Diversity in Cell Properties and Transport Behavior among 12 Different Environmental *Escherichia coli* Isolates

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Escherichia coli is a commonly used indicator organism for detecting the presence of fecal-borne pathogenic microorganisms in water supplies. The importance of *E. coli* as an indicator organism has led to numerous studies looking at cell properties and transport behavior of this microorganism. In many of these studies, however, only a single strain of *E. coli* was used even though research has shown that significant genetic variability exists among different strains of *E. coli*. If this genetic diversity results in differences in cell properties that affect transport, different strains of *E. coli* may exhibit different rates of transport in the environment. Therefore, the objectives of our study were to investigate the variability in surface characteristics and transport behavior of *E. coli* isolates obtained from six different sources: beef cattle, dairy cattle, horse, human, poultry, and wildlife. Cell properties such as electrophoretic mobility, cell size and shape, hydrophobicity, charge density, and extracellular polymeric substance composition were measured for each isolate. In addition, the transport behavior of each isolate was assessed by measuring transport through 10-cm packed beds of clean quartz sand. Our results show a large diversity in cell properties and transport behavior for the different *E. coli* isolates. This diversity in transport behavior must be taken into account when making assessments of the suitability of using *E. coli* as an indicator organism for specific pathogenic microorganisms in groundwater environments as well as modeling the movement of *E. coli* in the subsurface.

The land-application of human and animal wastes can result in infiltration into the subsurface of pathogenic microorganisms such as bacteria, viruses, and protozoa. Once in the subsurface, fecal-borne pathogenic microorganisms may pose a threat to public health if they reach groundwater drinking supplies (Unc and Goss, 2004). Indeed, recent studies have shown that nearly half of all the drinking water wells tested in the United States have been contaminated by fecal material (Macler and Merkle, 2000). Common sources of fecal pathogens found in the environment include faulty septic systems; land-application of biosolids; leaking sewer lines; and agricultural activities such as the use of manure for crop fertilization, pasturing of livestock, animal feeding operations, and leaky manure storage lagoons (Gerba and Smith, 2005; Macler and Merkle, 2000).

Because testing for specific pathogenic microorganisms is both costly and time consuming, these organisms are rarely targeted for measurement. Rather, most agencies responsible for monitoring water supplies test for the presence of nonpathogenic microorganisms commonly found in fecal material such as total coliforms, fecal coliforms, *E. coli*, or enterococci (Leclerc and Mosel, 2001). The presence of these microorganisms indicates that the water may be contaminated with fecal material and therefore could pose a risk to human health. The importance of *E. coli* as an indicator organism has led to several studies looking at cell properties and transport behavior of individual strains of this important microorganism (Bolster et al., 2006; Bradford et al., 2006; Castro and Tufenkji, 2007; Jiang et al., 2007; Levy et al., 2007). A potential limitation to these studies, however, is that research has shown that significant genetic variability exists among strains of *E. coli* isolated from different host species (Carson et al., 2001; Dombek et al., 2000) and even from the same host species (Galland et al., 2001; Kudva et al., 1997; Vali et al., 2004). If these genetic differences result in differences in surface characteristics that affect transport, different strains of *E. coli* may exhibit different rates of transport in the environment. Indeed, Yang et al. (2004) observed a wide range in cell motility for 280 different *E. coli* strains isolated from a bovine feedlot. Transport experiments with two of these isolates later showed important differences in transport behavior (Morrow et al., 2005). We have also observed variations in surface characteristics and transport behavior between two *E. coli* strains isolated from two different sources in response to various starvation periods (Haznedaroglu et al., 2008).

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Given the importance of understanding *E. coli* transport through porous media, further investigations are needed to look at the range in cell properties and transport behavior for different strains of *E. coli*. Therefore, the objectives of our study were to investigate the variability in cell properties and transport behavior of *E. coli* isolates obtained from six different sources: beef cattle, dairy cattle, horse, human, poultry, and wildlife. Cell properties such as electrophoretic mobility, cell size and shape, hydrophobicity, charge density, and extracellular polymeric substance composition were measured for each isolate. In addition, transport behavior of each isolate was assessed by measuring *E. coli* transport through 10-cm packed beds of clean quartz sand. The results of this study will help determine the potential variability in cell properties and transport behavior of *E. coli*, an important indicator of water quality.

Materials and Methods

Bacteria Preparation

Escherichia coli isolates used in this study were kindly provided by Dr. Sloane Ritchey (Eastern Kentucky University). Isolates were obtained for poultry (PL), horse (HO), beef cattle (BC), and dairy cattle (DC) from positively identified fecal samples combined from several animals located at Spindletop Farm, Lexington, KY. Presumed human (HU) isolates were obtained from raw sewage samples. Isolates were also collected from a stream with no known human impact and were assumed to represent wildlife (WI). Two isolates from each source were used in this study as indicated by the number following the acronym (e.g., HU1 and HU2). Sample collection and isolation procedures were followed as described by Turco (1994). Stool samples were shaken for 10 to 30 min on a reciprocal shaker in a phosphate buffer and serially diluted in physiological saline as necessary. The extracts were filtered onto a 0.45-μm grid membrane filter and the filters were incubated on mFC agar (Becton, Dickinson and Company, Sparks, MD) at 44.5°C overnight. Cobalt blue colonies were selected and subsequently plated on EC-MUG (Becton, Dickinson and Company) media to confirm that they were *E. coli*. For the raw sewage and wildlife samples, 1 to 10 mL of the water samples was filtered directly onto 0.45-μm membrane filters. Plating and incubation procedures were performed as mentioned above. All isolates were stored at –80°C in Luria-Bertani (LB) broth (Fisher Scientific, Fair Lawn, NJ) with 15% glycerol added.

On the day before experimental analysis, the *E. coli* isolates were cultured in LB broth at 37°C until reaching mid-exponential growth phase (4 h) and harvested. A refrigerated bench-top centrifuge equipped with fixed angle rotor was used to pellet the cells with an applied 6000-times *g*-force for 15 min at 4°C. The cell pellet was re-suspended in 0.01 mol L^{-1} KCl solution prepared with deionized (DI) water and reagent-grade KCl (Fisher Scientific) with no pH adjustment (pH 5.6–5.8). This process was repeated twice to secure the removal of the growth medium. Cells were allowed to adjust to the KCl solution for 18 h before each experiment. Identical protocols for cell preparation were followed in both the cell characterization and transport experiments.

Cell Properties

The electrophoretic mobility of bacterial cells was determined by diluting the incubated and rinsed cell pellet to an optical density of 0.20 to 0.25 in 0.01 mol L^{-1} KCl solution at 546 nm wavelength. Measurements were conducted at 25°C using a zeta potential analyzer (Brookhaven Instruments Corporation, Holtsville, NY). Measurements were repeated 10 times and averaged values were reported. The experimentally determined electrophoretic mobility values were converted to zeta potential values using the Smoluchowski equation (Elimelech et al., 1995).

For size measurement analysis, phase contrast images were taken using an inverted microscope (IX 70; Olympus, Japan) and camera at an approximate concentration of 10^8 cells mL^{-1} in 0.01 mol L^{-1} KCl solution. Images containing more than 40 cells were processed by a software package (SimplePCI, Precision Instruments Inc., Minneapolis, MN) and individual cell lengths and widths were determined. Measured values were used to calculate cell sphericity (width/length).

Hydrophobicity analysis of the cells was done by the microbial adhesion to hydrocarbon (MATH) test as described elsewhere (Pembrey et al., 1999). Briefly, the partitioning of cells between a hydrocarbon, n-dodecane (laboratory grade, Fisher Scientific), and the electrolyte solution (0.01 mol L^{-1} KCl) was determined spectroscopically. The relative hydrophobicity of each cell type was quantified as the percent of total cells partitioned into the hydrocarbon phase.

Potentiometric titrations of cells were conducted to determine the relative acidity and surface charge density of the bacterial surfaces. An auto-titrator (798 MPT Titrino, Metroohm, Switzerland) was used to determine the amount of base consumed by suspended cells during the titration between pH 4 and 10. Bacterial suspensions with concentrations of 3×10^8 cells mL^{-1} to 3.2×10^9 cells mL^{-1} in 0.01 mol L^{-1} KCl were titrated by concentrated NaOH (0.3 M). Surface charge density was calculated from the resultant acidity values (Shim et al., 2002; Walker et al., 2005).

The extracellular polymeric substance (EPS) composition, specifically the total protein and the polysaccharide content, was analyzed based on an established extraction method (Azeredo et al., 1999; Chen and Walker, 2007). Briefly, the pellet of harvested bacterial cells was suspended in formaldehyde-NaCl solution followed by various centrifugation steps and final exposure to cold ethanol-KCl solution. The analysis of protein was performed using the Lowry method (Switzer and Garrity, 1999) with Bovine Serum Albumin (BSA, 1 mg mL^{-1}; Fisher BioReagents, Fisher Scientific) as the standard. Samples were measured spectroscopically (BioSpec-mini, Shimadzu Corp., Kyoto, Japan) at a wavelength of 500 nm. The analysis of total sugars was performed using the phenol-sulfuric acid method described previously (Dubois et al., 1956) with Xanthan gum (Practical Grade, Fisher Scientific) as the standard. Samples were measured spectroscopically at a wavelength of 488 nm.

Column Experiments

Transport experiments were conducted through water-saturated columns packed with clean quartz sand (Unimin, New

Caanan, CT). Before column packing, the quartz sand was sifted through 350-µm and 250-µm sieves (U.S.A. Standard Testing Sieves, ATM Corp., New Berlin, WI). The sand was then boiled in a 2-L flask containing 1 mol L^{-1} hydrochloric acid (Fisher Scientific) for 2 h and then rinsed with DI water until the rinse water pH was ~5.6. The sand was then dried in an oven overnight at 105°C, re-rinsed in DI water the following day, and dried again overnight in an oven. Finally, the sand was sterilized by autoclaving at 121°C and 15 psi for 20 min then rehydrated by boiling in filtered DI water for 2 h.

Columns were wet packed with the clean, autoclaved sand into 4.8-cm diameter Chromaflex Chromatography Columns (Kontes Glass Co., Vineland, NJ) filled with the 0.01 mol L^{-1} KCl electrolyte solution. Porosity was 0.35 ± 0.015 as determined gravimetrically. Column lengths ranged from 9.9 to 10.1 cm. After packing was completed, columns were operated in a downward direction using a peristaltic pump, and approximately 10 pore volumes of the electrolyte solution were passed through each column to equilibrate the sand pack. Columns were operated at a flow rate of 1 mL min^{-1} for a Darcian velocity of ~0.16 m d^{-1}. All column experiments were run in duplicate. A 1.0-pore-volume pulse of *E. coli* with an optical density of 0.20 to 0.22 (~1 × 10^8 cells mL^{-1}) was injected at the top of each column, followed by bacteria-free electrolyte solution. Effluent was collected every 5 min using a Spectra/Chrom CF-1 fraction collector (Spectrum Chromatography, Houston, TX). Concentrations of *E. coli* were determined by measuring the optical density of the samples at a wavelength of 546 nm with a UV-visible spectrophotometer (BioSpec-mini, Shimadzu, Kyoto, Japan). Bromide was added in several columns as a conservative tracer so that a dispersion coefficient for the columns could be determined. The breakthrough curve of Br was nearly identical in all columns indicating similar packing throughout the experiments.

Model Development

Numerous models exist for describing the transport of bacteria through one-dimensional laboratory columns. Preliminary modeling of our breakthrough curves (BTCs) revealed that the commonly used first-order deposition model was inadequate for describing the observed transport behavior of the bacteria through our laboratory columns. Therefore, we used a second-order, or time-dependent, deposition model which assumes that only a finite number of bacterial deposition sites exist and that as these sites fill, deposition rates decrease (Bolster et al., 1999b):

$$\frac{\partial C}{\partial t}+\frac{\partial S}{\partial t}=D\frac{\partial^2 C}{\partial x^2}-v\frac{\partial C}{\partial x} \quad [1]$$

$$\frac{\partial S}{\partial t}=k_1\psi C-k_2 S \quad [2]$$

$$\psi=\frac{S_{\max}-S}{S_{\max}} \quad [3]$$

where C is the reduced concentration of bacteria in the aqueous phase ($C = c/c_0$ where c is the aqueous bacterial concentration [cells mL^{-1}] and c_0 is the initial concentration of cells [cells mL^{-1}] applied to the column), S is the reduced concentration of deposited bacteria in the column ($S = s\rho_b/\theta c_0$ where s is the concentration of deposited bacteria in the column [cells g^{-1}], ρ_b is the bulk density of the sediments [g cm^{-3}], and θ is porosity), x is the length of the column (cm), D is the hydrodynamic dispersion coefficient (cm^2 h^{-1}), v is the interstitial pore water velocity (cm h^{-1}), k_1 is the bacterial deposition rate (h^{-1}), k_2 is the entrainment rate (h^{-1}), ψ is the fraction of sites available for bacterial deposition, and S_{max} is the maximum bacterial retention capacity of the sediments. Equations [1] through [3] were solved by the predictor-corrector finite-difference method (Remson et al., 1971) for a semi-infinite column with a flux-averaged inlet boundary condition (Bolster et al., 1999a; Parker and van Genuchten, 1984). The transport model was fit to BTC data using Levenberg-Marquardt least-squares regression (Seber and Wild, 2003).

Model fits were assessed by calculating the model efficiency, E (Nash and Sutcliffe, 1970):

$$E=1-\frac{\sum_{i=1}^{n}(r_i)^2}{\sum_{i=1}^{n}(C_i-C_{avg})^2} \quad [4]$$

where r_i is the ith residual between model prediction and observation, C_i is the ith measured reduced concentration of bacteria in the effluent, and C_{avg} is the average of the observed effluent concentrations. A model efficiency of 1 means that the model is a perfect fit to the data whereas an E value less than zero means that the model provides a poorer fit to the data than if the data were predicted based solely on the average of the measured data.

Statistical Analysis

One-way analysis of variance (ANOVA) was conducted to identify statistically significant differences in cell properties and the percent of injected bacteria eluted from the 10-cm sand columns. Mean separations were performed using Fisher's least significant difference (LSD) method. Correlation coefficients were calculated between measured cell properties, percent recovery of bacteria, and fitted model parameters. All statistical analyses were performed using SAS Version 9.1 (SAS Institute, 2003) and differences were considered significant at $p < 0.05$.

Results

A large range in cell properties was observed for the different *E. coli* strains, with many of the differences being statistically significant (Table 1). Hydrophobicity, as measured by the MATH assay, ranged from 0.6% for HU2 to 61% for HO1. Zeta potential ranged from –3.5 mV for BC1 to –49 mV for WI2, whereas surface charge density ranged from 9.6 × 10^2 µC cm^{-2} for DC1 to 1.3 × 10^4 µC cm^{-2} for WI1. Total protein content of the EPS ranged from 7.4 mg per 10^{10} cells for HO1 to 11.7 mg per 10^{10} cells for BC1. Total sugar content of the EPS ranged from 0.074 mg per 10^{10} cells for DC2 to 0.22 mg per 10^{10} cells for HU1. Cell size was also shown to vary.

Table 1. Measured values of the percent bacteria recovered in the 10-cm column transport experiments and selected cell surface properties (standard deviations are in parentheses).

Isolate†	Percent recovery	Hydrophobicity	Zeta potential	Length	Width	EPS protein	EPS sugar	Surface charge density
		%	mV	———μm———		———mg/10^{10} cell———		μC cm^{-2}
BC1	95a (0.05)	46b (4.8)	−3.5a (1.5)	1.6b,c (0.39)	0.88b (0.15)	12c (0.90)	0.18d,e (0.0023)	2363
BC2	44d (7.6)	27c (11)	−29e (2.1)	1.6b,c (0.48)	0.72c (0.17)	7.9e,f (0.78)	0.11h (0.024)	2938
DC1	34e,f (0.66)	30c (3.1)	−10c (2.4)	1.7a,b (0.63)	0.86b (0.20)	17b (0.0048)	0.13g (0.0022)	955
DC2	34e,f (0.083)	46b (10)	−6.8b (1.3)	1.6b,c (0.34)	0.67c,d (0.15)	8.1e,f (0.0030)	0.074i (0.0024)	3926
HO1	61c (0.67)	61a (1.3)	−40f (2.9)	1.5b,c,d (0.48)	0.67c,d (0.16)	7.4f (0.19)	0.11h (0.00026)	5329
HO2	1.7h (0.86)	56a (7.1)	−24d (4.2)	1.7a,b (0.63)	0.62d,e (0.20)	12c (0.15)	0.17f (0.0019)	5103
HU1	79b (2.6)	17d (1.4)	−39f (4.9)	1.9a (0.94)	0.97a (0.23)	9.4d,e,f (0.17)	0.22c (0.00092)	2555
HU2	39d,e 1.9)	0.57e (0.25)	−48g,h (3.9)	1.5b,c,d (0.43)	0.63d,e (0.16)	7.8e,f (0.078)	0.18d (0.00038)	2478
PL1	41d,e (7.9)	2.0e (0.50)	−30e (3.2)	1.3d (0.26)	0.73c (0.15)	10c,d,e (0.29)	0.15g (0.00012)	982
PL2	44d (0.10)	2.4e (0.50)	−30e (2.2)	1.5c,d (0.45)	0.71c (0.12)	11c,d (0.0047)	0.17e,f (0.00014)	2879
WI1	17g (2.3)	4.9e (0.41)	−46g (4.8)	1.5b,c,d (0.42)	0.57e (0.21)	16b (0.56)	0.27b (0.0013)	13,287
WI2	26f (6.0)	5.3e (0.41)	−49h (1.9)	1.4c,d (0.42)	0.58e (0.19)	49a (4.4)	1.0a (0.0060)	7978

† BC1, beef cattle 1; BC2, beef cattle 2; DC1, dairy cattle 1; DC2, dairy cattle 2; HO1, horse 1; HO2, horse 2; HU1, human 1; HU2, human 2; PL1, poultry litter 1; PL2, poultry litter 2; WI1, wildlife 1; WI2, wildlife 2.

‡ Within each column, values with the same letter are not significantly different at the $p = 0.05$ level.

Cell length varied from 1.3 μm to 1.9 μm, for isolates PL1 and HU1, respectively, whereas cell width ranged from 0.57 μm to 0.97 μm for isolates WI1 and HU1, respectively.

In many cases, statistically significant differences in cell properties were observed for the two isolates obtained from the same source. For instance, within-host variability in hydrophobicity was statistically significant for beef cattle, dairy cattle, and human isolates (Table 1). Within-host differences in zeta potential were statistically significant for all isolates except for poultry. For EPS protein content, within-host variations were statistically significant for the beef cattle, dairy cattle, horse, and wildlife isolates, whereas within-host differences for EPS sugar content were statistically different for all six hosts used in this study. Statistical comparisons for surface charge density were not performed because duplicate data were not collected.

Correlations between the different cell properties measured in this study were generally low and not statistically significant (Table 2). Of the statistically significant correlations observed in this study, nearly all were between properties which had shared parameters so the correlations were inflated artificially. For instance, cell sphericity is calculated from cell width whereas surface charge density is calculated from cell width and length. Therefore, the only meaningful correlation between cell properties is the strong inverse correlation between zeta potential and the EPS sugar to protein ratio (r = -0.751; p = 0.0048).

In addition to cell properties, a wide range in transport behavior among the different *E. coli* isolates was observed (Fig. 1, Table 1). Percent recovery of cells eluted from the columns ranged from 1.7% for HO2 to 95% for BC1, with many of the differences being statistically significant. Like cell properties, statistically significant differences ($p < 0.05$) in percent recovery were observed within some host types. For instance, while only 1.7% of HO2 cells were recovered, 61% of HO1 cells were recovered from the 10-cm sand columns ($p < 0.05$). For the beef cattle isolates, percent recovery ranged from 44 to 95% ($p < 0.05$). On the other hand, percent recoveries observed for the poultry and dairy cow isolates were not statistically significant from each other. The percent recovery of bacteria was significantly correlated with cell width (r = 0.742; p = 0.0058) and cell sphericity (r = 0.736; p = 0.0063) but not with any other cell property ($p > 0.05$).

Like percent recovery, the shape of the BTCs varied sharply among isolates. Distinct shouldering was observed in the BTC for HU1, HU2, and WI2, whereas this behavior was not observed in the remaining nine isolates. Breakthrough of the bacteria occurred concurrently with that of the conservative tracer for two isolates (BC1 and HU1) whereas breakthrough for the remaining 10 isolates was delayed compared to the conservative tracer. Despite this large variability in BTC behavior, the second-order deposition model provided good fits to all BTCs, with model efficiencies ranging from 0.959 to 0.999 (Fig. 1; Table 3). (Effluent data for HO2 were not fit with the model due to poorly defined BTC data for this organism.) Fitted values for k_1 ranged from 0.15 to 7.5 h^{-1} for BC1 and WI1, respectively, whereas for k_2, fitted values ranged from 0.026 to 3.0 h^{-1} for WI1 and BC1, respectively. Values for S_{max} ranged from 0.25 to 4.5 for HU1 and BC1, respectively. The only statistically significant correlation between fitted model parameters and cell properties was the correlation between k_1 and cell width (r = -0.602; p = 0.050). (Due to the large uncertainty associated with fitted S_{max} values for BC1 (Table 3), these values were excluded from the correlation analyses.)

Discussion

Although each *E. coli* isolate was subjected to the exact same storage and growth conditions, we observed significant variability in measured cell properties, bacterial recovery, and fitted transport parameters for the different isolates and, in several cases, between *E. coli* isolates obtained from the same host type. Yang et al. (2004) reported finding 89 distinct operation taxonomic units from 280 different *E. coli* isolates obtained from a bovine feedlot. They also observed a broad range in cell motility between these isolates, though no correlation between genotypic and phenotypic characteristics was observed. In a follow-up

Table 2. Correlation matrix for percent recovery, cell properties, and model parameters obtained by fitting the column breakthrough data with the second-order deposition model. Statistically significant ($p \leq 0.05$) correlations are in italic.

	Percent recovery	Hydrophobicity	Zeta potential	Length	Width	Sphericity (W/L)	Ratio (sugar/protein)	Surface charge density	k_1	k_2
Hydrophobicity ($n = 12$)	0.150									
Zeta potential ($n = 12$)	0.235	0.545								
Length ($n = 12$)	0.193	0.387	0.203							
Width ($n = 12$)	***0.742***	0.129	0.467	0.561						
Sphericity ($n = 12$)	***0.736***	−0.0889	0.450	−0.0243	***0.811***					
Ratio ($n = 12$)	0.223	−0.493	***−0.751***	0.048	−0.032	−0.106				
Surface charge density ($n = 12$)	−0.443	−0.107	−0.492	−0.148	***−0.637***	***−0.691***	0.194			
k_1 ($n = 11$)	***−0.725***	−0.097	−0.163	−0.420	***−0.602***	−0.446	−0.472	0.347		
k_2 ($n = 11$)	***0.747***	0.395	0.519	0.049	0.437	0.532	−0.010	−0.196	***−0.622***	
S_{max} ($n = 10$)	***−0.946***	−0.293	0.128	−0.401	−0.581	−0.450	−0.345	0.418	0.618	***−0.878***

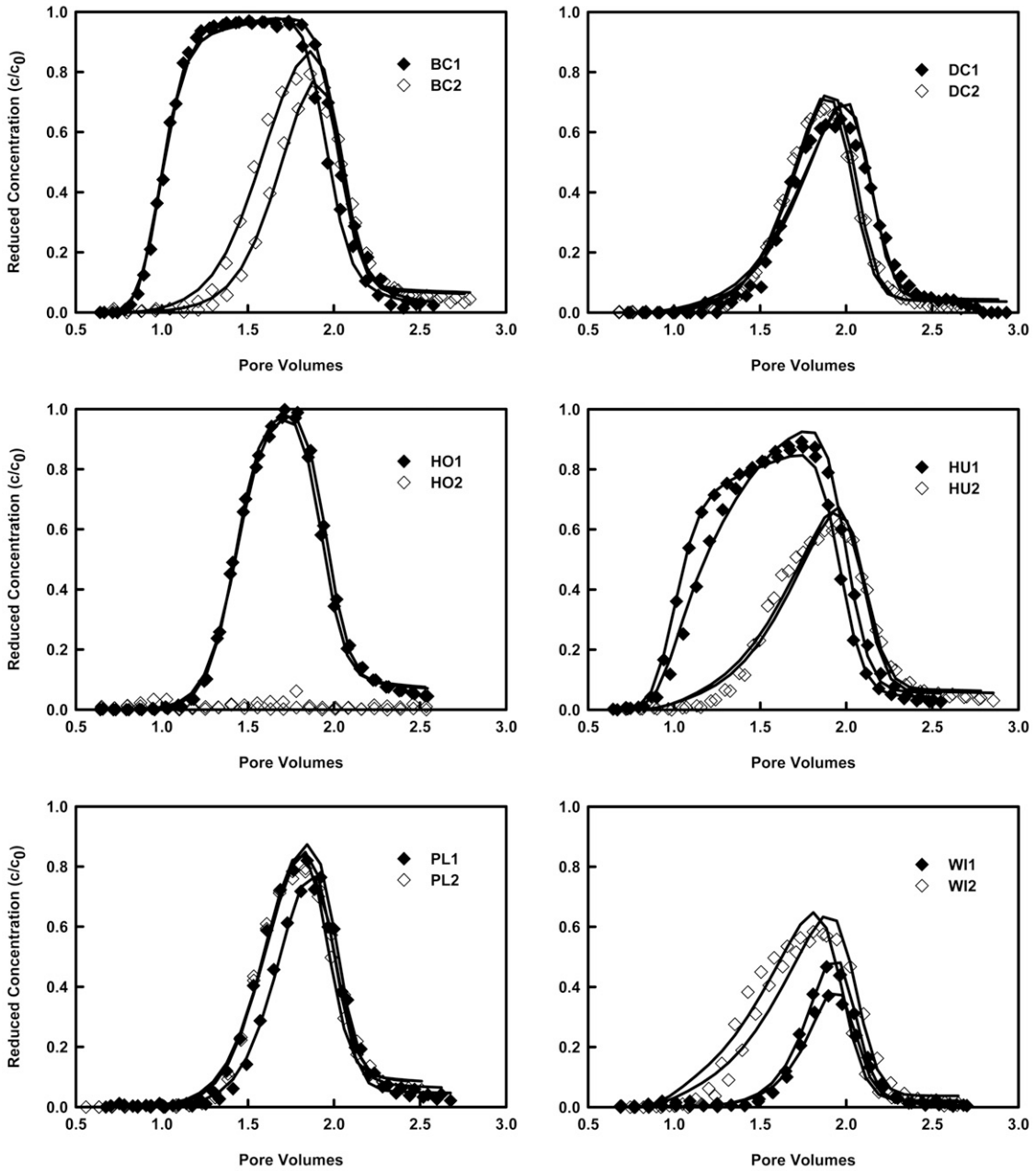

Fig. 1. Observed breakthrough data (symbols) and model fits (solid line) for 12 environmental *E. coli* isolates. Breakthrough curve (BTC) data for HO2 were not modeled because the BTC was poorly defined for this organism.

Table 3. Fitted parameter values and model efficiencies (*E*) for replicate (a and b) columns. Standard errors are in parentheses.

	k_1		k_2		S_{max}		E	
Isolate	**a**	**b**	**a**	**b**	**a**	**b**	**a**	**b**
BC1	0.15 (0.057)	0.15 (0.040)	3.0 (1.6)	2.8 (1.1)	4.5 (186)	1.5 (14)	0.996	0.998
BC2	4.0 (0.31)	5.8 (0.34)	0.13 (0.035)	0.14 (0.022)	0.55 (0.011)	0.70 (0.008)	0.985	0.990
DC1	4.5 (0.38)	4.9 (0.51)	0.069 (0.026)	0.072 (0.033)	0.77 (0.014)	0.77 (0.017)	0.973	0.961
DC2	4.7 (0.27)	5.3 (0.31)	0.055 (0.018)	0.061 (0.018)	0.68 (0.0078)	0.72 (0.0080)	0.990	0.989
HO1	5.8 (0.25)	5.4 (0.22)	0.24 (0.027)	0.24 (0.026)	0.41 (0.0041)	0.41 (0.0042)	0.998	0.998
HO2	ND	ND	ND	ND	ND	ND	ND	ND
HU1	1.0 (0.074)	0.4 (0.018)	0.24 (0.067)	0.21 (0.030)	0.25 (0.011)	0.25 (0.0089)	0.994	0.999
HU2	3.7 (0.30)	3.2 (0.25)	0.11 (0.031)	0.11 (0.033)	0.74 (0.016)	0.70 (0.016)	0.970	0.972
PL1	5.0 (0.28)	5.9 (0.31)	0.14 (0.026)	0.08 (0.016)	0.58 (0.0076)	0.68 (0.0063)	0.993	0.993
PL2	5.3 (0.36)	4.9 (0.37)	0.15 (0.032)	0.18 (0.041)	0.58 (0.0089)	0.58 (0.010)	0.990	0.987
WI1	7.5 (0.44)	6.5 (0.51)	0.030 (0.010)	0.026 (0.012)	0.91 (0.0054)	0.97 (0.011)	0.989	0.976
WI2	2.3 (0.21)	2.6 (0.23)	0.033 (0.033)	0.060 (0.039)	0.61 (0.019)	0.67 (0.024)	0.966	0.957

study, Morrow et al. (2005) reported that zeta potential ranged from –4.9 to –29 mV for these isolates, a range similar to what we observed in our study. In addition, they reported that the strains ranged from hydrophilic to hydrophobic, results again consistent with our findings. Morrow et al. (2005) also reported nearly a two-fold difference in deposition rates to Pyrax between the three different *E. coli* strains evaluated in their study. Thus, the large amount of diversity we observed among 12 different environmental *E. coli* isolates is consistent with previously published studies which have found significant inter-strain variability in cell properties and transport behavior for *E. coli*.

Hydrophobicity and surface charge are often reported to be the dominant factors controlling the transport of bacteria through porous media (Bolster et al., 2006; Jacobs et al., 2007; Walker et al., 2004; Walker et al., 2005). In this study, however, we did not observe bacterial recovery to be significantly correlated with either hydrophobicity or surface charge. Rather, we found that bacterial recovery was most strongly correlated with cell width and shape (i.e., sphericity). Based on colloid-filtration theory, cell size is expected to be important in determining bacterial attachment rates to sediments and has been confirmed in a handful of studies. Gannon et al. (1991) and Fontes et al. (1991) both observed percent recovery of bacteria to increase with decreasing cell length. Harvey and Garabedian (1991), on the other hand, found that the average length of an injected, indigenous bacterial population increased down-gradient from the injection point, indicating that the longer cells transported more readily through the aquifer. Because the optimal size for transport will depend on factors such as bacterial density, sand size, and flow velocity, the effect of increasing cell size on cell transport will depend on experimental conditions (Harvey and Garabedian, 1991). In our study, we observed bacterial recovery and the deposition rate, k_1, to be correlated with cell width but not cell length. Because colloid-filtration theory is based on spherical particles, it is difficult to know how well the theory predicts particle collisions for rod-shaped bacteria and which dimension (i.e., length or width) is best suited for the calculation of k_1. Our results would suggest that cell width may be better suited than cell length for calculating k_1. Pore-scale simulations of Xu et al. (2008) indicate that nonspherical particles orient themselves so that their major axis (i.e., length) is parallel to flow, suggesting cell width may indeed be the preferred dimension for estimating collision efficiencies.

Cell shape has also been shown to affect bacterial transport, and our observation that bacterial recovery was greater for the more spherical cells is consistent with the findings of others. Weiss et al. (1995) measured the cell-size distribution of a bacterial suspension before and after passage through a sand-packed column. The authors found that for 12 of the 14 organisms studied, the eluted cells were more spherical than the cells in the influent suspension, suggesting that the more spherical cells were favored for transport. Fontes et al. (1991) observed higher recovery of a coccus-shaped bacterial strain compared to a rod-shaped strain, and Dong et al. (2002) compared the transport behavior of two bacterial strains through intact columns and reported greater recovery of the more spherical cells. More recently, Salerno et al. (2006) compared the transport behavior of spherical and rod-shaped latex microspheres with the same surface charge and found that the rod-shaped particles were preferentially retained in glass beads compared to the spherical particles. Additional research is required to determine how cell size and shape affect bacterial depositional processes so that further refinements of colloid-filtration theory can be made to account for nonspherical particles.

Despite the large variability we observed in the BTC shape between the different *E. coli* isolates, the second-order deposition model provided good model fits to all BTCs. Traditionally, second-order deposition models have been used to describe the blocking of attachment sites by previously deposited bacteria (Bolster et al., 1999b; Camesano et al., 1999). Under these conditions, S_{max} can be taken to represent the maximum surface coverage on the sediment surface available for bacterial attachment. From the fitted S_{max} values, we can calculate the corresponding maximum percent surface coverage, θ_{max}, of the sediment surfaces by (Bolster et al., 2001):

$$\theta_{max} = S_{max} \frac{c_0 a_b n d_c}{6(1-n)} * 100 \qquad [5]$$

where a_b is the surface area of the bacteria and n is the porosity. Based on the fitted S_{max} values, the maximum percent surface coverage in our experiments ranged from 0.08 to 0.25% (excluding BC1 and HO2). Such low values would suggest that if attachment is indeed the primary mechanism of removal in our system, then attachment is only occurring at a very small number of sites on the surface collectors, a finding which is not surprising given that both bacteria and the quartz sand are negatively charged at the experimental pH. The attachment of colloidal particles to

like-charged surfaces has been attributed, in part, to the presence of surface charge heterogeneities on the collector surface due to bulk chemical impurities or surface impurities not removed during the sand-washing process (Litton and Olson, 1993; Song et al., 1994; Tufenkji and Elimelech, 2004). These surface charge heterogeneities can carry a neutral or positive charge compared to the bulk negative charge of the quartz sands, thereby leading to localized sites favorable for the attachment of the negatively-charged bacteria. Because the total area of these surface charge heterogeneities is likely to be small (Song et al., 1994), the filling of these sites will occur even at low overall surface coverage.

An alternative explanation for the observed time-dependent behavior of the BTCs is the filling of straining sites (Bradford et al., 2006; Foppen et al., 2005; Xu et al., 2008). Straining sites are defined as locations within the porous media where bacteria are captured in pore spaces too narrow to allow the passage of the bacteria or at grain-to-grain contact points (Bradford et al., 2002). In our study, it is unlikely that straining played a major role due to the size of the bacteria and sand grains. For instance, results of Xu et al. (2008) indicate that for nonspherical particles, straining plays a minimal role in systems where the ratio between bacteria width and grain size is below 0.008; in our study the ratio between bacteria width and grain size was below 0.004. In addition, Foppen et al. (2005) observed that straining was unimportant in the removal of *E. coli* in columns packed with 200 to 500-μm sand, sand sizes which bracket the sand used in our study (250–350 μm). Tufenkji et al. (2004) observed minimal straining for latex microspheres with diameters of 1.9 μm or less in clean quartz sand ranging in size from 150 to 250 μm. Furthermore, if straining were the dominant factor controlling bacterial deposition in our study, we would expect that the bacterial deposition rate, k_1, to be positively correlated with cell width (Xu et al., 2008), yet we observed the opposite. Without column dissection data, however, we cannot completely rule out the possibility that straining played a role in the retention of *E. coli* in our laboratory columns and note that Bradford et al. (2006) found straining to be important for *E. coli* in 240-μm sand, which is just below the size used in our study.

Even though our model did an adequate job of describing the transport of *E. coli* through the 10-cm columns, our inability to correlate the fitted model parameters with any of the measured cell properties suggests one of the following: our model possesses limitations, *E. coli* transport is dependent on a combination of cell properties, or *E. coli* transport is governed by cell properties not measured in this study. (Although we did find k_1 to be correlated with cell width, this correlation—while statistically significant—was not very strong.) What our modeling results do demonstrate, however, is that a large diversity exists in the transport behavior among the different *E. coli* isolates and that model parameters obtained for one *E. coli* strain will likely be inadequate for describing the transport behavior of other strains of *E. coli*. However, our results should be viewed in their proper context, that of an idealized laboratory setting. Factors controlled for in this study, but expected to affect surface properties and transport behavior of *E. coli* in the environment, include starvation state of the cells (Haznedaroglu et al., 2008), growth conditions (Yang et al., 2006), sediment characteristics such as size and hydraulic conductivity (Levy et al., 2007), presence of organic matter in solution and on the sediment phase (Johnson and Logan, 1996), presence of metal-oxyhydroxide coatings on the sand grains (Foppen and Schijven, 2005), and soil moisture content (Jiang et al., 2007), among others. Nevertheless, our findings provide useful information regarding the potentially large inter-strain variability in cell properties and transport behavior that exists with this important microbial indicator of water quality.

Conclusions

Because USEPA (1986) recommends the use of *E. coli* as an indicator of fecal contamination in freshwater systems, its fate and transport in the subsurface needs to be better understood. Recognizing the importance of understanding the fate and transport of *E. coli*, recent studies have focused on identifying transport processes and obtaining model parameters specific for *E. coli* as well as comparing the transport behavior of *E. coli* with specific pathogens. In many of these studies, however, only a single strain of *E. coli* was used (e.g., Bolster et al., 2006; Bradford et al., 2006; Foppen et al., 2007; Foppen et al., 2005; Foppen and Schijven, 2005; Hijnen et al., 2005; Jiang et al., 2007; Levy et al., 2007; Meschke and Sobsey, 2003; Walker et al., 2005); yet based on our findings, as well as previously published reports (Castro and Tufenkji, 2007; Haznedaroglu et al., 2008; Morrow et al., 2005; Yang et al., 2006; Yang et al., 2004), a large diversity in cell properties and transport behavior exists among different *E. coli* strains. Therefore, generalizations about the transport behavior of *E. coli* based on results from a single strain of *E. coli* should be made with caution. In addition, this large variability suggests that the modeling of *E. coli* in the environment will require a distribution of bacterial attachment rates, even when modeling *E. coli* movement from a single fecal source. Further study is needed to assess the diversity in transport behavior of *E. coli* isolates under more realistic conditions, such as intact soil and aquifer materials, as well as under more representative media and growth conditions.

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