Targeting *de novo* L-cysteine biosynthesis (DeNoCB) in *Pseudomonas aeruginosa* for novel antimicrobial strategies

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Bacterial DeNoCB is a key metabolic hub and a promising antibiotic target due to its absence in humans, yet it remains poorly explored in *P. aeruginosa*[1,2]. Four key DeNoCB enzymes - CysK (PA2709), CysM (PA0932), CysE (PA3816), and CysH (PA1756) - were identified and physiologically/biochemically characterized. Their roles were investigated using deletion mutants grown in minimal media with different S-sources (sulfate, thiosulfate, or L-cysteine). Single deletions of *cysM* or *cysK* did not result in cysteine auxotrophy, whereas the double Δ*cysM*Δ*cysK* mutant showed no growth for up to 18 h, followed by recovery at later time points, suggesting the presence of alternative pathway(s)[3]. CysE was found to be essential for DeNoCB, by supplying the key intermediate *O*-acetylserine, as the Δ*cysE* mutant failed to grow on both sulfate and thiosulfate. Finally, the Δ*cysH* mutant was confirmed to be a cysteine auxotroph, but only when growing on sulfate.

Notably, both Δ*cysH* and Δ*cysE* exhibited reduced virulence in the *Galleria mellonella* infection model compared to the wild-type strain, thus emerging as promising antimicrobial targets.

Recombinant production and functional characterization of all four enzymes enabled compound library screening and inhibitor validation, both *in vitro* and in bacterial cultures. Preliminary results identified hit compounds targeting CysE (IC_{50} = ~30 μM, >60% growth inhibition) and CysH (IC_{50} = ~50 μM, >30% growth inhibition), while assays on CysK and CysM are ongoing.

Overall, these findings provide new insights into *P. aeruginosa* metabolic and regulatory networks, highlighting the key role of sulfur metabolism in bacterial physiology and pathogenicity.

References

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