

## ABSTRACT

**THESIS:** Bacteriophage Identification from Dairy Environments

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*Bacillus* spp. are within a family of bacteria known to form spores and create biofilms for survival in extreme environments [1]. The spores and biofilms are resistant to temperature extremes, radiation, antibiotics, and many disinfectants. When the spores are ingested by or exposed to susceptible animal hosts, they can germinate and create biofilms or cause infections such as endophthalmitis, bacteremia, pneumonia, or gastroenteritis [2]. The virulence potential makes these bacteria problematic in the food industry as pathogens and spoilers. Biological control of *Bacillus* spp. may be possible with bacteriophage. As a protective measure, dairy farmers could utilize a known bacteriophage after pasteurization to ensure dairy product quality and safety. The goal of this research was to isolate and characterize unknown bacteriophage found in raw dairy milk. By performing host range, DNA sequencing, polymerase chain reaction (PCR), and imaging, phage candidates were identified that may have promise as agents for biological control of *Bacillus* spp. of concern to dairy scientists and consumers. Raw milk samples were processed for phage extraction and enrichment. Phage were subjected to DNA extraction, purification, sequencing, and transmission electron microscopy. Results revealed that the phage isolated in this study demonstrated the ability to infect several common *Bacillus* spp. (*B. cereus*, *B. amyloliquefaciens*, *B. subtilis*, and *B. thuringiensis* subsp. *berliner*), established

that levels of CaCl<sub>2</sub> were highly influential for phage uptake, and indicated the presence of shared amplicons between the unknown phage and both *Bacillus* phage deep blue (NC\_031056) and *Bacillus* phage BCP8-2 (NC\_027355), both of which are within the *Myoviridae* family but reside in different subfamilies. Experiments also revealed that glycogen precipitation was more efficient for DNA precipitation before sequencing, and TEM imaging unveiled that the isolated phage appeared to have an icosahedral-shaped capsid head, visible sheath, and appeared to be approximately 100nm in overall length. The capsid alone measured 70nm in diameter. Tail fibers were less resolved due to the 40,000X magnification and decreased image clarity. The phage shared some characteristics with both T2 and T4 bacteriophage within the *Myoviridae* family [3, 4]. Further host range studies, genomic analyses, and PCR will reveal if this phage is novel and a possible future biological control agent, and/or whether the phage harbors antibiotic resistance genes that could have food safety and public health implications.