

## Occurrence of *Bacillus thuringiensis* Producing Parasporin, Cancer Cell-Killing Cry Proteins, in Vietnam

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A total of 63 *Bacillus thuringiensis* isolates were obtained from urban soils in Vietnam. Of these, 34 were allocated to 12 H serogroups. None of the isolates showed larvicidal activities against three lepidopterans. Only three isolates killed larvae of the mosquito, *Aedes aegypti*. Noninsecticidal crystal proteins of the four isolates showed cytotoxic activities against HeLa cells. Hemolytic activities were associated with parasporal proteins of the three mosquitocidal isolates, but not with those of the four parasporin-producing isolates. These cancer cell-killing proteins proved closely related to parasporin-1 (Cry31Aa), not only immunologically but also phylogenetically.

It has been generally accepted that the majority of *Bacillus thuringiensis* populations in natural environments of Japan produce parasporal inclusions with no insecticidal activities. Interestingly, recent investigators have provided evidence that parasporal inclusion proteins of certain noninsecticidal *B. thuringiensis* isolates occasionally exhibit *in vitro* cytotoxic activities preferential for human cancer cells (6). Proteins with this unique function are now categorized into a group designated parasporin (7). Currently, parasporins are classified into four families (3). Interestingly these four parasporins have all been isolated from *B. thuringiensis* isolates obtained from natural environments in Japan.

Previous investigators have reported that natural environments of tropics and subtropics of Southeast Asia are a good reservoir of *B. thuringiensis* populations with a great diversity of serological and biological characteristics (1). This study examines Vietnamese soils for the presence of *B. thuringiensis*, and reports on the occurrence of parasporin-producing organisms in this region.

Isolation of *B. thuringiensis* was done according to the method of Ohba and Aizawa (8). *B. thuringiensis* was

recovered from all of the seven soil-sampling sites in urban areas of the city of Hanoi. The frequency of *B. thuringiensis* colonies was 1.6% among 4031 colonies of the *B. cereus* group. H serotyping of *B. thuringiensis* isolates was done with the reference H antisera against H1-55. At least 12 H serogroups were detected in *B. thuringiensis* soil populations of the Vietnam. Of the 63 isolates under study, 34 (54%) were sero-positive for the reference antisera, five were untypable, and 24 were untestable.

*B. thuringiensis* isolates were examined for larvicidal activities against four insect species: *Bombyx mori*, *Plutella xylostella*, *Spodoptera litura*, and *Aedes aegypti*. Qualitative one-dose assays were done with spore/parasporal inclusion mixtures as described previously (5). None of 63 isolates exhibited larvicidal activities against the three lepidopterans. Only three isolates, one of which belonged to serovar *colmeri* (H21), and the other two to serovar *konkukian* (H34), showed mosquito larvicidal activities against *A. aegypti*. Proteinase K-digested parasporal inclusion proteins of the three mosquitocidal isolates exhibited strong cytotoxic activities against sheep erythrocytes.

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We then examined proteinase K-digested parasporal proteins of the 14 selected isolates for *in vitro* cytotoxic activities against the human uterus cervix cancer cell, HeLa. These isolates produced huge spherical parasporal inclusions with no insecticidal activities. The tests were done with a one-dose assay technique according to the method of Mizuki et al. (6). Marked cytopathological changes, characterized by cell rounding and ballooning, were induced in HeLa cells by the proteins from four isolates, while no anti-cancer-cell activities were associated with those from ten other isolates. No hemolytic activities were associated with cancer cell-killing proteins of the four isolates.

When tested with Ouchterlony double immunodiffusion, cancer cell-killing proteins of the four isolates formed single immunoprecipitin lines against parasporin-1 antibodies, but not against antibodies of the three other existing families, parasporin-2, 3, and 4. In PCR experiments with *cry31Aa* (*parasporin-1*)-specific primers, 1-kb DNA fragments were obtained from all four isolates. Nucleotide sequencing revealed that these DNA fragments were >99% homologous to the corresponding region in *cry31Aa*.

Our results clearly show that the parasporal inclusion proteins of the four *B. thuringiensis* isolates from Vietnam belong to the parasporin-1 family (7). This is supported by the following facts: (i) the proteins have *in vitro* cancer cell-killing activities, (ii) they are immunologically closely related to parasporin-1, and (iii) homology >99% is revealed in partial DNA sequences between *parasporin-1* (*cry31Aa*) and genes encoding the proteins of the four isolates. It should be noted that the reference strains of cancer cell-killing *B. thuringiensis* were all from natural environments in Japan (2-4, 9). Our findings strongly suggest that the parasporin producers are widely distributed, not only in Japan but also in other regions of Asia.

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