Protein *TolC* on the Transport of *Escherichia coli* within Saturated Quartz Sands

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Abstract

The outer membrane protein (OMP) TolC is the cell surface component of several drug efflux pumps that are responsible for bacterial resistance against a variety of antibiotics. In this research, we investigated the effects of OMP TolC on *E. coli* transport within saturated sands through column experiments using a wide type *E. coli* K12 strain (with OMP TolC), as well as the corresponding transposon mutant (*tolC::kan*) and the markerless deletion mutant ($\Delta tolC$). Our results showed OMP TolC could significantly enhance the transport of *E. coli* when the ionic strength was 20 mM NaCl or higher. The deposition rate coefficients for the wild type *E. coli* strain (with OMP TolC) was usually >50% lower than those of the *tolC*-negative mutants. The measurements of contact angles using three probe liquids suggested that TolC altered the surface tension components of *E. coli* cells and lead to lower Hamaker constants for the cell-water-sand system. The interaction energy calculations using the extended Derjaguin-Landau-Verwey-Overbeek (XDLVO) theory suggested that the deposition of the *E. coli* cell primarily occurred at the secondary energy minimum. The depth of the secondary energy minimum increased with ionic strength, and was greater for the TolC-deletion strains under high ionic strength conditions. Overall, the transport behavior of three *E. coli* strain within saturated sands could be explained by the XDLVO calculations. Results from this research suggested that antibiotic resistant bacteria expressing OMP TolC could spread more widely within sandy aquifers.

1. Introduction

Groundwater accounts for ~48% of global drinking water supply ¹. Groundwater, however, is susceptible to microbial contamination ²⁻⁴. It was reported that since 1920, about 50% of the waterborne disease outbreaks in the US were caused by contaminated, untreated or inadequately treated groundwater ⁴. Each year, groundwater contamination is responsible for an estimated 750,000 to 5.9 million illnesses and 1400-9400 deaths in the US ². A thorough understanding of the transport of microbial cells within groundwater systems is essential to the assessment of the public health risks associated with groundwater microbial contamination as well as the design, evaluation and implementation of appropriate mitigation measures.

The outer membrane of Gram-negative bacteria such as *Escherichia coli* and *Salmonella* is comprised of lipopolysaccharide (LPS), proteins and filamentous structures such as

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fimbriae/pili and flagella⁵. Several recent studies suggested that various bacterial surface structures could significantly influence the transport behavior of bacterial cells by affecting the attachment of the cells to aquifer solid matrix⁶⁻¹¹. Haznedaroglu et al.¹¹ reported that flagella could enhance the deposition of *Salmonella enterica* and thus lower its mobility within quartz sands. For *E. coli* O157:H7, Kim et al.^{9, 10} observed that the surface macromolecules such as LPS could alter cell surface properties, cell-solid energy interactions and subsequently cell transport behavior in saturated porous media. Lutterodt et al.⁸ suggested that the positive charges originating from OMP Ag43 could promote the attachment of *E. coli* cells to the negatively-charged quartz surfaces and lower their mobility within quartz sands. Walczak et al.^{6, 7} showed that tetracycline-resistant and tetracycline-susceptible *E. coli* strains that were isolated from various environmental sources displayed distinct OMP profiles and the tetracycline-resistant *E. coli* strains consistently showed higher mobility within saturated quartz sands. The OMP TolC, which has a molecular mass of ~52 kDa, was identified as a potential factor in reducing the deposition of *E. coli* to the surface of quartz sands. The recent studies on the relationship between cell outer membrane protein and transport within saturated porous media, however, were usually quantitative and speculative⁶⁻⁸.

TolC is the membrane component of several types of multidrug efflux pumps (e.g., the AcrAB-TolC pump), which are responsible for bacterial resistance to a variety of antibiotics such as tetracycline, erythromycin and ampicillin¹²⁻¹⁵. The possibility that TolC could enhance the mobility of bacterial cells within the saturated porous media has broad environmental implications with regard to the spread of antibiotic resistant bacteria within groundwater aquifers and warrants further investigation. The primary goal of this research is to quantify the effects of OMP TolC on the transport of *E. coli* within saturated sands in an unambiguous manner using TolC-deletion mutants. The XDLVO theory was then applied to examine how OMP TolC influenced *E. coli* mobility through altering the energy interactions between the *E. coli* cells and the surface of sands.

2. Materials and Methods

2.1 The *E. coli* strains

In this research, the wild type strain was *E. coli* K12 (strain W25113), which was used to make the Keio Collection of single-gene knockouts¹⁶. The strain JW5503 (*tolC::kan*) was obtained from the Keio collection: in this strain the *tolC* open reading frame was replaced with a kanamycin cassette (amplified from plasmid pKD13) flanked by FLP recombination sites¹⁶. To construct the markerless deletion of *tolC* (i.e., to excise kanamycin resistance), *E. coli* JW5503 was transformed with plasmid pCP20 and the kanamycin resistant colonies were selected at 30°C. pCP20 has temperature sensitive replication and thermal induction of FLP recombinase expression^{17, 18}. The transformants were cultured at 43°C, after which the loss of both pCP20 and the kanamycin resistance cassette were confirmed via polymerase chain reaction (PCR) and ampicillin/kanamycin sensitivity testing. The markerless strain lacking TolC was referred to as $\Delta tolC$.

The *E. coli* cells preserved in 20% glycerol under -80°C was streaked onto Luria-Bertani (LB) (Fisher Scientific) agar plates. After overnight incubation at 37°C, cells from a freshly formed colony were transferred to culture tubes containing 15 ml LB broth. The culture tubes were shaken at 90 rpm and incubated at 37°C for 6 hours. The starter culture was used to inoculate LB broth (1:500 dilution ratio). Following the incubation at 37°C for 18 hours on an orbital shaker (90 rpm), the *E. coli* cells were harvested by centrifugation (4000 × g, 10 minutes, 4°C). To remove the growth medium, the bacterial pellet was rinsed 4 times with the appropriate electrolyte solution. The concentration of cells was then adjusted to ~ 4

$\times 10^7$ cell/ml for the column transport experiments. The pH of the cell suspensions was ~ 5.7 .

2.2 Column transport experiments

The silica sands used for the column experiments were purchased from US Silica and had a size range of 0.210-0.297 mm. The sands received from the manufacturer were alternately cleaned using concentrated nitric acid to remove metal hydroxides and diluted NaOH solution to remove natural clay particles¹⁹, rinsed with deionized water and dried at 80 °C. The porosity of the sand was 0.37 as determined using the bulk density method²⁰.

A pair of glass chromatography columns (Kontes, Vineland, NJ) measuring 15 cm in length and 2.5 cm in diameter were vertically oriented and then wet-packed with the clean silica sands. Care was taken to eliminate the possibility of trapped air bubbles. Once packed, >30 pore volumes (PV) of appropriate background electrolyte (i.e., 1, 5, 20, 50 or 100 mM NaCl) was injected into the columns to equilibrate them. The downward flow was driven by gravity and the Darcy velocity was maintained at 0.31 cm/min using peristaltic pumps (MasterFlex, Vernon Hills, IL). The flow velocity that was employed in the column experiments is on the high end of natural groundwater flow, and is on the same order of magnitude as that encountered in riverbank filtration²¹.

Upon the completion of the equilibration step, the transport experiments were initiated by injecting the *E. coli* cell suspensions to the top of the columns and concentrations of the bacterial cells in the effluent were determined through measuring the absorbance at a wavelength of 220 nm using a Shimadzu UV-1700 spectrophotometer. The injection of *E. coli* cell suspension (~ 3.5 PV) lasted for 60 min, after which the columns were flushed with bacteria-free background electrolyte solution until the absorbance of effluent returned to the background values.

The clean-bed deposition rate coefficients (k_d) of the *E. coli* cells within the saturated sand packs were estimated from the steady-state breakthrough concentrations in the effluent²²⁻²⁴:

$$k_d = -\frac{v}{\varepsilon L} \ln \left(\frac{C}{C_0} \right) \quad (1)$$

where ε is porosity, v is the specific discharge, L is the column length and C/C_0 is the normalized breakthrough concentration relevant to clean-bed conditions, which was obtained from the average bacterial breakthrough concentrations between 1.8-2.0 PV^{22,24}.

The retained *E. coli* cells can be remobilized when the ionic strength of the solution is lowered²⁵. For each *E. coli* strain, upon the completion of the column experiments using 100 mM NaCl, the 1 mM NaCl solution was injected to the columns and the concentrations of the released *E. coli* cells were monitored similarly using the spectrophotometer. The results obtained were used to evaluate the reversibility of *E. coli* retention within the sand packs.

2.3 XDLVO calculations

The mobility of *E. coli* cells within the saturated sands is determined by the energy interactions between the cells and the surface of the sands. According to the XDLVO theory, the energy interactions between the *E. coli* cells and the surface of quartz sands are the summation of the Lifshitz-van der Waals (LW) interaction, the electrostatic double layer (EDL) interaction and the Lewis acid-base (AB) interaction:

$$\Phi^{\text{Total}} = \Phi^{\text{LW}} + \Phi^{\text{EDL}} + \Phi^{\text{AB}} \quad (2)$$

The LW, EDL and AB interaction energies (Φ^{LW} , Φ^{EDL} and Φ^{AB}) for the cell-sand (sphere-plate geometry) system can be calculated using the following equations²⁵⁻³²:

$$\Phi^{\text{LW}} = -\frac{Aa_b}{6h} \left[1 - \frac{5.32h}{\lambda} \ln \left(1 + \frac{\lambda}{5.32h} \right) \right] \quad (3)$$

$$\Phi^{\text{EDL}} = \pi \epsilon_0 \epsilon_w a_b \left\{ 2\psi_b \psi_s \ln \left[\frac{1 + \exp(-\kappa h)}{1 - \exp(-\kappa h)} \right] + (\psi_b^2 + \psi_s^2) \ln [1 - \exp(-2\kappa h)] \right\} \quad (4)$$

$$\Phi^{\text{AB}} = 2\pi a_b \lambda_w \Delta G_{h_0}^{\text{AB}} \exp \left(\frac{h_0 - h}{\lambda_w} \right) \quad (5)$$

where A is the Hamaker constant; a_b is the radius of the bacterial cells; λ is the characteristic wavelength and was set as 42.5 nm; h is the separation distance between the cell and sand surface; ϵ_0 is the dielectric permittivity of vacuum, ϵ_w is the dielectric constant

of water; κ^{-1} is the Debye length ($\frac{0.302}{\sqrt{I}}$ nm at 22°C, I =ionic strength); ψ_b and ψ_s are the surface potentials of the bacterial cells and sand, respectively; λ_w (= 0.6 nm) is the characteristic decay length of AB interactions in water; h_0 represents the minimum equilibrium distance between the cell and sand surface due to Born repulsion and equals to 0.157 nm; and $\Delta G_{h_0}^{\text{AB}}$ represents the hydrophobicity interaction free energies per unit area corresponding to h_0 .

The values of a_b , ψ_b , ψ_s , A and $\Delta G_{h_0}^{\text{AB}}$ were required for the interaction energy calculations. To determine cell sizes (a_b), images of the *E. coli* cells suspended in 1 and 100 mM NaCl were obtained using a Nikon Eclipse 50i microscope that was equipped with a Photometric CoolSnap ES digital camera and MetaMorph software. The length and width of ~ 30 cells were determined using the ImageJ software and the equivalent radii of the cells were

calculated as $\sqrt{\frac{L_c \times W_c}{\pi}}$, where L_c and W_c represent the length and width of the cell, respectively^{33, 34}. In this research, zeta potential values were used in place of surface potentials for the XDLVO calculations³⁵. *E. coli* cell suspensions were prepared in a similar fashion as the column transport experiments and the quartz sands were pulverized and the colloid-sized quartz particles were suspended in the NaCl solutions³⁶. The zeta potential values of the bacterial cells and quartz particles were then measured using a ZetaPALS analyzer (Brookhaven Instruments Corporation).

The Hamaker constants were calculated from the LW surface tension parameters of bacteria (γ_b^{LW}), Water (γ_w^{LW}) and sand (γ_s^{LW})³⁷:

$$A = 24\pi h_0^2 \left(\sqrt{\gamma_b^{\text{LW}}} - \sqrt{\gamma_w^{\text{LW}}} \right) \left(\sqrt{\gamma_s^{\text{LW}}} - \sqrt{\gamma_w^{\text{LW}}} \right) \quad (6)$$

The values of $\Delta G_{h_0}^{\text{AB}}$ can be obtained from the electron-accepting (γ^+) and electron-donating (γ^-) surface tension parameters³⁷:

$$\Delta G_{h_0}^{AB} = 2 \left[\sqrt{\gamma_w^+} \left(\sqrt{\gamma_b^-} + \sqrt{\gamma_s^-} - \sqrt{\gamma_w^-} \right) + \sqrt{\gamma_w^-} \left(\sqrt{\gamma_b^+} + \sqrt{\gamma_s^+} - \sqrt{\gamma_w^+} \right) - \sqrt{\gamma_b^- \gamma_s^+} - \sqrt{\gamma_b^+ \gamma_s^-} \right] \quad (7)$$

where the subscripts of b , w and s represent bacteria, water and sand, respectively. For water, the values of γ_w^{LW} , γ_w^+ and γ_w^- are 21.8, 25.5 and 25.5 mJ m⁻², respectively³⁸. For quartz sands, the previously reported values of γ_s^{LW} (39.2 mJ m⁻²), γ_s^+ (1.4 mJ m⁻²) and γ_s^- (47.8 mJ m⁻²) were used in this research³². To determine the values of γ_b^{LW} , γ_b^+ and γ_b^- for each *E. coli* strain, bacterial lawns were produced by filtering cells onto porous membrane, which was subsequently dried at room temperature for ~15 minutes. The contact angles (θ) of two polar and one non-polar probe liquids with known surface tension parameters on the bacterial lawns were measured using a Rame-Hart goniometer^{26, 32, 37}.

$$\gamma_i^L (1 + \cos \theta) = 2 \sqrt{\gamma_i^{LW} \gamma^{LW}} + 2 \sqrt{\gamma_i^+ \gamma^-} + 2 \sqrt{\gamma_i^- \gamma^+} \quad (8)$$

where the subscript i represents water ($\gamma^L=72.8$, $\gamma^{LW}=21.8$ and $\gamma^+=\gamma^-=25.5$ mJ m⁻²), glycerol ($\gamma^L=64.0$, $\gamma^{LW}=34.0$, $\gamma^+=3.92$ and $\gamma^-=57.4$ mJ m⁻²) or diiodomethane ($\gamma^L=50.8$, $\gamma^{LW}=50.8$ and $\gamma^+=\gamma^-=0$ mJ m⁻²)³⁷, respectively.

3. Results and Discussion

3.1 Column transport experiments

The normalized effluent *E. coli* concentrations under various ionic strength conditions are shown in Figure 1 (left panels). For all the *E. coli* strains, the increase in ionic strength led to lower breakthrough concentrations and lower recovery of bacterial cells in the effluent. For instance, the breakthrough concentrations (between 1.8-2.0 PV) of the wild type strain decreased from 87.0(±1.7)% to 11.2(±0.6)% when ionic strength increased from 1 mM to 100 mM. Accordingly, integration of the breakthrough curves showed that 86.3(±2.9)% and 12.8(±0.6)% of the wild type *E. coli* cells traveled through the sand columns under 1 and 100 mM NaCl conditions, respectively.

Upon the completion of the column experiments using 100 mM NaCl, the 1 mM NaCl solution was injected into the columns and the concentrations of the released *E. coli* cells were monitored (Figure 1, right panels). The pulse-type release of the previously retained *E. coli* cells led to extremely high cell concentrations in the effluent. Integration of the release curves showed that, at the end of the release experiments, 28.0(±2.6)%, 50.9(±7.8)% and 60.4(±0.5)% of the cells remained immobilized for the wild type, *tolC::kan* and $\Delta tolC$ *E. coli* strains, respectively. In comparison, the column experiments performed using 1 mM NaCl showed that ~13.7(±2.9)%, 9.4(±0.9)% and 11.7(±1.7)% of the wild type, *tolC::kan* and $\Delta tolC$ cells were retained, respectively. The fact that higher fractions of the cells remained retained following the release experiments suggested that cell immobilization was only partially reversible.

The clean-bed deposition rate coefficients (k_d) were calculated from the early breakthrough concentrations using equation (1) (Figure 2). Student's *t*-test performed using Microsoft Excel showed that the deposition rate coefficients were similar ($p>0.05$) for the *tolC::kan* and $\Delta tolC$ strains under all ionic strength conditions (1-100 mM NaCl). This indicated that the insertion of the kanamycin resistance cassette into *E. coli* chromosome and the gain of kanamycin resistance had little effects on the mobility of *E. coli* within saturated quartz sands. When the ionic strength was between 1-5 mM, the wild type *E. coli* strain also had similar k_d values as the *TolC*-deletion *E. coli* strains (Student's *t*-test, $p>0.05$). The removal

of OMP TolC, however, led to significant increase in values of k_d (i.e., decrease in *E. coli* mobility) when the ionic strength was between 20 and 100 mM (Student's *t*-test, $p < 0.05$). This finding was consistent to previously published results that suggested that OMP TolC could enhance the transport of *E. coli* isolated from various natural sources such as dairy manure^{6,7}. To elucidate the mechanisms behind the effects of OMP TolC on the mobility of *E. coli*, we characterized the *E. coli* cells and examined the energy interactions between the *E. coli* cells and quartz sands using the XDLVO theory.

3.2 Cell characterization

Results from the cell size measurements under 1 and 100 mM NaCl conditions suggested that ionic strength had minimal effects (Student's *t*-test, $p > 0.05$) on the size of the *E. coli* cells. For instance, the equivalent diameter of the wild type *E. coli* cells was $2.04(\pm 0.19)$ μm in 1 mM NaCl and $2.01(\pm 0.17)$ μm in 100 mM NaCl, respectively. For each *E. coli* strain, all the size measurements were thus pooled together and a single diameter value was calculated. The average sizes of the wild type, *tolC::kan* and ΔtolC strains were $2.04(\pm 0.19)$ μm , $1.98(\pm 0.22)$ μm and $2.02(\pm 0.17)$ μm , respectively. The results from Student's *t*-test indicated that the removal of OMP TolC and/or the presence of kanamycin resistance did not significantly alter cell size.

When suspended in 1, 5, 20, 50 and 100 mM NaCl (non-buffered, pH ~5.7), the zeta potential values of the *E. coli* cells were negative, suggesting that their surfaces were negatively charged (Figure 3). Given the slightly acidic pH of the cell suspensions, these negative charges should originate from the deprotonation of acid-base functional groups such as carboxylic and phosphoric groups³⁹. With the increase in ionic strength, the zeta potential values of the *E. coli* cells generally became less negative due to the compression of the electric double layer³⁴. Additionally, all three *E. coli* strains displayed comparable zeta potential values under the same ionic strength conditions (Student's *t*-test, $p > 0.05$). The OMP TolC is a 471-residue trimer that contains an α -helical domain, a mixed α/β domain and a β domain⁴⁰. The α -helical domain, which forms a tunnel through the periplasm, is anchored to bacterial wall. The β -barrel, which extends to the outside of the outer membrane, has a length of ~4 nm⁴⁰. Because the exterior of the β domain is largely non-polar⁴⁰, it is expected that the deletion of TolC from the outer membrane would not significantly alter cell surface charge and zeta potential. Similarly, because the kanamycin resistance in the *tolC::kan* strain is conferred by the Tn5 neomycin phosphotransferase, an aminoglycoside modifying enzyme that exists and functions inside the *E. coli* cell¹⁸⁴¹, the kanamycin resistance had little effect on cell zeta potential.

The results of the contact angle measurements are shown in Table 1. Student's *t*-test indicated that there was significant difference ($p < 0.05$) in the diiodomethane contact angle between the wild type and TolC-deletion *E. coli* strains. It was interesting to note that the wild type, which was able to produce the non-polar OMP TolC, had a higher contact angle of the non-polar diiodomethane than the two TolC-deletion mutants. Similar trend was observed in Ong et al.²⁶ and Johanson et al.⁴². Ong et al. observed *E. coli* D21f1, which lacks the charge-containing LPS, had a higher contact angle of diiodomethane than the LPS-producing *E. coli* D21²⁶. Johanson et al. reported that *Enterococcus faecium* that produced the hydrophobic enterococcal surface protein (*esp*) had a higher contact angle of diiodomethane than the corresponding *esp*-deletion mutant⁴². The values of γ_b^{LW} , γ_b^+ and γ_b^- for each *E. coli* strain were then calculated from the contact angle measurements using equation (8) (Table 1). These values, together with the zeta potential values and cell size measurements were then used to quantify the energy interactions between the *E. coli* cells and the quartz sands.

3.3 XDLVO energy interactions between *E. coli* cells and quartz sands

The values of $\Delta G_{h_0}^{AB}$ were 23.8 mJ m^{-2} , 26.4 mJ m^{-2} and 23.8 mJ m^{-2} and the Hamaker constants were $0.78 \times 10^{-21} \text{ J}$, $2.72 \times 10^{-21} \text{ J}$ and $2.92 \times 10^{-21} \text{ J}$ for the wild type, *tolC::kan* and ΔtolC *E. coli* strains, respectively (Table 1). The values $\Delta G_{h_0}^{AB}$ showed that the AB force between the *E. coli* cells and the surface of quartz sands was repulsive. Equation (6) suggests that the Hamaker constant is a function of the LW surface tension components of the bacterial cell, water and quartz sands. Because the electron-accepting (γ^+) and electron-donating (γ^-) surface tension components for diiodomethane are both zero, the LW surface tension parameter for each *E. coli* strain was calculated from equation (8) that corresponded to diiodomethane. The difference in the Hamaker constants thus was the result of the difference in the diiodomethane contact angles measured on the bacterial lawns of the three *E. coli* strains. The results obtained in this research indicated that variations in cell surface structures such as OMPs could alter cell surface tension components and subsequently the energy interactions between the cells and the surface of quartz sands. Similar relationship was reported in several previous publications^{26, 42}. Ong et al.²⁶, for instance, observed that the wild type *E. coli* D21 and its LPS-deletion mutant (strain D21f2) had different surface tension values. As a result, for four different types of surfaces (i.e., mica, glass, polystyrene and Teflon), the Hamaker constants for strain D21 were approximately two times the magnitude of the Hamaker constants for strain D21f2. Johanson et al.⁴² reported that for *Enterococcus faecium*, the removal of the surface protein *esp* increased the Hamaker constant for the cell-water-quartz system from $4.2 \times 10^{-21} \text{ J}$ to $4.8 \times 10^{-21} \text{ J}$ and the value of $\Delta G_{h_0}^{AB}$ from 24.1 to 31.4 mJ m^{-2} .

The XDLVO energy interactions between the *E. coli* cells and the surface of quartz sands were calculated and shown in Figure 4. For all the ionic strength conditions, there existed sizeable energy barriers for the attachment of *E. coli* cells to quartz sands. The magnitude of the energy barrier was comparable for all three *E. coli* strains under the same ionic strength condition, but decreased from $\sim 6.4 \times 10^4 \text{ kT}$ to $\sim 5.4 \times 10^4 \text{ kT}$ when the ionic strength increased from 1 mM to 100 mM. The presence of the substantial energy barriers made it difficult for the *E. coli* cells to be deposited into the primary energy minimum and the immobilization of the *E. coli* cells should thus occur primarily through their entrapment within the secondary energy minimum^{25, 34, 38, 43}.

When the ionic strength was within 1-5 mM, the three *E. coli* strains shared similar XDLVO energy interaction profiles (Figures 4A and 4B). For the higher ionic strength range (20-100 mM), the XDLVO calculations showed that the two TolC-deletion strains had similar interaction energy profiles; and their secondary energy minimum was always deeper than the wild type strain. For each *E. coli* strain, the depth of the secondary energy minimum also increased with ionic strength. The XDLVO calculations were thus consistent to the experimental observations that i) the three *E. coli* strains had similar mobility under the low ionic strength conditions; ii) the TolC-deletion strains had higher deposition rates than the wild type strain under high ionic strength conditions; and iii) the deposition of the *E. coli* cells increased with ionic strength (Figure 2). Overall, our data suggested that the deposition rate coefficients (k_d) were negatively related to the depth of the secondary energy minimum.

The XDLVO calculations predicted the absence of the secondary minimum for the ionic strength of 1 mM NaCl (Figure 4A). Inspection of the XDLVO interaction energy profiles revealed that the lowest point was $\sim 0.5 \text{ kT}$. In theory, this indicated that there should be no cell immobilization at the secondary energy minimum. The experimental results showed that the transport of the *E. coli* cells was not conservative and $\sim 12\%$ of the *E. coli* cells were immobilized. Similar discrepancies between the interaction energy calculations and particle

transport results were previously reported^{29, 44}. For instance, Farahat et al. observed that *E. coli* cells could attach to quartz under pH > 4.5 when the XDLVO energy calculations showed the absence of secondary energy minimum²⁹. A wide range of factors such as heterogeneity in cell properties (e.g., the zeta potential of some cell might be less negative than the average values), charge heterogeneity and roughness on the surface of quartz sands, XDLVO forces as well as flow hydrodynamics could have contributed to the deposition of a fraction of *E. coli* cells when the average XDLVO profile predicted the absence of secondary energy minimum⁴⁵⁻⁴⁸.

The XDLVO calculations were also consistent to the results obtained from the release experiments. When the ionic strength was lowered from 100 mM NaCl to 1 mM, the depth of the secondary energy minimum decreased and a fraction of the *E. coli* cells that were previously retained was remobilized²⁵.

Compared to the DLVO theory, the extra force that is considered by the XDLVO theory is the AB force (equation 5). In this research, the AB force was repulsive and the inclusion of this force significantly increased the magnitude of the energy barrier. However, because the AB force decreased more rapidly with the separation distance than the LW and EDL forces, and because the secondary energy minimum was generally located 7 nm or further away from the sand surface, the AB force has very small effects on the depth of the secondary energy minimum. For *tolC::kan*, the depth of the secondary energy minimum was -7.34 kT and -7.57 kT for the XDLVO theory and DLVO theory, respectively.

For Gram-negative bacteria such as *E. coli*, outer membrane structures such as LPS and proteins can exert steric interactions for the cell-surface system^{34, 49}. Such steric interactions are not considered by the XDLVO calculations and can significantly influence the transport behavior of bacterial cells within porous media³⁴. The *E. coli* K12 strain that was used in this study did not express the O-antigen of the LPS and the length of the LPS was estimated as 3 ± 2 nm⁴⁹. The length of OMP TolC that is extended to the outside of the membrane is ~ 4 nm⁴⁰. The XDLVO calculations showed that the secondary energy minimum was usually located >7 nm from the surface of the sands (Figure 4). Therefore, the steric forces could increase the magnitude of the energy barrier, but should have negligible impact on the location and depth of the secondary minimum. This is confirmed by the calculation of the steric forces using the formula that was derived by Wang et al.³⁴ (Figure S1).

3.4 Environmental implications

The extensive use of antibiotics in both food animal production and human medicine has led to the selection of antibiotic-resistant bacteria in manure and wastewater⁵⁰⁻⁵³. Many previous studies showed that the improper storage, management and use of wastewater and manure could lead to the contamination of groundwater by a variety of bacteria that were resistant to the major lines of antibiotics that are currently being used to treat human diseases^{54, 55}. The spread of antibiotic resistant bacteria in the environment is raising serious public health concerns across the world^{56, 57}.

Drug efflux pumps represent one of the most effective and widespread mechanisms for antibiotic resistance in bacterial cells⁵⁸. Particularly, efflux pumps that span the cytoplasm, periplasm and outer membrane could transport antibiotics to the outside environment and therefore lead to bacterial resistance against high levels of antibiotics. The OMP TolC was reported to be the outer membrane component of several common efflux pumps (e.g., the AcrAB-TolC pump) that are responsible for bacterial resistance to various antibiotics such as tetracycline, macrolide and ampicillin¹²⁻¹⁵. It was observed that the total dissolved solid (TDS) of groundwater that was influenced by manure storage and application was often

1000 mg/L or higher (20 mM NaCl is equivalent to a TDS of 1170 mg/L)⁵⁹. Findings from this research suggested that antibiotic resistant bacteria with the OMP TolC could have higher mobility and display wider spread within sandy aquifers influenced by manure.

Several recent publications highlighted the diversity in the transport behavior of bacterial isolates obtained from various environmental sources within saturated porous media^{7, 60-62}. Such variations pose challenges to the modeling and prediction of the spread of bacteria within the groundwater system. There is a growing body of evidence, including the findings from this research, which suggests that cell surface structures such as LPS and OMP can have significant impact on the mobility of bacterial cells within aquifer materials^{6-8, 35, 42, 63}. It is likely that the mechanisms underlying the variations in the transport of environmental bacterial isolates are related to the variations in the cell surface structures⁸. The improved understanding of the relationship between the surface structure of bacterial cells and their attachment to natural mineral surfaces could enable us to better assess the public health risks associated with groundwater microbial contamination.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Breakthrough curves of (A) wild type, (B) *tolC::kan* and (C) $\Delta TolC$ *E. coli* cells suspended in 1, 5, 20, 50 and 100 mM NaCl, respectively (left panels). C represents *E. coli* concentrations in the effluent and C_0 represents influent *E. coli* concentrations. Upon the completion of the 100 mM NaCl experiments, the 1 mM NaCl solution was injected to the columns and the release kinetics of the previously retained bacterial cells are shown in the right panels.

Figure 2.

Average deposition rate coefficients (k_d) for the three *E. coli* strains under ionic strength conditions of 1, 5, 20, 50 and 100 mM. The error bars, which represent the standard deviations, are usually smaller than the symbols.

Figure 3.

Zeta potential values of the *E. coli* cells and the quartz sands in 1, 5, 20, 50 and 100 mM NaCl. Error bars represent the standard deviation of a minimum of 5 measurements.

Figure 4.

The calculated XDLVO interaction energy profiles as a function of separation distance for (A) 1 mM, (B) 5 mM, (C) 20 mM, (D) 50 mM and (E) 100 mM ionic strength conditions. The interaction energy was expressed in kT , where k is the Boltzmann constant, and T is absolute temperature in Kelvin. Figure insets show the secondary energy minimum.

Table 1

Contact angle measurements, surface tension components, Hamaker constant (A) and $\Delta G_{h_0}^{AB}$ for the three *E. coli* strains. Numbers in parentheses are the standard deviations of the contact angle measurements.

Properties		Wild type	<i>tolC</i> strain	$\Delta tolC$
Contact angle ($^\circ$) (n = 4)	Water	18.7($\pm$ 1.4)	19.0($\pm$ 3.0)	19.6($\pm$ 1.5)
	Glycerol	21.4($\pm$ 8.4)	25.9($\pm$ 2.0)	20.6($\pm$ 2.8)
	Diiodomethane	67.4($\pm$ 3.9)	55.4($\pm$ 8.7)	54.1 ($\pm$ 2.8)
Surface tension components (mJ m ⁻²)	γ_b^{LW}	24.33	31.2	32.0
	γ_b^+	6.51	3.63	4.3
	γ_b^-	47.93	48.3	44.8
Hamaker constant, A (10 ⁻²¹ J)		0.78	2.72	2.92
$\Delta G_{h_0}^{AB}$ (mJ m ⁻²)		23.8	26.4	23.8