Development of Two Phage Therapy-Based Strategies for the Treatment of American Foulbrood Disease Affecting Apis Mellifera capensis

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Abstract: American foulbrood (AFB) is the world's most devastating honeybee disease that has drastically reduced the population of Apis mellifera capensis since 2009. The outbreak has jeopardized the South African bee keeping industry as well as the agricultural sector dependent on honeybees for honey production and pollination, leading to significant economic losses. AFB is caused by Paenibacillus larvae, a spore-forming, Gram positive facultative anaerobic and flagellated bacterium. The use of antibiotics within beehives has selected for resistant strains of P. larvae, while the current practice of burning spore contaminated beehives and equipment contributes to the economic losses in the honeybee-keeping industry. Therefore, phage therapy is proposed as a promising alternative to combat P. larvae strains affecting A. mellifera capensis. The genomes of two P. larvae strains isolated from infected combs in the Western Cape have been sequenced and annotated using bioinformatics tools. Genome analyses has revealed that these P. larvae strains are lysogens to more than 6 different prophages and possess different type of clustered regularly interspaced short palindromic repeat (CRISPRs) regions per strain. Active prophages from one of the two P. larvae strains were detected and identified using PCR. Electron microscopy was used to determine the family of the identified active prophages. Lytic bacteriophages that specifically target the two P. larvae strains were purified from sewage wastewater, beehive materials, and soil samples to investigate their potential development as anti-P. larvae agents. Another alternative treatment being investigated is the development of a prophage endolysin cocktail. Endolysin genes of the prophages have been targeted, cloned and expressed in Escherichia coli. The heterologously expressed endolysins have been purified and are currently being assessed for their lytic activity against P. larvae strains and other commensal microorganisms that compose the honeybee larvae microbiota. The study has shown that phage therapy and endolysins have a great potential as alternative control methods for AFB disease affecting A. mellifera capensis.

Keywords: American foulbrood, bacteriophage, honeybee, Paenibacillus larvae

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