In this study we investigate how growth stage and depositional environment affect variability of cell properties and transport behavior of eight porcine *E. coli* isolates. We compared the surface properties for cells harvested during exponential and stationary growth phase and their transport behavior through columns packed with either uncoated or Fe-coated quartz sand. We then investigated correlations between measured cell properties and fitted bacterial attachment efficiencies. For both growth stages we found that bacterial attachment efficiencies in the uncoated quartz sand varied among the eight different isolates by over an order of magnitude whereas attachment efficiencies in the Fe-coated sands varied by a factor of less than two. With the exception of one isolate, growth condition had minimal impact on attachment efficiencies to the uncoated sands. A strong and statistically significant inverse relationship was observed between bacterial attachment efficiencies in the uncoated quartz sand columns and log-transformed zeta potential, whereas a mild yet statistically significant relationship between bacterial attachment efficiencies in the Fe-coated sands and cell width was observed. For the experimental conditions used in our study, we found that variability in *E. coli* transport was more dependent on the depositional environment than on growth conditions.

**Introduction**

Infiltration of fecal material into the subsurface can result in the contamination of groundwater supplies by pathogenic microorganisms such as bacteria, viruses, and protozoa, thereby posing a threat to public health. Studies have shown that fecal contamination is routinely detected in drinking water wells and groundwater is often reported as the source of waterborne disease outbreaks (1). To assess whether a groundwater source is at risk for fecal contamination, agencies responsible for monitoring water supplies generally test for the presence of nonpathogenic microorganisms commonly found in fecal material—referred to as indicator organisms—due to the difficulties and limitations of testing for individual pathogens. Indicator organisms are also used in watershed fate and transport models to predict pathogen movement through the landscape (2). While the use of indicator organisms for assessing the microbiological quality of drinking water has notable limitations, their use will likely continue in the foreseeable future (3). Therefore, a good understanding of the factors controlling their fate and transport in the environment is needed.

One of the most commonly used indicator organisms in groundwater systems is *E. coli*. (Though some strains of *E. coli* are pathogenic, the majority are not) Under the new Ground Water Rule, *E. coli* is one of only three indicators EPA has established for determining whether a groundwater source used as a public water supply is fecally contaminated (1). Given the importance of *E. coli* as an indicator of fecal contamination in groundwater systems, there have been a number of recent studies focusing on understanding the various biological, geochemical, and physical factors affecting *E. coli* transport through porous media (4). These factors include cell acclimation time (5, 6), growth conditions (7, 8), composition of the extracellular polymeric substances (EPS) (5, 9), surface charge and hydrophobicity (9–11), cell motility (11, 12), temperature (6), ionic strength (13), sediment size (14), hydraulic conductivity (15), presence of metal-oxide-hydroxide coated sand grains (13), and soil moisture content (16).

Of particular relevance concerning *E. coli* transport through porous media are recent studies that have shown that a large diversity exists in both cell properties and transport behavior among different *E. coli* isolates. For instance, Morrow et al. (17) reported a nearly 2-fold difference in bacterial deposition rates to Pyrax for three *E. coli* isolates. Bolster et al. (9) observed deposition rates to vary by a factor of 10 among 12 *E. coli* isolates obtained from six different sources. Lutterodt et al. (11) also observed bacterial attachment efficiencies to vary by a factor of 10 among six *E. coli* isolates obtained from a soil used for cattle grazing. Similarly, Foppen et al. (10) measured breakthrough of 54 different *E. coli* isolates from various sources and observed bacterial attachment efficiencies to range by nearly a factor of 10 resulting in a two-log variation in effluent bacteria concentration following passage through 7 cm columns packed with quartz sand. The cause of this variability in *E. coli* transport, however, remains unclear and warrants further research.

Our objective in this study was to further our understanding of the factors controlling variability in *E. coli* transport by conducting cell characterization and transport experiments using eight different *E. coli* isolates obtained from a swine lagoon. We compared the cell properties and transport behavior for cells harvested at two different growth stages (exponential and stationary) and two depositional environments (clean and Fe-coated quartz sand). We then investigated correlations between measured cell properties and fitted bacterial attachment efficiencies.

**Materials and Methods**

**E. coli Isolation and Isolate Selection.** Five 1 L samples of liquid swine effluent were collected from a swine lagoon located on the farm at Western Kentucky University. The samples were collected at five randomly chosen locations within the lagoon at a depth of ~1 m using 1 L sterilized Nalgene bottles attached to an adjustable sampling pole. The samples were returned to the laboratory and processed immediately. Ten ml from each 1 L bottle were serially diluted to 10^-6 in phosphate buffer. One hundred μL from each dilution was plated onto m-Tech plates and incubated at 44.5 °C for 24 h. Following incubation, each presumptive *E.
coli colony (i.e., purple colony) was plated on EMB and mFC agar. This yielded a total of 339 presumptive E. coli colonies. E. coli isolates were fingerprinted by BOX PCR analysis using the BOXAIR primer as previously described (18). Eight isolates were chosen from the major fingerprint groups to be used in the column studies. Each of the eight isolates was further confirmed to be E. coli by chemical analysis using Enterotube tests following the manufacturer’s directions and by PCR analysis of the uidA gene as previously described (19). All isolates were stored at −80 °C with 15% glycerol.

Isolate Typing and Evaluation of fimH and agn43. The eight isolates were placed in one of four phylogenetic groups (A, B1, B2, or D) using the triplex PCR method of Clermont et al. (20) as modified by Higgins et al. (21). The PCR-based lipopolysaccharide core typing methodology of Appelmelk et al. (22) was used to identify the outer core oligosaccharide type of each E. coli isolate. PCR analyses for the presence of agn43 and fimH gene clusters were carried out as previously described (23) except the initial denaturation step was extended to 15 min. Assays were carried out in Qiaagen HotStart Taq Master Mix (Qiagen, Valencia, CA) in a total volume of 25 µL with 2 µL of genomic DNA extract and 0.5 µM primer in a PTC-200 model thermal cycler (MJ Research/ BioRad, Hercules, CA). PCR mixtures (10 µL) were electrophoresed on 1% agarose gel 1 h at 75 V. Ethidium bromide stained gel images were captured with a FOTO/Analyst Investigator/Eclipse system (Fotodyne Inc., Hartland, WI). Primer sequences, product size, and targeted genes are shown in Supporting Information (SI) Table S1.

Cell Preparation. Prior to transport or surface characterization experiments, 40 µL of an overnight culture of E. coli was inoculated into 40 mL of Luria–Bertani (LB) broth and incubated at 37 °C until reaching either midexponential growth phase (3.5 h) or stationary phase (16 h). A refrigerated centrifuge equipped with fixed angle rotor was used to pellet the cells with an applied 3700 g force for 15 min at 4 °C. The cell pellet was resuspended in 1 mM 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 in order to ensure complete removal of the growth medium.

Cell Characterization. The electrophoretic mobility, hydrophobicity, surface charge density, extracellular polymeric substance (EPS) composition, and cell size were measured for each isolate using previously published methods (9). Greater details of the cell characterization methods can be found in the SI.

Transport Experiments. Transport experiments were conducted through water-saturated columns packed with either clean quartz sand (Unimin, New Caanan, CT) or Fe-coated quartz sand ranging in size from 710 to 850 µm in diameter. This sand size was chosen to minimize the impact of physical straining on the removal of bacteria from the aqueous phase. Sand was cleaned and portions of the acid-washed sand were coated with Fe-oxo-hydroxides using previously published methods (13). Streaming potential of the clean and Fe-coated sand was measured using an electro kinetic analyzer (Anton Paar GmbH, Graz, Austria) following the procedure described in Kim et al. (24).

Columns were packed by slowly pouring water-saturated autoclaved sand into 2.5 cm diameter Chromaflex Chromatography Columns (Kontes Glass Co., Vineland, NJ) filled with a 1 mM KCl electrolyte solution while vibrating the columns. Porosity ranged from 0.36 to 0.39 as determined gravimetrically. Column lengths ranged from 9.9 to 10.1 cm. After packing was completed, columns were operated in an upward direction using syringe pumps (model 200 syringe pump, KD Scientific Inc., New Hope, PA) 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 0.67 mL/min for a Darcian velocity of 0.36 cm/min. Bacterial solutions with an optical density of ~0.20 (~1 × 10^8 cells/mL) were injected into each column for 38 min (~1.2 pore volumes) followed by 2 pore volumes of bacteria-free electrolyte solution. Effluent was collected every 3 min using a Spectra/Chrom CF-1 fraction collector (Spectrum Chromatography, Houston, TX). Effluent concentrations of E. coli were determined by measuring the optical density of the samples at a wavelength of 546 nm. All experiments were duplicated on a separate day to ensure true replication. In several columns bromide was added as a conservative tracer so that a dispersion coefficient for the columns could be determined.

Data Analysis. The breakthrough curves (BTCs) for each treatment were fit with both first-order and second-order deposition models using weighted least-squares regression. Weights were calculated assuming a coefficient of variation in the observed BTC concentrations of 5%; a value equivalent to the median coefficient of variation observed between duplicate data points for all our column experiments combined. Bacterial attachment efficiencies (α), which describe a cell’s ability to become associated with the sand surface, were calculated using the hemispheres-in-cell model of Ma et al. (25) from the fitted deposition parameters obtained from the best-fit model for each treatment. (See SI for further details on the transport equations and modeling methodology used.) Correlation and regression analyses were used to determine whether fitted α values were significantly correlated with any of the measured cell properties. To account for multiple parameters, multiple regression analysis was performed. All regression analyses were performed using PROC REG in SAS 9.2 (SAS Institute, 2003).

Results and Discussion

Isolate Selection and Characterization. Using a threshold of 80% similarity in BOX fingerprint patterns, 25 unique profiles were differentiated from the 339 E. coli isolates. Eight isolates, representing 81% of the diversity of isolates, were selected for transport studies and cell surface characterization. Each of these eight isolates was confirmed to be E. coli by PCR amplification of the uidA gene and by the Enterotube II method (SI Tables S4 and S5). Based on the genotyping triplex assay of Clermont et al. (20), six of the isolates grouped with the B1 genotype and two with group A (SI Table S5). Clermont et al. (20) also found that isolates from swine feces were dominated by the B1 genotype with remaining isolates being genotype A.

The outer core oligosaccharide types of the eight E. coli isolates were identified as R1, R3, K12, and R1/R3 mixed type (SI Table S5). The identification of isolate SP2C04 as core type K12 was unexpected since this group is infrequent among natural populations. R1 core type dominates in natural populations and three of our isolates fit this core type. Isolate SP3B03 was positive for both R1 and R3 core groups. Appelmelk et al. (26) described an R1/R3 mixed type which reacted equally well with polyclonal α-R1 and with α-R3 antiserum. They suggest that the R3 reaction might be due to cross-reactions with non-LPS antigens.

Cell Properties. Measured cell properties of the eight porcine E. coli isolates analyzed in this study are shown in Table 1. In general, we observed a large range in cell properties among the different E. coli isolates. Zeta potential varied by a factor of nearly 25 for exponential-phase cells with values ranging from −2.0 to −46 mV and varied by a factor of nearly 100 for stationary-phase cells with values ranging from −0.8 to −70.5 mV. Hydrophobicity also varied by over an order of magnitude ranging from 6 to 81% for the exponential-phase cells and from 5 to 81% for the stationary-phase cells. Surface charge density varied by a factor of 6 and 11 for
TABLE 1. Measured Values of Percent Recovery and Selected Cell Properties (Standard Deviations Are in Parentheses) for Exponential- (exp) and Stationary-Phase (stat) Cells

<table>
<thead>
<tr>
<th>isolate</th>
<th>hydrophobicity (%)</th>
<th>zeta potential (mV)</th>
<th>surface charge density (µC/cm²)</th>
<th>cell length (µm)</th>
<th>cell width (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP1C10</td>
<td>26 (9.3)</td>
<td>-4.5 (0.04)</td>
<td>1.0 × 10⁴ (0.033)</td>
<td>1.53 (0.023)</td>
<td>0.86 (0.23)</td>
</tr>
<tr>
<td>SP1H01</td>
<td>35 (11.6)</td>
<td>-6.2 (0.04)</td>
<td>1.2 × 10⁴ (0.035)</td>
<td>1.61 (0.023)</td>
<td>0.81 (0.03)</td>
</tr>
<tr>
<td>SP2A12</td>
<td>13 (1.5)</td>
<td>-26 (4.5)</td>
<td>1.7 × 10³ (0.09)</td>
<td>1.61 (0.037)</td>
<td>0.76 (0.013)</td>
</tr>
<tr>
<td>SP2B07</td>
<td>11 (8.3)</td>
<td>-45 (4.5)</td>
<td>2.4 × 10³ (0.055)</td>
<td>1.54 (0.030)</td>
<td>0.76 (0.011)</td>
</tr>
<tr>
<td>SP2C04</td>
<td>7.0 (2.9)</td>
<td>-26 (0.76)</td>
<td>1.1 × 10⁴ (0.008)</td>
<td>1.73 (0.010)</td>
<td>0.88 (0.010)</td>
</tr>
<tr>
<td>SP3B03</td>
<td>11 (1.2)</td>
<td>-89 (1.6)</td>
<td>1.8 × 10³ (0.024)</td>
<td>1.74 (0.029)</td>
<td>0.83 (0.029)</td>
</tr>
<tr>
<td>SP4C08</td>
<td>41 (10.0)</td>
<td>-74 (2.19)</td>
<td>1.7 × 10³ (0.059)</td>
<td>1.59 (0.098)</td>
<td>0.86 (0.021)</td>
</tr>
<tr>
<td>SP4H03</td>
<td>6.0 (1.5)</td>
<td>-46 (0.49)</td>
<td>8.6 × 10³ (0.098)</td>
<td>1.66 (0.023)</td>
<td>0.92 (0.023)</td>
</tr>
</tbody>
</table>

Exponential- and stationary-phase cells, respectively, whereas cell size was relatively uniform among the different isolates with cell length only ranging from 1.5 to 1.8 µm and cell width ranging from 0.76 to 0.97 µm. The sugar and protein contents of the EPS varied by factors ranging from 4 (sugar content of stationary-phase cells) to 9 (sugar content of exponential-phase cells) (SI Table S6). Such large diversity in cell properties among different E. coli isolates is consistent with what has been reported in the literature under a variety of experimental conditions (5, 8–11, 17).

Increasing incubation time in LB broth from 3.5 to 16 h had varying effects on measured cell properties (Table 1). For example, increasing incubation time increased hydrophobicity for five of the isolates while decreasing it for three, with the largest change observed for SP2C04 which went from hydrophilic (7%) to very hydrophobic (81%). Similar to Walker et al. (8), changes in zeta potential were generally minimal with the exception of SP4H03 in which increasing incubation time resulted in zeta potential becoming significantly more negative (−46 to −71 mV). For seven of the isolates surface charge density values were lower, in most cases by a factor of 10 or greater, for the stationary-phase cells compared with the exponential-phase cells. This is likely due to the fact that the bacteria enter a starvation state by 16 h, and therefore are conserving their resources needed to fully determine the importance of growth stage on E. coli transport behavior.

The presence of the Fe-oxyhydroxide coatings on the quartz sand grains resulted in decreased effluent concentrations for seven of the eight isolates (Figure 2A and B), results consistent with previously published studies (13, 31–33). The one isolate, SP3B03, which did not experience decreased transport through the Fe-coated sand had a near-neutral charge (Table 1). The range in transport behavior among the different E. coli isolates through the Fe-coated sands was much less than what was observed for the uncoated sands with percent recoveries ranging from ~33% for SP2C04 to ~54% for SP3B03. Bacterial attachment efficiencies ranged by a factor of less than two for the Fe-coated sands with values ranging from 0.45 to 0.82 for the exponential-phase cells and from 0.52 to 0.87 for the stationary-phase cells (Figure 2C). For all isolates the α values for the stationary-phase cells were higher than those observed for the exponential-phase cells, though these differences were generally small and within the uncertainties of α.

The presence of metal-oxyhydroxide coatings on quartz sand is often assumed to result in a net positive charge on the sand surface thereby removing any electrostatic barrier to bacterial deposition. Under such favorable conditions, all of the cells that strike a collector are expected to attach to the collector and therefore variability in transport is expected to be reduced compared with unfavorable conditions. This
is indeed what we observed with our E. coli isolates and what has been reported for nonbiological colloids (34). However, all of our fitted $\alpha$ values were significantly lower than the expected value of one. In measuring the zeta potential of our Fe-coated sands we found that the surface charge was negative rather than positive ($-6.1$ mV), and DLVO calculations show that a barrier to deposition existed in the Fe-coated sands for seven of the isolates (SI Table S7), likely explaining, at least in part, why our observed values of $\alpha$ were less than one. The presence of the Fe-coatings did, however, reduce the energy barrier by 50% or more compared to the uncoated sand thereby resulting in less unfavorable conditions and increased deposition (SI Table S7). The one exception was with isolate SP3B03 in which no energy barrier existed for either sand type and explains why the $\alpha$ values were similar for the uncoated and Fe-coated sands for this one organism. The fact that $\alpha$ values for this organism were

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**FIGURE 1.** Observed (symbols) and fitted (solid line) breakthrough curves for eight porcine E. coli isolates through uncoated quartz sand columns for cells harvested in the exponential (A) and stationary (B) phases. Also included are the fitted values and 95% confidence intervals (C) of the bacterial attachment efficiencies ($\alpha$) for exponential- (closed symbols) and stationary-phase (open symbols) cells. Because we assumed that the only source of uncertainty in $\alpha$ was the uncertainty in $k$ obtained from the weighted least-squares fit, the uncertainties associated with our fitted $\alpha$ values may underestimate their true uncertainties.

**FIGURE 2.** Observed (symbols) and fitted (solid line) breakthrough data for eight porcine E. coli isolates through Fe-coated quartz sand columns for cells harvested in the exponential (A) and stationary (B) phases. Also included are the fitted values and 95% confidence intervals (C) of the bacterial attachment efficiencies ($\alpha$) for exponential- (closed symbols) and stationary-phase (open symbols) cells. Because we assumed that the only source of uncertainty in $\alpha$ was the uncertainty in $k$ obtained from the weighted least-squares fit, the uncertainties associated with our fitted $\alpha$ values may underestimate their true uncertainties.
Derjaguin like-charged collectors is in qualitative agreement with controlling bacterial transport through columns packed with comparing multiple (have not been shown to be correlated with zeta potential but). We observed a strong and statistically significant inverse correlation (r = −0.89; P < 0.001) between α and the log-transform of the absolute value of zeta potential in the uncoated sands (Figure 3A; SI Table S8). No other parameter was found to be significantly (P < 0.05) correlated with α for the uncoated sands (SI Table S8). Multiple linear regression analysis indicated that the best overall model (r² = 0.85; P < 0.001) for describing the variability in α was a 2-parameter model that included sugar content and log-transformed zeta potential; however, zeta potential was clearly the most important factor with a partial r² of 0.78 compared to 0.07 for sugar content.

The observation that zeta potential is the dominant factor controlling bacterial transport through columns packed with like-charged collectors is in qualitative agreement with Derjaguin–Landau–Verwey–Overbeek (DLVO) theory which predicts that bacterial attachment efficiencies to like-charged collectors will be higher for cells with a lower net negative charge due to a reduction in the electrostatic double-layer repulsion between the negatively charged bacteria and sand grains. This behavior is commonly observed for individual isolates when the surface charge has been modified by changes in ionic strength (7, 13, 35); however, when comparing multiple E. coli isolates, bacterial deposition rates have not been shown to be correlated with zeta potential (9–11). We cannot explain why this correlation was not observed in these studies but note that two of the three studies compared E. coli isolates obtained from multiple sources (9, 10). It is possible that additional confounding factors may be present when correlating bacterial transport to surface properties for E. coli isolates obtained from multiple sources.

For the Fe-coated sands a small yet statistically significant correlation (r = 0.54, P = 0.03) was observed between cell width and α (Figure 3B; SI Table S8). Using multiple linear regression analysis, no other parameters were found to add significant (P < 0.05) improvements for describing the observed variability in α. Because we used cell width in our calculation of η, this correlation suggests that cell width may play an additional role in bacterial deposition not currently incorporated into η. However, additional studies are needed to determine if this observed correlation is of both statistical and practical importance.

In addition to measuring various cell surface properties, we also evaluated the presence of two genes that encode for phase-variable surface proteins (fimH which encodes for type 1 fimbriae and agn43 which encodes for Antigen 43) that have been associated with bacterial adhesion to surfaces (36, 37). Consistent with Yang et al. (23), we found that all isolates were positive for fimH suggesting that this gene may be ubiquitous in livestock fecal E. coli strains and therefore its occurrence is not a useful tool for predicting transport behavior (SI Table S5). Conversely, only three of the eight isolates tested in our study were positive for the agn43 gene (SI Table S5) and two of these isolates, SP3B03 and SP1H01, had high attachment efficiencies to the quartz sand grains. Although the presence of the agn43 gene does not necessarily mean that the gene is expressed and that the Ag43 outer membrane protein is present (36), our results are consistent with both Yang et al. (12) and Lutterodt et al. (11) who found that the expression of this gene decreased E. coli transport through porous media.

**Implications for Modeling E. coli Transport.** Consistent with other studies, we observed a large range in the transport behavior of different E. coli isolates through porous media. Morrow et al. (17) attributed their observed differences among the three different isolates used in their study to differences in cell hydrophobicity. Bolster et al. (9) observed a mild inverse relationship between cell width and bacterial deposition rates for their 12 E. coli isolates. Both Lutterodt et al. (11) and Yang et al. (12) found that cell motility and Ag43 expression were the dominant factors controlling transport of various E. coli isolates through columns packed with a variety of materials. On the other hand, Poppen et al. (10) did not find any correlation between transport behavior and measured cell properties for the 54 E. coli isolates used in their study. In this study we found that cell retention in columns packed with clean quartz sand was inversely correlated with the zeta potential of the cells whereas a mild relationship between cell width and bacterial deposition rates in Fe-coated sands was observed. These results indicate that the factors controlling the large diversity in transport behavior among different E. coli isolates likely vary depending on the experimental conditions and source of the E. coli isolates.

This large diversity in cell properties and transport behavior among different E. coli isolates clearly demonstrates the inadequacy of using a single strain of E. coli to assess the general transport behavior of all E. coli strains. Furthermore, this variability shows the insufficiency of using a single deposition rate to model E. coli transport, especially for conditions unfavorable to deposition. As an example, we conducted an additional set of transport experiments with bacterial solutions containing both SP3B03 (the isolate with the highest α) and SP2C04 (the isolate with the lowest α) at three different concentration ratios (90:10, 50:50, and 10:90). The combined transport behavior of the cells was then predicted using a simple bimodal transport model where
the attachment efficiency for both isolates was weighted by the fraction of that isolate in solution (38). Figure 4 shows that by properly accounting for the bimodal distribution in α, we were able to adequately predict the BTC behavior of the combined solutions through the uncoated quartz sand columns. Though this is an admittedly oversimplified example, it does illustrate the importance of including a distribution of bacterial deposition rates when modeling E. coli transport from a single fecal source. Continued research is required to assess the impacts of biological variability (both inter- and intrastrain variability) on modeling the transport of E. coli in the environment.

Acknowledgments
We thank Stacy Antle for assistance with the transport studies. This research was part of USDA-ARS National Program 206: Manure and By-product Utilization. Mention of trade names or commercial products is solely for the description of experimental procedures and does not imply recommendation or endorsement by the USDA.

Supporting Information Available
Additional information on cell characterization methods and Tables S1 – S8. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited


ES1010253