CONSIDER FOR TALK

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Puzzling Virion Morphology and DNA Characterization Results from Microbacteriophages Discovered at Western Carolina University

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Western Carolina University (WCU) has been a member of the SEA-PHAGES program for three years, and during this time WCU students have isolated and archived 62 Actinobacteriophages. In previous years students used the host *Mycobacterium smegmatis* for bacteriophage isolation. This year two new hosts, *Microbacterium foliorum* and *Gordonia terrae*, were used. In the past only bacteriophages belonging to the family *Siphoviridae* have been isolated at WCU using *Mycobacterium smegmatis*. However, this year our electron microscopy (EM) results revealed that we had potentially isolated 1 *Myoviridae* and 3 *Podoviridae* bacteriophages using the host *Microbacterium foliorum*. The isolation of 3 potential *Podoviridae* bacteriophages was especially surprising given that only 1 *Podoviridae* bacteriophage has been reported to have been isolated using an Actinobacterial host by the entire SEA-PHAGES program. The purified DNA of 3 Microbacteriophages potentially representing all 3 tailed bacteriophage families (Andromedas-*Siphoviridae*, Neferthena-*Myoviridae*, and ColaCorta-*Podoviridae*) was sent to the University of Pittsburgh for whole genome sequencing. In contrast to what we expected, our sequencing results revealed that all 3 bacteriophages likely belonged to the family *Siphoviridae*. We were even more surprised to learn that bacteriophages Andromedas and ColaCorta belonged to the same subcluster (EA2) and were almost genetically identical. Our restriction enzyme analysis results from ColaCorta and Andromedas were also puzzling. The DNA of Andromedas and ColaCorta were not cut even by restriction enzymes whose target sequences were abundantly present in these genomes. Current studies are focused on clarifying the discrepancies between our sequencing results and EM and restriction enzyme analyses.