The *Pseudomonas aeruginosa sirB2* gene is a fitness determinant of anaerobic growth and its inactivation affects virulence and rugose small colony variant

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Pseudomonas aeruginosa (*Pa*) chronic infections in patients with cystic fibrosis (pwCF) are challenging to eradicate. Infection success relies on *Pa*'s ability to adapt to the complex CF lung environment. Transcriptional analysis of *Pa* communities from sputum samples indicates that *Pa* growth in CF airways is associated with a distinct transcriptional profile. Most of the genes modulated *in vivo* remain poorly characterized.

In this study, we characterized the gene of unknown function PA14_RS04555 (*sirB2*), whose expression is particularly stimulated in the CF lung environment and shares homology with virulence determinants in *Salmonella enterica*. Our research indicates that *sirB2* is transcriptionally controlled by the virulence regulators Vfr and AmrZ. Its deletion enhances *Pa* pathogenicity, increasing virulence in *Galleria mellonella* larvae and promoting bacterial translocation and biofilm formation in a differentiated human airway epithelial infection model. In vitro, we confirmed that *sirB2* inactivation triggers biofilm formation only when oxygen access is restricted. Under these conditions, the *sirB2* mutant leads to an increased emergence of hypervirulent rugose small-colony variants (RSCV) through the accumulation of secondary mutations in the *wsp* operon, thereby increasing the second messenger c-di-GMP levels. Our data indicate that RSCV emergence is linked to an imbalance in the NAD+/NADH ratio under oxygen-limited conditions. Indeed, the absence of the *sirB2* gene reduces fitness under anaerobic growth conditions with nitrate as the sole electron acceptor, and this phenotype is independent of the ubiquinone pool, suggesting that the *sirB2* gene is an important determinant of survival in the lungs of pwCF.

Further studies are underway to decipher the mechanism of action of the *sirB2* gene.