

# Isolating and Annotating Novel Bacteriophages That Infect *Gordonia rubripertincta*: A Comparison Between Evergreen22 (Fribs8) and KayGee

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Phage therapy is a promising option to mitigate antibiotic resistance. Phages, a type of virus, are genetically diverse and globally plentiful. Yet only 14,500 phages have been discovered, much less annotated. Using isolation, purification, amplification, and DNA extraction, this two-phase study discovered and sequenced a novel phage called Evergreen22 that infects *Gordonia rubripertincta*, an opportunistic bacterium. Another novel phage—KayGee—, in the same CT cluster as Evergreen22 was annotated using DNA Master, which indicated 73 genes. Following the 14-guiding principles recommended by Turner and colleagues (2021), each of the 73 genes for KayGee was manually refined using an additional 8 bioinformatic tools. The annotation was sent to PhagesDB at the University of Pittsburgh for validation and for addition to the metagenomic database. All three hypotheses were supported. A wet-lab temperature study showed the optimal infection temperature of Evergreen22 to be 35°C, implying it would be efficacious in humans (body temperature is 37°C). The study compared Evergreen22 and KayGee on plaque morphology, annotations and the temperature study, and showed the phages to be lytic phages that do not produce TRNAs (toxins). Both phages are suitable for phage therapy. Also, KayGee has two rare genes only seen in one other CT-cluster phage called Pons whereas Evergreen22 had one rare gene, adding to the phage taxonomy knowledgebase. Furthermore, both phages can be used in bioremediation, therapeutic phage cocktails, and food preservation. Future studies should conduct one-step growth curves for both phages to determine the burst size of each.

## Awards Won:

Third Award of \$1,000