

Thesis Title: Characterization of Conjugation-like Gene
Transfer Process in *Bacillus thuringiensis*
subspecies *israelensis*

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ABSTRACT

The plasmids pBC16 and pC194 from *Bacillus thuringiensis* subsp. *israelensis* strains A084-16-194 were transferred to 25 subspecies of *B. thuringiensis* by a conjugation-like process using broth mating technique. The frequencies of transfer varied considerably between different mating pairs, ranging from 1.1×10^{-9} to 9.8×10^{-5} . Additionally, chromosomal transfer could also be demonstrated in ten *B. thuringiensis* subspecies with very low frequencies (4.3×10^{-9} to 3.7×10^{-7}). The matings within a group of eight subspecies gave higher frequencies of transfer than the matings between the subspecies indicating that the frequencies of transfer was higher in

the intrasubspecific than the intersubspecific transfers. The results indicated that the capability to transfer plasmids among these various subspecies did not depend on the presence of specific plasmids nor specific plasmid patterns in either donor or recipient cells.

The cell clumping phenomena appeared to be specific between certain pairs of *B. thuringiensis*. Among few strains being tested, wild type strain 4Q2 and its cured strains 4Q272 and c4Q272, clumping could be divided into two groups i.e. clumping group I (4Q2) and clumping group II (4Q272 and c4Q272).

The transfers of pBC16 plasmid from strain A084-16-194 into strain c4Q272 were found to be inhibited by 0.2 mM EDTA, 0.25 M $MgCl_2$, 0.5 M $MgSO_4$ and 0.05 M $CaCl_2$. These chemicals, at the same concentrations, could be demonstrated to reduce (EDTA and $MgCl_2$) or inhibit ($MgSO_4$) the extent of clumping between strain A084-16-194 and c4Q272.

Beside the clumping phenomena, it was also found that strain in clumping group I (4Q2) possessed high level of penicillin G resistance. Strain 4Q2 was resistant to penicillin G at MIC of 12.5 mg, but strains in clumping group II (4Q272 and c4Q272) were quite susceptible to penicillin G with MIC of 0.06 and 0.03 ug, respectively.

Extraction of S-layer protein by treatment with a 6 M urea indicated that there appeared to be extra-high molecular weight protein in the extracts obtained from

B.t.i. strain 4Q2. This protein band was found to be absent in strains 4Q272 and c4Q272. The antibody toward this S-layer protein was prepared and used for locating of S-layer protein on *B.t.i.* cell by using indirect immunofluorescent technique. Immunodiffusion reaction and Western blot analysis confirmed the specificity of the anti-S-layer protein antibody.

It was found that the antibody against 4Q2 S-layer protein inhibited the plasmid transfer via the conjugation-like process between *B.t.i.* strain 4Q2-16 and c4Q272. The frequencies of transfer of pBC16 plasmid was found to reduce from 9.7×10^{-6} to less than 1×10^{-8} in the absence and presence of anti 4Q2-S-layer protein antibody, respectively. This antibody could also found to reduce the frequencies of transfer of pBC16 plasmid via conjugation-like process between *B.t.i.* strain A084-16-194 and c4Q272. The frequencies of transfer between the two latter strains were found to reduce from 2.2×10^{-5} to 1.2×10^{-6} in the absence and presence of the antibody, respectively.

Using antibody detection technique, S-layer protein gene from *B.t.i.* strain 4Q2 was cloned in pBluescriptKS and pUC12 of *Escherichia coli* DH5 α . Three positive clones namely PAC1, PAC2 and PAC3, containing the genes encoding for the S-layer protein were obtained. The PAC1 clone was subsequently subcloned to PAC11, PAC111 and PAC112. The presence of gene product was confirmed by using Western blot analysis.

The 1.5 kb fragment of the pAC111 plasmid was sequenced by using dideoxy chain-termination method. The nucleotide sequence showed the putative initiation codon (ATG), -10, -35 and Shine-Dalgarno sequence.

Southern blot hybridization using 5.2 kb fragment of the pAC11 as biotinylated probe indicated that the S-layer protein gene was located on chromosome of *B.t.i.* strains 4Q2, 4Q272 and c4Q272.