DksA–(p)ppGpp interplay is a key determinant of *Pseudomonas aeruginosa* extracellular and intracellular infection

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Pseudomonas aeruginosa is an opportunistic Gram-negative pathogen responsible for severe acute and chronic infections. Although traditionally considered extracellular, it can persist within host cells, contributing to immune evasion and antibiotic resistance. Despite its clinical relevance, the molecular mechanisms enabling intracellular survival remain poorly understood.

The stringent response (SR), mediated by the alarmone (p)ppGpp and the transcriptional regulator DksA, is a key global regulatory system controlling bacterial adaptation and virulence. Here, the interplay between (p)ppGpp and DksA in regulating virulence and intracellular persistence of *P. aeruginosa* was investigated. Using a reverse genetics approach, mutants lacking DksA proteins (Δ*dksA*), (p)ppGpp (Δ*rs*, lacking the *relA* and *spoT* genes), or both (Δ*dksA-rs*) were generated. All SR-mutant strains showed reduced motility, impaired production of virulence factors, and attenuated pathogenicity in *Galleria mellonella*.

Importantly, SR mutants displayed decreased cytotoxicity and impaired intracellular survival in human lung epithelial cells compared to the wild type, with the Δ*dksA-rs* showing the strongest defect. This phenotype was correlated with reduced expression of the Type III Secretion System effector ExoS and an impaired ability to evade intracellular degradative pathways. Confocal microscopy revealed increased colocalization of the Δ*dksA-rs* mutant with LAMP1-positive compartments, indicating impaired escape from lysosomal degradation. Consistently, inhibition of lysosomal acidification and autophagosome-lysosome fusion by bafilomycin restored mutant survival.

Overall, (p)ppGpp and DksA act both additively to promote *P. aeruginosa* virulence and intracellular persistence, highlighting the SR as a critical determinant of host-pathogen interaction and a promising target for the development of novel antimicrobial strategies.