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2023 SEA Symposium Abstract

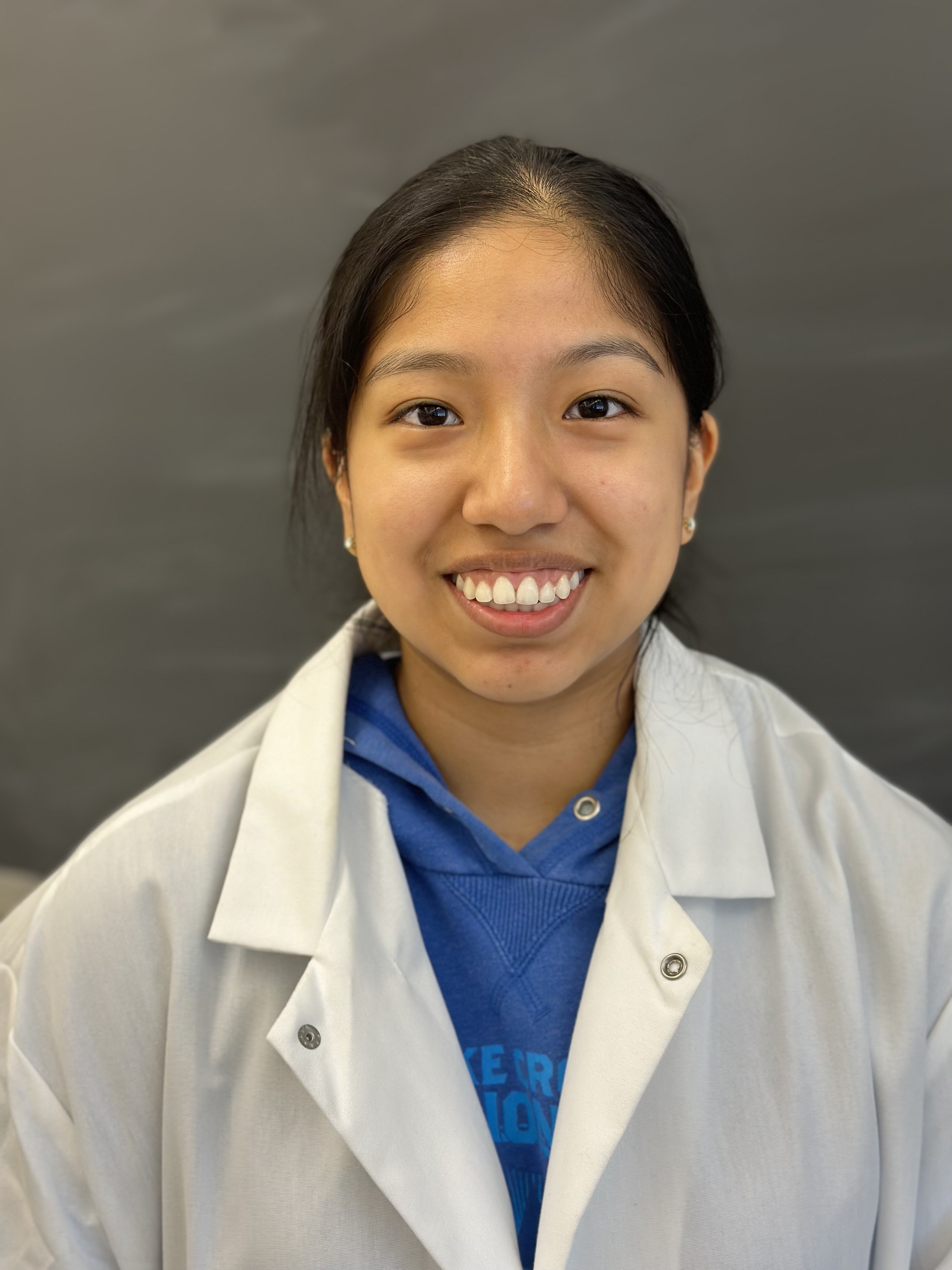
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Isolation, characterization and initial sequence comparisons of bacteriophages capable of infecting Azotobacter vinelandii

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During the fall semester of 2022, one section of the SEA-PHAGES introductory biology lab course (BIOL 222L) at Case Western Reserve University used *Azotobacter vinelandii* as a host species for the isolation of novel bacteriophages. *A vinelandii* is a globally distributed and genetically tractable diazotroph capable of fixing nitrogen aerobically. It is a gram-negative bacteria from the class Gammaproteobacteria. Understanding soil phage-host dynamics is key for evaluating the role of phages in biogeochemical cycles and in overall ecosystem functioning. An important first step in this project is isolating and charactering *A vinelandii* phages, since no molecularly characterized phages have thus far been described, although prophage genomes are predicted from whole genome sequencing of *Azotobacter* species. In all, we isolated at least 8 unique phages from soils from northern Ohio, 6 of which appear to be temperate. Electron microscopy revealed that they have diverse morphologies, including podoviridae, siphoviradae, and myoviridae. All of the podoviridae and siphoviradae appear to have icosahedral heads, while the lone myoviridae has a prolate head. We used Oxford Nanopore Technology to sequence all of these bacteriophages. After initial sequencing, base calling, quality control, assembly and polishing, each phage genome consisted of approximately 2-3 contigs. Genome sizes ranged from approximately 100 to 250 kB. Surprisingly, BLAST results indicate nearly no overlap between *Azobacter* phages and any of the phages found in the Actinobacteriophages database. Rather, there is considerable similarity to phages capable of infecting *Enterobacter* species. We are using genome alignments with these *Enterobacteriophages* to order and orient contigs for individual *Azobacteriophages*, and are designing primers for Sanger sequencing to complete the genome assembly. We are in the process of identifying core structural genes and integration cassettes for each genome, and will perform phylogenetic analyses on these sequences. We are in the process of making lyosgens for all of the temperate phages in order to test immunity relationships between them. We aslo noticed greater phage virulence when phages infected *A vinelandii* growing in nitrogen rich media compared to nitrogen deficient media (PYCa vs. Dean). We are testing this directly using Nitrogen deficient media supplemented with various concentrations of glutamine as a nitrogen source. Finally, we are examining synergistic interactions between lytic *Azobacteriophages* and antibiotics for host cell killing.