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Capacity of the bovine intestinal mucus and its components to support growth of *Escherichia coli* O157:H7

C. C. Aperce, J. M. Heidenreich and J. S. Drouillard†

Department of Animal Sciences and Industry, Kansas State University, Call Hall, Manhattan, KS 66506-1600, USA

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Colonization of the gastrointestinal tract of cattle by Shiga toxin-producing Escherichia coli *increases the risk of contamination of food products at slaughter. Our study aimed to shed more light on the mechanisms used by* E. coli *O157:H7 to thrive and compete with other bacteria in the gastrointestinal tract of cattle. We evaluated,* in vitro*, bovine intestinal mucus and its constituents in terms of their capacity to support growth of* E. coli *O157:H7 in presence or absence of fecal inoculum, with and without various enzymes. Growth of* E. coli *O157:H7 and total anaerobic bacteria were proportionate to the amount of mucus added as substrate. Growth of* E. coli *O157:H7 was similar for small and large intestinal mucus as substrate, and was partially inhibited with addition of fecal inoculum to cultures, presumably due to competition from other organisms. Whole mucus stimulated growth to the greatest degree compared with other compounds evaluated, but the pathogen was capable of utilizing all substrates to some extent. Addition of enzymes to cultures failed to impact growth of* E. coli *O157:H7 except for neuraminidase, which resulted in greater growth of* E. coli *O157 when combined with sialic acid as substrate. In conclusion,* E. coli *O157 has capacity to utilize small or large intestinal mucus, and growth is greatest with whole mucus compared with individual mucus components. There are two possible explanations for these findings (i) multiple substrates are needed to optimize growth, or alternatively, (ii) a component of mucus not evaluated in this experiment is a key ingredient for optimal growth of* E. coli *O157:H7.*

Keywords: cattle, *Escherichia coli* O157:H7, intestinal mucus

Implications

Escherichia coli O157:H7 is causing, every year, deaths and costly recalls worldwide, yet little is known of the relationship between *E. coli* O157:H7 and the gastrointestinal colonization of cattle. These studies offer insight regarding the potential of *E. coli* O157:H7 to utilize intestinal mucus and its components as substrates for growth in the gastrointestinal tracts of cattle. Factors influencing intestinal mucus secretion in cattle may be important determinants of *E. coli* O157:H7 colonization rates and, thus, important components in the development of innovative and efficient preharvest interventions to prevent food chain contaminations.

Introduction

Escherichia coli are commensal copiotroph bacteria found in the intestinal mucus layer (Montagne *et al.*, 2000; Moller *et al.*, 2003; Naylor *et al.*, 2003) of mammalian digestive tracts (Ihssen and Egli, 2005). Some strains of *E. coli* are pathogenic to humans, such as the well known Enterohemorrhagic (EHEC) *E. coli* O157:H7. Infection with EHEC often originates from the ingestion of contaminated food products. *E. coli* O157:H7 is non-pathogenic to cattle, and cattle are thus recognized as an important reservoir for the pathogen. Exposure in human populations can occur either directly through contact with cattle or their waste products, or indirectly through water, meat or other food products that have been contaminated by cattle feces. To control contamination in the food chain, it is essential to understand how this pathogen is able to grow and compete with other bacteria in the bovine gastrointestinal tract. Previous studies have shown that bovine intestinal mucus supports bacterial colonization and can selectively influence composition of the bacterial population (Deplancke and Gaskins, 2001), yet little is known of the nutrients and metabolic pathways used by *E. coli* O157:H7 (Miranda *et al.*, 2004). Intestinal mucus is comprised of mucins, glycoproteins, glycolipids, epithelial cell debris and electrolytes (Conway *et al.*, 2006). Degradation of the complex mucin components to simpler, more readily fermentable substrates requires multiple enzymes. Proteases convert the mucus from viscous to fluid state and endoglycosidases act at internal sites to release

† E-mail: jdrouill@ksu.edu

oligosaccharide fragments. Sialic acid is then removed from these fragments by neuraminidases, allowing the degradation of the remaining chain by exo-glycosidases and β-galactosidases (Corfield *et al*., 1992). *E. coli* does not produce polysaccharide-degrading enzyme (Chang *et al*., 2004), thus restricting its capacity for carbohydrate utilization to mono- or disaccharides (Mayer and Boos, 2005). Moreover, *E. coli* O157:H7 lacks neuraminidase activity (Hoskins *et al*., 1985), and therefore has limited ability to degrade complex mucin molecules. The organism is thus dependent upon other anaerobes present within the gastrointestinal tract to degrade mucin polysaccharides and release fragments beneficial for their growth (Jones *et al*., 2008). Analysis of bovine small intestinal contents revealed the presence of free mucus-derived carbohydrates, including galactose (1.43 mM), GlcNAc (0.89 mM), GalNAc (0.72 mM), fucose (0.64 mM), mannose (0.50 mM) and *N*-acetyl neuraminic acid (0.09 mM; Bertin *et al*., 2013), all of which can be metabolized by *E. coli* O157:H7. Nevertheless, to be maintained in the gastrointestinal tract of cattle, *E. coli* O157:H7 needs to compete with other bacteria to colonize the mucus layer. In the 1980s, it was believed that bacteria have a single preferred nutrient as a substrate for growth (Freter, 1988); however, recent studies have illustrated that *E. coli* relies on a diverse range of nutrients for its growth (Chang *et al*., 2004), allowing EHEC to proliferate in cattle and shed in their feces, thus providing opportunity for contamination of carcasses at harvest. Fox *et al.* (2009) demonstrated that galactose, gluconic acid, glucuronic acid, galacturonic acid, glucosamine and porcine mucin increased growth of Nal^R *E. coli* in fecal fermentations. In addition, Bertin *et al.* (2013) showed that EDL933, an EHEC strain, consumed the free mucus-derived carbohydrates present in bovine small intestinal content more rapidly than resident microflora. The following *in vitro* experiments were conducted to gain further insight on the mechanisms used by *E. coli* O157:H7 to thrive and compete with other bacteria of the bovine gastrointestinal tract. Fermentation assays were performed on small or large bovine intestinal mucus, or their constituents, with or without various enzymes, and in the presence or absence of fecal inoculum.

Material and methods

Intestinal mucus harvest

Intestinal tissues were collected from freshly harvested cattle and, immediately, transported to the Preharvest Food Safety Laboratory (Kansas State University, Manhattan, KS, USA). Sections of the ileum and colon were excised with sterile scissors and washed with a HEPES (*N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid)-Hanks buffer (8.0 g of NaCl, 0.4 g of KCl, 0.185 g of $CaCl_2 \cdot 2H_2O$, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.05 g of Na_2HPO_4, 0.35 g of KH_2PO_4, 2.6 g of HEPES; adjusted to pH 7.4) to remove digesta. Mucus was collected from each of the sections by gently scraping the epithelium with a sterile microscope slide. Harvested mucus was centrifuged twice at 27 000 $\times$ **g** for 20 min to remove cellular debris and impurities. Supernatant containing the crude mucus was dialyzed overnight at 4°C in HEPES-Hanks buffer, lyophilized and stored at −20°C.

Bacterial strains

Five Shiga toxin-producing *E. coli* O157:H7 strains (STEC; 01-2-1863, 01-2-7443, 01-2-10004, 01-2-10530 and 01-2-12329), isolated from feedlot pen fecal samples by Sargeant *et al.* (2003), were used in this experiment. Mutants resistant to nalidixic acid (Nal^R) were obtained by serial transfer into Luria-Bertani broth (Neogen Inc. Baltimore, MD, USA) containing increasing concentrations of nalidixic acid (from 0.2 to 50 mg/l; Sigma-Aldrich, St. Louis, MO, USA) following the procedure outlined by Schamberger *et al.* (2004).

Bacterial inoculum preparation

All five Nal^R STEC were plated onto tryptic soy agar containing 5% sheep's blood (Blood agar; Thermo Fisher Scientific, Waltham, MA, USA) and were incubated overnight at 37°C. A single colony was picked from each plate and grown overnight at 37°C in 10 ml tryptic soy broth (TSB; Thermo Fisher Scientific) to be transferred to five other 9-ml TSB tubes. After incubation at 37°C for 18 h, the five broths were combined in equal proportions to create a five-strain cocktail of Nal^R STEC.

Fecal inoculum preparation

Feces were collected by rectal palpation from a steer fed a concentrate-based diet and transported to the Preharvest Food Safety Laboratory in a pre-warmed thermos. Feces were blended in an Osterizer blender for 60 s with McDougall's buffer (pH 6.8; McDougall, 1948) in a 1 to 6 ratio (weight/volume) under a stream of CO_2, strained through two layers of cheese cloth, and the resulting fecal suspension was collected.

In vitro *fermentation assay*

Fecal inoculum or McDougall's buffer were added to tubes containing substrates to be tested in a 1 : 2 ratio (volume/volume) under a stream of CO_2 to maintain anaerobic environment. Tubes were inoculated with the five-strain bacterial inoculum to achieve a final concentration of 10^3 CFU/ml, gassed with CO_2, capped with butyl stoppers fitted with Bunsen valves, and incubated on a shaker at 40°C. A volume of 100 μl was extracted from each fermentation tube after 0, 6, 8, 12 and/or 24 h and diluted into 900 μl of Butterfield's phosphate buffer (26.22 g of KH_2PO_4/l; pH 7.2). Subsequent dilutions (100 μl) were plated in triplicate onto sorbitol MacConkey agar (Becton Dickinson, Franklin Lakes, NJ, USA), supplemented with cefixime (0.05 mg/l), potassium tellurite (2.5 mg/l), and nalidixic acid (50 mg/l; CTN-SMAC). Plates were incubated at 37°C for 24 h and non-sorbitol fermenting colonies were enumerated to determine Nal^R STEC concentrations.

Nitrogen and organic matter (OM) content of mucus

OM contents of mucus harvested from the small and large intestine were determined by ashing the samples (Undersander *et al*., 1993). Briefly, mucus samples were dried in aluminum pan overnight at 105°C to determine dry matter content. Pans were then heated to 450°C overnight, slowly cooled and transferred to a desiccator to be weighted.

OM percentage was obtained by subtracting ash content from a hundred. A bicinchoninic acid assay (Thermo Fisher Scientific) was performed to assess CP content of samples. *In vitro* fermentations performed in this study were carried out in tubes containing mucin from the small and large intestine at equal levels of OM (4.4 mg of OM/ml; 7.5 and 9.2 μg of protein/ml for small and large intestinal mucus, respectively) unless stated otherwise.

Increasing concentrations of mucus

Increasing concentration of mucus from the small intestine (0, 0.5, 1.0, 2.0, 4.4, 10 and 15 mg OM per ml of inoculum) were tested in an *in vitro* fermentation assay as described previously. In addition, aliquots obtained at different sampling times were plated in triplicate onto tryptic soy agar (TSA; Thermo Fisher Scientific) to allow enumeration of total anaerobic bacteria. The TSA plates were inoculated with 100 μl of the cultures and incubated at 40°C in a Coy rigid anaerobic chamber (Coy Laboratory Products, Grass Lake, MI, USA) containing 90% nitrogen, 5% CO_2 and 5% hydrogen.

Mucus and mucus components

Mucin from the ileum and the colon (4.4 mg of OM/ml), as well as mucus components such as the lipid L-α-phosphatidylserine, (1 mg/ml; CAS number: 840032P, Avanti Polar Lipids Inc., Alabaster, AL, USA), and the carbohydrates: D-gluconic acid (CAS number: G9005), D-glucuronic acid (CAS number: 6556-12-3), *N*-acetyl-D-glucosamine (CAS number: 7512-17-6), D-galacturonic acid (CAS number: 91510-62-2), sialic acid (CAS number: 131-48-6), galactose (CAS number: G5388) and mannose (10 mg/ml; CAS number: 3458-28-4, Sigma-Aldrich) were tested as growth substrate in the *in vitro* fermentation assays.

Enzymes and enzyme inhibitors

All enzymes and inhibitors were added to tubes containing small or large intestinal mucin and McDougall's buffer to be subjected to a fermentation assay. A protease from bovine pancreas (CAS number: 9001-92-7), an endoglycosidase from *Elizabethkingia meningoseptica* (PNGase F; CAS number: 83534-39-8), a neuraminidase from *Clostridium perfringens* (CAS number: 9001-67-6) and a lipase from *Candida antartica* (CAS number: 9001-62-1) all were purchased from Sigma-Aldrich and tested at a concentration of one unit per milliliter of McDougall's buffer. β-Galactosidase (CAS number: 9031-11-2; Sigma-Aldrich) and phenylethyl β-D-thiogalactopyranoside (PETG; CAS number: P-1692; Invitrogen, Grand Island, NY, USA) were inoculated at a concentration of 100 and 200 μM, respectively. Finally, bacterial protease inhibitor cocktail containing 4-(2-aminoethyl)benzenesulfonyl fluoride, EDTA disodium salt, bestatin, pepstatin A, and E-64 (P8465; Sigma-Aldrich) was added at two different concentrations, 0.25 and 2.5 ml/g *E. coli* O157 culture.

Statistical analysis

Colony forming units (CFU) counts were transformed to the $\log_{10}$ scale. Statistical analysis of fermentation assays were performed using the MIXED procedure of SAS (SAS 9.2, Cary, NC, USA). Sampling times, substrate types, presence or absence of fecal inoculum and their interactions were included in the model as fixed effects. Effect of increasing level of small intestinal mucus on *E. coli* O157:H7 and total anaerobes were analyzed using the MIXED procedure of SAS and linear contrasts. Sampling time, small intestinal mucus concentration, and the interaction of sampling time × small intestinal mucus concentration were included as fixed effects in the model. Differences were considered different at $P < 0.01$ and $P < 0.05$.

Results

Mucus anatomical origin and composition

The analysis of the nitrogen and OM content of the mucus collected at the ileum and colon revealed slight differences in composition with 1.068 μg of protein and 627 μg of OM per milligram of small intestinal mucus *v.* 0.910 μg of protein and 442.6 μg of OM per milligram of large intestinal mucus. Figure 1 depicts the growth of NaI^R *E. coli* O157:H7 in presence or absence of a fecal inoculum, with small intestinal mucus, large intestinal mucus, or no substrate overtime. It reveals no significant differences ($P > 0.10$) in growth when mucus from the ileum and colon were used as substrate. The bacteria increased from 10^3 CFU/ml of culture, at hour 0, to between 10^7 and 10^8 CFU/ml at hour 8. An overall time effect was observed on the growth of the bacteria ($P < 0.01$); however, there was no significant difference between hour 8 and hour 12. The presence or absence of fecal inocula in the culture did affect growth of *E. coli* O157:H7 ($P < 0.01$). Final concentration of the pathogen was 10^7 CFU/ml in tubes without fecal inoculum, compared with 10^5 CFU/ml in tubes containing fecal inoculum.

Increasing concentrations of mucus

Growth of total anaerobic bacteria and NaI^R *E. coli* O157:H7 was tested with increasing levels of small intestinal mucus. Growth of total anaerobes was significantly influenced by the levels of substrate addition at hours 6 and 12 (Table 1; SI

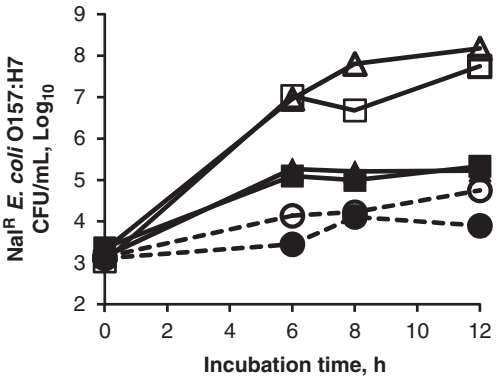

Figure 1 Growth of NaI^R *E. coli* O157:H7, with buffer (○), small (△) or large (□) intestinal mucus as a substrate, in presence (bold symbols) or absence of fecal inoculum (open symbols). s.e.m. = 0.21, effect of time, $P < 0.01$; effect of mucus origin, $P > 0.01$; effect of addition of fecal inoculum, $P < 0.01$.

Table 1 *Growth of Nal*^{R} Escherichia coli *O157:H7 and total anaerobes with increasing levels of small intestinal (SI) mucus over a 12 h fermentation at 40°C*

		SI mucus concentration (mg of OM/ml)							*P*-value	
	Hour	0	0.5	1	2	4.4	10	15	TRT	linear
E. coli O157:H7(CFU/ml, Log 10)	0	3.15	3.13	3.08	3.18	3.16	3.38	3.40	>0.05	>0.05
	6	4.08	4.29	4.86	4.66	5.01	4.90	5.74	<0.01	<0.01
	8	4.18	4.50	4.62	5.05	4.97	5.12	5.88	<0.01	<0.01
	12	4.64	4.73	4.64	4.90	5.06	5.15	5.84	<0.05	<0.01
Total anaerobe (CFU/ml, Log 10)	0	6.82	6.82	6.82	6.82	6.82	6.82	6.82	>0.05	>0.05
	6	7.88	7.89	7.96	7.91	8.95	9.03	8.98	<0.01	<0.01
	8	8.57	8.57	8.13	8.45	8.56	8.73	8.86	>0.05	>0.05
	12	7.71	8.04	8.00	8.10	8.11	8.62	8.72	<0.01	<0.01

s.e.m. = 0.3.

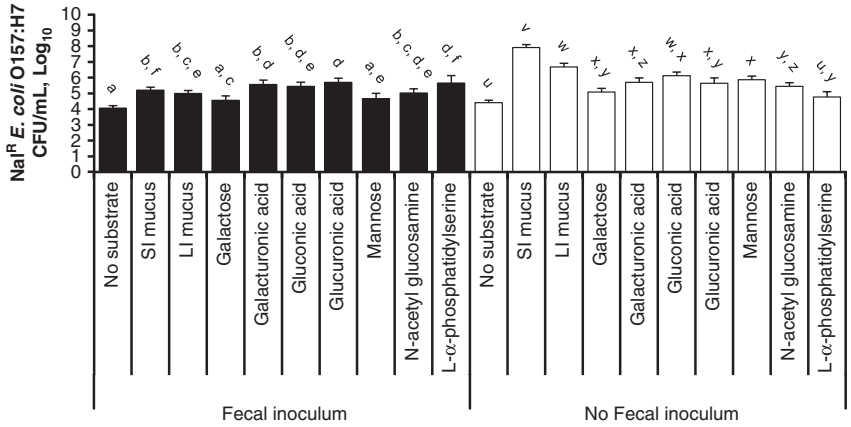

Figure 2 Growth of Nal^{R} *E. coli* O157:H7 after 8 h incubation at 40°C with mucus or single mucus components as a substrate for growth, in presence or absence of fecal inoculum. Means are compared within fecal inoculum groups. Means without a common superscript letter are different, $P < 0.05$.

concentration effect, $P < 0.01$). A linear increase in anaerobe concentration was observed with increasing concentration of small intestinal mucus (linear effect, $P < 0.01$). Cultures containing 15 mg of mucus OM/ml supported a 28% increase in anaerobe growth after 12 h incubation compared with cultures without mucus. Growth of Nal^{R} *E. coli* O157:H7 increased at every time point in response to increased concentrations of mucus (linear effect, $P < 0.01$). Counts of Nal^{R} STEC counts were increased by 72% in the presence of 15 mg of mucus OM/ml of culture compared with tubes containing 0 mg of mucus/ml after 12 h of fermentation.

Mucus and mucus components

Figure 2 depicts the growth of *E. coli* O157:H7 after 8 h of anaerobic incubation with or without fecal inoculum and either whole mucus or selected components of mucus as substrates. In presence of a fecal inoculum, all substrates increased the growth of *E. coli* O157:H7 ($P < 0.05$), with the notable exceptions of mannose and galactose. Growth was numerically greatest with L-α-phostatidylserine and glucuronic acid as substrates, but was not different from that obtained with whole mucus from the small or large intestines ($P > 0.179$). In the absence of fecal inoculum, all of the mucus components tested, with the exception of L-α-phosphatidylserine, increased growth of the bacteria in comparison to the batch containing no substrate ($P < 0.05$). Mucus originating from the large and small intestines supported greater growth than individual mucus fractions ($P < 0.05$), which is in contrast to results observed in the presence of fecal inoculum. Gluconic acid was the only single compound to yield growth similar to that obtained with whole large intestinal mucus ($P > 0.09$), but still was less than that observed for whole small intestinal mucus ($P < 0.0001$).

Figure 3 illustrates growth of *E. coli* O157:H7 in response to small intestinal mucus or sialic acid substrates, in presence or absence of a fecal inoculum. Nal^{R} STEC growth was significantly lower in sialic acid than in mucus ($P < 0.01$) in absence of fecal inoculum. When fecal inoculum was added to cultures with small intestinal mucus, there was a decrease in growth of Nal^{R} *E. coli* compared with similar cultures without fecal inoculum ($P < 0.0001$). When fecal inoculum was added to cultures containing sialic acid, growth of *E. coli* O157:H7 increased in comparison to similar cultures without fecal inoculum ($P < 0.0002$). The addition of neuraminidase to cultures containing sialic acid fermentation increased growth of the bacteria compared with tubes containing sialic acid with ($P < 0.025$) or without fecal inoculum ($P < 0.0001$),

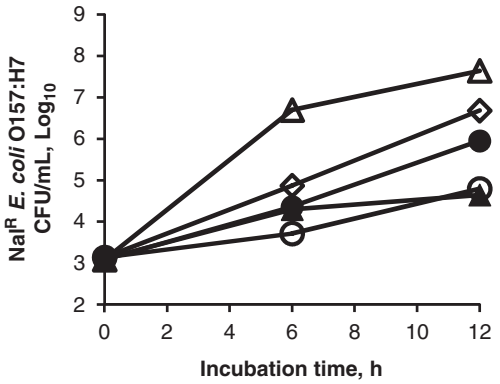

Figure 3 Growth of Nal^R *E. coli* O157:H7 in response to small intestinal mucus (△) and sialic acid (○), in presence (bold symbols) or absence of fecal inoculum (open symbols) and in response to sialic acid with neuraminidase (◇). Effect of time, $P < 0.01$; effect of substrate, $P < 0.01$; effect of addition of fecal inoculum, $P < 0.01$; interaction between substrate and fecal inoculum, $P < 0.01$.

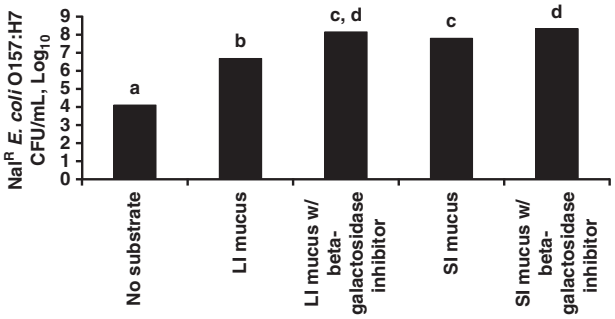

Figure 4 Growth of Nal^R *E. coli* O157:H7 after 8 h incubation at 40°C with no substrate, small (SI) or large (LI) intestinal mucus and in presence or absence of β-galactosidase inhibitor. Means without a common superscript letter are different, $P < 0.05$.

but growth still was less than that of cultures containing small intestinal mucus as substrate ($P < 0.0001$).

Enzymes and enzyme inhibitors

Figure 4 illustrates our attempt to evaluate the stimulatory effect of mucus degrading enzymes on growth of *E. coli* O157:H7. There were no effects of protease, endoglycosidase, lipase, β-galactosidase, neuraminidase or protease inhibitor addition to cultures (data not shown; $P > 0.05$). Conversely, addition of β-galactosidase enzyme inhibitor increased growth of Nal^R *E. coli* O157:H7 cultured with either small or large intestinal mucus ($P < 0.05$).

Discussion

Previous studies have shown heterogeneity in mucus composition and thickness across the human and rat gastrointestinal tracts (Atuma *et al.*, 2001; Freitas *et al.*, 2002; Robbe *et al.*, 2004). Bovine mucus is believed to display a certain level of heterogeneity between the different sections of the intestine, and this diversity is considered partly responsible for the bacterial tropism (Robbe *et al.*, 2004; Snider *et al.*, 2009). Our analysis of the ileum and colon mucus composition revealed no major differences in nitrogen or OM content. Moreover, pathogenic *E. coli* was equally capable of growing on large and small intestinal mucus. These observations led us to postulate that heterogeneity in bovine mucus is either less important than in other species or that differences in composition do not appreciably influence bacterial growth.

Growth of the pathogen was attenuated by the presence or absence of fecal inoculum in the assay. In presence of fecal inoculum, Nal^R *E. coli* growth was reduced by at least 2 log units, which likely is due to competition for nutrients between fecal bacteria and our introduced strains of Nal^R *E. coli* O157:H7. These observations are consistent with the principle of competitive exclusion (Tkalcic *et al.*, 2003), by which the presence of other bacteria in the medium limits substrate availability, thus reducing pathogen growth.

Another interesting observation was the linear increase in Nal^R *E. coli* O157:H7 and total anaerobes with increasing level of small intestinal mucus. Small intestinal mucus at 15 mg of OM/ml supported a 2 log increase in growth of both Nal^R *E. coli* O157:H7 and total anaerobes after 12 h incubation. Our results suggest that intestinal mucus stimulated pathogenic *E. coli* and total anaerobe.

Nal^R *E. coli* O157:H7 was able to grow on all mucus components tested in the absence of fecal inoculum, indicating that the bacteria were able to metabolize all compounds evaluated. Whole mucus resulted in the greatest degree of growth, despite the ability of the pathogen to grow on all substrates. Whole mucus may represent a combination of substrates that more closely meet requirements of the pathogen or, alternatively, a component of mucus not tested in this experiment may be a key ingredient for optimal growth of Nal^R *E. coli* O157:H7. For example, an *E. coli* mutant deficient in the catabolic pathway for L-fucose demonstrated a marked decrease in colonization of the rectal mucus (Snider *et al.*, 2009). Similar results were observed in mice and *in vitro* with *E. coli* MG1655 (Chang *et al.*, 2004; Fabich *et al.*, 2008). Fucose is only a minor component of calf ileal mucus (Montagne *et al.*, 2000), and thus was not evaluated in this study. In retrospect, it could be a key constituent supporting optimal growth of Nal^R *E. coli* O157:H7. Fox *et al.* (2009) indicated that, in presence of fecal inoculum, addition of galactose, gluconic acid, glucuronic acid, glucosamine, galacturonic acid, mannose, galactosamine, fucose, and porcine mucin, all supported growth of Nal^R *E. coli* O157:H7. However, mannose, fucose and galactosamine addition did not support growth greater than the control. In the current experiment, in the presence of fecal inoculum, mannose and galactose were the only two components tested that yielded growth not different from the control. In addition, growth observed with whole bovine mucus was similar to that observed by Fox *et al.* (2009) using porcine mucin, suggesting that compositional differences may not appreciably influence bacterial growth.

Cultures of Nal^R *E. coli* O157:H7 alone with sialic acid as substrate resulted in modest bacterial growth, whereas in the presence of fecal inocula growth of the pathogen closely

resembled that obtained with whole mucus. The *E. coli* strains used in our experiment appeared to have limited ability to use sialic acid, but seem capable of using intermediate products or metabolites synthesized by other fecal bacteria in the degradation of sialic acid. This observation could explain partly why feeding distiller's grains to feedlot cattle stimulates *E. coli* O157:H7 shedding (Jacob *et al.*, 2008, 2009). Distiller's grains are rich in yeasts, which contain up to 3% of their dry mass as sialic acid, (Malhotra and Singh, 2006). In such conditions, it is conceivable that a derivative of sialic acid is the active component that stimulates proliferation of the pathogen in cattle fed dried distiller's grains. In addition, the EHEC strain O157:H7 used by Bertin *et al.* (2013) was able to grow on *N*-acetyl neuraminic acid to level comparable to that obtained with *N*-acetyl glucosamine, which may indicate a strain specific ability to metabolize sialic acid.

One of our initial hypotheses was that *E. coli* O157:H7 would have little ability to use whole mucus to support its growth because the organism is not known to produce endoglycosidase (Chang *et al.*, 2004), but our results suggest otherwise. First, Nal^R *E. coli* O157:H7 grew best on whole mucus compared with individual compounds. It is possible that the mucus harvested for this experiment had already been partially cleaved by the gastrointestinal microflora and was readily available to Nal^R *E. coli* O157:H7. This may explain why addition of protease, endoglycosidase, lipase or neuraminidase to the medium did not improve growth of the pathogen. Only β-galactosidase inhibitor had an effect on the growth of the bacteria and, surprisingly, increased the Nal^R *E. coli* O157:H7. Presence of β-galactosidase activity is used in chromogenic medium as a means of distinguishing *E. coli* O157:H7 from other *E. coli* which are β-galactosidase negative and glucuronidase positive. Therefore, we were expecting the addition of β-galactosidase inhibitor to decrease growth of the organism. It is possible that galactosides are more stimulatory to growth of Nal^R *E. coli* O157:H7 than degradation products derived from galactosides, or that the inhibitor itself was used as a source of nutrient by the bacteria. The latter explanation seems unlikely, based on the very small amount of inhibitor (200 μM) added in this assay. Additional experiments are needed to further investigate this effect.

In conclusion, this series of experiments provided information regarding metabolism of mucus and mucus components by pathogenic *E. coli* O157. We were unable, however, to identify a single component as a key stimulator or inhibitor of growth of these bacteria. In order to develop innovative and efficient preharvest intervention measures, it is important to further investigate the relationship between *E. coli* O157:H7, or other STEC, and the gastrointestinal colonization of cattle.

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