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Bordetella Pertussis Cloning and Conjugation

Alec McMahon

The pathogenic microbe, *Bordetella pertussis*, is most recognizable for causing the disease commonly known as whooping cough. A whole-cell vaccine for *B. pertussis* was first developed in 1914 and it later became one part of the DTP combined vaccine in 1948. Since the development of these vaccines, *B. pertussis* has lost its prominence in research labs and remained understudied as a result. This research was performed to continue identifying gene 00815, one of many ambiguous genes in *B. pertussis*. Genetic comparisons with other related bacterial genes have identified this gene to be involved in the production of lipopolysaccharide (LPS) membrane components. To test this relationship, cloning was used to develop a mutant for observation and characterization. Gibson assembly and traditional cloning were both used to insert DNA fragments into a cloning plasmid designed to integrate into the *B. pertussis* chromosome to create the mutant. The edited plasmid will be inserted into *E. coli* that can undergo conjugation with *B. pertussis* to transfer the plasmid. Early results indicate that the Gibson assembly method is unsuccessful at creating a mutant. Experiments are underway to determine the success of traditional cloning in creating the 00815 mutant.