BEEF INDUSTRY SAFETY SUMMIT
March 1-3, 2016
Austin, TX

Project Title: **Development and Evaluation of Bacteriophage Cocktail against *Salmonella* Isolated from the Beef Feedlot Environment**

Presenter: **Yicheng Xie[1]**, Jason J. Gill[1,2]

[1]Department of Animal Science, Texas A&M University, College Station, TX, 77843-2471
[2]Center for Phage Technology, Texas A&M University, College Station, TX, 77843-2128

Presenters email address: seasonxie@tamu.edu
Mailing address: NMR Facility, Office N318A, Texas A&M University, College Station, TX 77845

Category: Pre-Harvest

Published: Unpublished to date

Objective: Bacteriophages may provide an intervention for controlling *Salmonella* in beef. The objective of this study is to determine patterns of phage resistance in *Salmonella* in order to formulate an effective phage cocktail capable of overcoming bacterial mutation to phage resistance. Phages will then be evaluated for efficacy alone and in combination in an *in vitro* assay.

Experimental Design & Analysis:

Panels of *Salmonella* bacterial isolates and *Salmonella* phages were collected in 2014 from South Texas beef feedlots and from municipal wastewater. A subset of eight feedlot-isolated phages capable of infecting a feedlot-isolated *S.* Kentucky strain were selected for further study. Phage-resistant mutants of *S.* Kentucky were selected by co-culturing the bacterium with each phage individually and isolating surviving bacterial colonies. Phage-resistant strains were assessed for their sensitivity to an 18-phage panel to determine if resistance to one of the test phages conferred resistance to other phages in the collection. Experiments combining phages capable of infecting phage-resistant mutants are ongoing. Briefly, a standardized bacterial inoculum is placed into broth medium in 96-well microtiter plates and challenged with varying concentrations of single phages or phage mixtures, and growth is observed at various time points or at an overnight endpoint. These assays provide a measure of phage virulence and also assess the development of phage resistance in the bacterial host.

Key Results:

Eight phages isolated from beef feedlots were used to select for phage resistance in a *S*. Kentucky strain isolated from cattle feces. While phenotypically resistant *S.* Kentucky colonies were observed for all eight phages, genetically stable *S.* Kentucky mutants were only obtained for three phages: phage 12, phage 25 and phage 27. When tested against the 18-phage panel, the wild-type *S.* Kentucky was sensitive to all 18 phages. The phage 12-resistant mutant retained sensitivity to 14 of the 18 phages, the phage 27-resistant mutant was sensitive to 11 of 18 phages, and the phage 25-resistant mutant was sensitive to only 4 of the 18. The phage 25-resistant mutant exhibited a rough phenotype and atypical colony morphology on XLD media. Bactericidal efficacies of these phages alone and in combination are currently being evaluated in an *in vitro* culture assay.

How can this information be applied in the industry?

Asymptomatic *Salmonella* carriage in beef cattle is a significant food safety concern and the beef feedlot environment is a reservoir of this pathogen. Bacteriophages may play a role in the ecology of *Salmonella* in the feedlot environment and may also prove useful as a means of controlling this pathogen in beef. In this study, phage-resistant strains were found to remain sensitive to other phages which implies that potential phage cocktail combinations could be developed to overcome phage resistance when applying phages as an antimicrobial intervention.