Functional Secretion of Organophosphorus Hydrolase Enzyme Using Recombinant Bacillus subtilis

Zhila Hafezieh; Samaneh Khodi; Ali Mohammad Latifi*

Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences,Tehran,Iran

iranrose2002@yahoo.com

Background & Objectives: Organophosphorus pesticides are the most common chemicals to protect crops and livestock. These are acetylcholine esterase inhibitors and have been reported as the third cause of poisoning and the main cause of mortality due to poisoning in Iran. Many microorganisms have been found that are able to break down toxic organophosphorus compounds. The most identified Organophosphorus hydrolase is called OPH that is found in Flavobacterium ATCC 27551 and P. diminuta MG. Since the OPH is an intracellular enzyme, for the first time in this study, in order to overcome the mass transfer problem and increasing the biodegradation rate, recombinant secreting organophosphorus hydrolase (OPH) was designed.

Methods: opd gene was amplified using Vector pET-28a (+)-inaVN-opd as a template of opd gene followed by cloning in the pW980 vector (resulted in pWOP) so for the first time by this Methods a designed secretory system was applied in *Bacillus subtilis* WB600 to secret OPH. To investigate the expression site, SDS-PAGE analysis of cells harboring pWOP and supernatant was applied. In order to study the ability of recombinant cell to utilize organophosphorus pesticides, Mineral Salts Medium supplemented with chlorpyrifos was used. Activity was assessed by following decrease in absorbance of Cp by spectrophotometry at OD280.

Results: The results showed the presentation of OPH in extracellular environment, and the ability of recombinant B. subtilis to utilize chlorpyrifos as the sole source of energy, without growth inhibition. Location of OPH was confirmed by comparing activity of supernatant to membrane and cytoplasm fractions.

Conclusion: It can be concluded that designed system can be used efficiently to secret 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: Secretory Protein; Organophosphorus Hydrolase; *Bucillus subtilis*; Chlorpyrifos; Biodegredation