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Arthrobacter globiformis B-2979 CookieBear lysogens exhibit mixed immunity to known temperate phages

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Viruses are infectious particles that replicate within specific hosts. We focus on bacteriophages, which infect bacterial cells. Bacteria can be infected by phages through two mechanisms: the lytic cycle, which leads to cell lysis, or the temperate cycle, which results in host genome integration. Our research aims to characterize a potential temperate phage, CookieBear, which infects the soil microorganism Arthrobacter globiformis B-2979. Genome sequencing identified CookieBear as a member of Cluster AY, with its closest relatives being Raphaella (99%), Auxilium (99.2%), and Richie (98.2%). Based on genome annotation, we hypothesized the presence of temperate (CookieBear\_37; tyrosine integrase) and lytic (CookieBear\_28; endolysin) genes. To determine whether CookieBear can undergo the temperate cycle, we attempted to isolate a lysogen, a bacterium with the phage genome integrated. We successfully recovered three stable lysogens and confirmed through phage release assays. Immunity assays demonstrated that CookieBear lysogens resist self-infection. An expanded immunity panel revealed that the lysogens permit infection by the lytic Cluster FE phage Piku but prevent infection by temperate phages from Clusters AS1 (Eesa) and FL (Hirko). Notably, another Cluster AY phage, Anekin, infected the lysogen at levels comparable to the control. Future work will focus on comparative genome analysis to identify factors involved in mixed immunity and provide insights into Cluster AY temperate phage infections.