**Divide and conquer: how *Pseudomonas aeruginosa* temporally and spatially organizes the collective to control virulence**

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Pseudomonas aeruginosa is a hospital-endemic pathogen that causes tens of thousands of infections each year. It is highly antibiotic resistant and difficult to eradicate from healthcare environments. Specifically, P. aeruginosa forms biofilms on healthcare equipment, like the tubes of catheters and ventilators, which is a major source of patient transmission. Biofilm formation in P. aeruginosa is regulated by quorum sensing (QS), a mechanism of inter-bacterial communication that underpins virulent behaviors. QS in P. aeruginosa is coordinated primarily by a network of LuxI-type synthases and LuxR-type receptors, where the synthases produce homoserine lactone (HSL) autoinducer molecules and the receptors act as transcription factors when bound by their cognate ligands. LasR is a LuxR-type receptor that canonically upregulates another QS pair, the RhlIR system. RhlI synthesizes C4HSL, which binds the LuxR-type transcription factor RhlR. However, QS progression is different in clinical strains of P. aeruginosa; there are frequent mutations in lasR that render the receptor non-functional, and consistent mutations in the rhlI gene that had not previously been characterized. Notably, rhlI mutations are often concurrent with lasR inactivating mutations, suggesting that there is a biological advantage to harboring these variants. Previously, we showed that RhlI variants identified within a cohort of clinical P. aeruginosa strains restore virulence phenotypes in a ∆lasR background by re-calibrating C4HSL levels. We also observed that these rhlI mutations promote the formation of a small colony variant (SCV) subpopulation in ∆lasR strains that is hyper biofilm forming. RNA-Seq analysis of these SCVs revealed upregulation of a two-component signal transduction system, PhoPQ, which has been implicated in virulence and antibiotic resistance in numerous bacterial genera. Deletion of phoQ in a ∆lasR strain resulted in the loss of pyocyanin production and a delay in SCV formation. Moreover, overexpression of rhlR in the ∆lasR∆phoQ background complemented these virulence phenotypes, suggesting that the transcription factor PhoP regulates the rhl operon in the absence of lasR. To our knowledge, this is the first report of cross-regulation between the RhlIR and PhoPQ systems. This work expands our understanding of virulence gene regulation in P. aeruginosa, and particularly the formation of hyper biofilm forming SCV subpopulation.