Characterization of a Fic Protein from *Bordetella Bronchiseptica* with Guanylyltransferase Activity

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Keywords: *Bordetella*; guanylyltransferase; AMPylation

Filamentation induced by cAMP(Fic) proteins regulates diverse cellular processes in bacteria. While Fic proteins predominantly utilize ATP to post-translationally modify target proteins, some utilize other nucleotide derivatives to alter the activity of their target. *Bordetella sp.* causes respiratory tract infections, including whooping cough in humans. A combination of waning immunity to *B. pertussis* and the emergence of human-adapted *B. bronchiseptica* strains have resulted in recent epidemics of whooping cough-like illnesses worldwide – highlighting the presence of novel *Bordetella* proteins critical for virulence and/or fitness. Such proteins would be key candidates for a more effective vaccine designed for newly circulating *Bordetella* strains. Interestingly, we discovered a Fic protein, BbFic in *Bordetella bronchiseptica*, that fits the transcriptional profile of such predicted virulence factors. Unlike most Fic proteins that preferentially bind and utilize ATP as a nucleotide source, BbFic weakly binds ATP and instead shows preferential usage for GTP. We thus report the enzymatic and biophysical characterization of BbFic as a bona fide guanylyltransferases, and present structural insights into BbFic-nucleotide interaction. We solved the crystal structure of apo BbFic at 3.1 Å and using AlphaFold predicted a putative function of BbFic. Using molecular docking and mutagenesis, we elucidated a mechanism for GTP recognition, which implicates two arginine residues within its nucleotide-binding pocket (Flap). Furthermore, our bioinformatics analyses of the entire Fic protein to identify similarity networks using BbFic as an index protein identified a sub-cluster of proteins that also function as guanylyltransferases. The importance of our work is two-fold: 1) BbFic represents a new category of fitness genes predicted to play a role in new host adaptations for *Bordetella*, and 2) BbFic frames the groundwork for understanding Fic-mediated GMPylation as a novel post-translational modification in signal transduction.