CONSIDER FOR TALK

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A study of phage with attitudes: defensive Gordonia phage Sidious and MagicMan and crazy Rhodococcus phage Whack and SleepyHead

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Through use of multiple bacterial hosts in the SEA PHAGES classroom, UMHC, isolated and sequenced two singleton *Rhodococcus* phage and six novel *Gordonia* phage belonging to clusters A15, CT, CZ7, DB, DC, and DU. Two of the *Gordonia* phage, Sidious and MagicMan, are temperate and encode viral defense systems. Sidious is the sole member of subcluster CZ7. Its genome is 51,789 bp in length, has a GC content of 66.6%, and encodes 84 putative genes. Sidious shares an immunity repressor with cluster CZ1 phage BatStarr and Nymphadora and *G. terrae* lysogens of Sidious are homoimmune with these phage. Sidious lysogens are also immune to infection by phage that do not have related immunity repressors: Yeezy and BaxterFox (CZ3); BetterKatz (DI) and Sitar (DE1). This heterotypic immunity may be due to Sidious genes gp41 and 40, which encode a putative abortive infection system, RexA and RexB, respectively. MagicMan is a cluster DB phage with a 47,598 bp genome with 67% GC content. The genome encodes 70 putative genes including an integrase (gp37), immunity repressor (gp38), and putative BrnT-like toxin (gp35). An obvious anti-toxin gene was not identified; however gp36 is divergently transcribed relative to gp35 and the gene product has a C-terminal ribbon-helix-helix domain, consistent with the antitoxin BrnA. *Rhodococcus* phage SleepyHead and Whack are temperate, singleton phage. The SleepyHead genome is 43,943 bp in length, has 61% GC content, and encodes 67 putative genes, including 37 orphams. SleepyHead encodes an immunity cassette that includes a reverse oriented immunity repressor (gp40), a peptidase (gp39) and tyrosine integrase (gp38). The peptidase has a strong HHPRED match to ImmA Zn-dependent peptidases and could function as an anti-repressor. There are 11 reverse genes between the integrase and the minor tail proteins that include 2 transposases, a ribonuclease and a membrane protein. *Rhodococcus* phage Whack has a 49,660-bp genome with 61.9% CGC content. Whack is also a singleton, sharing only 25% of its 77 putative genes with its closest relative, singleton *Rhodococcus* phage, REQ2. Nearly half of Whack’s genes (43%) belong to orphams, including the immunity repressor and tyrosine integrase. Downstream of the integrase are 7 reverse oriented genes that include 3 DNA binding proteins, 2 membrane proteins and a lipoprotein.