Cloning of *nahB* Gene and Expression of NahB Dehydrogenase from *Pseudomonas putida* for Structural and Functional Studies

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Polycyclic aromatic hydrocarbons (PAHs) are released through the environment by anthropogenic activities related to the use of petroleum and its derivatives. Most of these compounds are mutagenic and carcinogenic. Bioremediation is a strategy for PAHs elimination which uses enzymes or microorganisms displaying the capacity to metabolize these compounds. Naphthalene is one of the most commonly found PAHs in the environment and its degradation by *Pseudomonas sp.* is an alternative procedure for the decontamination of the environment with this contaminant. In *Pseudomonas putida* G7, the *nahB* gene is responsible for codification of the NahB protein, which is one of the enzymes involved in the degradation of naphthalene. In the present work we focus on the amplification and cloning of *nahB* gene and expression of NahB enzyme. The main objective of our work is the characterization of the structure-function relationship of NahB by X-ray diffraction and biochemical assays. The bacteria *Pseudomonas putida* G7 was cultivated in minimum media with naphthalene and its DNA was extracted. Specific primers for the *nahB* gene were constructed and a protocol for the amplification of the gene by PCR was standardized. The fragment was cloned in pCR 2.1-TOPO vector and the recombinant plasmid was transformed in *E. coli* TOP10 competent cells. The clones were analyzed by colony PCR. The plasmidial DNA was extracted for sequencing and the gene was, later, sub-cloned in prokaryote expression vectors pET28a-TEV and pET28a-GST-TEV. These were later transformed in BL21 pRARE competent cells for protein expression.

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