Identifying Novel Mechanisms of Quorum Sensing Receptor Protein RpfR: Relevance to the BDSF Quorum Sensing Signaling Pathway

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Burkholderia cenocepacia is a multi-drug resistant bacteria, prevalent in fatal pulmonary infections associated with cystic fibrosis. It uses a signaling molecule known as BDSF which binds protein RpfR during quorum sensing - a process that coordinates various aspects of virulence. Last year, this project attempted to characterize the activity of RpfR homologues in Burkholderia cenocepacia (BcRpfR), Cronobacter turicensis (CtRpfR) and Escherichia coli (EcRpfR). This year, it focuses on BcRpfR, specifically examining evolved mutants of RpfR that have an improved ability to form biofilms, a model for evolution during chronic infections in cystic fibrosis. Specifically, in V.cholerae, it was found that BcRpfR and CtRpfR exhibited net diguanylate cyclase (DGC) activity and, upon addition of BDSF, an increase in phosphodiesterase (PDE) activity. GtrR, another protein present in the BDSF pathway, was found to inhibit RpfR's DGC activity, allowing GtrR-RpfR complexes to act as virulence promoters. RpfR mutants with elevated biofilm levels, Y355D and A106P, showed heightened levels of DGC activity and similar levels of PDE activity relative to WT (wild type) RpfR. Upon interacting with GtrR, Y355D and A106P demonstrated a downshift in DGC activity, however not to the extent shown by WT RpfR. Investigating the Y335D and A106P interactions with GtrR revealed higher Kd values than WT which supports an exploitation of the RpfR-GtrR interaction in the mutants. Survival assays further support this biofilm-virulence trade off. Overall, this project has established the role of GtrR in Burkholderia pathogenesis - a major step in finding a solution to infections caused by these deadly strains of bacteria.

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