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PAPER

Impact of growth conditions on transport behavior of *E. coli*

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The aim of this investigation is to determine the effect that growth solution has on the cell surface properties and transport behavior of eleven *Escherichia coli* isolates through saturated porous media. The two growth solutions used were a standard laboratory growth medium (LB) and a dairy manure extract solution. In general, cells grown in manure extract were more hydrophobic, had a more negative zeta potential, had lower amounts of surface macromolecules, and had lower attachment efficiencies than isolates grown in LB. An inverse relationship between the natural log of zeta potential and the attachment efficiency of the isolates for the cells grown in LB media was the only statistically significant correlation observed between transport behavior and cell characteristics of the isolates. This study shows the need to consider growth conditions when studying bacteria to better mimic the environmental stresses that bacteria undergo in the natural environment. This approach could lead to a better understanding of the behavior of manure-derived bacteria in aquatic and terrestrial environments.

Introduction

Contamination from fecal matter is the leading cause of impairment of potable water sources in the US.[1] *Escherichia coli* are the most commonly identified fecal indicator bacteria (FIB) originating from agricultural and urban runoff,[2] and are used as an indicator of water quality. While the indicator bacteria can be used to determine whether fecal contamination occurred, the association between *E. coli* and the targeted pathogenic bacteria has been found to be weak.[3] These pathogens include *Campylobacter*, *Salmonella*, and virulent strains of *Escherichia coli*.[4,5] Pathogenic bacteria shed from livestock have the potential to reach potable water sources, including groundwater, from farms *via* storm runoff or in manure that has been treated for reuse.[6] Another route for introduction is manure from farm animals used as a fertilizer for agricultural crops,[7] which could contaminate the farm produce with pathogenic bacteria.[8] Hence, understanding the fate and transport of various strains of *E. coli* originating from agricultural waste is essential for assessing water quality and protecting drinking, recreational, and agricultural water sources from contamination.

Previous work has utilized highly idealized conditions to investigate microbial fate and transport. For example, when studying *E. coli* in the laboratory, growth media such as LB (lysogeny broth) is often used to provide a consistent nutrient source for the organisms to grow. Such growth media are designed and tested to ensure reproducible and predictable growth conditions.[9] This laboratory technique has led to an improved understanding of the genetics of *E. coli*,[10] demonstrating how pathogens can infect people,[11] mechanisms of bacterial transport,[12] and biofilm formation.[13,14] However, nutrient-rich growth media are not representative of the

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Environmental impact

Pathogenic bacteria shed from livestock have the potential to reach potable water sources. Since many of these pathogens are below the detection limits of current bacteria tracking techniques, fecal indicator bacteria (FIB) are used to assess how far the microorganisms could travel in the environment. These bacteria are generally grown in laboratory culture media such as LB media to study the fate and transport properties of FIB. Therefore, it is of critical importance to know whether laboratory culture media such as LB can accurately assess the fate and transport properties of environmental isolates. This study found that the isolates grown in LB had different cell surface and transport properties than cells grown in an environmentally representative growth solution (manure extract). This study demonstrates that the practice of using a single representative strain grown in standard laboratory culture media is insufficient to model the transport of bacteria from a manure source.

conditions that the organisms will be exposed to once expunged from the intestines of farm animals. Therefore, to understand the fate and transport of *E. coli* originating from agricultural waste– or any other microorganism of interest–it is essential that the organisms are grown in more representative media.

Previous studies have made an effort to link cell surface properties to the transport of the microorganisms in the environment. Notably cell type,[15–19] size,[20–22] hydrophobic interactions,[15,18,21,23,24] surface charge,[18,22,23,25–28] and surface macromolecular content[29–31] have been measured. However, relatively few studies have investigated the link between cell characteristics and transport behavior of cells grown in manure solutions. Cellular characteristics such as zeta potential and hydrophobicity have been shown to differ for cells grown in either manure or other idealized laboratory environments such as bovine intestinal conditions.[32,33] Yang *et al.* reported that among the isolates that they tested, four had higher attachment efficiency when grown in manure, while two isolates had lower attachment efficiency.[33] These trends show that the fate and transport of bacteria isolates are affected by composition of the growth media.

In addition to the growth conditions of cells, the presence of manure in bacterial transport can have an effect on their adhesion properties. The attachment of the bacteria to soil particles is greatly reduced when the cells are suspended with manure as opposed to cells suspended in an electrolyte suspension.[34–36] The attachment of the cells suspended in manure to negatively charged soil may be hindered,[7] since they may tend to attach better to positively charged inorganic particles that may aid in their transport.[37] These reported trends suggest the presence of manure during bacterial transport may allow pathogenic bacteria to be transported to a greater extent. This suggests studies without manure may be underestimating movement of bacteria in groundwater.

To address this notable gap in the literature, a study was developed to compare and evaluate the differences in the fate and transport of cells that were grown in either a dairy manure extract solution or more traditional laboratory media (LB). Eleven *E. coli* isolates from dairy manure were extensively characterized using physical and chemical techniques. Additionally, transport studies were conducted with the organisms after growth in one of the two test media. Results allow for an assessment of whether the typical laboratory practice of using a standard growth media is adequate for simulating and predicting bacterial fate and transport in manure contaminated aquatic environments.

Materials and methods

E. coli isolation and isolate selection

Five 1 L samples of dairy manure slurry (*i.e.*, mixture of manure, urine, and straw) were collected from a manure pit located on the farm at Western Kentucky University. The samples were collected at five randomly chosen locations within the pit 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 as previously described except the initial denaturation step was extended to 12 min.[38] Briefly, Rep-PCR using 0.5 μM of the BOX AIR primer (5′-CTACGGCAAGGC GACGCTGACG-3′) (BOX-PCR) was carried out in a 25 μl reaction containing 1X EconoTaq PLUS master mix (Lucigen, Middleton, WI) and 2 μl of diluted cell suspension. The PCR was conducted on a PTC-200 model thermal cycler (MJ Research/ BioRad, Hercules, CA) and PCR mixtures (10 μl) were electrophoresed on 1% agarose gel supplemented with 40% synergel (Diversified Biotech, Boston, MA) for 4 h at 75 V. Ethidium bromide stained gel images were captured in quadrants with a FOTO/Analyst Investigator/Eclipse system (Fotodyne Inc., Hartland, WI). Bands were identified and the data were statistically analyzed using Fingerprinting II Software Version 3.0 (BioRad, Hercules, CA). DNA fingerprints were compared using the Jaccard method for calculating similarity coefficients; cluster analyses of similarity matrices were performed by an unweighted pair group method with arithmetic mean (UPGMA) and isolates were grouped into unique BOX-PCR profiles based on 80% similarity in a BOX-PCR fingerprint pattern as previously described.[39] Eleven isolates were chosen from the major fingerprint groups to be used in the column studies. Each of the isolates was further confirmed to be *E. coli* by chemical analysis using Enterotube following the manufacturer's directions and by PCR analysis of the *uidA* gene as previously described.[40] All isolates were stored at −80 °C with 15% glycerol.

Isolate typing and evaluation of fimH and agn43

The eleven isolates were placed into one of four phylogenetic groups (A, B1, B2, or D) using the triplex PCR method of Clermont *et al.*[41] as modified by Higgins *et al.*[42] The PCR program was 15 min at 94 °C, 29 cycles at 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s. PCR analyses for the presence of *agn43* and *fimH* were carried out as previously described.[43] The touchdown PCR conditions for the *agn43* reaction consisted of an initial cycle of 95 °C for 15 min, 10 cycles of 94 °C for 30 s, 72 °C to 62 °C for 60 s, and 72 °C for 30 s, and 35 cycles of 94 °C for 30 s, 62 °C for 60 s, and 72 °C for 30 s. The PCR program for *fimH* was 15 min at 95 °C, 35 cycles of 94 °C for 30 s, 60 °C for 60 s, and 72 °C for 30 s followed by a final extension at 72 °C for 5 min. All assays were carried out in Qiagen 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 Table 1.

Manure extract preparation

Manure extract was prepared by mixing 40 g fresh dairy manure with 1 L of synthetic precipitation solution (EPA Method 1312)[44]

Table 1 Gene Targets and Primer Sequences

	Target	Primer	Oligo (5′ to 3′)	Product Size (bp)	Ref.
Genotyping					
chuA	Gene required for heme transport in enterohemorrhagic O157:H7	ChuA.1 ChuA.2	GACGAACCAACGGTCAGGAT TGCCGCCAGTACCAAAGACA	279	Clermont 2000
TspE4C2.1	Anonymous DNA fragment	TspE4C2.1 TspE4C2.2	GAGTAATGTCGGGGCATTCA CGCGCCAACAAAGTATTACG	152	
yjaA	Gene of unknown function	YjaA.1 YjaA.2	TGAAGTGTCAGGAGACGCTG ATGGAGAATGCGTTCCTCAAC	211	
Characterization					
fimH	Type 1 fimbriae; common adhesion factor associated with colonization	fimH-F fimH-R	CGTGCTTATTTTGCGACAGA CTCCGGTACGTGCGTAATTT	400	Yang 2004
agn43	Most abundant phase variable outer membrane protein	Agn43F Agn43R	GACTATGACCGGATTSTGGCAGGCT GTGGCTCCAGCATCAGRTTGTCAG	498	Yang 2004

for one hr using a roller. The resulting slurry was centrifuged at 2900 × g for 20 min. The decanted solution was filtered twice through a 0.45 μm filter and stored at 4 °C in the dark. Immediately prior to use, 0.01% yeast extract was added and the mixture filtered through a 0.45 μm filter.

Cell preparation

The eleven isolates selected were individually streaked on LB-Miller agar plates and grown in a 37 °C incubator overnight. Single colonies were picked from the LB plates and allowed to grow in 5 mL vials at 37 °C for 12 h to achieve stationary phase. The cells were diluted (1 : 100 ratio with deionized water (DI)) and 200 μl of the diluted cell culture were placed into a 200 mL flask of either manure extract, with 0.01% weight/volume of yeast extract, or LB media. This subsequent culture was incubated at 37 °C until stationary phase. Next, the liquid cell culture was centrifuged to separate the cells from the growth media at 3700 × g for 15 min at 4 °C (Eppendorf 5810R, Germany). The cell pellet was resuspended in 10 mM KCl solution prepared with DI and reagent grade KCl (Fisher Scientific, Pittsburgh, PA) with no pH adjustment (pH 5.6–5.8). This process was repeated twice in order to ensure complete removal of the growth medium prior to further analyses.

Cell surface characterization

The extracellular polymeric substance (EPS) composition, cell size, electrophoretic mobility, hydrophobicity, and surface charge density were measured for each isolate using previously published methods.[28] All of the cell surface analyses described below used 10 mM KCl as the background solution, unless stated otherwise.

To quantify the extracellular polymeric substance on the cell surface, the pellet of harvested bacterial cells was suspended in formaldehyde-NaCl solution. After two hr the fixed cells were centrifuged, washed with DI water, and sonicated for three min. The cell pellet was then placed in a −80 °C freezer followed by freeze drying for six hr before the colorimetric test.[45] After diluting the pellet in DI water triplicate assays were made for sugar and protein analysis. The protein and sugar were measured spectroscopically (BioSpec-mini, Shimadzu Corp., Kyoto, Japan) using the Lowry Method[46] at 500 nm and the phenol-sulfuric acid method[47] at 480 nm, respectively.

Cell size was measured from images taken using a phase contrast microscope (Micromaster; Fisher Scientific, Pittsburgh, PA) and camera. Images containing significant number of cells (each with >50) were processed by image processing software (Matlab, The MathWorks, Inc., Natick, MA) and individual cell lengths and widths were determined.

A zeta potential analyzer (Brookhaven Instruments Corporation, Holtsville, NY) was used to determine the electrophoretic mobility of the cell isolates. After suspending the cells in 10 mM KCl, as described above, the bacterial solution was diluted to an optical density of ~0.2 at a wavelength of 546 nm. The measurements were repeated five times for three assays taken from the diluted bacterial stock solution and the average was reported. Electrophoretic mobility values that were measured were converted to zeta potential values using the Smoluchowski equation.[48]

Hydrophobicity analysis of the bacteria was done by using the microbial adhesion to hydrocarbon (MATH) test.[49] The bacteria were first diluted to an optical density of ~0.2 at a wavelength of 546 nm. One mL of a hydrocarbon, n-dodecane (Fisher Scientific), was added to three assays of 4 mL of the diluted bacteria suspension and each of the assays were vortexed for 2 min. The partitioning of cells between n-dodecane and the electrolyte solution (10 mM KCl) was then determined spectroscopically at 546 nm after 15 min. The relative hydrophobicity was calculated as the percent of total cells partitioned into the hydrocarbon phase.

The surface charge density and relative acidity of the bacterial surfaces were determined by conducting potentiometric titration with the isolates. Bacterial suspensions with concentrations of 10^8–10^9 cells mL^{-1} in 10 mM KCl were titrated by concentrated NaOH (0.1 N) from pH 4–10 using an auto-titrator (798 MPT Titrino, Metrohm, Switzerland). Surface charge density and acidity were calculated by knowing the amount of base consumed to change the pH of the solution from 4 to 10.[50]

Transport experiments

Transport experiments were conducted using water-saturated columns packed with clean quartz sand (Unimin™, New Caanan, CT) that passed through a 850 μm sieve and was collected on a 710 μm sieve (USA Standard Testing Sieves, ATM Corp., New Berlin, WI). The sand was boiled in a 2 L flask containing 1 M 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 at 105 °C. Finally, the sand was sterilized by autoclaving at 121 °C and 15 psi for 20 min. Columns were wet packed by slowly pouring the clean, autoclaved sand into 2.5 cm diameter Chromaflex™ Chromatography Columns (Kontes Glass Co., Vineland, NJ) while vibrating the columns. Columns ranged in length from 9.9 to 10.1 cm. After packing was completed, a 10 mM electrolyte solution was pumped through the columns in an upward direction using syringe pumps (Model 200 syringe pump, KD Scientific Inc., New Hope, PA), and approximately 10 pore volumes (PV) 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^{-1} for a Darcian velocity of ~0.36 cm min^{-1}.

Following equilibration, a 38 min pulse (~1.2 PV) of an *E. coli* bacterial solution was passed through each column followed by 75 min of a bacteria free electrolyte solution. Concentrations of *E. coli* in the influent and effluent were determined by plating the appropriate dilutions on mFC Agar plates (Difco Laboratories Inc., Detroit MI) and incubating overnight at 37 °C. The *E. coli* concentration in the influent was ~2 (±1)×10^6 colony forming units (CFUs) per mL. Three bulk effluent samples were collected from each column to determine the total number of cells which passed through the 10 cm columns. The three samples were collected after ~0.8, 2.2, and 3.2 PV. The fractional recovery of each *E. coli* isolate was calculated from the total number of cells collected in the second sample and the total number of cells introduced into the column. The dimensionless bacterial deposition rate, κ, was then calculated from the relative recovery by:[51]

$$\kappa = \left[-\ln(fr) + \left(\frac{\ln(fr)^2}{Pe} \right) \right] \quad (1)$$

where *fr* is the fractional recovery defined as the number of bacteria recovered in the column effluent normalized by the total number of bacteria introduced into the system and *Pe* is the hydrodynamic Peclet number of the fluid (dimensionless). The Peclet number in our columns is estimated to be 110 based on bromide tracer tests conducted in an earlier study using similarly packed columns.[28] The bacterial attachment efficiency was calculated from the deposition rate using the hemispheres-in-cell model derived by Ma *et al.*[52]:

$$\alpha = \frac{2d_c}{3(1-\varepsilon)} \frac{\kappa}{L} \frac{1}{\eta} \left[\frac{3-\varepsilon}{3-3\varepsilon} - \frac{2(3-\varepsilon)}{\pi(3-3\varepsilon)} \cos^{-1}\left(\frac{3-3\varepsilon}{3-\varepsilon} \right)^{1/2} + \frac{2}{\pi} \sqrt{2\left(\frac{3-\varepsilon}{3-3\varepsilon} \right)^{-1/2} - 1} \right]^{-1} \quad (2)$$

where α is the bacterial attachment efficiency, d_c is the sand grain diameter (cm), ε is porosity, L is column length (cm), and η is the single-collector efficiency calculated using the correlation equation of Ma *et al.*[52] assuming a bacterial density of 1055 kg m^{-3} and a Hamaker constant of 6.5 × 10^{-21} J.[53] Every transport experiment was repeated on a separate day using a different culture to ensure true replication.

Statistical analysis

Differences in cell surface properties, fractional recoveries, and sticking efficiencies between LB- and manure-grown cells were statistically analyzed using Fisher's LSD method with no adjustment for multiple comparisons. All statistical analyses were performed using JPM ver 7.0 (SAS Institute, 2007) and differences were considered significant at $P < 0.05$.

Results and discussion

Isolate selection and genetic characterization

Selective plating and BOX PCR analysis of DNA extracts were conducted on the *E. coli* obtained from dairy pit slurry. Using a threshold of 80% similarity in BOX fingerprint patterns, 11 unique profiles were differentiated from the 339 *E. coli* isolates. Eleven isolates, representing 94% of the diversity of dairy *E. coli* isolates, were selected for transport studies and cell surface characterization. The genotyping triplex assay of Clermont *et al.*[41] was used to place the 11 *E. coli* isolates into one of the four main phylogenetic groups (A, B1, B2 and D). Based on this analysis, five of the dairy isolates grouped with the B1 genotype, four grouped with the D genotype and two with group A (Table 2). All of the isolates were positive for the *fimH* and all except two (DP2D08, DP4B09) were positive for *agn43*, two well-known adhesion factors.[21,32] These results are consistent with a previous study of 1,346 *E. coli* isolates from livestock manures and agriculturally impacted waters.[39] In that study, *fimH* (present in 80% to 95% of isolates) and *agn43* (present in 40% to 100% of isolates) were the most commonly detected genes of eleven targets associated with adhesion, transport and virulence.

Cell surface properties of the bacterial isolates

An extensive list of measured cell properties of isolates grown in LB or manure are displayed in Table 3 and 4. For eight of the eleven isolates, hydrophobicity was greater for the manure extract grown cells compared with the LB grown cells (six of these were significant at $P < 0.05$). The range of hydrophobicity values for the cells grown under the two different conditions were similar, 25–85% and 24–84% for isolates grown in the dairy manure extract and LB, respectively. This trend is similar to previous work with *E. coli* grown in manure *vs.* a more nutrient rich environment,[32] when the strains were more hydrophobic when grown in manure, or did not change at all.

All of the isolates had a negative zeta potential, ranging between −5 to −32 mV, and −6 to −46 mV for the cells grown in LB and manure, respectively. The zeta potentials for six isolates were significantly more negative ($P < 0.05$) when grown in manure as compared with cells grown in LB. This greater magnitude of charge renders these cells more stable in suspension and

Table 2 Type distribution and genetic characterization of E. coli isolates. The genotypes as well as the presence and absence of a few key adhesion factors are listed for each isolate. ± denotes presence and absence of gene, respectively

	DP1A04	DP1F07	DP1H11	DP2D08	DP2G06	DP3B05	DP3D01	DP3F07	DP4B04	DP4B09	DP4 F12
Triplex Geno-type	D	D	B1	B1	B1	B1	B1	A	D	D	A
uidA	+	+	+	+	+	+	+	+	+	+	+
fimH	+	+	+	+	+	+	+	+	+	+	+
agn43	+	+	+	–	+	+	+	+	+	–	+

potentially capable of greater transport when conditions are unfavorable for bacterial attachment to surfaces.[54] Only one isolate, DP4F12, was significantly less negative ($P < 0.05$) when grown in manure. Four of the isolates showed no significant difference in zeta potential between the two growth solutions. These organisms had zeta potential values less negative than −10 mV, except for DP2G06, which had values of −28 and −27 mV for cells grown in LB and manure, respectively. The range of zeta potential values of these isolates is similar to previously published research irrespective of growth conditions.[21,22,28,32]

Table 3 displays the calculated values of surface charge density. This is a measure of the distribution of polar functional groups exposed on the cell surface as determined from the experimentally determined acidity level and subsequently calculated charge density.[50] While there was no noticeable trend in surface charge density values between the two growth conditions, the range in values between isolates was greater for the cells grown in the manure extract than in LB (80–977 μC cm^{-2} *vs.* 123–533 μC cm^{-2}, respectively). These ranges in values are typical of *E. coli* isolates.[22,28]

For each isolate the extracellular polymeric substances (EPS) content was measured (Table 4). Levels of total EPS, as well as sugar and protein, were significantly lower ($P < 0.05$) for cells that were grown in manure as compared to those grown in LB. Notably, bacteria grown in LB media had approximately an order of magnitude higher sugar content than cells grown in the manure extract (0.78–2.55 mg sugar/10^{10} cells and 0.09–0.30 mg sugar/10^{10} cells, respectively). A similar trend was observed for protein, with cells grown in LB having 26.6–94.7 mg protein/10^{10} cells, and cells grown in manure extract having 0.63–10.47 mg protein/10^{10} cells.

These measured cell properties for the 11 *E. coli* dairy isolates confirm that *E. coli* exhibit very different behavior when grown in LB broth as compared to dairy manure extract (Tables 3 and 4). Four isolates (DP1A04, DP1F07, DP1H11, and DP3D01) showed complementary trends, including a higher absolute zeta potential value, increased hydrophobicity, and decreased surface charge density in manure, compared with cells grown in LB media. The cell properties of DP2D08 were similar to the previously mentioned four isolates except the surface charge density was higher when grown in the manure extract. DP3F07 and DP4B04 did not vary between the media types with regard to zeta potential and hydrophobicity, but both had higher surface charge density when grown in manure. DP2G06 cell properties did not vary between the media types with regard to zeta potential and hydrophobicity, but had a slightly lower surface charge density when grown in the manure extract. DP3B05 became more hydrophobic, had a higher absolute value of zeta potential, and a lower surface charge density when grown in manure extract. DP4B09 was more hydrophobic, had a similar zeta potential, and had a higher surface charge density when grown in manure extract. DP4F12 had similar hydrophobicity, lower zeta potential, and higher surface charge density when grown in manure extract. Each isolate had much lower level of EPS on a per cell basis, for both sugar and protein content, when grown in manure extract than when they were grown in LB media (Table 4).

Growth media influence on transport

The fractional recovery (*fr*) of the *E. coli* isolates eluted from the 10 cm columns ranged from 0.32 to 0.67 for cells grown in dairy

Table 3 Comparison of the cell surface properties of *E. coli* isolates obtained from dairy cattle manure grown in the lab using LB media and manure extract. The values displayed include the average value of the experiments and the standard deviations are in parentheses

	Hydrophobicity (%)		Zeta Potential (mV)		Surface Charge Density (μC cm^{-2})	
Isolate	LB	Manure	LB	Manure	LB	Manure
DP1A04	46 (9.8)	75[a] (2.7)	−7.2 (0.3)	−30[a] (2.2)	244	179
DP1F07	38 (11)	76[a] (7.7)	−8.6 (0.1)	−25[a] (0.4)	192	166
DP1H11	34 (3.0)	74[a] (20)	−33 (0.7)	−46[a] (5.2)	224	80
DP2D08	24 (8.6)	80[a] (1.8)	−12 (0.1)	−21[a] (1.6)	127	292
DP2G06	67 (8.2)	75 (4.1)	−28 (1.1)	−27 (1.7)	123	104
DP3B05	84 (2.5)	60[a] (3.5)	−5.8 (0.9)	−31[a] (3.8)	212	82
DP3D01	34 (6.9)	50[a] (5.3)	−5.4 (2.5)	−20[a] (4.7)	396	116
DP3F07	77 (6.7)	75 (1.3)	−9.6 (2.1)	−9.1 (0.1)	160	276
DP4B04	31 (3.3)	25[a] (0.8)	−6.1 (1.1)	−8.5 (0.8)	127	646
DP4B09	25 (11)	63[a] (5.0)	−7.1 (2.3)	−6.8 (0.3)	225	977
DP4F12	83 (3.6)	85 (2.9)	−14 (3.6)	−7.9[a] (0.2)	533	731

[a] Differences between cells grown in manure extract and LB that exceed Fisher's LSD of 13.8 for hydrophobicity and 4.57 for zeta potential ($P = 0.05$).

Table 4 Comparison of the extracellular polymeric substances on *E. coli* isolates obtained from dairy cattle manure grown in the lab using LB media and manure extract. The values displayed include the average value of the experiments and the standard deviations are in parentheses

	Mean Sugar (mg/10^{10} cell)		Mean Protein (mg/10^{10} cell)	
Isolate	LB Media	Manure Extract	LB Media	Manure Extract
DP1A04	1.34 (0.01)	0.12[a] (0.003)	43.1 (0.7)	2.33[a] (0.4)
DP1F07	1.40 (0.05)	0.09[a] (0.001)	52.2 (1.4)	1.98[a] (0.3)
DP1H11	0.84 (0.01)	0.15[a] (0.003)	26.6 (0.1)	1.90[a] (0.7)
DP2D08	1.05 (0.04)	0.12[a] (.0006)	39.0 (0.9)	0.65[a] (0.05)
DP2G06	2.24 (0.2)	0.30[a] (.0007)	91.4 (0.5)	0.63[a] (0.3)
DP3B05	2.36 (0.02)	0.16[a] (0.02)	85.6 (2.2)	2.27[a] (0.5)
DP3D01	1.33 (0.01)	0.25[a] (0.005)	39.2 (0.3)	3.57[a] (0.7)
DP3F07	1.55 (0.04)	0.27[a] (0.003)	56.1 (2.5)	3.05[a] (0.5)
DP4B04	0.78 (0.02)	0.28[a] (0.005)	28.8 (2.5)	10.47[a] (0.5)
DP4B09	1.13 (0.02)	0.13[a] (0.005)	40.4 (2.4)	2.86[a] (0.2)
DP4F12	2.55 (0.06)	0.30[a] (0.01)	94.7 (5.3)	0.63[a] (0.06)

[a] Differences between cells grown in manure extract and LB that exceed Fisher's LSD of 0.312 for sugar concentration and 2.65 for protein concentration ($P = 0.05$).

manure extract and 0.36 to 0.65 for cells grown in LB broth (Table 5). Calculated bacterial attachment efficiencies (α) ranged from 0.33 to 0.82 for manure extract grown cells and 0.33 to 0.73 for LB grown cells (Table 6). Nine of the eleven isolates had lower fractional recoveries (3 to 32% lower) and higher attachment efficiencies (6 to 86%) when grown in LB broth compared with dairy manure extract. Differences between growth conditions exceeded Fisher's LSD ($P < 0.05$) for *fr* for five isolates (Table 5) and α for six isolates (Table 6).

The range in bacterial attachment efficiencies among the different *E. coli* isolates observed in this study was lower than the range observed for eight isolates obtained from a swine lagoon using similarly packed columns.[28] In that study α values varied by over an order of magnitude for stationary phase cells grown in LB broth whereas in this study α values varied by a factor of less than three. One possible explanation for the reduced range in α is that a 10 fold higher ionic strength electrolyte solution was used in the current study. When conditions become more favorable for deposition, as would occur with an increase in ionic strength, the diversity in α among *E. coli* isolates is expected to decrease.[28]

In order to explain the differences in transport behavior we observed among the different *E. coli* isolates, the correlation between the attachment efficiency and the different cell surface properties reported above was investigated. The only statistically significant correlation observed was between the zeta potential

Table 5 Measured fractional recoveries for *E. coli* grown in LB broth and dairy manure extract

Isolate	Manure	LB	Δfr[a]
DP1A04	0.45	0.47	0.019 (4.2)
DP1F07	0.54	0.40	−0.14 (−26)*
DP1H11	0.67	0.64	−0.033 (−5.0)
DP2D08	0.60	0.58	−0.017 (−2.9)
DP2G06	0.59	0.53	−0.052 (−8.9)
DP3B05	0.47	0.38	−0.090 (−19)*
DP3D01	0.46	0.36	−0.10 (−22)*
DP3F07	0.63	0.43	−0.21 (−32)*
DP4B04	0.47	0.41	−0.062 (−13)
DP4B09	0.49	0.41	−0.082 (−17)*
DP4F12	0.32	0.36	0.042 (13)

[a] Differences in fractional recovery (*fr*) between the two growth treatments (Δfr) with percent differences between treatments in parentheses. Significant differences which exceed Fisher's LSD of 0.068 ($P = 0.05$) are denoted with an asterisk.

Table 6 Calculated attachment efficiencies for *E. coli* grown in LB broth and dairy manure extract

Isolate	Manure	LB	$\Delta\alpha$[a]
DP1A04	0.427	0.423	−0.004 (−0.9)
DP1F07	0.332	0.488	0.156 (46.8)*
DP1H11	0.209	0.237	0.028 (13.2)
DP2D08	0.292	0.307	0.015 (5.1)
DP2G06	0.319	0.320	0.001 (0.3)
DP3B05	0.403	0.494	0.091 (22.5)*
DP3D01	0.448	0.566	0.118 (26.3)*
DP3F07	0.234	0.453	0.219 (93.4)*
DP4B04	0.393	0.454	0.061 (15.4)
DP4B09	0.368	0.477	0.109 (29.6)*
DP4F12	0.583	0.519	−0.064 (−11.0)

[a] Differences in attachment efficiencies (α) between the two growth treatments ($\Delta\alpha$) with percent differences between treatments in parentheses. Significant differences which exceed Fisher's LSD of 0.10 ($P = 0.05$) are denoted with an asterisk.

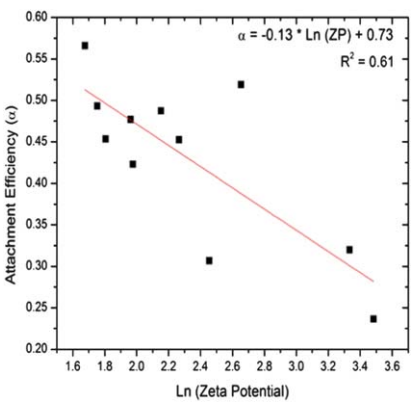

Fig. 1 Relationship between the natural log of the average zeta potential and the average attachment efficiency for isolates grown in LB media. The best fit linear equation and R-squared value are displayed in the top right corner of the plot.

and attachment efficiency of the cells grown in LB media (Fig. 1). Interestingly, the best-fit line relating attachment efficiency with the natural log of the zeta potential has a slope (−0.13) very similar to the one reported previously for swine *E. coli* isolates conducted under similar experimental conditions but with a 10-fold lower ionic strength solution (−0.11). This relationship, however, did not hold for cells grown in dairy manure extract. This suggests that cell characteristics controlling the transport of *E. coli* through porous media vary depending on composition of the growth media. Thus, results from experiments utilizing *E. coli* grown in commercial media may not be directly applicable to *E. coli* movement in the environment.

Conclusions

This study has investigated the differences in the cell surface and transport properties of cells that were grown in dairy manure extract and more traditional laboratory media (LB). Notably the cells grown in manure were more hydrophobic, had a more negative zeta potential, less EPS, and had a lower attachment efficiency than the isolates grown in LB. The cell surface properties and attachment efficiencies of the 11 *E. coli* dairy isolates demonstrated the standard laboratory practice of using a single isolate and standard growth media (LB) is insufficient for simulating transport of *E. coli* from a manure source. Future research is needed to adequately model the fate and transport of bacteria grown in more representative growth solutions. Using isolates from other livestock grown in their respective manure sources should be considered to give a more accurate portrayal of the transport properties of the indicator bacteria used to track the pathogens to ensure they do not reach ground and surface water.

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