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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