Project Title: Nutritional Basis for *E. coli* O157 Colonization of the Bovine Rectum

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Background

E. coli O157 causes over 73,000 foodborne illness cases annually and 60 deaths per year. The *E. coli* O157 problem begins with cattle. Indeed, bovine-origin food products are the primary vehicle of infection, cattle are the principal reservoir, and bovine fecal shedding is the primary mode of food contamination. Because of the importance of the bovine reservoir and fecal shedding to the disease epidemiology, the colonization of cattle by *E. coli* O157 has been extensively studied. Recently, the rectum was identified as a site of tropism for *E. coli* O157 colonization of adult cattle. However, the underlying factors impacting bovine colonization *by E. coli* O157 are not known. The overall objective of this research is to understand the bovine rectum-bacterial relationship with a goal to reduce bovine shedding of *E. coli* O157. This goal is consistent with NCBA's priority to 'build the knowledge base on the ecology and epidemiology of *E. coli* O157:H7 from preharvest to the harvest facility.'

The stated objectives for this work were:

1) Pre-harvest basic research to elucidate nutritional bases for colonization of the bovine rectum by *E. coli* O157. Factors fundamental to rectal colonization are incompletely understood and previous studies have focused on attachment factors. The principle method employed within this objective is in vivo co-colonization of cattle with wild-type *E. coli* O157 EDL933 and a collection of isogenic mutants deficient in sugar catabolism pathways. The occurrence and degree of a colonization defect in mutant strains will inform the investigator what nutrients are fundamental to the rectal colonization by *E. coli* O157. Knowledge of such nutrient preferences obtained from these experiments provides a basis for the next objective.

2) The second objective is aimed at intervention development. Prebiotic strategies are proposed that hypothesize that alteration of the nutrient availability of the rectal microenvironment by instillation of an abundance of a sugar non-essential to *E. coli* O157 will favor proliferation of commensal *E. coli* that prefer that specific sugar. Probiotic strategies are proposed that aim to co-colonize other bacteria that possess superior abilities to grow and/or catabolize a nutrient preferred by *E. coli* O157. Both prebiotic and probiotic strategies, in this context, are hypothesized to limit or eliminate *E. coli* O157 colonization by means of a phenomenon known as competitive exclusion or colonization resistance.

Methodology

Cattle: At the end of the project, twelve cattle had been purchased and cannulated and two cattle (previously cannulated) were borrowed from another, non-related, completed investigation to round out some study numbers.

Cannulation surgery: The developed model utilizes adult cattle with surgically-placed duodenal cannulas.

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Experiments: All completed experiments were 12-15 day, competitive co-colonization experiments between wild-type *E. coli* O157 EDL933 and isogenic catabolic mutants. Experiments focus on the systematic investigation of the nutritional basis of colonization. The wild-type *E. coli* O157 EDL933 is resistant to streptomycin and nalidixic acid. Catabolic isogenic mutants are resistant to streptomycin and chloramphenicol. Strains are co-colonized at 1010 cfu/animal via the cannula and are resistant to the streptomycin-treated local environment. Differential enumeration of shedding in rectal mucus samples is accomplished by plating 10-fold dilution series onto sorbitol-MacConkey (SMAC)-Strep-Nalidixic acid for wild-type bacteria and SMAC-Strep-Chloramphenicol for the isogenic mutant.

Statistical Methods: Differential enumeration of wild-type and mutant strains occurs at days 0,1,2,3,5,7,9,12, and 15. For each set of 5 cows, the bacterial counts are averaged by day, plotted, and displayed in a time-course with error bars representing one standard deviation. Statistical significance is assessed with the non-parametric Wilcoxson Signed Ranks Test, deemed appropriate for this study by statistics colleagues. It essentially examines small data sets not thought to have a normal distribution and examines data for trends of deviations from a central point (usually 0) when absolute values are used.

Findings

E. coli O157 WT vs. *E. coli* O157 Δu xaC showed the uxaC mutation blocks catabolism of both hexuronates, glucuronate, and galacturonate.
Figure 1 is similar to what was seen in similar colonizations in a mouse model. Glucuronate and galacturonate may be important for rectal colonization of *E. coli* O157.

E. coli O157 WT vs. *E. coli* O157 Δ *nagEman*XYZ is a dual mutation. Subsequent trial with manXYZ (see trial 6) only may shed light on interpretation of this trial's results. The nagE mutation was significant in mice under similar experimental methods (Fabich, et al). These sugars may be of modest importance in the bovine rectal colonization of *E. coli* O157.

E. coli O157 WT vs. *E. coli* O157 Δ *uxa*B showed that galacturonate likely not important in colonization of the bovine rectum by *E. coli* O157. When interpreted with trial #1, where catabolism of both galacturonate and glucuronate was blocked and significant separation was seen, it may indicate that the important hexuronate in this case is glucuronate.

E. coli O157 WT vs. *E. coli* O157 Δ *nagEman*XYZ showed that the mutant outcompetes wild-type in week 2. Although not visually apparent, a slight competitive advantage of the wild-type is present at days 3,5, and 7. These points are nearly statistically significant by the Wilcoxson Signed Ranks Test. When interpreted with trial #2 (and trial #6 subsequently), these data may indicate a modest importance for N-acetyl glucosamine.

E. coli O157 WT vs. *E. coli* O157 Δ *uxa*B revealed results very similar to trial #3. Galacturonate not essential to colonization of the bovine rectum by *E. coli* O157. Again, when examined in context of trial #1, this seems to indicate that glucuronate catabolism was the essential component of the defective colonization by the mutant in trial #1.

E. coli O157 WT vs. *E. coli* O157 Δ showed the averaged data visually exhibits a colonization deficiency of the mutant. Again, as it is a double mutant, it is best interpreted in light of trial #6 (manXYZ) coming up. N-acetyl glucosamine may be sugar of very modest importance to rectal colonization by *E. coli* O157.

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E. coli O157 WT vs. *E. coli* O157 Δ *uxa*B showed the averaged data visually exhibits no colonization deficiency of the mutant. Galacturonate is not essential. However, interpreting trial 3 and 5, along with this merged data, in comparison to trial 1, seems to indicate a modest importance for glucuronate.

E. coli O157 WT vs. *E. coli* O157 Δ *man*XYZ showed glucosamine is not essential to the colonization of *E coli* O157 in the bovine rectum. When considered in context with trials #2 and #4, where double mutant nagEmanXYZ was competed, it seems to again confirm the attribution of the colonization deficiency to the N-acetyl glucosamine catabolism.

E. coli O157 WT vs. *E. coli* O157 Δ *nan*AT showed sialic acids may be of very modest importance to rectal colonization of *E. coli* O157 in the early initiation of colonization. Sialic acids are not important for the maintenance of colonization. Many aspects of this trial are similar to the findings of similar trials in mice (Fabich, et al, vide supra).

E. coli O157 WT vs. *E. coli* O157 Δ *gal*K showed that galactose is important to the rectal colonization of *E. coli* O157. Of the eight trials performed on six competitive colonizations, this colonization defect was most substantial and most clear-cut.

One of the first things that became apparent during the sugar catabolism experimental trials is that many mutants tested in these experiments had little to no colonization defects. Although on its face this fact may be a disappointment, to those of us heavily involved in understanding the nutritional ecology of a bacterial pathogen, it tells us two key things:

1.That the experimental model is robust to detect mutants that show both lack and presence of colonization defects.

2.That the mutants tested can compete with their wild-type parent in vivo.

Although the following nutrient preferences represent interpretations across trials (which was not statistically validated), it does show that *E. coli* O157 does have some nutrient preferences.

Galactose > Glucuronate > N-acetyl glucosamine > Galacturonate = Glucosamine = Sialic acids

Briefly, the other six mutants were deficient in catabolism of: fucose, ribose, N-acetyl galactosamine, gluconate, sorbitol, maltose, and maltodextrins. Although a review of those trials goes beyond the scope of this report, the sugar preferences in those six/seven carbohydrate sources are as follows:

Maltose/maltodextrins > Fucose > Ribose > N-acetyl galactosamine = Gluconate = Sorbitol

Combining all 12-13 carbohydrate sources into a single data set is difficult, especially when trying to rank the degrees of deficiency exhibited by some mutants. However, the following ranking seems reliable, when examining all previous data:

Large colonization defects: Maltonse/maltodextrins > Fucose > Galactose
Intermediate colon. defects: Glucuronate > Ribose > N-acetyl glucosamine
No colonization defects: Galacturonate = Glucosamine = Sialic acids = N-acetyl galactosamine = Gluconate = Sorbitol

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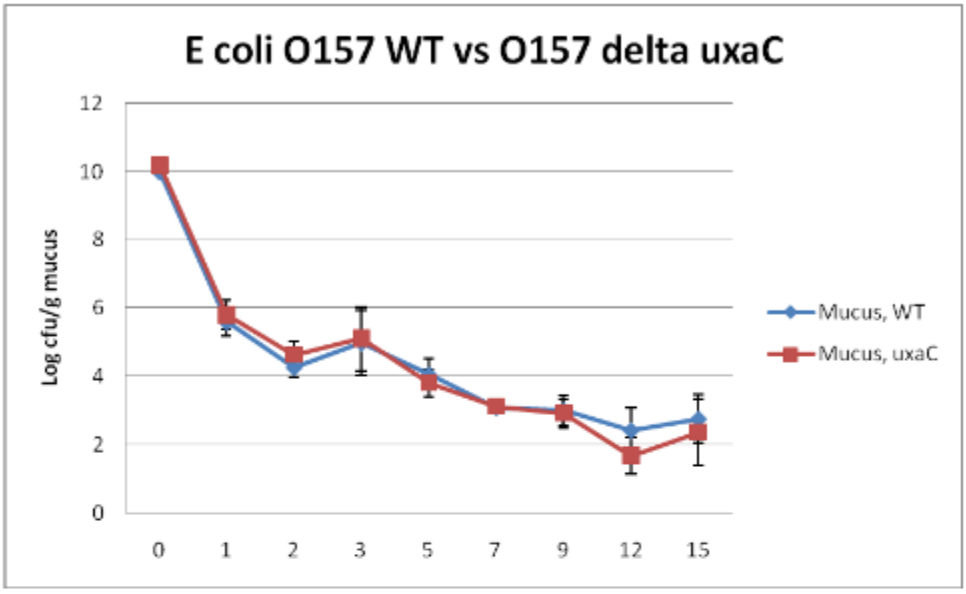
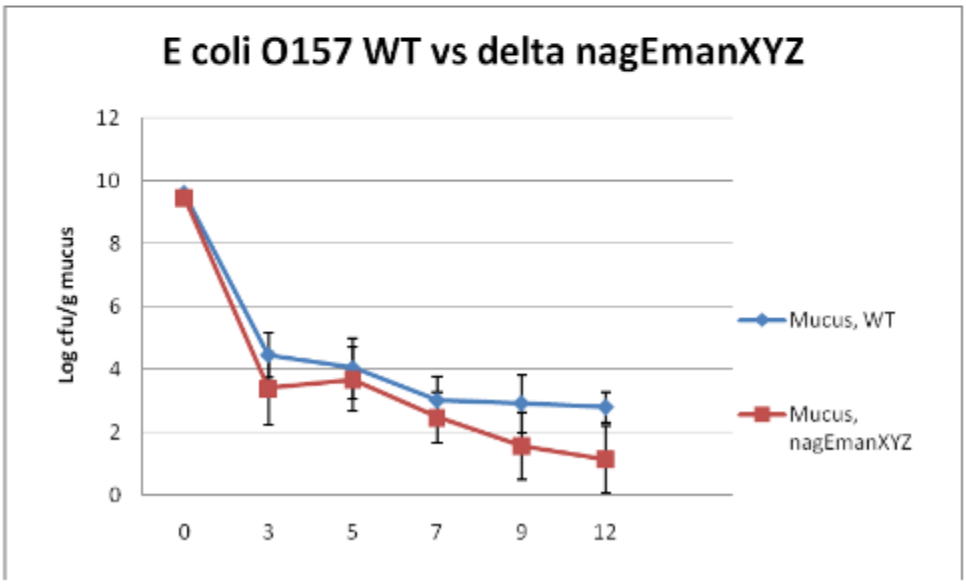
Implications

Experiments were accomplished by colonizing the bovine intestine with high numbers of both a normal strain of *E. coli* O157 (parent or wild-type) and a mutant strain that was identical to the parent, with the exception of a targeted elimination of a key gene involved in degrading very specific and very diverse sugars. Eight colonization trials of 10 cattle tested six different sugar degradation pathways. Of the six sugars tested, the nutrient preference was Galactose > Glucuronate > N-acetyl glucosamine > Galacturonate = Glucosamine = Sialic Acids. These findings, combined with similar findings of previous experiments, lay a foundation to tailor rational intervention strategies aimed at disrupting the acquisition of critical sugars *E. coli* O157 needs to colonize the bovine rectum.





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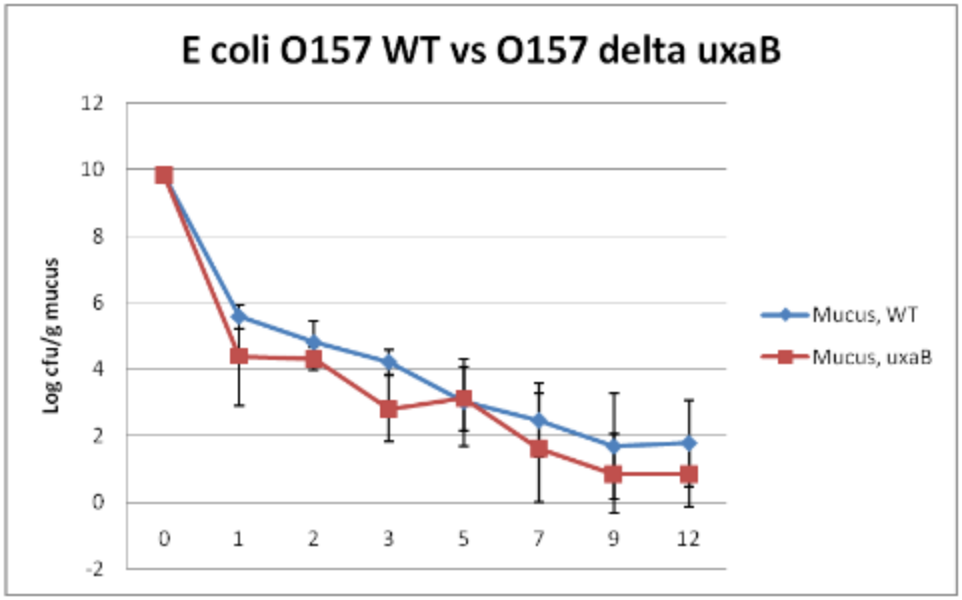

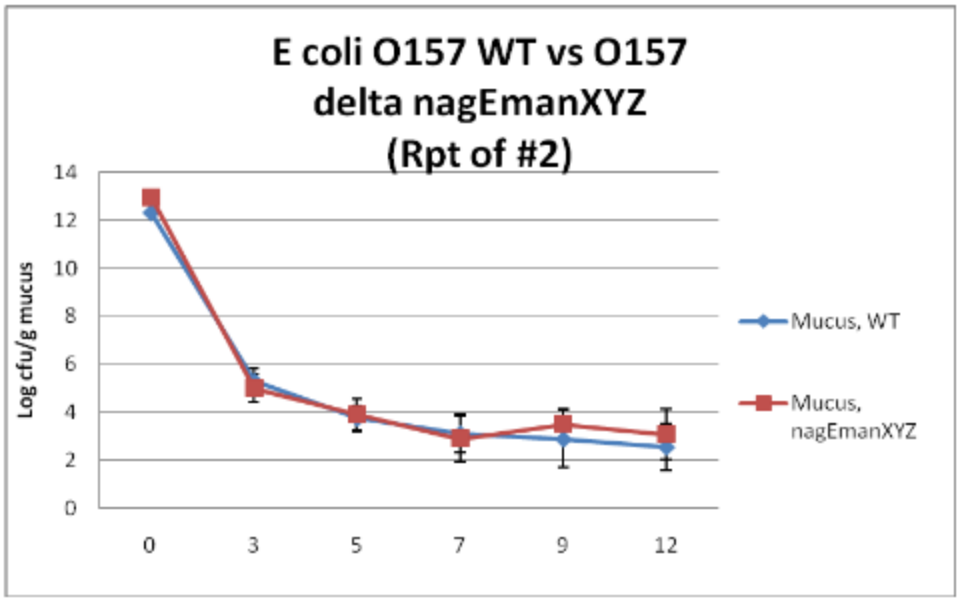

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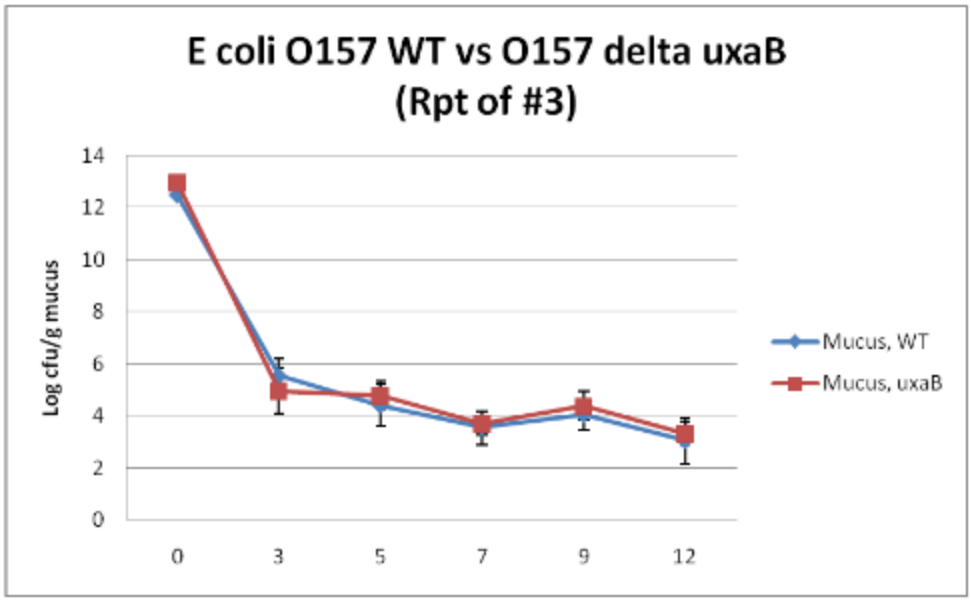

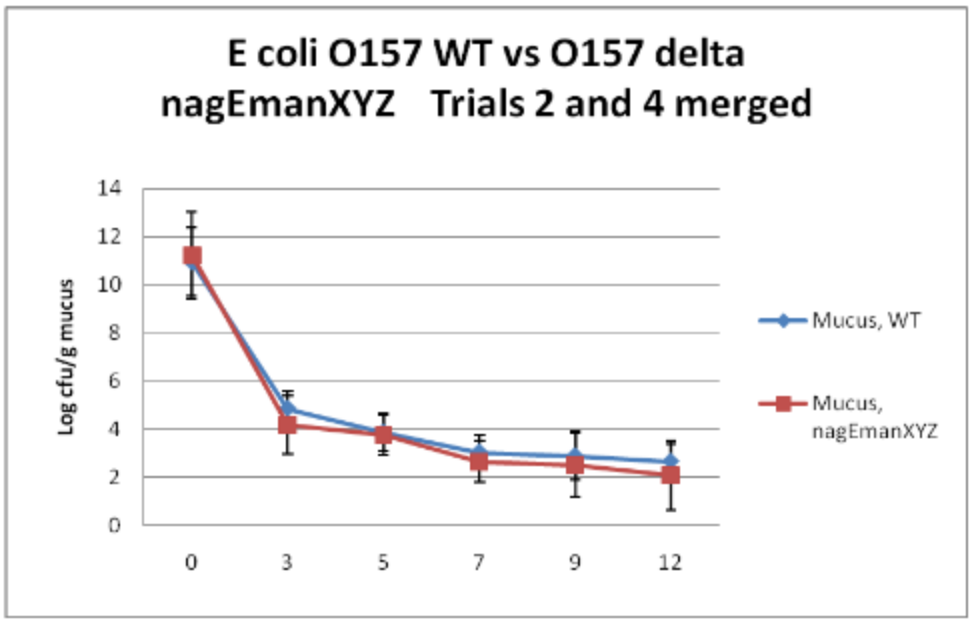

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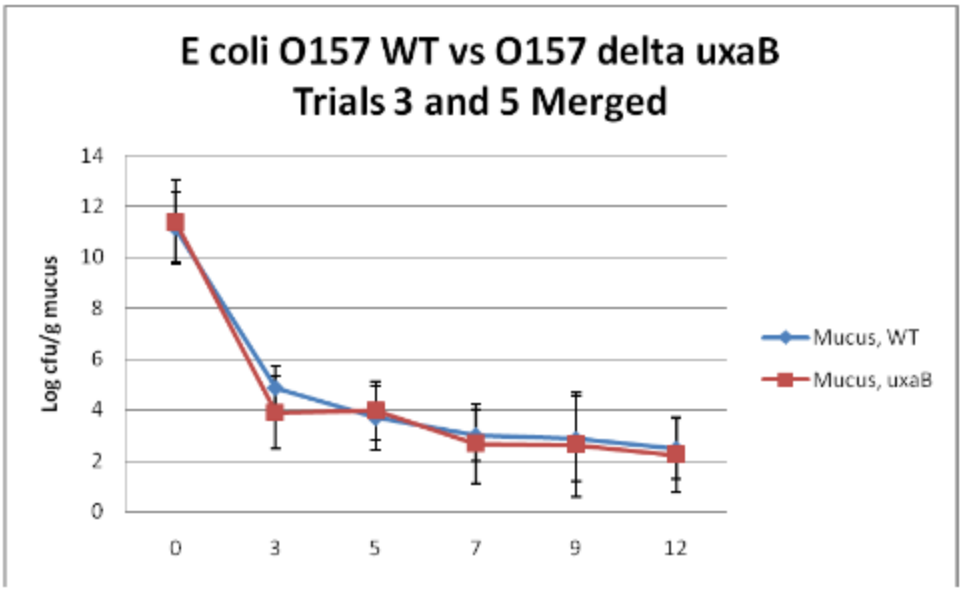

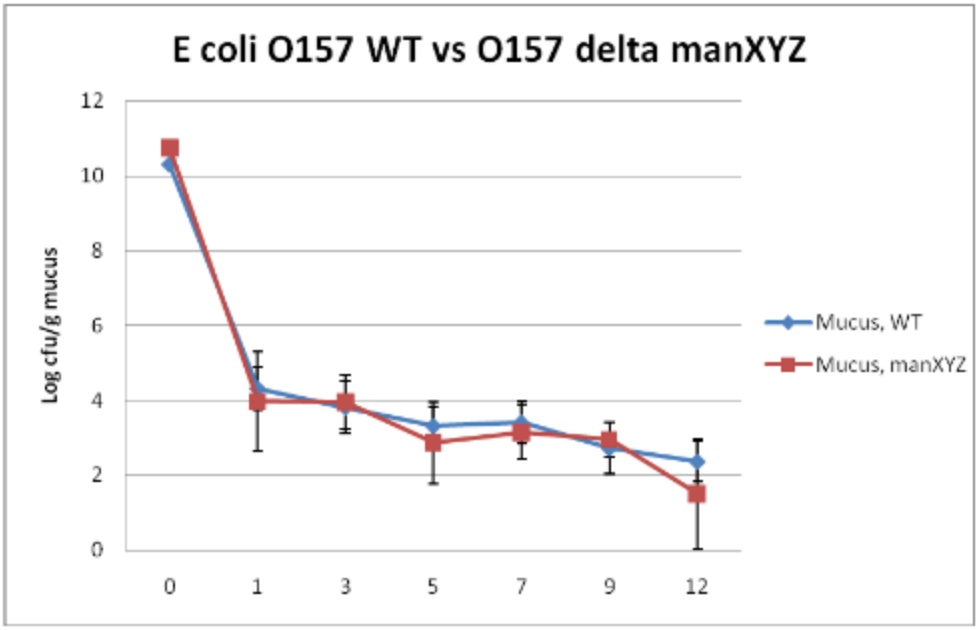

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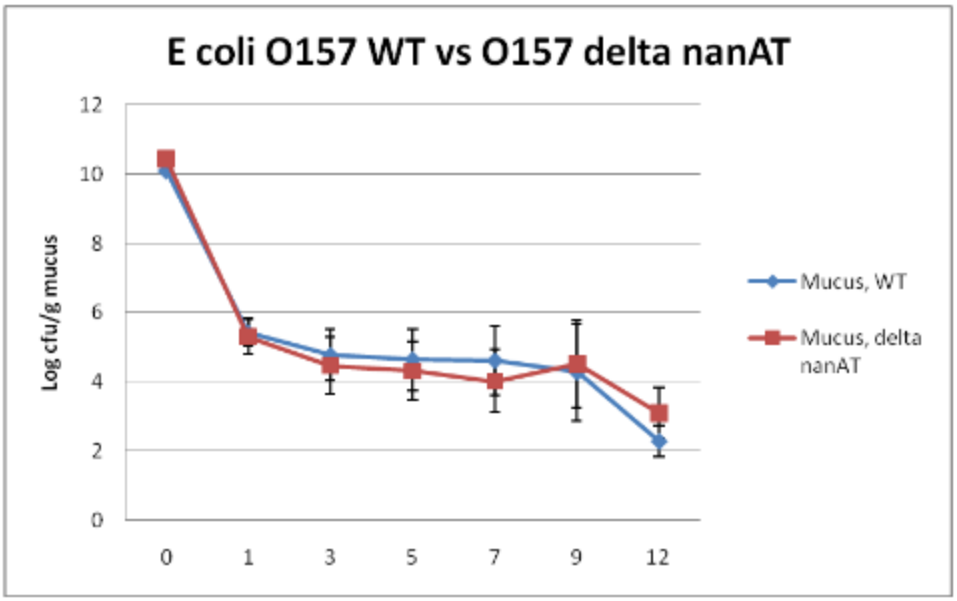

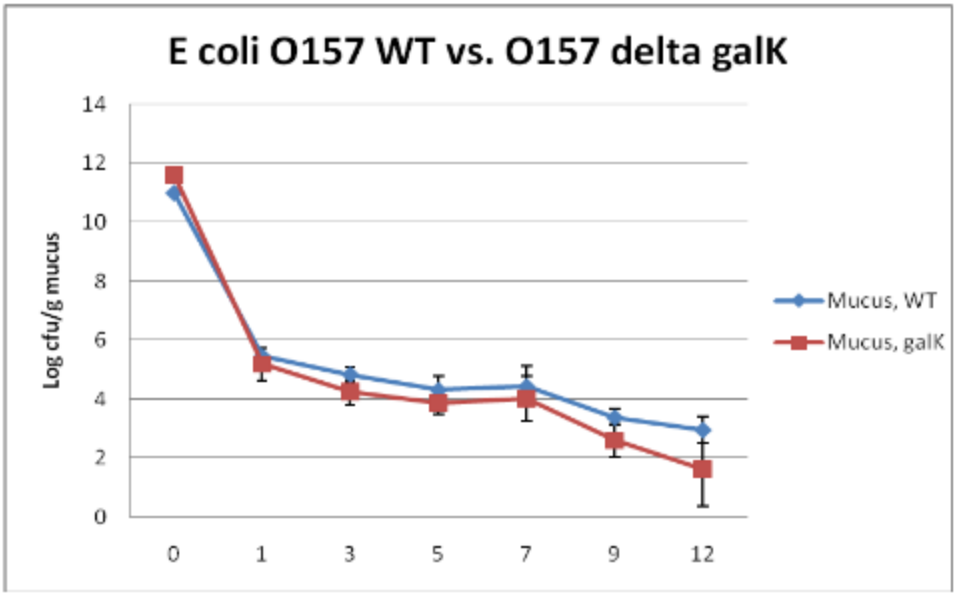

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