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2022 SEA Symposium Abstract

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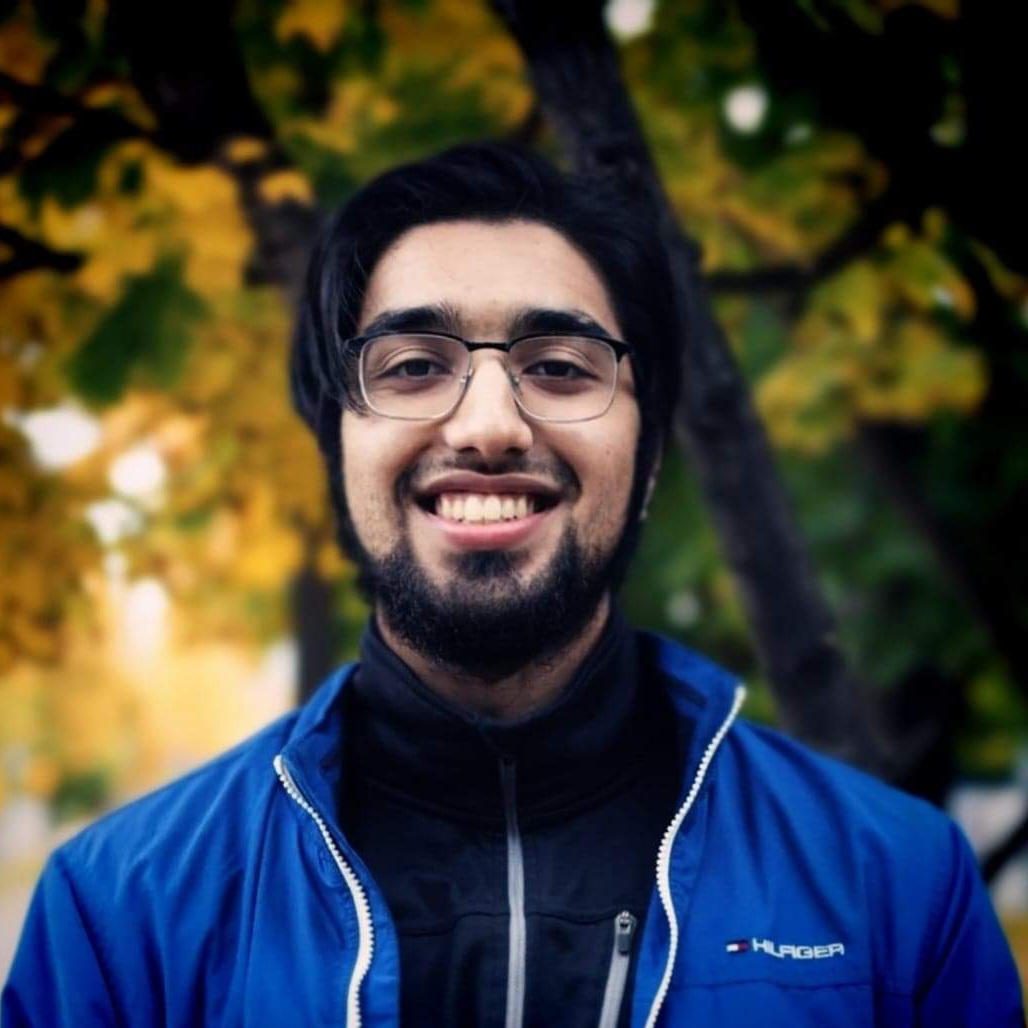
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Prophage Liberation! A method to increase the titer of the temperate phages Khorsid and Winseler.

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Bacteriophages can exist in a lysogenic or lytic state. The lysogenic state is a dormant state where upon infection, phage DNA is incorporated into the bacterial host genome. As the cell divides the prophage DNA is passed on to the daughter cells. When the cell is under stress, the prophage is induced and its genome excises from the bacterial chromosome and enters the lytic life cycle.   
  
Most phages are temperate and exist in an equilibrium of lytic and lysogenic cycles. Extremely temperate phages are challenging to purify and amplify and thus underrepresented among sequenced phages. The aim of this study was to develop a method to increase prophage induction and phage release in liquid cultures of the temperate phages Khorsid and Winseler.   
Khorsid and Winseler infect *Arthrobacter globiformis* and were isolated and purified in Ottawa, ON Canada in September 2021. Both phages proved extremely difficult to amplify and neither produced a lysate above 10^9 PFU. Although a TEM image was obtained for Khorsid, neither phage yielded enough genomic DNA for restriction mapping, and the ultrastructure of Winseler remains a mystery.   
  
Antibiotics are used to kill bacteria, but at sub-lethal doses induce several stress responses, including DNA damage, a trigger for prophage induction in most bacteria. Carbadox, an antibiotic frequently used for livestock, is a topoisomerase inhibitor that causes double stranded DNA breaks and has been shown to effectively induce prophages in some species. We therefore wondered if the addition of Carbadox to Khorsid and Winseler lysogens, or to liquid infections of *A. globiformis* with Khorsid and Winseler phages, might shift the equilibrium between lysis and lysogeny to favour a lytic life cycle, thereby increasing phage release and the resulting lysate titer.  
  
We have determined the minimal inhibitory concentration (MIC) of Carbadox on *A. globiformis* to be 10 µg/mL. Using this concentration of Carbadox during a liquid infection with Khorsid causes a 10^4 increase in PFU, suggesting we can use this method to isolate sufficient genomic DNA for restriction mapping and sequencing. Similar experiments with a Khorsid and Winseler lysogen did not yield as large of an increase. We are currently optimizing this method by varying host or lysogen density and concentration of Carbadox, and we will focus on increasing the titer of Winseler.   
  
Future work will create a streamlined Phage liberation! protocol in which optimal conditions that favour the lytic cycle can be rapidly determined for specific phage-host pairs, and sufficient genomic DNA can be isolated for future study. Our eventual sequencing of Khorsid and Winseler will allow us to also determine if these extremely temperate phages increase the diversity of Actinobacteriophages.