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2025 SEA Faculty Meeting Abstract

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Determining the Optimal MOI, Lysis Time, Post Lysis Growth Rate and Life Cycle of Novel Gordonia rubripertincta Phages

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With the rise of antibiotic resistance, new treatments for bacterial infections are needed. Phage therapy is a promising alternative treatment that requires thorough characterization of phages for development. As part of the SEA PHAGES program at NSU, four novel *Gordonia rubripertincta* phages were isolated and annotated from 2021-2023: Alyssamiracle, Fribs8, Genamy16, and NovaSharks. Fribs8 is in cluster CT, which has been determined to have a lytic life cycle, while the other three phages are in cluster DV, which has an unknown life cycle. The goal of this research was to further characterize these phages by determining their optimal multiplicity of infection (MOI), lysis time, post lysis growth rate and life cycle. *G. rubripertincta* was grown in combination with each phage at MOIs of ~ 0.1, 1, 10 and 100 in triplicate. The absorbance at OD600 was measured every 10 min for 48 h using a Victor X4 (Perkin Elmer) 96-well microplate reader. Results showed that the optimal MOI was 100 for Fribs8, Genamy16 and NovaSharks, and 10 for Alyssamiracle, with lysis times of 5.7 h, 7 h, 7.5 h and 5.4 h, respectively. Interestingly, bacterial growth was seen after lysis. The growth rate after lysis was determined and showed that the higher the MOI, the higher the growth rate, suggesting a faster recovery possibly due to lysogens or resistant bacteria emerging. To determine whether the phages had a lytic or temperate life cycle, A spot titer was performed and potential lysogens were collected and purified from the mesa of the 10^0 spot. A phage release assay was performed on four purified colonies from each sample to determine if any lysogenic phage were present. The three cluster DV phages tested negative in the phage release assay, indicating a lytic life cycle while, unexpectedly, the cluster CT phage demonstrated phage release in 1 of the 4 samples tested. These results suggest that the post lysis growth are most likely due to resistant bacteria emerging and not lysogens. Together, these results contribute to the larger understanding of *Gordonia* phages and may aid in the development of effective phage therapy for antibiotic resistant *G. rubripertincta* infections in the future.