Physiological Characterization of Low-Molecular-Mass Penicillin Interactive Enzymes of Mycobacteria

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Abstract

Penicillin interactive enzymes (PIEs) constitute a group of enzymes that either bind to beta-lactams (PBPs) or hydrolyse beta-lactams (beta-lactamases). However, little is known about the physiological nature of those PIEs in mycobacteria. To study their physiological behaviour, three LMM-PIEs of mycobacteria were selected, namely, MSMEG_2433 (putative DD-CPase of *M. smegmatis*) and its homologue DacB2 (from *M. tuberculosis*) and MSMEG_4455 (putative beta-lactamase of *M. smegmatis*). The genes of their membrane-bound forms were cloned and expressed in *E. coli*. Interestingly, *in vivo* expressions of MSMEG_2433 and DacB2 were able to restore the cell shape oddities of *E. coli* septuple PBP deletion mutant suggesting their ability to perform as DD-CPase *in vivo*. Also, the beta-lactam resistance in mutant Δ*ampC* mutant of *E. coli* was augmented upon ectopic expression of MSMEG_2433 and MSMEG_4455 indicating *in vivo* beta-lactamase activity. To establish the biochemical reasons behind such physiological behaviours, the soluble forms of these PIEs were purified and analysed. Harmonizing with the observed physiological behaviours, soluble MSMEG_2433 and DacB2 exhibited DD-CPase activities with the pentapeptide substrates. Surprisingly, sMSMEG_2433 and sDacB2 also showed considerable deacylation efficiency with the beta-lactam substrate, a distinct character of beta-lactamase. However, the deacylation efficiency (an indicator of efficient beta-lactam hydrolyzing efficiency) of sMSMEG_4455 was very high as compared to both DD-CPases (sMSMEG_2433 and sDacB2). *In silico* analyses suggested the presence of an 'Ω-like loop' near the active site of all these enzymes, which is a characteristic feature of beta-lactamases and is involved in enhancing the deacylation efficiency of beta-lactamases. Therefore, to understand the physiology of the 'Ω-like loop', significant amino acids were mutated (E75A in MSMEG_2433 and D167E in DacB2, & Y194A in MSMEG_4455) at 'Ω-like loop' region. The deacylation efficiency of the mutant proteins was altered considerably that reduced both DD-CPase and or beta-lactamase activities. The effects of such mutations also affected the physiological behaviours like cell shape and beta-lactam resistance. Overall, the study reveals the presence of dual activity PIEs in both *M. smegmatis* and *M. tuberculosis*, and a very efficient penicillinase in

M. smegmatis where the enzymatic efficiencies are governed by specific amino acids of the 'Ω-like loop'.