A comprehensive study of three lesser-known penicillin interacting enzymes of *Mycobacterium smegmatis*

Abstract

Beta-Lactam antibiotics are among the most effective antimicrobial agents; however, the emergence of beta-lactamase-mediated resistance has greatly reduced their efficacy. While beta-lactamases from pathogenic bacteria are well studied, their diversity and physiological relevance in environmental mycobacteria remain poorly understood. This thesis investigates three underexplored penicillin-interacting enzymes (PIEs) from *Mycobacterium smegmatis*—MSMEG_1586, MSMEG_3978 (BlaE), and MSMEG_6194—to elucidate their biochemical functions, substrate preferences, and evolutionary relationships. Membrane-bound variants of the enzymes were expressed in *Escherichia coli* and *M. smegmatis* strains to assess their effects on beta-lactam susceptibility and cell morphology. MSMEG_1586 and MSMEG_3978 enhanced beta-lactam resistance, particularly to cephalosporins, whereas MSMEG_6194 did not alter antibiotic resistance but complemented cell shape defects in a PBP-deficient mutant, indicating a role in cell wall maintenance rather than drug resistance. Biochemical and computational analyses revealed distinct mechanistic features among these enzymes. MSMEG_1586, though resembling class C beta-lactamases, exhibited an expanded substrate range, showing high activity toward cefotaxime and the unusual capacity to hydrolyse aztreonam. Structural modelling suggested that the absence of the canonical R2 loop and the presence of a unique Asn265 in the omega-loop contribute to this broadened specificity. MSMEG_3978, in contrast, displayed kinetic behaviour characteristic of an extended-spectrum class C beta-lactamase (Bush–Jacoby group 1e). Site-directed mutagenesis identified Tyr170 as a key residue involved in the deacylation process, confirming its mechanistic role in catalysis. Unlike these beta-lactamases, MSMEG_6194 functioned as a DD-carboxypeptidase, lacking beta-lactamase activity due to the absence of a catalytic glutamic acid residue in its omega-loop. Remarkably, introducing this residue restored beta-lactamase activity while diminishing DD-CPase function, revealing a functional switch and evolutionary link between the two enzyme families. Overall, this study demonstrates the remarkable functional diversity of penicillin-interacting enzymes in *M. smegmatis*. These findings highlight how subtle structural variations modulate enzyme specificity and catalytic versatility, offering valuable insights into the molecular evolution of beta-lactamases and related enzymes in mycobacteria.