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Cloning and Expression of *cry1Aa*, *cry1Ab*, *cry1C*, and *cry1Da* Genes from *Bacillus thuringiensis* var. *aizawai*

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Keywords*: Bacillus thuringiensis* subsp. *aizawai;* insecticidal crystal protein (ICP); response surface methodology (RSM).

Four cry genes (*cry1Aa, cry1Ab, cry1C* and *cry1Da*) were cloned separately from a commercial product Xentari (based on *Bacillus thuringiensis* var. *aizawai*) and expressed in an acrystalliferous *B. thuringiensis* (Cry⁻B) respectively. Total protein produced by recombinant strains of *cry1Aa, cry1Ab, cry1C* and *cry1Da* were 640, 1700, 1400 and 1500 μg/mL respectively which caused more than 93% mortality against 3rd-instar larvae of *Plutella xylostella* (Lepidoptera) and 96% mortality against 2nd-instar larvae of *Trichoplusia ni* (Lepidoptera). The LC_{50}, expressed in ppm of the total protein against 2nd-instar larvae of *T. ni* from recombinant strains of *cry1Aa, cry1Ab, cry1C* and *cry1Da* after 72 h incubation, were 24.9, 13.4, 7.3 and 13.2 μg/mL respectively. Response surface methodology (RSM) was applied to find the optimal ratio of protein combination from recombinant strains of *cry1Ab* and *cry1C* against 2nd-instar larvae of *T. ni*. Results showed that the optimal ratio of protein mixture from *cry1Ab* and *cry1C* was 27.8 and 24.6 μg/mL respectively, which gave a mortality of 97.8% against 2nd-instar larvae of *T. ni*.

The conventional method for multifactor experimental designs is time-consuming and cannot detect the true optimum, notably because of interactions among factors. Response surface methodology (RSM) is one of the worthwhile techniques to identify the explanatory variable in the system (3, 4, 6). Generally, RSM can be used to evaluate the relative significance of several factors, optimization of microbiological media culture conditions, and synthesis of metabolites, etc. Insecticides derived from the soil bacterium *Bacillus thuringiensis* are becoming an increasingly important component of ecologically sound pest management. Insecticidal crystal proteins from *B. thuringiensis* are extremely toxic to many pests and have been a primary focus of much recent research (5, 7). In this report we tentatively explore the synergism between recombinants by RSM. RSM was applied to find optimal ratio of protein combination from recombinant strains containing *cry*1Ab and *cry*1C genes (1, 2) against 2nd-instar larvae of *Trichoplusia ni.* Multiple regression analysis was carried out with Statistical 7.0 (Statsoft Inc., Tulsa, Ok, USA).

We have four conclusions as follows: 1) Four *cry* genes (*cry1Aa*, *cry1Ab*, *cry1C* and *cry1Da*) were cloned separately (Fig. 1) from a commercial product Xentari (based on *Bacillus thuringiensis* subp. *aizawai*) and expressed in an acrystalliferous *B. thuringiensis* (*cry*-B) respectively. 2) Total protein produced by recombinant strains of *cry1Aa*, *cry1Ab*, *cry1C* and *cry1Da* were 640, 1700, 1400 and 1500 µg/mL respectively, which caused more than 94% mortality to 3rd-instar larvae of *Plutella xylostella* and 96% mortality to 2nd-instar larvae of *Trichoplusia ni*. 3) The LC_{50} expressed in ppm of the total protein against 2nd-instar larvae of *T. ni* from recombinant strains of *cry1Aa*, *cry1Ab*, *cry1C* and *cry1Da* after 72h incubation were 24.50, 13.39, 8.57 and 12.47 µg/mL respectively. 4) RSM can be applied to find the optimal ratio of protein combination from different recombinant strains against insect-larvae tested (Fig. 2).

In the present study, the RSM is developed to improve the potency of *B. thuringiensis* toxin that may contribute to the success of resistance management. It is expected that RSM can also be employed to broaden the host spectrum of *B. thuringiensis* toxin as long as with optimal combination.

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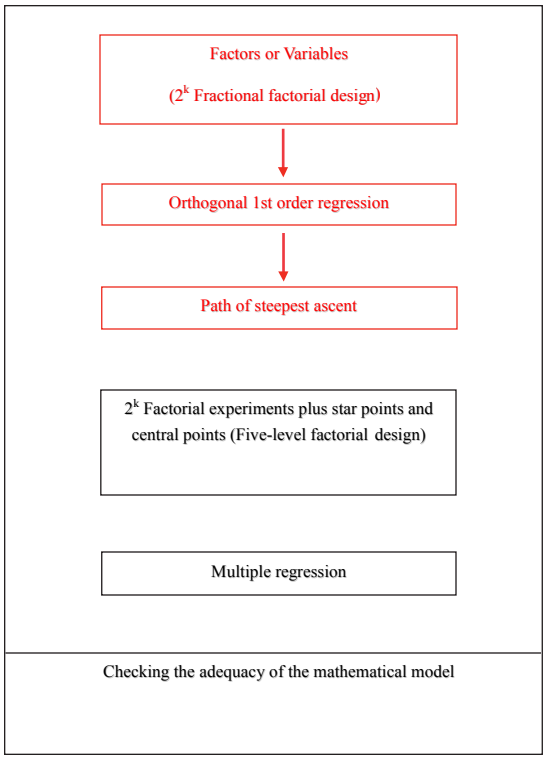

FIG. 2. The systematic diagram of response surface methodology.

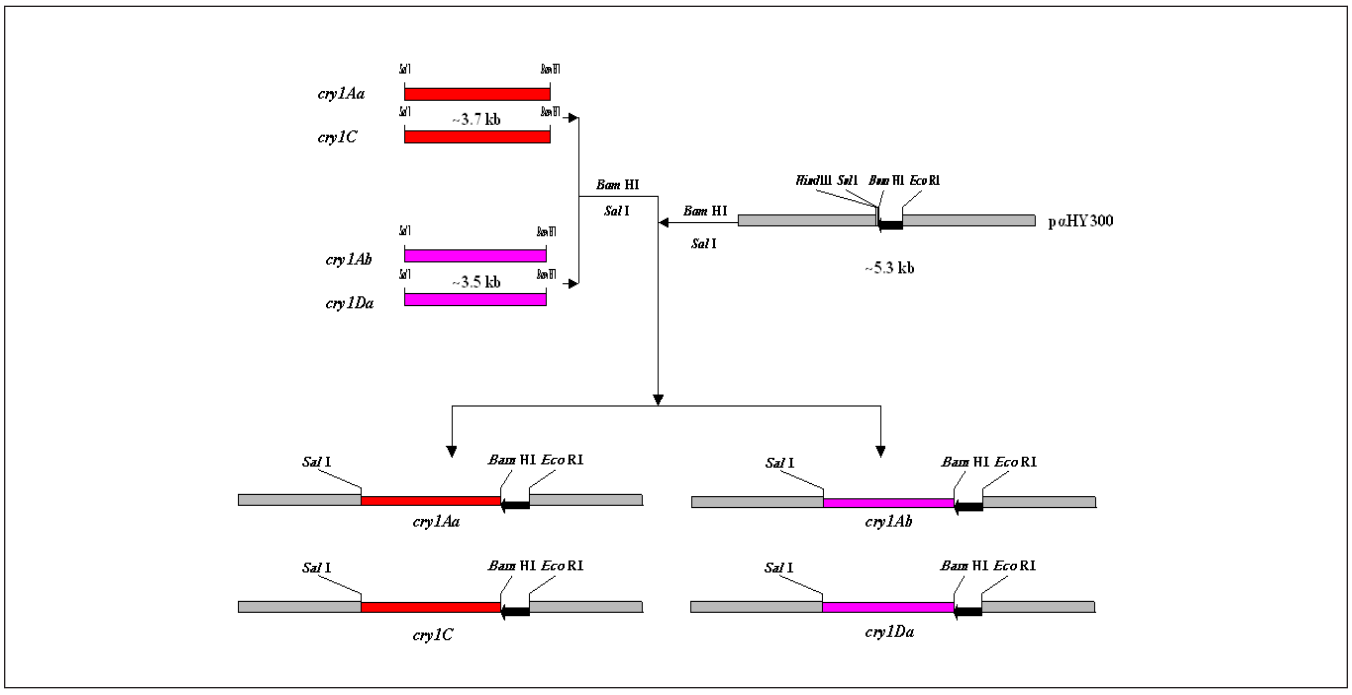

FIG. 1. Construction of *cry1Aa*, *cry1Ab*, *cry1C*, and *cry1Da* genes with shuttle vector pαHY300.