Project Title: Characterization and Evaluation of Bacteriophages for the Control of *Salmonella* in the Beef Feedlot Environment

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Background

In the U.S., commercial ground beef has been reported to be contaminated with *Salmonella* despite the adoption of antimicrobial interventions on the carcass. *Salmonella* was found in lymph nodes of asymptomatic cattle at slaughter, meaning asymptomatic beef cattle could yield beef trim contaminated with *Salmonella*, which poses a potential food safety risk. Although hypothesized, *Salmonella* transmission routes have not yet been comprehensively described in the published literature; however, exposure to *Salmonella* within the feedlot environment seems to serve as the origin of this colonization.

Bacteriophages (phages) are viruses that infect bacteria. These viruses are the most abundant form of life in the biosphere, and are ubiquitous and highly successful predators of bacteria. Phages are present in soil, fresh water, and open oceans and are associated with plants and animals as a part of their normal flora. Phages are non-pathogenic and non-toxic to humans. Phages were initially proposed as biological control agents for the treatment of human disease shortly after their initial discovery in the early 1900s. The use of phages in human medicine was not fully adopted, but widespread antimicrobial resistance in a variety of pathogenic and nuisance bacteria has rekindled Western interest in phage therapy.

The objective of this study was to extend the knowledge on phages for the control of lymph node colonization by *Salmonella* in beef cattle, as a means to improve the safety of fresh beef. Due to their ubiquity within the feedlot environment, it has been hypothesized that phages may play a role in modulating populations of *Salmonella* in the feedlot, and phages against *Salmonella* may have utility as an antimicrobial intervention to reduce *Salmonella* carriage in feedlots with high *Salmonella* prevalence.

Methodology

Three feedlots located in South Texas were selected for sample collection. Previous studies have identified these feedlots to be consistently positive or negative for *Salmonella* carriage in the lymph nodes of cattle presented for harvest. In each feedlot, three pens were selected for sampling. Triplicate subsamples were taken from dropped feces, animal pen soils, feed from feed bunks and water from drinking troughs. Triplicate subsamples were pooled to produce one composite sample per pen, per sample type (solid or liquid). Twenty-nine novel bacteriophages were isolated from the three feedlots and characterized by molecular methods, their host range and their ability to clear liquid culture.

Findings

While over 40 plaques were initially picked following testing and sample enrichment, only 29 phage isolates survived through the sub-culturing process and could be propagated to usable

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product. Of these phages, 12 of 29 came from feedlot 1, an operation with a historically high *Salmonella* prevalence, and 13 phages were isolated from feedlot 2, a lot with a historically moderate prevalence. While over a dozen plaques were picked from feedlot 3, only 4 survived the sub-culturing process and could be utilized in this study. Feedlot 3 is an operation with a historically low *Salmonella* prevalence.

The diversity of the phages in the collection was quite high, with 14 major groups. Three phage groups, groups 2, 5 and 7, accounted for over half of the total phage isolates. Two groups, groups 5 and 9, had members isolated from more than one feedlot, indicating that stable phage types may be geographically distributed across wide regions. This may also reflect some level of common contact between these sites.

Host range testing was carried out to identify the ability of each phage to infect various *Salmonella* strains. Host ranges of the phages were highly variable, with the broadest host range phage infecting 16 of the 20 *Salmonella* strains tested, and the narrowest host range phage infecting 4 *Salmonella* strains. No single phage was able to infect all 20 *Salmonella* strains used in this experiment. Phage sensitivity of the *Salmonella* strains was found to be highly diverse as well. *S.* Typhimurium LT2 and Typhimurium 3003 were at least partially sensitive to 15 of 18 phages tested while *S.* Bergen showed no sensitivity to any phage in this collection. Phage sensitivity patterns as observed in this study can vary within a species or serotype. These data demonstrate that phage sensitivity is not strongly linked to host serotype, suggesting that many of these phages recognize bacterial surface features other than the major features used by the *Salmonella* serotyping system.

Implications

The broadest host range phage, infecting 80% of the *Salmonella* strains tested, indicates therapeutic candidate phages can be found in the beef cattle feedlot environment. However, completion of genomic DNA assembly and annotation is required to more fully understand the biology of these phages and make inference on their lifestyle. Several phages were unable to clear *Salmonella* cultures at low to moderate concentrations but were able to reduce culture turbidity, suggesting that phage resistance might occur readily among the *Salmonella* strains tested in this study. Using multiple phages against one *Salmonella* strain would reduce the development of phage resistance and increase their antibacterial activity in culture and in the field.

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Table 1. Host ranges of 18 *Salmonella* phages isolated from south Texas feedlots against a panel of 20 *Salmonella* strains. Bacterial strains are denoted by the serovar and strain identifier. Phage sensitivity is represented as the average score (ranging from 0-4) from three replicate experiments, with a score of 0 indicating the bacterial strain is completely insensitive to the phage and 4 indicating the strain is as sensitive as the phage's normal propagation host. Cells are also heat mapped to represent phage sensitivity, with darker green representing higher sensitivity.

		Bacterial Strain																					
Phage isolate	RFLP Group	Anatum 3001	Anatum 3022	Anatum Fd1001A1	Anatum S2028C3	Bergen H1042-1	Cerro H2006-1	Dublin 3030	Enteritidis SGSC2475	Enteritidis 3115	Heidelberg SGSC2480	Kentucky Fc1033C3	Montevideo 3002	Montevideo S2029C2	Muenchen S2028C1	Newport 3000	Newport 3026	Reading 3023	Typhimurium 3003	Typhimurium 3116	Typhimurium LT2	infects at all	strongly infects
1	1	1.3	1.3	0.0	0.0	0.0	1.3	0.0	2.7	1.3	0.0	0.0	0.0	4.0	0.0	4.0	4.0	1.3	4.0	4.0	4.0	12	6
3	2.1	4.0	0.0	0.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	4.0	0.0	4.0	4.0	0.0	0.0	0.0	0.7	0.0	0.0	6	5
5	2	2.7	0.0	0.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	4	2
6	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.3	0.0	0.0	4.0	0.0	0.0	4.0	2.3	3.7	0.0	1.0	0.0	4.0	7	4
8	14	1.7	0.0	4.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	1.0	0.0	0.7	0.7	0.0	0.0	0.0	0.3	0.0	0.7	8	1
9	2.2	2.3	0.0	0.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	4.0	0.0	3.7	3.3	0.0	0.0	0.0	0.7	0.0	0.0	6	3
12	6	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.3	0.0	0.0	3.0	0.0	0.0	3.3	4.0	3.7	0.0	1.3	0.0	3.3	8	4
13	7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	3.0	0.0	4.0	3	1
15	4	3.3	2.7	0.0	3.3	0.0	0.0	4.0	4.0	2.3	1.7	3.0	4.0	4.0	3.0	0.7	3.3	2.7	3.0	0.0	1.0	16	7
17	5	4.0	0.7	0.0	4.0	0.0	0.0	0.0	4.0	1.4	0.3	2.7	3.7	4.0	4.0	1.3	4.0	0.7	0.3	0.0	1.3	15	7
18	8	1.7	2.0	1.3	1.3	0.0	0.0	4.0	4.0	2.7	4.0	0.0	4.0	1.3	0.0	3.0	1.3	1.3	4.0	0.0	0.7	15	5
21	5.1	1.0	0.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.3	4	1
22	9	4.0	0.7	0.3	4.0	0.0	0.0	0.0	4.0	0.0	0.3	4.0	4.0	4.0	4.0	0.0	4.0	0.7	1.7	0.0	2.3	14	8
24	10	4.0	0.0	0.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	4	3
25	11	4.0	1.0	0.0	3.0	0.0	0.7	0.0	4.0	0.0	0.0	4.0	4.0	4.0	4.0	0.0	4.0	0.0	0.3	0.0	1.3	12	7
26	9.1	0.0	0.0	4.0	0.0	0.0	0.7	1.0	0.3	0.7	0.3	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.3	8	1
27	12	3.7	0.7	0.0	4.0	0.0	1.3	0.0	4.0	0.0	0.3	4.0	3.7	3.7	4.0	2.7	4.0	0.0	0.3	1.3	4.0	15	9
28	13	3.0	0.7	0.0	3.0	0.0	0.7	0.0	3.7	0.0	0.0	2.3	2.7	2.7	2.7	2.0	4.0	0.7	0.3	0.7	3.0	15	2

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