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Abstract

Tuberculosis (TB), a disease caused by the bacterium *Mycobacterium tuberculosis*, is a significant global threat to human health. The emergence of drug resistant *M. tuberculosis* necessitates the identification of new drug targets for the development of new, shorter regimens. The peptidoglycan (PG) core of the *M. tuberculosis* cell wall is a potential source of drug targets because it is unique to bacteria and plays a vital role in a multitude of cellular processes and host-mediated immune responses. PG is constantly remodelled by PG synthases and hydrolases in response to external stimuli. This research focuses on *N*-acetylmuramyl-L-alanine amidases (amidases), PG hydrolases that are implicated in bacterial growth, cell division, virulence and antibiotic tolerance. More specifically, this PhD aims to characterize the *M. tuberculosis* Ami1 (Rv3717) homologue and to highlight its potential as a drug target. Genotypic characterization of a previously generated *M. tuberculosis* mutant strain lacking *ami1* (H37*Δami1S*) was conducted prior phenotypic assessments. The deletion of *ami1* had no significant effect on growth rate and cell division in standard 7H9 media. In contrast, the growth rate of H37*Δami1S* was significantly reduced when grown in Sauton's minimal media with (1%) or without glycerol as a carbon source. We then surmised that Ami1 possibly plays a role in intracellular survival, where host-derived carbon sources support bacterial growth. The survival of H37*Δami1S* was reduced in IFN-γ stimulated U937 macrophages.

H37*Δami1S* displayed increased susceptibility to rifampicin when assessed by broth microdilution. This observation was credited to a weakened, more permeable cell wall. Consistent with this, H37*Δami1S* exhibited an increased ethidium bromide uptake. Subsequently, we hypothesized that H37*Δami1S* may display alterations in antibiotic tolerance/persistence. In a 7-day time-course experiment, H37*Δami1S* displayed increased susceptibility to vancomycin, ethionamide and isoniazid as evidenced by declining bacterial survival. We interrogated the isoniazid-associated phenotype further, by assessing the transcription of all three amidase-encoding genes. Only *ami1* was induced following exposure to isoniazid whereas the expression of *ami3* and *ami4* remained at basal levels. The regulation of the *ami1* gene was explored further through bioinformatics, which revealed two putative transcriptional regulators predicted to bind upstream of *ami1*, namely Rv1423 and Rv1776c.

Protein homology modelling detected HTH DNA binding domains in both proteins. These proteins were then cloned for recombinant expression in the pET29a+ system for purification. Rv1776c was successfully expressed and purified. Electrophoretic mobility shift assays yielded preliminary data that suggested that Rv1776c binds the promoter region of the *ami1* gene. Attempts to optimize binding were unsuccessful. To further evaluate the role of Rv1776c and Rv1423 in regulating *ami1* gene expression, we over-expressed the regulators, using the tetracycline operator, and assessed effect on cell wall stability, via an ethidium bromide diffusion assay. Over-expression of Rv1776c was not achieved despite increasing concentrations of anhydrotetracycline, suggesting possible downstream regulation of Rv1776c; however, over-expression of Rv1423 was achieved. An increase in ethidium bromide uptake was observed in strains over-expressing Rv1776c and Rv1423. Increasing anhydrotetracycline concentrations in both strains resulted in marginal decreases in the transcription of *ami1*. Overall, this study has demonstrated that Ami1 plays a vital role in how *M. tuberculosis* utilizes a carbon source during normal growth and survival *in vitro*. Moreover, the transcription of *ami1* is specifically and directly responsive to isoniazid exposure, possibly via two transcriptional repressors. This work therefore supports further characterization and development of Ami1 as novel drug target in *M. tuberculosis*.