Thesis Title	Sequence Analysis and Expression of Chitinase Gene from *Bacillus thuringiensis* subsp *pakistani* in *Bacillus thuringiensis* subsp *israelensis*
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ABSTRACT

A 6.3 kb *Hin*dIII fragment harbouring the chitinase gene (*chi*) from *Bacillus thuringiensis* subsp *pakistani* (*B.t.p.*) was cloned in pGEM7 and the recombinant plasmid was designated as pCHIP1. Subcloning of a 4.1 kb *Hin*dIII/*Cla*I fragment in both orientations resulted in pCHIPH and pCHIPH/R which enabled the cells to produce enzyme. Therefore, the complete *chi* gene was suggested to locate in this fragment, which was subsequently subjected to DNA sequencing. It was found that the *chi* gene consisted of 1,905 nucleotides which encoded for a protein of 635 amino acids with molecular weight 71,092.54 Da. Comparison of sequence data showed that this *chi* gene and its enzyme had the highest score of homology to *B. circulans* chitinase A1 (*chiA*) gene and enzyme (Watanabe *et al.*, 1990) with 71 and 75% at nucleotide and amino acid levels respectively. A *B.t.p. chi* gene was then subcloned into pBC16 to obtain pCHIPH/R-02 and further introduced into *Bacillus thuringiensis* subsp. *israelensis* (*B.t.i.*) strain 4Q2-72 by electroporation. The

kinetic study of chitinase enzyme production during growth in half strength nutrient broth containing 0.1% colloidal chitin (1/2 NBC) showed that *B.t.* 4Q2-72 (pCHIPIII/R-02) and *B.t.p.* at 72 hr of cultivation produced chitinase at 14.822 and 10.091 mU/ml which were 1.8 and 1.3 times respectively, higher than that of *B.t.*4Q2-72 host which produced enzyme at 8.024 mU ml. Chitinase enzyme seemed to be inducible enzyme since its activity increased when media was supplemented with colloidal chitin. It was shown that under uninduced condition (1/2 NB), *B.t.*4Q2-72 (pCHIPIII/R-02) produced chitinase enzyme about 4 mU higher than that of *B.t.*4Q2-72 (5.9 mU/ml compared to 1.867 mU/ml) whereas under induced condition (1/2 NBC) about 5 mU higher activity was observed in *B.t.*4Q2-72 (pCHIPIII/R-02) which produced chitinase at 15.05 mU/ml than that of *B.t.*4Q2-72 which produced chitinase at 9.95 mU/ml. Chitinase enzyme was also characterized by activity detection in renaturing SDS-PAGE. Both *B.t.p.* and *B.t.*4Q2-72 displayed 4 chitinase activity bands and their molecular weight were about 67, 60, 46, and 32 kDa. However, chitinase from cloned gene in *E. coli* displayed only 2 activity bands which have molecular weight about 67 and 60 kDa. No immunohomology among *B. helsenformis*, *B.t.p.*, and *E. coli*DH5α and *B.t.* 4Q2-72 transformants was detected by immunodiffusion. The plasmid pCHIPIII/R-02 seemed to be relatively stably maintained in *B.t.*4Q2-72 host when cells harboring this plasmid were grown in media containing 2 different concentrations of either 15 or 30 μg/ml tetracycline in which 94 and 96 % of cells still maintained its plasmids till 4 weeks. Whereas the stability of plasmid in transformants grown in tetracycline free media was reduced rapidly in the first week to 85% and then slightly decreased to 81-82% throughout the period of growth till 4 weeks. *B.t.*4Q2-72 and its transformant were similary able to germinate and reveling in *Culex quinquefasciatus* and *Aedes aegypti* larval cadavers. But the efficacy seemed to vary depending on cadaver strains. The

effect of chitinase enzyme on killing activity was performed by toxicity assays against *Culex quinquefasciatus* larvae. The washed spore preparations of *B.t.* 4Q2-72 (pCHIPII/R-02) were as toxic as the parental strain with LC_{50} of 1.96 x 10 cells/ml and 1.92 x 10 cells/ml, respectively. The toxicity of chitinase alone against *Culex* larvae was not significantly shown in which chitinase enzyme at 1.495 mU/ml caused 16.67 % mortality and at 4.517 mU/ml caused 20 % mortality. The LC_{50} of spore suspension mixed with chitinase at 0.747 and 1.495 mU/ml was 1.5 and 2.0 fold increased, respectively when compared to spore suspension alone. Whereas toxicity assay of whole cell culture broth of *B.t.* grown in NYSM or *B.t.* (pCHIPII/R-02) grown in NYSM or NYSM plus 0.1% colloidal chitin showed that, the LC_{50} of transformant grown under induced condition was 4.28 cells/ml which was about 10 times more toxic than host and transformant grown in uninduced medium which gave LC_{50}s of 3.62 x 10 and 3.29 x 10 cells/ml, respectively. From all of these results, the enzyme chitinase is suggested to enhance killing activity against mosquito larvae.