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KULWADEE PHANNACHET : CLONING, EXPRESSION AND PURIFICATION OF A *BACILLUS THURINGIENSIS* PUTATIVE PORE-FORMING FRAGMENT. THESIS ADVISORS : CHANAN AUGSUTHANASOMBAT Ph.D., SAKOL PANYIM Ph. D., GERD KATZENMEIRE Ph.D. 113 p. ISBN 974-589-414-1

The CryIVB δ-endotoxin produced by *Bacillus thuringiensis* subsp. *israelensis* is highly toxic to *Aedes aegypti* mosquito larvae. The toxin effects of the different δ-endotoxins is proposed to be due to the formation of pores in the midgut epithelial cell membrane, leading to the death of the target larvae by starvation and septicemia. The crystal structure of both the CryIAa and CryIIIA toxins reveal a possible apparatus for pore formation of a bundle of seven hydrophobic and amphipathic helices in domain I, which could penetrate the membrane to form a transmembrane pore. This thesis describes cloning, expression and purification of that portion of the CryIVB mosquito-larvicidal toxin gene coding the putative pore-forming (PPF) fragment comprising the predicted helix bundle (α1-α7) and some of domain II. The PPF-encoding sequence was PCR amplified and cloned into the pPinP expression vector yielding the PPF fragment fused at N-terminus with the avidin affinity tag, which is biotinylated in *E.coli* cell. The soluble form of the 47kDa-biotinylated PPF fusion protein, which cross-reacts with CryIVB antibodies and streptavidin, was affinity purified by using the PinPoint Purification System via batch biotin-avidin binding method. After Factor Xa digestion, the 13kDa-biotinylated tag was removed from the PPF fragment. N-terminal sequencing reveals that the N-terminus of the 29kDa PPF fragment corresponds to that of the full length 130kDa CryIVB toxin. In additions, the 18kDa fragment produced by trypsin digestion reveals two internal cleavages occur at near the N-terminus and in the predicted loop region between α5 and α6 of the PPF fragment. Sized exclusion FPLC analysis reveals that the 29kDa PPF fragment is eluded from the column later than the expected elution volume but the 18kDa five-helix bundle can not be eluded out indicating that hydrophobic interaction between the PPF protein and the column matrix may have occurred.