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 signaling molecules known as Burkholderia Diffusible Signaling Factors (BDSF) which bind protein RpfR during quorum sensing - a process that coordinates virulence. This project characterizes the activity of RpfR homologues in Burkholderia cenocepacia (BcRpfR), Cronobacter turicensis (CtRpfR) and Escherichia coli (EcRpfR) to identify the role of BDSF in various aspects of RpfR activity, examine the role of RpfR in Burkholderia pathogenesis and ascertain protein interactions involving RpfR. Via mechanistic studies employing RpfR mutants containing mutations in the PAS, GGDEF and EAL domains, a new model for RpfR has been created. Specifically, it was identified that the trans isomer of BDSF, lauric acid, does not affect CtRpfR and EcRpfR activity. Furthermore, basal levels of DGC activity were attributed to CtRpfR while EcRpfR's GGDEF domain proved to be inactive. Upon addition of BDSF, CtRpfR exhibits heightened PDE activity and an upregulation in the expression of swarming motility genes, while EcRpfR remains unresponsive. Closer inspection of BDSF-RpfR binding led to the identification of two novel bindings sites, NA (120-126) and NA2 (139 -157). In addition, BcRpfR mutants Y355D and A106P exhibited heightened levels of DGC and PDE activity, indicating the importance of RpfR in Burkholderia pathogenesis. Finally, novel evidence of the N-terminal domain moderating fatty acid cleavage during RpfF-RpfR interaction has been shown. This project has illuminated key aspects of RpfR activity and identified multiple novel sites for drug intervention in the BDSF quorum sensing pathway.

Awards Won:
Third Award of $1,000