Investigating the role of the protein LysX2 of *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis (*Mtb*), the causative agent of tuberculosis, survives within the host through sophisticated mechanisms of environmental adaptation and resistance to immune-mediated killing, largely supported by the unique properties of its cell wall. Among the factors influencing cell wall composition, MprF-like proteins play a key role by modulating surface charge through the aminoacylation of phospholipids. *Mtb* encodes two members of this family, LysX and LysX2. While LysX has been extensively characterized, the function and mechanism of LysX2 remain poorly understood.

In this study, we investigated the role of LysX2 in its natural host, *Mtb*, by generating a knock-out strain using the ORBIT technique. Our results demonstrate that LysX2 is required for rapid adaptation to mildly acidic pH. Despite this deficiency, the KO strain compensates through the overexpression of *rv1169c*, encoding the PE11 protein involved in maintaining cell wall integrity. RNA-seq analysis further revealed the upregulation of oxidative stress response genes, including *sigH*, *katG*, and *rv2466*, in the KO strain.

Functional assays showed that the LysX2-deficient strain exhibits increased sensitivity to oxidative and nitrosative stress, as well as to vancomycin, indicating enhanced cell wall permeability and altered surface properties. High-performance thin-layer chromatography (HPTLC) lipid analysis revealed the accumulation of triglycerides under acidic conditions, consistent with a stress adaptation response. Finally, infection experiments using the murine RAW264.7 macrophage cell line demonstrated that the KO strain is significantly more susceptible to macrophage-mediated killing than the wild type.

Collectively, these findings identify LysX2 as a critical virulence factor in *Mtb*, contributing to resistance against key macrophage defense mechanisms. Further studies are needed to elucidate its molecular target and mechanism of action, potentially opening new avenues for therapeutic intervention.