Thesis Title Characterization and Expression of the Surface Layer Protein Gene in *Bacillus thuringiensis* subsp. *israelensis* Strain c4Q272

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ABSTRACT

The gene for a surface layer protein from *Bacillus thuringiensis* subsp. *israelensis* strain 4Q2 which had been cloned into *Escherichia coli* DH5α (pAC11) was transferred into *B. t. i.* strain c4Q272, a mutant strain of *B. t. i.* strain 4Q2 which does not produce S - layers.

The recombinant plasmids were constructed using a shuttle plasmid, pBS1672, pACS111 and pACS11, containing a 1.5 kb fragment and 5.2 kb fragment of S - layer protein gene. The recombinant plasmids pACS111 and pACS11 were transformed into *E. coli* DH5α by competent cell technique. Then the recombinant plasmids, pACS111 and pACS11, were transformed into *Bacillus subtilis* MI - 113 and *B. t. i.* strain c4Q272 via electroporation. The expression of the S - layer protein in recipient cells was confirmed by Western blot analysis using an anti - S - layer protein antibody of strain 4Q2. Southern

blot hybridization was used to confirm the presence of the S - layer protein gene in the transformants, using a biotinylated 1.5 kb fragment of the gene as a probe.

The S - layer protein gene was transferred from *B. t. i.* strain e4Q272 to strain 4Q272 via a conjugation - like process by millipore mating technique. *B. t. i.* strain 4Q272 does not produce S - layer whereas strain 4Q2 produces a S - layer. It was found that the frequency of transfer was between 7.97×10^{-3} to 1.23×10^{-3} and 5.95×10^{-1} to 1.89×10^{-5}, respectivelty.

This study it may be concluded that there is no correlation between synthesis of S - layer protein from plasmids pACS111 or pACS11 and penicillin resistance in *B. t. i.*. *B. t. i.* strains 4Q2 and 4Q2 - 16 possessed high levels of penicillin G resistance, with a mean inhibitory concentration, at MIC of 10.0 mg, but strains e4Q272 and 4Q272 were susceptible to as little as 0.010 to 0.019 μg of penicillin G.

Investigation of plasmid stability in transformants and transconjugants showed that the plasmid carrying the 5.2 kb fragment was more stable than the plasmid containing 1.5 kb fragment. The recombinant plasmid pACS11 was more stable in *B. t. i.* over 30 days period than that in *B. subtilis* MI - 113 (stable only in 21 days period).