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Genetic characterization of super-infection immunity in AZ cluster phages

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uOttawa PhageHunters have found and sequenced nineteen AZ cluster phages that infect *Arthrobacter globiformis* and *Arthrobacter sulfureus*. These phages form stable lysogens and we have examined super-infection immunity of eight lysogens using nine AZ phages. Lysogens of the Pixelle and Amyev phage provide super-infection immunity against many of the other AZ cluster phages.

We observed that some phages rapidly acquire genetic mutations that allow them to bypass this super-infection immunity, and we have purified ten ObiToo and Crewmate defense escape mutants (DEMs) that allow complete infection on Pixelle and Amyev lysogens. Six DEMs have been sequenced and all have mutations in the intergenic region upstream of a conserved DNA binding protein (gp50 in Crewmate) that has strong homology to the RpoS stress-activated sigma factor from *E. coli*. Five of these mutants lie within a short six base-pair palindromic sequence close to the -35 region of the putative gp50 promoter. We hypothesize that these mutants alter the transcription of this gene and downstream genes allowing bypass of super-infection immunity.

The RpoS-like DNA binding protein is conserved in all AZ phages, and we propose it defines the start of an immunity cassette that ends in a conserved serine integrase (gp55 in Crewmate), and also contains a conserved Spartan-like protease (SprT-like protease; gp53 in Crewmate). SprT-like proteases are activated by DNA binding and in eukaryotes play a role in DNA repair.

We have created plasmids that inducibly express the RpoS-like DNA binding protein and SprT-like protease in *Arthrobacter* and are testing a model in which the binding of the protease to this promoter region is required for expression of lysogeny genes and prophage-mediated immunity.