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MATHUS SUANJAN : CLONING AND EXPRESSION OF *mpdB* GENE RESPONSIBLE FOR METHYL PARATHION DEGRADATION USING A VECTOR DESIGNED FOR PROTEIN SECRETION. THESIS ADVISORS : ASST.PROF.JESDAWAN WICHITWECHKARN,Ph.D.,ASST.PROF.BUDSARAPRON NGAMPANYA,Ph.D.,AND RUJIKAN NASANIT,Ph.D. 125 pp.

Methyl parathion (MP) pesticide widely used in many developing countries is highly toxic, imposing threats to human health and environment. The MP-degrading *Burkholderia cepacia* indigenous to Thailand have been reported to produce methyl parathion hydrolase (MPH), an enzyme capable of degrading this insecticide. Its gene, *mpdB*, was previously cloned in *E. coli*, and the expression, purification and characterization of the recombinant MPH were performed. However, because of some difficulties and disadvantages of intracellular enzyme production such as protein insolubility and complicated cell lysis, this research then aims at an attempt to secrete MPH into either periplasmic space or culture medium. This was done by subcloning the *mpdB* gene into *E. coli* using expression vector pHisFlag-1, a vector designed for periplasmic expression. The coding sequence for polyhistidines added to its C-terminus would allow easier purification by metal affinity column. Using microtiter plate MPH assay, the recombinant clone was shown to produce MPH with enzymatic activity for degrading MP to the yellow-colored hydrolytic product, *p*-nitrophenol (PNP). The expressed enzyme was observed as yellow halo around the colony on MP agar plate assay, indicating the secretion of MPH. The recombinant MPH, as a polyhistidine C-terminal fusion protein, were separated from periplasmic fraction and culture medium fractions and purified by cobalt column. The specific activities of these purified proteins are 9.03 and 3.44 U/mg protein, respectively. The purified recombinant MPH was shown to have the molecular weight of about 36 kDa, corresponding to the 35 kDa of MPH and 0.84 kDa of polyhistidine-tag. The zymogram analysis revealed yellow color at the position of the 36-kDa protein band, confirming that the band was MPH.

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