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SIRIPORN SIRISAENGLOET : DETECTION OF δ-ENDOTOXIN GENES FROM LOCAL ISOLATES OF BACILLUS THURINGIENSIS BY POLYMERASE CHAIN REACTION. THESIS ADVISORS: CHANPEN WIWAT Ph.D., AROME PONGPAN M.S., WICHET LEELAMANIT Ph.D. 120 p. ISBN 974-664-068-2

Thirty-seven local isolates of *Bacillus thuringiensis* were collected from soil, water, and insect cadavers from various regions in Thailand. A rapid and accurate method for identification of *cry* genes of *B. thuringiensis* strains was performed by polymerase chain reaction. Four pairs of novel general primers specific to regions of high homology within genes encoding for four major classes of *B. thuringiensis* crystal proteins were designed. General Primers GP1, 5′-TAGGAGAAGCGCTAGCG-3′ (F) and 5′-TCTGCTTCCCATTCCGG-3′ (R); GP2, 5′-TTATTTGCACAGGCAGC-3′ (F) and 5′-ACTAAAGGAACCCCAGA-3′ (R); GP3, 5′-TGATGTTCGGCTATACC-3′ and 5′-ACACTGGGATTGTTCCT-3′; and GP4, 5′-GTATGTCAGGATTCCCA-3′ and 5′-CCAGCACTCCAATTAGA-3′ were used to generate PCR products specific for *cry1*, *cry2*, *cry3*, and *cry4* genes, respectively. Differentiation among each class was made on the basis of the electrophoretic pattern of the PCR products. The general primers gave products of about 433, 770, 620, and 584 bp. for the *cry1*, *cry2*, *cry3*, and *cry4* genes, respectively. Known *B. thuringiensis* strains as well as unidentified local isolates were analyzed by PCR. The results showed seven distinct *cry*-type profiles. Of these, the *cry3* gene profile was the most frequently found. Some isolates possessed unusual PCR products and multiple insecticidal crystal protein genes. A unique combination of *cry* genes in a single isolate was also found. These results indicated the presence of strains that may harbor novel *cry* genes as well as strains with new combinations of *cry* genes. The DNA templates prepared by the QIAGEN Genomic-tip system, alkaline lysis method, and direct colony method gave the same results in PCR, indicating that all methods were suitable for PCR analysis.Confirmation of the presence of the cry-type genes was performed by Southern blot hybridization using *cry* genes as probes. The results corresponded with the results of PCR screening. In addition, scanning electron microscopy also confirmed the presence of the crystal protein of *B. thuringiensis*.