Pajaree Saitonuang 2015: Characterization of Membrane Binding Sites of *Bacillus thuringiensis* Cyt2Aa2 Toxin. Master of Science (Genetics), Major Field: Genetics, Department of Genetics. Thesis Advisor: Mrs. Anchanee Kubera, Ph.D. 71 pages.

Cyt2Aa2 is a cytolytic toxin produced from *Bacillus thuringiensis* subsp. *darmstadiensis*. It is specifically toxic against Dipteran larvae *in vivo*, and also active to several cell types such as erythrocytes**.** The active toxin is proposed to bind to cell membrane, leading to the formation of membrane pore by toxin oligomerization and eventually cell lysis. This study aimed to characterize the role of expected lipid binding residues (I139, S159, L160, S161, A162, D209 and V215) of Cyt2Aa2. Alanine scanning mutants were constructed by site-directed mutagenesis and expressed in *E. coli*. These mutants were expressed as inclusion bodies which were solubilized in 50 mM carbonate buffer pH 10.5. All mutants, except I139A and V215A could retain as activated toxins after proteinase K cleavage. Three mutants, S159A, L160A and S161A showed high hemolytic activity but low toxicity against *A. aegypti*. Membrane interaction assays showed that these mutants bound and formed complexes as oligomer of toxin on rat red blood cells. Substitution by valine at A162 and asparagine at D209 could interfere membrane binding and oligomerization of the toxin. These mutants could not completely break RBC even at high concentration and showed no hemolytic activity. The mutant A162V showed no toxicity against *A. aegypti*, but D209N showed low toxicity against *A. aegypti*. Our data suggested that amino acids on loop between β4 and β5, and β7 of Cyt2Aa2 toxin were involved in membrane binding as well as complex formation. Substitution of amino acids in these regions altered the biological activity. Selectivity of the toxin to certain target cells might be improved by amino acid replacement in these regions.

_____ _____ __ / __ / ___

Student's signature Thesis Advisor's signature