

Efficient Degradation of DFP Using Recombinant *E.coli* Displaying Organophosphorus Acid Anhydrolase

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Background & Objectives: Organophosphorus Acid Anhydrolase enzyme catalyzes the hydrolysis of toxic organophosphorus cholinesterase-inhibiting compounds including insecticides and nerve gases such as sarin and soman. DFP-hydrolyzing enzymes have reported in diverse organisms such as squid, protozoa, mammals, clams, and soil bacteria including *Alteromonas* strain JD6.5, *A. undina* and *A. haloplanktis*. The high costs of enzyme purification and decrease of its stability limits its practical application in large scales. The purpose of this study is to overcome the mass transfer problem and increase the detoxification rate of organophosphorus compounds.

Methods: For the first time in this study a new anchor system derived from the N-terminal domain of ice-nucleation protein from *Pseudomonas syringe* InaV (InaV-N) was used to display OPA onto the surface of *E. coli*. The designed sequence was cloned in the vector pCDFDute-1 and then was expressed in *E. coli*. SDS-PAGE was used to trace the location of expressed InaVN-OPA. In order detect the function of protein displayed, recombinant bacteria was grown in minimal salt medium agar supplemented with DFP. The amount of degraded sample was assayed using FPLC.

Results: The tracing of recombinant protein using SDS-PAGE showed the presentation of InaVN-OPA on the outer membrane, and ability of recombinant *E. coli* to utilize diisopropylfluorophosphate (DFP) as the sole source of energy, without growth inhibition, indicated its significant activity. Location of OPA was detected by comparing activity of outer membrane fraction to inner membrane and cytoplasm fraction.

Conclusion: It can be concluded that InaV-N can be used efficiently to display foreign functional protein and these results highlight the high potential of engineered bacterium to be used in bioremediation of OPs-contaminated sources in the environment.

Keywords: DFP; Organophosphorus Acid Anhydrolase; Surface Display; Anchor