Expression and Characterization of Recombinant Cry1Ac Crystal Proteins with Foreign Proteins in *Bacillus thuringiensis* subsp. *kurstaki* Cry⁻B

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Bacillus thuringiensis produces insecticidal parasporal inclusions (crystal protein) used as a major ingredient of most microbial insecticides. Although many B. thuringiensis strains and their crystal proteins have been isolated and characterized, such findings have limitation of usefulness. For enhanced toxicity, fast effects, and the delay of resistance development, research on genetic manipulation of crystal genes and proteins by genetic engineering should be continued. The purpose of this study was to understand the molecular genetics of expression of recombinant crystals in B. thuringiensis, and the final goal was to improve usefulness of both crystal proteins and B. thuringiensis through the expression of the multi-functional recombinant crystals in B. thuringiensis.

As a primary study, expression of the cry1Ac gene under the control of the native or α-amylase gene promoter was investigated in the B. thuringiensis Cry⁻B strain. The cry1Ac gene under the control of either the native promoter (pProAc) or the α-amylase promoter (pAmyAc) was successfully expressed in the Cry⁻B strain. The transformant harboring pProAc, ProAc/CB, expressed the 130 kDa protein starting from 24 h after inoculation, just as was the case for the wild type strain of B. thuringiensis subsp. kurstaki HD-73. The pAmyAc-transformant (AmyAc/CB) began to express the gene just 6 h after inoculation, but the activity of the α-amylase promoter was relatively weaker than that of the native promoter (ProAc/CB).

As the first attempt for production of recombinant crystals, the expression of a recombinant gene comprised of the cry1Ac and GFP genes in the Cry⁻B strain was examined. The GFP gene was inserted into the

XhoI site, located behind the proteolytic cleavage site, in the middle region of the cry1Ac gene (pProAc-GFP). The ProAc-GFP/CB produced mRNA transcripts encoding the cry1Ac-GFP recombinant gene, but did not produce an inclusion body. Although the expression level was relatively low, the transformant did express recombinant protein forms but displayed a heterogeneity of molecular weights. The sporulated cells and spore-crystal mixtures of ProAc-GFP/CB exhibited insecticidal activity against Plutella xylostella larvae. Accordingly, the current results suggest that a recombinant protein including the B. thuringiensis crystal protein can be functionally expressed in B. thuringiensis.

Second, the expression of a fusion protein between Cry1Ac and Cry11A was examined. The cry11A gene was inserted behind the XhoI site in the middle region of the cry1Ac gene, but the C-terminal fragment (structure fragment) of Cry1Ac was removed in fusion construction (pProAcN-11A). ProAcN-11A/CB formed small ovoidal crystals with an approximately 60 kDa protein, unexpectedly. However, ProAcN-11A/CB exhibited significant dual toxicity to larvae of two insect species, P. xylostella and Culex pipiens. The results suggest that the expression and crystallization of a recombinant protein with a toxic fragment of Cry1Ac and Cry11A was incomplete in B. thuringiensis although ProAcN-11A/CB produced parasporal inclusions and had a dual toxicity.

Finally, the expression of a recombinant crystal with Cry1Ac and EGFP was investigated. Unlike the GFP fusion in the middle region of Cry1Ac, the fusion in the N-terminus of Cry1Ac and EGFP (EGFP-Cry1Ac) was attempted under the control of the cry1Ac promoter. ProMu-EGFP/CB produced parasporal inclusions, dissimilar to MuEGFP/CB and ProAc-GFP/CB. This parasporal inclusion was of bipyramidal-shaped crystals in size ranging from 200 to 300 nm. The recombinant protein of ProMu-EGFP/CB was approximately 150 kDa, which was confirmed by the immunoblot analysis. The LC_{50} value of ProMu-EGFP/CB was two fold higher when compared to that of ProAc/CB to P. xylostella larvae. Through the EGFP-Cry1Ac fusion expression, the possibility of successful expression and crystallization between B. thuringiensis crystal protein and a foreign protein was validated.