Title:

Isolation and characterization of *Listeria* phages for biosanitation of biofilms containing *Listeria monocytogenes*

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Abstract:

Listeria monocytogenes is a foodborne pathogenic bacterium that can form biofilms and colonize and persist in biofilms formed by pioneer bacteria. The resistance of *L. monocytogenes* to various physical and chemical stressors and its ability to form biofilms have been hypothesized to contribute to its persistence in the food industry. Biofilms are the most common mode of growth of bacteria and contribute to contamination of food with pathogenic bacteria in food processing environments. Biofilm-inhabiting bacteria are more resistant to antimicrobials and sanitizers than are planktonic (free-swimming) bacteria. Bacteriophages are viruses that infect bacteria and have potential utility as antimicrobials and bio-sanitizers.

The goal of the present work is to isolate and characterize broad host range *Listeria* phages and to test their ability to degrade *L. monocytogenes* containing biofilms. Eight broadly infective *Listeria* phages distinguishable by their host ranges have been isolated from poultry and ready to eat meat processing plants using four different isolation approaches. The approaches that were used were single strain enrichment, enrichment with a cocktail of *Listeria* strains, and cyclic sequential enrichment with and without adsorption. The latter two approaches respectively select for broadly infective phages and efficiently adsorbing broadly infective phages. The phages have been tested by performing efficiency of plaquing, liquid culture lysis, and adsorption efficiency experiments. Pulsed field gel electrophoresis has been done to estimate the genome sizes, which were found to be approximately 135 kilobase pairs in length. The selected phages are broadly infective, at a

multiplicity of infection (input) of 1 completely inhibit growth of more than half of the 21 *Listeria* strains against which they were screened (comparable to P100) and are more efficiently adsorbing than P100 at 21°C in BHI containing 2mM $CaCl_2$. Transmission electron microscopy shows that the phages that have been imaged are myoviruses with similar morphology to Listex P100.