Phospholipid Deletion Alters Membrane Composition, Growth and Stress Response in Mycobacterium smegmatis

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Mycobacterium tuberculosis, the bacterial agent of tuberculosis, has become resistant to many traditional antibiotics due to its thick, lipid-rich membrane. Phosphatidylethanolamine (PE) is a key phospholipid within the mycobacterial membrane and is essential for maintaining the cell's stability and function. This study examines the role of phosphatidylserine decarboxylase (Psd) in PE synthesis by investigating the pssA-psd operon, which encodes for sequential enzymes in the final steps of PE synthesis. The deletion of psd was expected to disrupt PE production, altering membrane lipid composition and inhibiting cellular growth. A psd deletion mutant and complement strain were generated and compared against the wild-type strain using growth assays, environmental stress tests, and lipidomic analysis. Lipidomic analysis revealed a complete loss of PE in the mutant. The mutant also exhibited reduced growth and increased sensitivity to environmental stress. Furthermore, complementation did not restore PE production, highlighting the cell's reliance on the entire operon for PE synthesis. These findings demonstrate that Psd is critical for maintaining membrane lipid balance in mycobacteria, highlighting the enzyme as a target for anti-tubercular drug development. Structural analysis of Psd using Alphafold3 and ChimeraX revealed that the catalytic serine group is located within a deep, hydrophobic binding pocket. Based on the surrounding residue environment, an ideal inhibitor would be a lipophilic small molecule featuring a planar ring structure (to stack with Phe207) and positive functional groups (to target Glu230). Such a molecule would effectively inhibit Psd and block PE production, providing an effective strategy for targeting tuberculosis.

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