STUDY ON CRYSTAL PROTEIN GENE FROM *BACILLUS THURINGIENSIS*

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M.Sc. (BIOTECHNOLOGY)

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ABSTRACT

The *cry1C* gene was cloned from *Bacillus thuringiensis* subsp. *aizawai* SP41. Its open reading frame (ORF) comprised 3,567 bp encoding 1,189 deduced amino acid residues with a predicted molecular mass of 134.7 kDa. The upstream region from *cry1C* open reading frame presented the DNA sequence corresponding to those of overlapping promoters, BtI and BtII. The translationally fused gene between either *cry1C* or its mutated promoters which included ribosome binding site and part of *cry1C* gene (encoded for four amino acid residues) and truncated β-galactosidase (*lacZ*) gene from 17th codon from its N-terminus, was constructed. The promoter and reporter genes were joined by linker nucleotides which encoded for an additional three amino acid residues. The fusion constructs were introduced into acrystalliferous (Cry') *B. thuringiensis* subsp. *israelensis* strain c4Q2-72 by electroporation. Enzyme activity of the different *cry1C* promoter-*lacZ* fused genes in c4Q2-72 transformants was analysed during 10 and 11 h (T.10 to T.11) before and after onset of sporulation phase, respectively. β-galactosidase activity in pCryC1-*lacZ* (contained active BtI and BtII promoters) was detected at T.0 (onset of sporulation) and activity rapidly increased after T.4 and reached maximum at T.11 (47,764.9 Miller units/mg of protein). At T.11, constructs with mutated BtI (pCryCABtI-*lacZ*) or BtII (pCryCABtII-*lacZ*) produced 3.5 (13,231.3 Miller units/ mg of protein) or 6 fold (8,014.7 Miller units/ mg of protein) lower activity than that of wild type, respectively. From activity monitoring, BtII was expressed about 4 h later than BtI which was expressed after cells entered sporulating stage. Hence, both BtI and BtII promoters were essentially required for efficient expression of *cry1C* gene in *B. thuringiensis*.

KEY WORD: *CRY1C GENE/ BACILLUS THURINGIENSIS/ OVERLAPPING PROMOTERS/ PROMOTER ACTIVITY*

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