RESEARCH ABSTRACT
PORK SAFETY

Title: Application of a novel inhibition mechanism to control *E. coli* O157:H7 and other non-O157:H7 pathogenic *E. coli*. **NPB Project #10-086.**

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Scientific Abstract:

A novel phenotype was recently identified whereby specific strains of *Escherichia coli* inhibit competing *E. coli* via a mechanism that was designated "proximity-dependent inhibition" (PDI). PDI-expressing *E. coli* (PDI+) inhibit a broad range of susceptible *E. coli* strains (PDI-), including enterohemorrhagic (EHEC) and enterotoxigenic pathogens. In this study every strain from a genetically diverse panel of *E. coli* O157:H7 (n=8) and additional EHEC strains (*E. coli* O26, O103, O111, O121, O145) were susceptible to the PDI phenotype with an average 7 log decrease in population size when co-cultured with a microcin-producing strain. Live-dead staining was consistent with inhibition by death of susceptible cells. Comparative genome analysis identified the genetic component of PDI, which is a plasmid-borne operon that encodes a novel microcin. The plasmid was fully sequenced and is currently being annotated. The microcin operon includes genes presumptively required for secretion, immunity, microcin synthesis, and microcin activation. Transfer of the plasmid to a PDI- strain resulted in transfer of the PDI phenotype and deletion of different components of the operon resulted in loss of the inhibition phenotype. Deletion of *tolC* also resulted in loss of the PDI+ phenotype and this confirmed that the putative microcin is most likely secreted via a type I secretion pathway. Deletion of an unrelated plasmid gene (*traM*) had no effect on the PDI+ phenotype indicating that our gene deletion methods do not confound the experimental results. Quantitative RT-PCR experiments are currently underway to determine the kinetics of expression, but initial work is consistent with expression being maximized during rapid population growth. The ability to inhibit a diversity of *E. coli* strains indicates this microcin, called Microcin-E25, may influence community composition of the gut flora and it may be useful for control of important enteric pathogens.