Identifying genes that affect colony morphology in bacterial pathogen *Clostridioides difficile*

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The pathogenic bacterium *Clostridioides difficile* is a leading cause of healthcare-associated gastrointestinal infections, with symptoms ranging from diarrhea to potentially fatal toxic megacolon, pseudomembranous colitis, or sepsis. *C. difficile* exhibits phenotypic heterogeneity through phase variation, a mechanism that many bacteria use to alter surface-exposed structures. Phase variation serves as a bet-hedging strategy in the face of a changing or stressful environment, such as a host immune response. The *cmr* switch, one of eight known phase variable elements in *C. difficile*, regulates expression of the atypical two-component signal transduction system (TCS), CmrRST. This TCS consists of a sensor kinase (CmrS) and two response regulators (CmrR and CmrT) that are capable of binding DNA and controlling downstream gene expression. Expression of *cmrRST* results in the formation of rough colonies, cell chaining, increased surface motility, biofilm formation, and increased virulence in the hamster model of infection. Our group is taking several approaches to identify the genes involved in these phenotypes: RNA-Seq, suppressor screens, and transposon mutagenesis, the latter two of which are described in this work. Given that the Δ*cmrT* strain cannot form rough colonies, a suppressor screen was conducted in this background to identify mutations that restore rough colony morphology. We found that an early stop codon mutation in the gene CDR20291_2442 restores rough colony morphology and increases surface spreading. Complementing a functional, wild-type version of CDR20291_2442 into the suppressor strain reverts to the Δ*cmrT* phenotype, suggesting that the wild-type CDR20291_2442 may function to maintain smooth colonies. Further, through transposon mutagenesis in the smooth Δ*cmrR*Δ*cmrT* strain, we isolated ~30 more isolates that restore rough colony morphology. Future work characterizing all our identified mutants will help reveal how *C. difficile* employs phenotypic heterogeneity to modulate virulence.