

Characterization of Recombinant PA1225: a Hypothetical NAD(P)H-Quinone Oxidoreductase from *Pseudomonas aeruginosa* Strain PAO1

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The product of gene *pa1225* in *Pseudomonas aeruginosa* PAO1 is currently annotated as a putative NAD(P)H-quinone oxidoreductase. A BLAST search revealed this gene does not have a match to any experimentally validated gene with an E value below 1e-5. This raises the possibility that the gene product PA1225 may be a novel enzyme. Interestingly, PA1225 in *P. aeruginosa* PAO1 is repressed 89 times in the presence of the LysR regulator PA4203. The latter also represses by x times nitronate monooxygenase (NMO), a detoxifying enzyme that oxidizes the mitochondrial toxin propionate 3-nitronate (1). Thus, PA1225 has potential as drug target against *P. aeruginosa*, an opportunistic gram-negative bacterium exhibiting multi-antibiotic resistance that thrives in water, immunocompromised humans, and hospital settings (2).

In this study, *pa1225* was amplified by PCR from the genomic DNA of *P. aeruginosa* PAO1 and ligated into vector pET20(b)+. The resulting recombinant plasmid was used to transform *Escherichia coli* strain Rosetta(DE3)pLysS for expression of PA1225. Optimization of recombinant protein expression, purification with ion-exchange chromatography, and kinetic characterization of the protein are currently ongoing and the results will be presented.

Keywords: *Pseudomonas aeruginosa*, PAO1, NAD(P)H-quinone oxidoreductase, LysR regulator, Rosetta(DE3)pLysS, recombinant enzyme, catalysis, residues.

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1. Vercammen, K., Wei, Q., Charlier, D., Dötsch, A., Häußler, S., Schulz, S., Francesca, S., Giovanni, G., Spain, J., Morten, R., Nielsen-Tolker, T., Dingemans, J., Ye, L., Cornelis, P. (2015). *Pseudomonas aeruginosa* LysR PA4203 Regulator NmoR Acts as a Repressor of the PA4202 nmoA Gene, Encoding a Nitronate Monooxygenase. *J. Bacteriol.*, 197(6), 1026-1039.
2. Winsor GL, Lam DK, Fleming L, Lo R, Whiteside MD, Yu NY, Hancock RE, Brinkman FS (2011). *Pseudomonas* Genome Database: Improved Comparative Analysis and Population Genomics Capability for *Pseudomonas* Genomes. *Nucleic Acids Res.*, 39, D596-600.