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2025 SEA Symposium Abstract

University of Maine, Honors College

Orono ME

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Tristan: The Phage Awakens—A New Hope Against Mycobacteria: Isolation of a Novel Bacteriophage Tristan

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Nontuberculous mycobacterial infections (NTMs) are caused by mycobacteria other than *Mycobacterium tuberculosis*, leading to a range of highly antibiotic resistant chronic pulmonary and extrapulmonary diseases, particularly in immunocompromised individuals. Mycobacteriophages (phages), viruses that infect and can kill mycobacteria, offer potential therapeutic applications. This study focused on the isolation and characterization of a novel mycobacteriophage, Tristan, that was isolated from soil samples in Orono, Maine, using enriched isolation techniques. On the bacterial host *M. smegmatis* mc²155, Tristan exhibited turbid plaques, and the identification of an immunity repressor (gp72) in the genome, confirms a temperate lifestyle. Electron microscopy revealed morphology consistent with the *Siphoviridae* family. Annotation of the phage genome revealed a genome length of 53,385 base pairs, 63.3% GC content, and an A2 cluster assignment among 119 members. The genome has 93 protein-coding ORFs where the left arm encodes forward transcribed structural genes (gp1 to gp33) and the right arm encodes reverse transcribed genes (gp34 to gp87). Tristan, like other cluster A2 phage, possesses lysins located before the terminase which do not align with the usual syntenic region. Many genes of unknown function are found in the right arm which are all reverse transcribed and typically encode DNA replication proteins. Future research should focus on exploring potential applications, including these non-canonical gene locations and genes of unknown function, for phage therapy targeting NTMs. This study contributes to the growing knowledge of mycobacteriophage diversity, particularly within subcluster A2. Comparative genomics will further elucidate evolutionary relationships among cluster A phages.