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Grabbing A1 phages by the tail: Characterization of amino acid variation and tertiary structure of the tape measure protein

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Bacteriophages were isolated from soil samples using the bacterial host *Mycobacterium smegmatis* mc2155. Instead of using a restriction enzyme digest to characterize phages before selecting which genomes to sequence, we applied subcluster-specific PCR primers targeting the tape measure protein (TMP). By comparing the sequenced products we determined whether phages matching to subclusters previously discovered at the University of West Alabama (UWA) were novel or replicated. The F2 subcluster is highly conserved across the TMP segment, and we could not determine whether our four F2 phages were unique or identical. Conversely, TMP segments from other subclusters were informative and indicated that multiple, unique phages from the same subcluster have been isolated at UWA. Our PCR results suggested that Sumter was an A1 phage. We chose to sequence this genome because UWA had not annotated an A1 phage genome before. The second phage, Candle, was selected because a cluster was not conclusively identified by PCR. The genomes of Sumter and Candle are 52,656 bp and 71,390 bp long respectively. Annotation in PECAAN and DNA Master identified 90 protein coding genes in Sumter and 96 in Candle. Next, we looked for patterns in sequence variation within the A1 subcluster. To do so, we determined synonymous codon usage bias using CAICAL and visualized protein sequence conservation in WebLogo for the following proteins: major tail protein, TMP, minor tail protein, DNA primase, helix-turn-helix DNA binding domain, membrane protein, and immunity repressor. Conservation of amino acids across members of the A1 subcluster was apparent in segments of the major tail and minor tail proteins. The other proteins investigated showed higher amino acid variability. We predicted protein tertiary structure for the seven genes listed above by using I-TASSER to match our amino acid sequences to structures in Protein Data Bank (PDB). All phages found by UWA students were visualized using a transmission electron microscope including members of the *Siphoviridae* and *Myoviridae*. Looking collectively at the sequenced phages from UWA, we confirmed the previously identified correlation between phage tail length and TMP length.