CONSIDER FOR TALK

7th Annual SEA-PHAGES Symposium Abstract

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From Compost to Clusters: Rhodococcus Phages from Coastal Virginia

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Belonging to the order *Actinomycetales, Rhodococcus* bacteria display a broad geographic distribution and have been found in diverse environments including wastewater treatment plants, mills, farmlands, caves, and even deep under ice in sub-glacial lakes. There is considerable interest in *Rhodococcus* given their metabolic properties for use in detoxification and even medicinal chemistry. In order to isolate potentially novel phages that infect *Rhodococcus*, undergraduates in the College of William & Mary SEA PHAGES course took samples of soil, mud, sludge, compost, and manure from around coastal Virginia and performed enrichment with a *Rhodococcus globerulus* culture. Of over 50 different locations (including a waste treatment plant, paper mills, and livestock grazing areas) and at least three replicate cultures, we found that one specific compost pile and nearby soil with runoff from the compost was the only source of phage that would form plaques on a *Rhodococcus globerulus* lawn. These phage produced small clear and large tubed plaques and high titer lysates, and had a latent period of 1-1.5 hours. We subsequently visualized the isolates under transmission electron microscopy to find phages with approximately 60 nm diameter heads and 170 nm tails. Preliminary sequence screening of the phages using a "shotgun" cloning approach suggested high similarity among the isolates; we therefore selected those with the most distinct plaque morphology for further analysis. Four phage isolates sequenced by University of Pittsburgh revealed a 46.6 Kbp genome, with approximately 65 genes, the majority of which coded for proteins of unknown function. The four genomes shared 99% similarity with one another and 98% to the RER2 *Rhodococcus* phage. All of the known *Rhodococcus* phages appear to fall into 3-4 clusters (with six additional singletons). One of these clusters (RER2-like), in particular, contains almost 70% of the known *Rhodococcus* phages including the William and Mary isolates. Another isolate taken from the same compost soil, but in the middle of winter, and sequenced by students in a “Phage Lab Continuation” course at William and Mary, also belongs to the RER2 family and shows similarity to the other four phage isolates from William and Mary. However, it is distinctly different in that it lacks several intergenic regions, an entire gene, and 89 bp at the beginning of the integrase gene. RNA-Seq experiments are currently underway to examine phage and bacterial gene expression in response to infection.