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Immunity Testing as a Probe for Phage Diversity Prior to Full-Genome Sequencing

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Bacteriophages follow one of two distinct life cycles upon infection of a permissible bacterial host. Lytic phages inject phage DNA into the bacteria and hijack the metabolic systems of the host to make new viral particles. The release of progeny viral particles causes the host cell to die which results in the presence of visible plaques on a lawn of host bacteria. In contrast, temperate phages infect the host and the phage genome integrates into the host bacteria genome via use of the phage integrase. Subsequent suppression of phage gene transcription is mediated by the cognate phage repressor. Each time the bacteria host (now called a lysogen) replicates, the integrated phage genome (prophage) is replicated along with the host. Under certain conditions, the phage genome excises from the host and the phage enters into the lytic cycle which often results in turbid plaques on a bacteria lawn. In 2017, students at UAB isolated five new *Corynebacterium* phages infecting the host *C. xerosis* (Juicebox, KobeBeanBryant, StAB, SamW, Troy). Stable lysogens were isolated from all five phages and cross-infection studies showed that phages with similar genomes (SamW and Troy) were homoimmune, while phages with dissimilar genomes (SamW and KobeBeanBryant) were heteroimmune. In fall 2018, we isolated 17 additional phages infecting the host *C. xerosis*. Preliminary genome characterization using restriction endonuclease digestion showed unique banding patterns for many of the phages so immunity testing using the 5 lysogens from 2017 was used as a secondary criteria for sequencing selection. Five phages (Adelaide, Bran, Dina, Lederberg, Stiles) were selected for full-genome sequencing and annotated in spring of 2019. Four of the five sequenced 2018 *C.xerosis* phages contained a tyrosine integrase gene indicating most *C.xerosis* phages may be temperate. Thus, we sought to isolate stable lysogens for each of the 17 newly isolated *C. xerosis* phages and completed additional immunity experiments to further probe the diversity of *C.xerosis* phages prior to full genome sequencing.