CONSIDER FOR TALK

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Speeding on Kroos Control and We Got GC in Low Places: Gordonia phages Kroos and Tanis

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Two new bacteriophage, Kroos and Tanis, were isolated on *Gordonia terrae* 3612. Kroos is a 57,974bp siphoviridae with a 74nm capsid and 315nm tail. It belongs to subcluster DE1, has 85 ORFs, and shares 88% nucleotide identity with Brandonk123. Kroos has a GC content of 68.1%, consistent with its host and the DE cluster. No tRNAs were found but all expected genes were, and function was identified for 28. Unlike most DE phages, Kroos has no reverse genes. In cluster DC, closely related to DE, phages Wizard and Twister6 have 17 phams that have synteny with Kroos. Kroos’ genes are generally not shared beyond *Gordonia* phages, as only 5.8% are in phams with 3 or more host species, most commonly *Gordonia*, *Mycobacterium* and *Actinoplanes*. Tanis is a 59,727bp siphoviridae containing 93 genes, of which all are in the forward direction. Tanis has a 55nm capsid and 325nm tail, is in cluster DJ and has 51% GC content - which is consistent with the DJ cluster, but not *G. terrae* (67.8%). Tanis does not contain any tRNA genes that could compensate for this disparity. Tanis shares 95.7% identity with Gravy and Kerry (DJ). In addition to functions shared with Kerry and Gravy, six ORF functions were inferred in Tanis. Interestingly, Tanis contains a major capsid protein/capsid maturation protease, which is consistent within DJ, but not other *Gordonia* clusters. Tanis and the DJ cluster appear to be most closely related to CC, an exclusively *Rhodococcus* cluster. In contrast to Kroos, 31.3% of Tanis’ genes are in phams with 3 or more host genera. The most conserved pham (37551) has 346 members across 4 hosts. After *Gordonia* phages, Tanis shares 47.3% of phams with *Rhodococcus*, 29% with *Arthrobacter*, 28% with *Streptomyces*, 16.2% with *Microbacterium* and 12.9% with *Mycobacterium* phages. We wondered whether Tanis’ shared genes could be due to frequent host switching, and if that may explain the lower than expected GC content. Actinobacteriophages predicted on PhagesDB to be lytic have a lower GC content (59.6%) than phages predicted to be temperate (64.9%; p=0.001). Predicted lytic phages also have significantly larger genomes than temperate (70,140bp vs 52,832bp; p=0.004). There is no relationship between phage lifestyle, GC content, or genome length and the presence or abundance of tRNA genes. Recent studies show the average GC content of uncultivated actinobacteria is 47%, suggesting cultivation may have led to an inappropriate label of “high GC” to the actinobacteria. Our findings that lytic actinobacteriophages have significantly lower GC content can be explained if lytic phages circulate throughout a population of actinobacteria ranging from mid to high GC content. Temperate phages may circulate less frequently, and our use of high GC hosts may select for high GC temperate phage. Lastly, additional phages were sequenced, representing *Gordonia* phage clusters CQ, CR, CU, DC, DE, DI, and DJ, and are under annotation.