"Functional characterization of AmpC β-lactamase and role of LMM-PBPs in peptidoglycan composition, β-lactam resistance and ampC regulation in *Pseudomonas aeruginosa*"

By

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Summary for PhD study

Pseudomonas aeruginosa is one of the most problematic versatile Gram-negative bacteria in causing opportunistic human infections which are particularly difficult to treat because of its intrinsic resistance to antibiotics, as a consequence of many intervening resistance mechanisms involving the ability to overproduce the chromosomally encoded cephalosporinases, Pae-AmpC, which are periplasmic enzymes, belong to group I class C serine β-lactamases and are also responsible of bacterial resistance in many bacteria. In *P. aeruginosa*, *ampC* expression is regulated mainly by AmpG permeases, AmpD amidases, AmpR, NagZ, and two competing AmpR-binding muropeptides [UDP-MurNAc-pentapeptides (*ampC* suppressor) and 1,6-anhydromuropeptides (*ampC* inducer)]. Low molecular mass penicillin-binding proteins [LMM-PBP; e.g. PBP4 (DacB), PBP5 (DacC), PBP7 (PbpG)] are a group of periplasmic enzymes that have DD-carboxypeptidase and/or DD-endopeptidase activities which participate in cell separation, peptidoglycan (PG)

maturation and recycling. Binding of β-lactams (e.g. penicillin) with LMM-PBPs causes an increase in anhydromuropeptides and periplasmic AmpC overproduction to hydrolyze that external unwelcome inducer. This study aims to highlight and to characterize the functions of Pae-AmpC and the role of LMM-PBPs PBP4, PBP5 and PBP7 in PG composition and bacterial resistance in *P. aeruginosa*; also, to study the role of these LMM-PBPs in Pae-*ampC* regulation and to see if they are needed for the recovery of rod shape of imipenem-induced round cells in *P. aeruginosa.* To fulfill this study we characterized several Pae-AmpC forms (wild type and mutants) in wild type and mutants of *E. coli* and in *P. aeruginosa* PAO1 strain which were tested for their PG composition by HPLC analysis and for bacterial resistance by disc diffusion method. Also, we constructed single and combined mutants of *dacB*, *dacC*, *pbpG* and *ampC* in PAO1 strain which were tested for their PG composition, *ampC* expression by RT-PCR, β-lactams susceptibility and their PBPs pattern by Bocillin-FL binding test. We analyzed PG composition and PBPs pattern in imipenem-induced round cells and their rod shape recovered cells in PAO1. We found that some Pae-AmpC mutants had a very low β-lactamase activity (AmpC-F4:C3 and AmpC-F4:C6); the mature form of Pae-AmpC had a high β-lactamase activity and a secondary DD-endopeptidase and DD-carboxypeptidase activities; only *dacB* single and combined mutations produced high *ampC* expression and β-lactam resistance; only *dacC* single and combined mutations produced maximum increase of PG pentapeptides. The triple mutant of *dacB*, *dacC* and *pbpG* displayed the largest increase in *ampC* expression and β-lactams resistance. Microscopic examination of all the constructed Pae mutants showed that they still retain their rod shape morphology similar to their parental PAO1 strain. Also, we found that activities of DacB, DacC and PbpG are not essential for recovery of rod shape in imipenem-induced spheres in *P.*