3937592 PYBS/D : MAJOR : BIOPHARMACEUTICAL SCIENCES;
Ph.D. (BIOPHARMACEUTICAL SCIENCES)
KEY WORDS : CHITINASE GENE / *CRY3A* / *BACILLUS THURINGIENSIS* / *BACILLUS CIRCULANS* NO.4.1 / GENE EXPRESSION

MONTHON LERTCANAWANICHAKUL : CO-EXPRESSION OF CHITINASE AND *CRY3A* ENCODING GENES IN *BACILLUS THURINGIENSIS*. THESIS ADVISOR : CHANPEN WIWAT Ph.D., AMARET BHUMIRATANA, Ph.D., SOMSAK PANTUMWATANA, Ph.D. 228 p. ISBN 974-664-978-7

Bacillus thuringiensis subsp. *israelensis* (*B.t.i.*) strain c4Q272 (Cry^-) harboring chitinase gene from *Bacillus circulans* No.4.1, pHYB43 was more structurally stable and produced higher chitinase than *B.t.i.* harboring chitinase gene from *Aeromonas hydrophila*, pHYA2. A chitinase gene was subcloned into a new shuttle vector pBCX, namely pBX43. It was found that *B.t.i.* strain c4Q272 harboring pBX43 produced approximately 3 times higher chitinase than harboring pHYB43 . *E. coli* or *B. subtilis* harboring a full length of a chitinase gene expressed chitinase of molecular mass 66 kDa, whereas, the *B.t.i.* transformants produced a 44 kDa chitinase which might be a processed form of an enzyme precursor. As well, the *cry3A* gene from *B.t. tenebrionis* was subcloned into pHY300PLK, pBCX and pTFP, namely pHY3A, pBX3A and pTP3A. *B.t.i.* harboring pHY3A or pBX3A produced the Cry3A protein at 0.252 mg/ml and 0.312 mg/ml, respectively, but *B.t.i.* harboring pTP3A did not. However, the segregrational stability of pBX3A and pTP3A in *B.t.i.* strain c4Q272 was lower than pHY3A when cultured under non-selective conditions. Thus, the recombinants chitinase-*cry3A* encoding genes were constructed in pHY3A, namely pH3A43, pH3A43(E1) and pH3A43 (E5). *B.t.i.* harboring those plasmids produced chitinase at 40 mU/mg and only *B.t.i.* harboring pH3A43 produced Cry3A protein at 0.053 mg/ml. It was found that pHY3A, pBX3A, pH3A43 and pH3A43(E1) exhibited a higher concentration of *cry3A*-specific mRNA than pTP3A and pH3A43(E5). The *B.t.i.* harboring the *cry3A* gene expressed the Cry3A protein of molecular mass 66 kDa. Both chitinase- and *cry3A* encoding genes were structurally stable in *B.t.i.* The segregrational stability of pHY3A was approximately 30 passages; whereas, pBCX gradually decreased. The plasmids, pTFP, pHYB43, pBX43, pTP3A, pH3A43, pH3A43(E1) and pH3A43(E5), were stably maintained at approximately 2-3 passages under non-selective conditions. It was observed that the *B.t.i.* harboring larger recombinant plasmids produced lower gene products and exhibited shorter segregrational stability than the smaller ones. *B.t.* subsp. *tenebrionis* and *B.t.i.* transformants did not show adverse effects on the beetle larvae, *Zophobus atratus*. However, chitinase could increase toxicity of *B.t.* subsp. *aizawai* against insect larvae. *B.t.* subsp. *aizawai* harboring recombinant chitinase genes (pHYA2 or pHYB43) showed higher insecticidal activity than parental *B.t.* subsp. *aizawai* toward gypsy moth larvae (*Lymantria dispar*). The case of *B.t.i.* harboring *cry1Aa* (pHY1Aa) or chitinase-*cry1Aa* (pH431Aa) did not show significantly different insecticidal activity. This was probably due to the low chitinase or Cry1Aa proteins of the transformant which contained a couple genes in a plasmid. It might be important to improve their expression by further gene manipulation.