Linking Microbial Community Structure to Function in Representative Simulated Systems

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Pathogenic bacteria are generally studied as a single strain under ideal growing conditions, although these conditions are not the norm in the environments in which pathogens typically proliferate. In this investigation, a representative microbial community along with *Escherichia coli* O157:H7, a model pathogen, was studied in three environments in which such a pathogen could be found: a human colon, a septic tank, and groundwater. Each of these systems was built in the lab in order to retain the physical/chemical and microbial complexity of the environments while maintaining control of the feed into the models. The microbial community in the colon was found to have a high percentage of bacteroidetes and firmicutes, while the septic tank and groundwater systems were composed mostly of proteobacteria. The introduction of *E. coli* O157:H7 into the simulated systems elicited a shift in the structures and phenotypic cell characteristics of the microbial communities. The fate and transport of the microbial community with *E. coli* O157:H7 were found to be significantly different from those of *E. coli* O157:H7 studied as a single isolate, suggesting that the behavior of the organism in the environment was different from that previously conceived. The findings in this study clearly suggest that to gain insight into the fate of pathogens, cells should be grown and analyzed under conditions simulating those of the environment in which the pathogens are present.

The common paradigm for studying pathogenic bacteria in the lab has been to grow the cells as a single strain in a nutrient-rich medium and then to harvest the organism and evaluate its phenotypic and/or genotypic characteristics (1). However, this traditional approach overlooks the critical fact that these pathogens do not exist under such idealized conditions (2). Microorganisms survive in complex communities with multiple species of bacteria, archaea, fungi, and protozoa, although bacteria make up most of the biomass (3–5). It is therefore imperative to study the microbial community as a biological system and to establish the pathogen's effect on the microbial community in the environment.

One method that avoids the shortcomings of studying individual microorganisms is to sample bacteria directly from the environment under investigation. This approach allows for the study of microorganisms in their natural environmental settings and has produced a wealth of data on microbial communities in the human microbiome (6–10) and in aquatic systems (11–14). However, there is no realistic capacity to control parameters within *in vivo* systems. *In vitro* systems, on the other hand, combine the complexity of environmental conditions with proper controls in the laboratory. Several *in vitro* models have been developed to simulate environments where pathogens may be found, including the guts of humans (15–17) and aquatic systems (18–20). These *in vitro* studies have allowed researchers to gain a better understanding of the behavior of microorganisms in environmentally relevant systems.

The function of *in vitro* gut environments has been tested by determining the microorganisms present (17, 21–23) and by monitoring fatty acid production (15, 17, 21, 24) and enzymatic activity (17, 21). For aquatic systems, such as surface water, wastewater, and groundwater, researchers have generally used synthetic formulas to recreate the aquatic chemistry found in those environments and to alleviate variability (18, 25–28). These *in vitro* environments have never been combined so as to study the life

cycle of microbial communities from the human colon to water treatment to groundwater.

Therefore, three simulated environments—a human descending colon, a two-chambered septic tank, and a groundwater system—were built in order to determine how microorganisms change when the external conditions are altered. These systems were chosen because they represent a pathway through which pathogens may be transported from a human host into groundwater. The aims of this study were to simulate environmentally relevant systems in the lab, to evaluate the structure and function of the microbial community present in each of these systems, and to evaluate the same characteristics with the addition of *Escherichia coli* O157:H7.

MATERIALS AND METHODS

Bacterial cell and medium selection. *Escherichia coli* O157:H7 (EDL933) was acquired from the USDA (Mark Ibekwe, USDA-ARS-USSL, Riverside, CA) for use as the model enteric pathogen in this study. The microbial community was taken from human fecal material donated by a healthy 25-year-old male volunteer who had not had antibiotics for a year. The sample used in the model colon was separated from the fecal matter by a 5-cycle differential centrifugation process and was stored in glycerol stocks in a -80°C freezer (Revco Plus; Thermo Scientific, Asheville, NC) as described previously (29). Briefly, 10 g of fecal matter was placed in 200 ml of 50 mM sodium phosphate buffer. After shaking, the sample was centrifuged at $200 \times g$ for 15 min (model 5804R centrifuge; Eppendorf,

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FIG 1 Schematic and flow diagram of the experimental apparatus and design. A, microbial community (and *E. coli* O157:H7 in select experiments); B, colon medium; C, PEG; and D, dialysis tubing (where the microbial community is inoculated). PEG flows between the reactor wall and the dialysis tube (E) and is tested for fatty acids (F). Cells are taken out for characterization (G). A two-chamber septic tank is inoculated with the colon effluent (H), and cells are taken out for characterization (I). Groundwater is inoculated with the effluent from the septic tank and is characterized (J).

Hamburg, Germany). The supernatant was placed in a clean flask and was kept on ice while the pellet was again transferred to the buffer and resuspended, and the process was repeated four more times. The bacteria in the supernatant were then extracted by centrifuging at $3,700 \times g$ for 15 min and suspending the pelleted bacteria in 5 ml of a previously autoclaved medium. This medium is similar in composition to digested food entering the large intestine (colon medium) (30) and contains, per liter, 4.5 g NaCl, 2.5 g K_2HPO_4 , 0.45 g $CaCl_2 \cdot 2H_2O$, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.005 g $FeSO_4 \cdot 7H_2O$, 0.05 g ox bile, 0.01 g hemin, 0.4 g cysteine, 0.6 g pectin, 0.6 g xylan, 0.6 g arabinogalactan, 0.6 g amylopectin, 5 g starch, 2 ml Tween 80, 3 g Bacto peptone, and 3 g casein. Additionally, 1 ml of a vitamin mixture containing, per liter, 1 mg menadione, 2 mg D-biotin, 0.5 mg vitamin B₁₂, 10 mg pantothenate, 5 mg nicotinamide, 5 mg *para*-aminobenzoic acid, and 4 mg thiamine was added after autoclaving. The cells suspended in the medium were allowed to grow in a shaker-incubator (at 200 rpm and 37°C; model 4639; Barnstead/Lab-Line, Melrose Park, IL) for 12 h. Subsequently, 2 ml was transferred to 200 ml of the same autoclaved medium and was allowed to grow for 6 h. Next, 500 μ l of cells was added to 500 μ l of a 50% glycerol solution, and the solution was stored in the -80°C freezer until use. All chemicals utilized were ACS research grade.

In vitro human colon. A human colon was simulated by a custom reactor maintained at pH 5.5 and 37°C within an incubator (Barnstead MaxQ 4000; Thermo Scientific, Asheville, NC), since these are the conditions of the proximal colon (31). The model consists of a 50-cm-long glass tube with a 5-cm internal diameter, a rubber stopper at each end, and an internal tube sustaining a cylindrically shaped dialysis membrane (diameter, 33 mm; pore size, 12,000 Da; Fisher Scientific, Pittsburgh, PA) (Fig. 1).

Each colon experiment lasted for 1 week. At the beginning of each week, the microbial community was taken from the stored glycerol stocks, inoculated into 200 ml colon medium (30), and placed in a shaker-incubator at 200 rpm and 37°C for 24 h. The bacterial suspension was then pumped into the dialysis tube of the model colon (Fig. 1, D) at a rate of 5 ml/min (Masterflex pump; Cole-Parmer, Vernon Hills, IL) (15) (Fig. 1, A). A polyethylene glycol (PEG) mixture, acting as a dehydrating medium, was used to simulate intestinal adsorption; it contained, per liter, 26.25 g polyethylene glycol 4000, 0.36 g $NaHCO_3$, and 0.7 g NaCl (15) (Fig. 1, C). The PEG mixture was fed into the area between the dialysis and the glass tubing in the model colon at a rate of 2.1 ml/min (Fig. 1, E), before the bacteria and the medium were allowed to flow into the model,

and continuously until the end of the experiment, as described in previous research (15). To simulate typical adult feeding times, 100 ml of the colon medium mixture was pumped into the dialysis tube three times a day, 6 h apart, at a rate of 5 ml/min (15) (Fig. 1, D). This weeklong experiment was repeated three times to ensure reproducibility.

Subsequently, three experiments were conducted in which the microbial community was spiked with the model pathogen at a 1:1,000 ratio by volume and was inoculated into the model colon reactor. In these experiments, a single colony of *E. coli* O157:H7 was aseptically picked from a plate, put into a 5-ml colon medium mixture, and shaken in a shaker-incubator (at 200 rpm and 37°C). After 16 h, 200 μ l of the *E. coli* O157:H7 suspension was added to the 200-ml microbial-community culture before the model was inoculated. The rest of the experiment was conducted as described above.

SCFA analysis. The effluent from the model colon (Fig. 1, F) and the PEG solution (Fig. 1, E) were sampled at each feeding time, and the samples were stored at -20°C until analysis. The short-chain fatty acids (SCFAs) butyric acid, propionic acid, and acetic acid were detected by gas-liquid chromatography (GC) (Agilent, Santa Clara, CA) using previously published methods (32) with the detector temperature set to 200°C. SCFAs were measured by integration under a fitted flame ionization detection curve.

Laboratory-scale septic tank. About 20% of all American households (26 million) and 25% of planned developments use septic tanks to dispose of their wastewater (33). These are generally self-regulated, with each state having distinct maintenance standards. It is for these reasons that a septic tank was modeled. The septic system was made by compartmentalizing an acrylic fish tank as a scaled-down version of a two-compartment septic tank (144 and 72 liters) with a 2:1 volume (34) (Fig. 1, H). The 100 ml of effluent collected from the colon was diluted with deionized water (DI) at a 17.5:1 ratio (1.75 liters) to simulate toilet flushing. The diluted colon effluent was poured into the first compartment of the model septic tank along with synthetic gray water (3.5 liters) at a 1:2 (by volume) ratio three times a day. This ratio was selected because approximately two-thirds of household wastewater comes from sources other than toilet flushing (35). The synthetic gray water, on a per liter basis, is composed of 20 mg humic acid, 50 mg kaolin, 50 mg cellulose, 0.5 mM $CaCl_2$, 10 mM NaCl, and 1 mM $NaHCO_3$ at pH 8 (27). The septic water was transferred from the first partition into the second compartment once the water reached 80% of the septic tank's height (64 cm), which equated to a 3-week residence in the first compartment.

Water quality tests on the model septic tank were conducted once per week to ensure that the water quality was similar to that of a full-scale functioning septic system (35) (Fig. 1, I). These included tests for pH, conductivity, hardness, total organic carbon (TOC), and total suspended solids (TSS). Conductivity and pH were measured using a conductivity probe (YSI 3200 conductivity instrument; YSI, Yellow Springs, OH) and a pH probe (XL15; Fisher Scientific, Pittsburgh, PA), respectively. Colorimetric titration was used to determine a hardness value (36). TOC was measured using a TOC-5050 analyzer (Shimadzu, Japan) (36), while TSS was measured by using a vacuum to force the water sample through a previously weighed 0.45- μ m glass fiber filter. After the filter was dried at 105°C in an oven, it was weighed again to quantify the amount of TSS (36).

Simulating groundwater conditions. Samples from the second compartment of the septic tank (900 ml) were centrifuged at $3,700 \times g$, and the pelleted bacteria were resuspended in 900 ml of synthetic groundwater (37, 38). The resuspended bacteria were placed in two 500-ml tissue culture flasks wrapped in aluminum foil to limit light exposure and were shaken at 70 rpm at room temperature, as was done in previous research (18) (Fig. 1, J).

Pyrosequencing. In order to determine the structure of the microbial community in each of the simulated systems, DNA was extracted from the samples using the Mo Bio (Carlsbad, CA) total microbial DNA extraction kit according to the manufacturer's protocol with one exception. The protocol calls for 1 ml of sample; however, this would yield too small a

quantity of DNA from the septic tank and groundwater samples. Therefore, 300-ml samples from the septic tank and groundwater systems were first centrifuged at $3,700 \times g$ and then resuspended in 3 ml of 10 mM KCl solution before extraction of the DNA. These DNA samples were then shipped to the Research and Testing Laboratory (Lubbock, TX) for bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) using Gray28F (5' GAGTTTGATCCTGGCTCAG) and Gray519r (5' GTNTTA CNGCGGCKGCTG) (39). The Roche 454 FLX instrument, titanium reagents, and procedures were based on the manufacturer's protocols (454 Life Sciences, Branford, CT). QIIME (quantitative insights into microbial ecology) software (40) was used to analyze the sequences. The sequences were first denoised, assigned operational taxonomic units (OTUs) with a 97% pairwise identity threshold, and finally given taxonomic identities.

Phenotypic characterization of the microbial community. The phenotypic characterization conducted included hydrophobicity, electrophoretic mobility (EPM), cell size, extracellular polymeric substance (EPS) content and composition, and calculation of the attachment efficiency of the entire microbial community through porous media. At a consistent time each week, samples were taken from the colon, septic tank, and groundwater systems. The bacteria from the systems (colon effluent, second compartment of the septic tank, and groundwater) were washed via centrifugation ($3,700 \times g$) and were suspended in 10 mM potassium chloride (KCl).

Hydrophobicity was measured by the microbial-adhesion-to-hydrocarbon (MATH) test, which gives a quantitative measure of the amount of cells partitioning in the hydrocarbon versus the electrolyte phase (41). The average microbial size, notably the length, width, and subsequently calculated average equivalent spherical radius, was determined from three images ($n > 20$) by utilizing a phase-contrast microscope and imaging software (Matlab; MathWorks, Natick, MA) (41). Electrophoretic mobility measurements were conducted using a zeta potential analyzer (ZetaPALS; Brookhaven Instruments Corp., Holtsville, NY) (42). EPSs were quantified as total proteins and total sugars by fixing the cells in a 0.22% formaldehyde solution, separating the cells from the EPS, lyophilizing the EPS, and measuring the sugar and protein contents by using previously described colorimetric methods (37). The phenotypic measurements described above were conducted in triplicate.

Attachment efficiency, a measure of the adhesive nature of cells, was determined by using a 14-cm saturated column (Kimble Chase, Vineland, NJ) packed with quartz sand (spherical diameter, 275 μ m; Iota; Unimin Corp., New Canaan, CT). For these transport experiments, washed cells were diluted using 10 mM KCl to an optical density of 0.2 at 546 nm. The bacterial solutions were pumped into the column for 3 pore volumes (PV), followed by 5 PV of bacterium-free electrolyte solution. The effluent was measured using an in-line spectrometer at 546 nm (Turner; Barnstead International, Dubuque, IA) to determine the deposition of the microbial community (43) and its attachment efficiency in the column (44).

Statistical and data analysis. Student paired *t* tests with normal distribution in Excel (Microsoft, Redmond, WA) were used to analyze the week-to-week variation of the structure and function of the microbial community. One-way analysis of variance (ANOVA) with normal distribution and homogeneity of variance was used to look at significance in the variances between the results from the different simulated systems. Differences were considered significant at a *P* value of <0.05 .

RESULTS

Composition of the microbial community (phylum). Samples were taken from the colon, septic tank, and groundwater systems to analyze the microbial communities' structure, fate, and transport at the same time once per week to ensure reproducibility. The microbial communities' structure was analyzed with pyrosequencing, and in the analysis the following measures were taken to enhance the comparability of amplicon sequencing data: removal of all singletons and rare operational taxonomic units (OTUs) and addition of sequences from technical replicate sequencing runs.

FIG 2 Average percentages of the four most prominent bacterial phyla (A, *Actinobacteria*; P, *Proteobacteria*; F, *Firmicutes*; B, *Bacteroidetes*) in each of the model systems (C, colon; CP, colon with model pathogen; ST, septic tank; STP, septic tank with model pathogen; GW, groundwater; GWP, groundwater with model pathogen).

For the first 3 weeks of experiments, the microbial community was tested without *E. coli* O157:H7. In the following 3 weeks, the model pathogen was introduced into the colon and was subsequently added to the aquatic systems. The results of the microbial community structure analysis at the phylum level are displayed in Fig. 2. The model colon had a composition that was within the normal range according to the work of Arumugam et al. (7), where the community was composed mostly of firmicutes and bacteroidetes. In the *in vitro* colon, the microbial community without the pathogen was composed mostly of bacteroidetes ($94\% \pm 2\%$), firmicutes ($5\% \pm 3\%$), and proteobacteria ($0.8\% \pm 0.6\%$). The same did not hold true for the community with the pathogen: the relative amounts of firmicutes and actinobacteria increased ($74\% \pm 18\%$ and $4\% \pm 2\%$, respectively) at the expense of the bacteroidetes ($20\% \pm 17\%$). Meanwhile, the relative amount of proteobacteria remained consistent ($1.5\% \pm 0.8\%$). The relative amounts of proteobacteria were comparable in the septic tank ($85\% \pm 5\%$) and the groundwater system ($83\% \pm 5\%$) without the pathogen ($P > 0.05$). The similarity in the relative amounts of proteobacteria between the aquatic systems held true for the septic tank ($60\% \pm 9\%$) and groundwater ($56\% \pm 3\%$) with the pathogen ($P > 0.05$). Although proteobacteria dominated the bacterial composition in both aquatic systems, there was a significant difference ($P < 0.05$) between the experiments in the absence and presence of *E. coli* O157:H7. In the septic tank, the relative amount of bacteroidetes was higher with the pathogen ($25\% \pm 4\%$) than without it ($3\% \pm 1\%$), while the relative amounts of firmicutes were relatively consistent ($15\% \pm 4\%$ with the pathogen and $13\% \pm 4\%$ without it). The opposite trend was true for the groundwater system: the relative amount of firmicutes was greater with the pathogen ($26\% \pm 7\%$) than without it ($7\% \pm 6\%$), while the relative amounts of bacteroidetes were consistent ($18\% \pm 5\%$ with the pathogen and $11\% \pm 11\%$ without it).

Microbial community activity in the model colon. The composition of short-chain fatty acids was relatively consistent in the colon environment over time (Fig. 3). Without *E. coli* O157:H7 present, the acetic/butyric/propionic acid composition ratio was

FIG 3 Comparison of the concentrations of short-chain fatty acids (AA, acetic acid; PA, propionic acid; BA, butyric acid) over a 5-day period in the model colon without (A) and with (B) *E. coli* O157:H7.

5:4:1. When the pathogen was introduced into the microbial community, the acetic/butyric/propionic acid ratio changed to 2:6:2. In both cases, the proportion of propionic acid was lowest, whereas the proportion of acetic acid decreased, and that of butyric acid increased, with *E. coli* O157:H7 present. These data sets were significantly ($P < 0.05$) distinct from each other.

Physical-chemical characteristics of the microbial community. The microbial community from the colon was significantly ($P < 0.05$) more hydrophilic and had lower electrophoretic mobility, attachment efficiency, and EPS content than the microbial communities harvested from the model aquatic systems (Table 1).

With the addition of *E. coli* O157:H7, the colon's microbial community had statistically lower ($P < 0.05$) electrophoretic mobility and attachment efficiency, and higher sugar and protein contents, than those of the aquatic systems. There was no statistical difference between the physical-chemical characteristics of the microbial communities in the septic tank and groundwater systems in the presence and absence of *E. coli* O157:H7. The addition of the pathogen significantly ($P < 0.05$) increased the hydrophobicity and EPS level of the bacteria grown in the colon, reduced the EPS levels in the aquatic systems, and enlarged the average cell size in all three systems.

Water quality of the septic tank. In order to confirm that the model septic tank performed in the same way as its real-world counterpart (45), standard water quality tests were performed on the effluent once per week for the 7 weeks during which the system was operational, and the results were compared to values reported in the literature (35). The average results and standard deviations for selected tests are displayed in Table 2.

DISCUSSION

The composition of the microbial community within the model colon was compared to those of *in vitro* and *in vivo* colon environments from previous studies by using 16S rRNA-based pyrosequencing. While there are known issues associated with the 16S rRNA pyrosequencing technique, such as PCR bias (46), sequencing errors (47, 48), and problems with the quantification of data (49), this study followed the guidelines established by Zhou et al. for increasing data comparability (50). Specifically, rare OTUs and singletons were removed, three biological replicates under each condition were tested, and all samples were collected and analyzed in the same manner to ensure more-reproducible data. The relatively large amount of bacteroidetes in the microbial community in the absence of the pathogen agrees with the findings for previously studied *in vitro* models (17, 23, 51, 52) and human gut microbiomes (7, 53). The majority of the real colon microbiomes have a higher concentration of firmicutes than bacteroidetes (7, 53). This microbial composition is similar to that of the *in vitro* colon in this study with the addition of *E. coli* O157:H7. In a mouse colon model, the production of firmicutes was amplified with the addition of an enterohemorrhagic *E. coli* pathogen to the microbial community in the gut (54, 55). Previous research has shown that the diversity of the indigenous microbial community is an important factor in determining whether invasive *E. coli* will be able to colonize an environment (56). It is broadly accepted in the literature that microbial communities with high biodiversity are more resistant to changes in their structure and function than those with lower biodiversity (56–59). In one of these studies,

TABLE 1 Measured values for selected cell surface properties and the attachment efficiency of the cells in column transport experiments

Sample	Hydrophobicity (%)	Electrophoretic mobility ($\text{cm s}^{-1} \text{V}^{-1}$)	Radius (μm)	EPS content ($\text{mg}/10^{10}$ cells)		Attachment efficiency
				Sugar	Protein	
Colon	12.5a (6.5) ^a	−1.67a (0.20)	0.46a (0.02)	6.30a (2.9)	31.1a (6.5)	0.22a (0.09)
Septic tank	40.1b (2.8)	−1.10b (0.37)	0.51a (0.05)	15.3b (3.1)	120b (58)	0.47b (0.2)
Groundwater	55.1b (6.3)	−1.35b (0.05)	0.48a (0.03)	15.1b (6.3)	126b (29)	0.56b (0.1)
Colon with pathogen	36.3b (10)	−1.95a (0.25)	0.57b (0.1)	17.8b (7.9)	78.5b (26)	0.34a (0.04)
Septic tank with pathogen	51.8b (8.4)	−1.24b (0.18)	0.60b (0.07)	5.15a (3.4)	47.6a (15)	0.47b (0.09)
Groundwater with pathogen	34.8b (9.5)	−1.38b (0.22)	0.57b (0.08)	4.69a (4.4)	44.7a (17)	0.55b (0.1)

^a Standard deviations are given in parentheses. Within each column, values with the same letter are not significantly different. (A P value of <0.05 was considered significant.)

TABLE 2 Water quality analysis of the model septic tank

Water quality test ^a	Value for model septic tank
Alkalinity (mg/liter as CaCO ₃)	300 (25) ^b
pH	6.8 (0.5)
Conductivity (μS/cm)	1.39 (0.05)
TOC (mg/liter)	152.6 (10)
Hardness (mg/liter as CaCO ₃)	51 (5)
TSS (mg/liter)	28 (7)
BOD (mg/liter)	61 (8)
Total organisms (cells/ml)	10 ⁶ (10 ⁵)

^a TOC, total organic carbon; TSS, total suspended solids; BOD, biological oxygen demand.

^b Standard deviations are given in parentheses.

where *E. coli* O157:H7 changed the community structure, the researchers explained the shift by suggesting that there was limited functional redundancy (56). The lack of functional redundancy in a microbial community allows the pathogen to establish an ecological niche.

The microbial activity of the colon in this study was compared to those of previous *in vivo* and *in vitro* gut environments by quantifying the short-chain fatty acids in the model colon. The total fatty acid content in the colon was insensitive to the presence or absence of the pathogen, although acetic acid levels decreased significantly ($P < 0.05$), while butyric acid levels increased significantly ($P < 0.05$), in the presence of *E. coli* O157:H7. Previous studies have suggested that microbial composition and fatty acid differences could be attributed to diet (15, 60–63), pH (64), the peptide supply (64), and the relative amount of oxygen in the system (65, 66). However, these parameters were consistent in this study, suggesting that the altered community structure is impacting the fatty acid composition of the model colon (64, 67). Previous research has shown that acetic acid impairs *E. coli* O157:H7 infection of the gut (68), while the introduction of *E. coli* O157:H7 could increase the relative amount of butyric acid (69). This would enhance the production of virulence genes, allowing the pathogen to establish a niche in the colon. An established microbial community may be robust enough to negate the effects of *E. coli* O157:H7 on microbial community structure by increasing the production of acetic acid while reducing that of butyric acid (24). The shift in short-chain fatty acid production in this study implies that the pathogen is now fully integrated into the microbial community and is influencing the microbial community to reduce acetate production and increase butyric acid production in order to allow the pathogen to produce virulence factors and find an ecological niche.

The microbial structure and water quality were assessed for the aquatic systems to ensure that they accurately represented the environments simulated. The septic tank was inoculated with the microbial community from the model colon, yet the composition differed greatly from that of the inoculum. Previous research has shown that proteobacteria tend to survive and thrive better than bacteroidetes and firmicutes in domestic wastewater systems (70), a pattern similar to that for this model septic system. Not only were the microbial data similar to those for an actual wastewater system, but all the water quality values for the model septic tank fell into the range of values for a real septic tank process (35). Regardless of the presence or absence of the pathogen, the micro-

bial communities of the groundwater samples were composed mostly of proteobacteria, with higher percentages of firmicutes and bacteroidetes than those found in the model septic tank effluent. The structure of the microbial community in the groundwater experiments was similar to that in previous research where groundwater was contaminated with livestock wastewater (71) or subjected to other perturbed environmental conditions (11).

The extensive phenotypic cell characteristics tested—hydrophobicity, electrophoretic mobility (EPM), radius, extracellular polymeric substance (EPS) content, and attachment efficiency—were chosen because they have been linked to the fate and transport of microorganisms in the environment (42, 72–74) and can be used for comparative evaluation of different environmental microbial communities. In the absence of *E. coli* O157:H7, cells from the colon had lower hydrophobicity, EPM, EPS, and attachment efficiency values than microbial communities in the aquatic systems. In the presence of *E. coli* O157:H7, the change in community structure led to some significant alterations in the microbial community's phenotypic response. In the colon, the change in community structure led to an increase in hydrophobicity, which has been shown to affect microbial deposition onto surfaces and the formation of biofilms (75, 76). The perturbation also changed the quantities of EPSs in all systems: EPS production increased in the colon, but decreased in the aquatic systems, in the presence of *E. coli*. This decrease in EPS production may make the microbial community less likely to attach to surfaces and to form biofilms in aquatic systems. The average size of the microbial community increased significantly in all three systems when *E. coli* O157:H7 was present, which could affect the community's transport through porous media.

There were a few statistically significant correlations among the cell properties of the three environmental systems. As in previous work using regression analysis, attachment efficiency was found to be correlated to electrophoretic mobility (72–74) and hydrophobicity (77). Additionally, attachment efficiency was inversely proportional to the EPS sugar-to-protein ratio ($P < 0.05$) (74). These relationships show that the cell surface characteristics (hydrophobicity, EPM, and EPS ratio) of the heterogeneous microbial communities can be correlated with the transport properties (attachment efficiency) of the cells in all of the representative systems in this study.

In order to better explain the impact of the microbial community in the three model systems (colon, septic tank, and groundwater) on *E. coli* O157:H7, the results of this study were compared with those for *E. coli* O157:H7 grown under idealized lab conditions in a 2009 study by Haznedaroglu et al. (78). That study investigated the fate and transport of *E. coli* O157:H7 and *Salmonella enterica* serovar Pullorum SA1685 under idealized conditions (with 10 mM KCl, the same ionic strength and salt condition used for cell phenotypic analysis in the current work) (78). This comparison of the characteristics of the bacteria grown in the presence and absence of a microbial community can provide indirect insight into the impact of the microbial community on *E. coli*. The hydrophobicity of *E. coli* in the Haznedaroglu study was significantly lower ($19\% \pm 2\%$) (78) than that of *E. coli* evaluated along with the microbial community (35% to 52%) from each of the model systems. This increase in hydrophobicity for *E. coli* within a microbial community over that for the isolate may further impact the fate of the cell, since hydrophobic interactions have been shown to affect microbial deposition onto surfaces and

to influence the formation of biofilms (75, 76). Another phenotypic measure of the *E. coli* O157:H7 isolate versus *E. coli* O157:H7 grown within a microbial community is EPM, which has been linked to the stability and attachment of bacteria. Notably, higher absolute values of EPM correlate with more-stable organisms (79, 80). The EPM for the individual isolate in the Haznedaroglu study was much less negatively charged ($-0.14 \text{ V s}^{-1} \text{ cm}^{-1}$) (78) than that for *E. coli* O157:H7 with the microbial community (-1.95 to $-1.24 \text{ V s}^{-1} \text{ cm}^{-1}$). This suggests that the microbial community of which *E. coli* is a part in the current study will be more stable in suspension than the individual isolate. Next, the EPS content was evaluated via the sugar/protein ratio so as to compare those for the individually grown *E. coli* isolate and *E. coli* grown in the microbial community. The EPS sugar and protein contents were evaluated because they have been reported as indicators of cell conditions (81). It was observed that the sugar/protein ratio was greatly enhanced in the presence of the microbial community (0.10 to 0.23) over that for *E. coli* O157:H7 in the Haznedaroglu study (0.03) (78). The increase in the amount of sugar relative to protein in the EPS has been related to biofilm formation previously (82), suggesting that *E. coli* within the microbial community is more likely to be part of a biofilm than isolated *E. coli* O157:H7.

The surface charge of the bacteria, as quantified by EPM, suggests that *E. coli* in the microbial community is more stable and would be more mobile than the *E. coli* isolate alone (as in the Haznedaroglu study [78]). However, the hydrophobicity and EPS analyses suggest that there are substantial hydrophobic interactions at play in the presence of the microbial community that may lead to greater attachment of the cells. The impact of these mechanisms can be compared in the transport data. The Haznedaroglu study did not report attachment efficiency but rather used deposition rates to predict the transport distance at which 3 log removal (99.9%) of the *E. coli* isolate flowing in porous media would be achieved (78, 83–85). This is a method commonly used to assess fate and transport (86, 87). Haznedaroglu et al. found that isolated *E. coli* O157:H7 could travel 3.7 m before removal of 99.9% of the cells (78). Similar calculations were conducted on the microbial consortia with *E. coli* O157:H7, which consistently required a shorter distance to achieve 3 log removal. This suggests that the cells in the microbial community are much more adhesive than the *E. coli* isolate alone. Based on this study, 0.1% of the *E. coli* bacteria have the potential to travel beyond 1.4 m, 0.9 m, or 0.8 m if released into the groundwater environment directly from the model colon, septic tank, or synthetic groundwater system, respectively. These calculations suggest that *E. coli* is impacted by the presence of a microbial community in that it becomes less likely to be transported in the subsurface than an isolated cell without the complexity of the consortium.

In this study, three *in vitro* systems were developed to replicate representative environmental conditions. These *in vitro* systems successfully simulated the community structures and functions of their *in vivo* counterparts. This approach has allowed for the investigation of the microbial community's response to the introduction of *E. coli* O157:H7. The differences described here (hydrophobicity, electrophoretic mobility, EPS levels, and general transport calculations) suggest that *E. coli* O157:H7 within a microbial community may be less mobile in aquatic environments and more prone to biofilm formation than the isolated strain. This study is an initial step in understanding how microbial communities behave in the environment and how resilient the structure

and function of the consortia are when invasive species are introduced. Other pathogenic bacteria may have different effects on native microbial communities. Thus, more research is needed to determine the long-term effects of pathogens on microbial communities in complex environments.

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