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THE ROLE OF CURLI AND CELLULOSE IN THE TRANSPORT AND SURVIVAL OF *ESCHERICHIA COLI* ON A CENTRAL NEW YORK DAIRY FARM

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ABSTRACT Enterobacterial pathogens such as *Escherichia coli* are persistently deposited in the environment through the spreading of manure wastes onto agricultural soils, representing serious water quality and human health concerns. In this experiment, *E. coli* isolates were collected from a dairy farm in Central New York at three distinct locations: (i) cow housing, (ii) calf housing, and (iii) field drain (tile) effluent. These environmental *E. coli* isolates were analyzed for the cell surface components cellulose and curli, traits that have been linked to increased environmental survival and transport through soil. Our results showed a high amount of diversity amongst *E. coli* isolates at each spatial location. Isolates collected from cow housing and calf housing displayed highly variable curli and cellulose-producing community profiles from one sampling week to another. However, isolates collected from the drain tile effluent consistently displayed similar curli and cellulose production communities over all sampling dates. These results indicate that the subsurface soil and presence of drain tiles tend to select for a certain subset of *E. coli* strains, perhaps better adapted for environmental survival and/or transport.

Keywords: *Escherichia coli*, curli, cellulose, *rdar*, transport

INTRODUCTION *Escherichia coli*, including the pathogenic strain 0157:H7, is commonly found in the intestinal tract and feces of mammals. Fecal deposits of *E. coli* are continually introduced into the environment through livestock grazing and the spreading of manure on agricultural fields (Ogden et al. 2001; Jamieson et al. 2002). The protection of surface, drainage and groundwater from *E. coli* contamination is of particular interest as several recent disease outbreaks have been linked to pathogenic bacterial contamination of water sources (O'Conner 2001; CDC 2008; CDPHE 2008). Despite efforts to prevent water-related diseases, enterobacterial outbreaks continue to occur. Our failure to prevent such outbreaks is due in part to our lack of understanding

the factors that influence survival and transport of pathogenic bacteria in the environment (Filip et al. 1988; Stevik et al. 2004; USEPA 2006).

When *E. coli* are shed from their host, they enter a harsh environment where they are subject to many stresses, including limited nutrient availability, osmostic stress, variations in temperature and pH, and predation (Marshal 1980; Savageau 1983; Winfield & Groisman 2003). In spite of these obstacles, *E. coli* can survive in the natural environment for extended time periods, from several days up to more than one year (Filip et al. 1998; Kudva el al. 1998; Entry et al. 2000, Fremaux et al. 2007). Survival rates have been shown to be influenced by the method (i.e., surface or injected) and form (i.e., slurry or solid wastes) in which the source is applied to the field (Nicholson et al. 2005). Precipitation and infiltration facilitates the transport of these pathogens through the soil matrix and into the underlying drainage systems or groundwater. Vinten et al. (2002) observed approximately 20% of the total *E. coli* cells, applied to a field in a manure slurry, in the drainage water.

Some *E. coli* produce patterned, aggregative colonies characterized by the extracellular components curli, fimbriae and cellulose. This phenomena is called the *rdar* morphotype, named for the cells' ability to form red, dry and rough colonies on Congo Red agar. This *rdar* morphotype is expressed in response to environmental cues, such as low osmolarity, nutrient limitations, and low temperatures (Collinson et al. 1993, Gerstel & Romling 2001), and has been shown to enhance microbial resistance to desiccation (White 2008). *Rdar* morphology is highly conserved between *E. coli* and *Salmonella* (Römling et al. 1998). It is hypothesized that the primary role of the *rdar* morphotype is to enhance survival of enterobacteria in nutrient-limited nonhost environments, although its exact role remains poorly understood.

This study examined the curli and cellulose producing abilities of *E. coli* isolates from three locations (i.e., cow housing, calf housing, and drainage tile) on a central New York dairy farm. The objectives of this study were to analyze morphotype distribution at each spatial location and determine if *E.coli* strains exhibiting curli and cellulose production, (as observed in the *rdar* morphotype) were more likely to be found in the field drain effluent.

MATERIALS AND METHODS

Sampling Location Samples were collected from a dairy farm within sixty kilometers of the research facilities at Cornell University in Ithaca, New York. The study farm was selected based on the presence of flowing subsurface drainage (tile) lines in crop fields where manure spreading occurred during the proposed sampling period. The soils in the crop field consist of fine-loamy, mixed active, mesic Glossaquic Hapludalfs and coarse-loamy, mixed, active, mesic Typic Fragiudepts which are common to the Central New York Finger Lakes region. Environmental samples were taken from the farm's calf housing, cow housing, and a tile drain every seven days for three weeks, between the months of October and November 2008.

Sample Collection Pre-weaned calf housing was sampled by swabbing sterile 4x4 gauze saturated in 30 ml of sterile double strength skim milk deep into moist bedding or wet flooring of four systematically selected individual calf pens/hutches, or four different

areas of a group calf housing facility. Cow housing samples were taken by swabbing wet areas of the floor of four high traffic areas of the lactating cow facilities, such as, alleyways to and from the parlor, holding areas, or around feed bunks and waterers.

Field tile drain outflows were sampled using Moore swabs. Three individual swabs, each having a one to two foot tail, were fastened approximately six to twelve inches apart to a clean six foot fiberglass rod. The rod was placed inside the outlet of the tile drain and clamped to the top of the discharge pipe. This placement allowed the swabs to sit on the bottom of the tile drain so water percolating through the soil and into the drain could flow through the swabs. Three Moore swabs were installed in the tile drain on days one, seven, and fourteen. They were collected on days seven, fourteen, and twenty-one. Collections consisted of retrieving the swabs from the drainage outlet pipe and placing each swab in individual sterile Whirl-Pak bags (Nasco, Fort Atkinson, WI). All samples were received by the research lab on the day of collection and refrigerated at 4°C until processed within 36 hours.

***E. coli* Culturing** The species *Escherichia coli* was isolated from environmental samples using sterile drag swabs soaked in double strength skim milk. The 4x4 gauze sponges were separated from each pooled sample. For the isolation of *E. coli*, environmental and More swabs were enriched in 100 ml of Difco GN Hajna broth (BD, Sparks, MD) and incubated at 37°C for 18–24 hours. Following incubation, the inoculated broth was streaked onto MacConkey (MAC) plates (Northeast Laboratory Services, Waterville, ME), in order to isolate generic *E. coli*. Approximately fifty *E. coli* isolates per sample were selected from MAC plates and stored on Tryptic Soy Agar (TSA) slants at 4°C.

Congo Red Morphotype Identification From TSA slants, each isolate was stabbed onto a YESCA-Congo Red agar plate (Hammar et al. 1996) as a qualitative means of assessing curli and cellulose production. Plates were sealed with parafilm and incubated for 7 days at 30º C. After incubation, colony growth formed by each strain (morphotype) was captured with a digital camera (Canon Powershot A590).

Statistical Analysis Using JMP 8.0 statistical software (SAS, Cary NC), statistical analysis was performed on morphotype distribution at each sampling location per sampling date. Kruskal-Wallis one-way analysis of variance by ranks was used to assess if differences existed amongst morphotype distributions. Kruskal-Wallis is a non-parametric method used for testing equality of populations among groups. Strain morphotype data were grouped by sampling date, resulting in three sets of temporal-based data at each spatial location. Using ranks assigned to each morphotype, Kruskal-Wallis ranks determined whether distribution of morphotypes were equal amongst all three sampling dates, with the null hypothesis being that morphotype distribution is equal amongst all sampling dates. Chi-square tests were used to determine significance (P=0.05) between populations.

RESULTS AND DISCUSSION

Morphotypes Observed *E. coli* strains collected on a dairy farm in central New York were analyzed for curli and cellulose production. At all sampling locations, strains produced one of three distinct morphotypes when plated on Congo red agar, each indicating unique curli and cellulose expression abilities: red, dry and rough (*rdar)*, red,

dry and smooth (*rdas*) or smooth and white (*saw*). Red, dry and rough morphotypes indicate the production of both curli and cellulose, as the curli fibers bind the Congo red dye present in the media staining red. The presence of cellulose is known to give rise to the rough ridges (Zogaj et al. 2003) observed in the morphotype. When curli were

Figure 1. Examples of each Congo red morphtype classification used in this study. From left to right: *rdar* (curli +, cellulose +), *rdas* (curli +, cellulose -), *saw* (curli -, cellulose -).

present with no cellulose, strains would stain red but maintained a smooth morphotype. Strains which displayed little or no curli or cellulose production were placed into a separate category, smooth and white ($curli^-$, $cellulose^-$).

Calf and Cow Housing The cow housing area, including the cow and calf housing pens, produced a distribution of morphotypes which varied significantly amongst sampling dates. Figures 2 and 3 show the morphotypic distribution of each sampling date at the cow housing and calf housing locations, respectively. In the calf housing, the *rdar* morphotype comprised more than 75% of the strains collected on the first sampling date but made up less than 30% of the total population in the succeeding two weeks of sampling. Similar variability was observed in the adjacent cow housing pens. Cow housing pen morphotype distributions during the first two sampling dates appeared to be fairly equal while the third sampling date yielded very different results, with the curli and cellulose deficient strains (*saw*) as the predominant (~80%) morphotype as compared

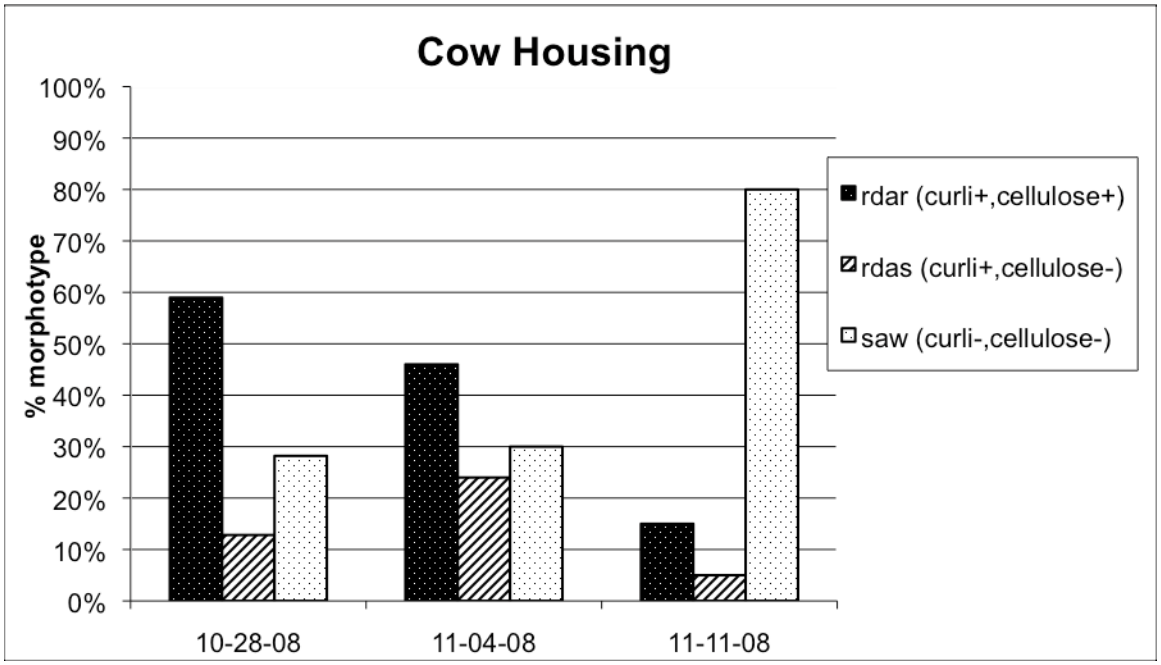

Figure 2. Morphotype distribution of samples collected at Cow Housing.

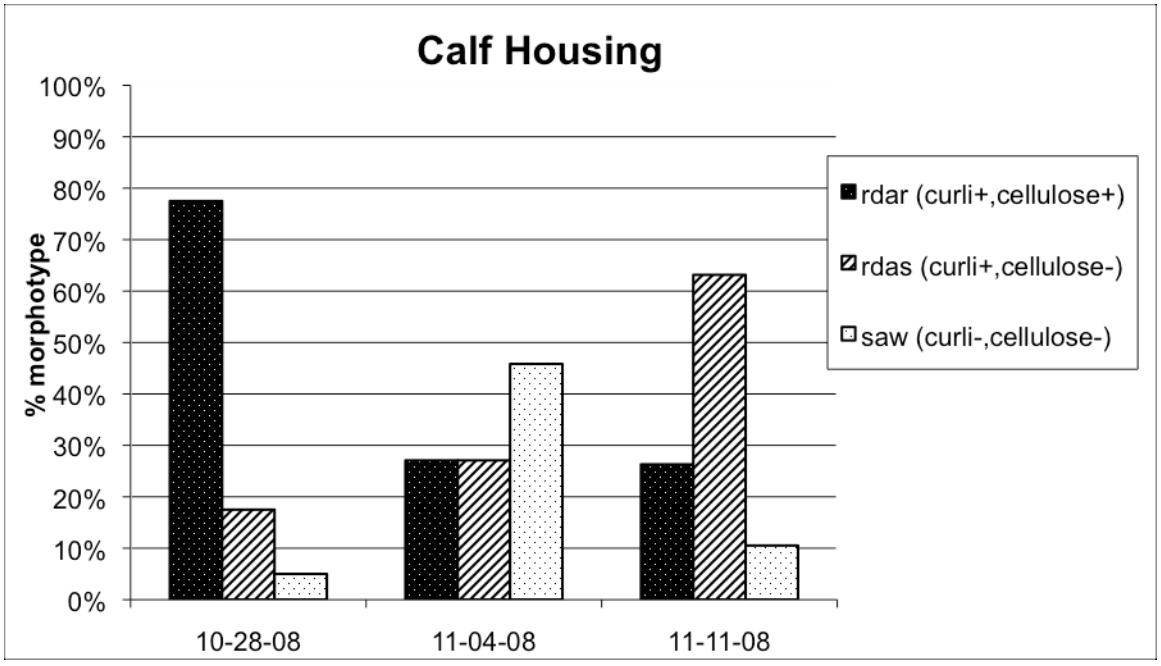

Figure 3. Morphotype distribution of samples collected at Calf Housing.

with ~30% from the first two sample dates. In both the cow and calf housing locations, strain population distribution, with respect to curli and cellulose expression, was highly variable from one sampling date to the next. In these locations, it does not appear that the any one morphotype was particularly favored, as there was a large amount of diversity amongst strains ($P<0.0142$). While these findings could be caused by temporal differences in environmental conditions, they are consistent with previous findings that

have shown high amounts of subspecies diversity in environmental *E. coli* samples (Yang et al. 2004).

Field Drain Strains isolated from the subsurface field drainage pipe outflow displayed more consistent morphotype distributions as shown in Figure 4. This was the only sampling location in which results did not significantly differ (P=0.05) over all three sampling dates (*P*-value = 0.722). The *rdar* morphotype was consistently the dominant morphotype at between 52-60% of total population, with the *rdas* morphotype between 10-17% and the *saw* morphotype between 28-33%. It thus appears that the strains isolated from the drainage pipe outflow had a more stable morphotypic make-up. Strains expressing *rdar* morphotypes appear to be more likely to survive after field application of manure and transport in the subsurface. These findings support the current hypothesis that *rdar* morphotypes enhance survival of *E. coli* in nonhost environments.

Further work is needed to verify that *E. coli* strains found in the drainage tile effluent are genetically similar to the strains from the cow housing manure that was surface-applied on the field. This will ensure that there is an actual survival mechanistic selection process occurring in strains coming from the cow housing, and which are ultimately transported to the drainage tile effluent following manure application.

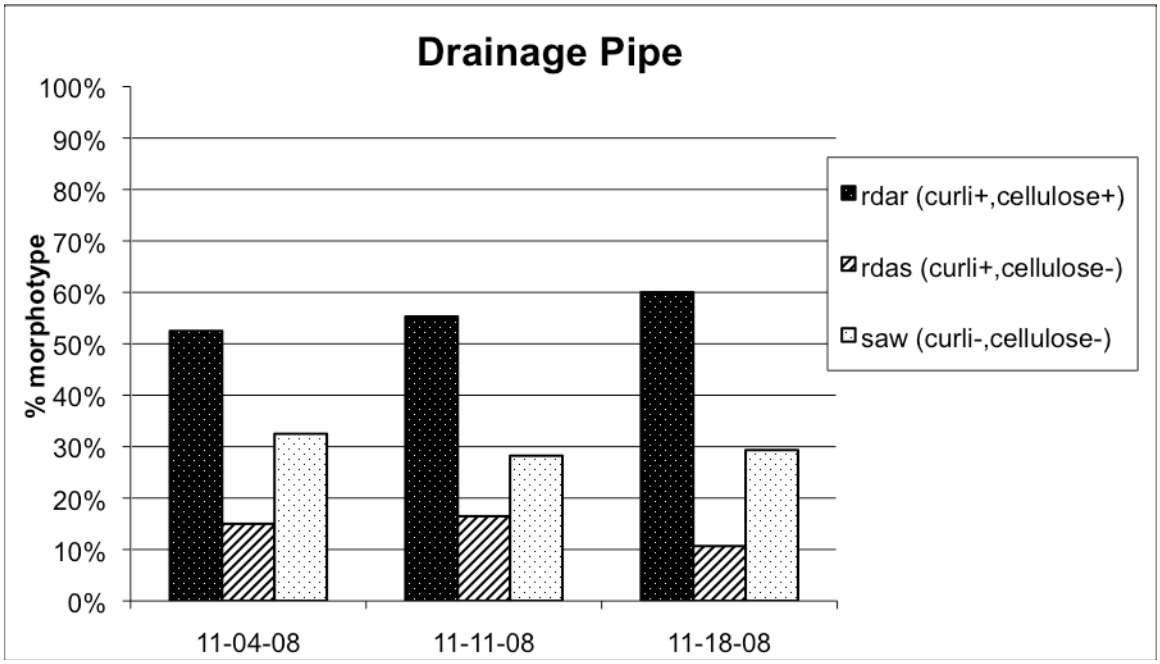

Figure 4. Morphotype distribution of samples collected in drainage pipe effluent.

CONCLUSIONS The results of this study indicate that curli and cellulose production, as observed in the *rdar* morphotype, appeared to be more consistently associated with *E. coli* strains found in the subsurface drainage waters in comparison to strains isolated from the cow housing areas. The subsurface drainage appeared to have reduced much of the temporal sampling diversity, perhaps as a result of the subsurface environment selecting for a more stable bacterial community. However, future studies at other locations and over longer time periods are needed to fully confirm these hypotheses. Knowing which *E. coli* strains are more likely to be transported to drainage systems based on simple

phenotypic characteristics will yield better management strategies to control the spread of pathogens in the environment from subsurface drainage discharges.

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