

"Cloning, expression and purification of two *ampC* mutants obtained during cloning of *Pseudomonas aeruginosa ampC*-PCR product in DH5 α - E coli"

By

Alaa Ropy Mahmoud Sayed

Supervisor:

Prof. Dr. Juan Alfonso Ayala Serrano

Center of Molecular Biology "Severo Ochoa" (CBMSO-UAM)
Madrid, Spain

University:

Universidad Autonoma de Madrid (UAM)

Faculty of Science

Spain



Summary for the master study

AmpC overexpression is an important key player in the β -lactam resistance in Gram-negative bacteria like *Pseudomonas aeruginosa*. It is either chromosomally encoded or plasmid-mediated. Infections with *Pseudomonas aeruginosa* are hard to treat because of complex resistance mechanisms displayed by this microorganism against the currently used antibiotics. These mechanisms involve increasing the efflux system, decreasing the influx system, target mutations and overproduction of AmpC β -lactamase.

This study aims to clone, purify and characterize two Pae-AmpC mutants, pGEM-13 (C¹⁵²→T point mutation) and pGEM-19 (T⁷²⁸→C point mutation). The PCR product of these *ampC* mutants were cloned and expressed using pET28b vector in *E. coli* BL21(DE3) cells. Both of the expressed AmpC mutants were partially purified using Ni-affinity chromatography. Purified fractions with high activity were pooled and concentrated. AmpC proteins were detected by SDS-PAGE and western blot. Also, β-lactamase activities on nitocefin were reported.

We found that 250 mM imidazole was sufficient to elute AmpC protein from Ni-affinity column. The purified fractions displayed two AmpC bands which may correspond to precursor and mature forms of AmpC protein. β-lactamase activities of the purified AmpC mutants on nitocefin displayed high activity using the pooled and the concentrated fractions beyond purification.

This work is in the way to study the antibiotic resistance in *Pseudomonas aeruginosa* in relation to the functional and the enzymatic characterization of *ampC* mutants and the determination of their crystal structure.