THE ISOLATION OF POLYHEDROSIS-LIKE VIRUS FROM *BACILLUS THURINGIENSIS*

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SUMMARY: Investigations have been undertaken in order to detect the existence of phages in three Bacillus thuringiensis strains; aizawai HD-134, galleriae HD-234, and kurstaki HD-341; their relations to the bacterial virulence and their relations to polyhedrosis virus. The results obtained showed that each bacterial strain contains different frequencies of phage particles which appeared as number of plaques following UV lysis induction. Bacillus thuringiensis can serve as an intermediate host for polyhedrosis virus. Its existence in the temperate or vegetative state in the lysogenic bacteria increased the efficiency of Bacillus thuringiensis in pest control.

Key Words: Isolation-polyhedrosis virus, bacillus thuringiensis.

INTRODUCTION

Pathologists are interested in defining mechanisms of pathogenicity among diverse bacterial pathogens. The critical question of what in the genome of the pathogen led to the fact that pathogenesis has been largely left unstudied (9).

The instability in the virulence of many pathogens suggested two hypotheses:

a- Repression or induction of the pathogen enzymes.

b- Lysogenic or phage conversion.

Lysogenic or phage conversion which causes phagen virulence was reported by Freeman (6) and Freeman and Morse (7) in *Coryne-bacterium diphtheria*.

Co-existence of a phage with its host bacterium within the lesion of diseased plants promises possibilities of obtaining bacterial cultures that carry phages, as stated by Kawamura (8), in *Pseudomonas solanacearum* phage. But, plant pathogens in nature are not always present together with their phages since they often consist of strains that lack the ability to produce phages.

Ali *et al.* (1) had found that high frequencies of lysogenic *Pseudomonas solanacearum* cells are parallel with their virulence. They concluded that pathogenicity might be due to lysogenic conversion with interaction between bacteriophage and enzymes activities and not to enzymes induction or repression.

Ali *et al.* (2) had found that the highly pathogenic *Erwinia carotovora* strains were highly lysogenic and those which were unstable lysogenic or lyse were avirulent.

The purpose of the present study is to detect the existence of phages in three *Bacillus thuringiensis* strains, their relations to the bacterial virulence and their relations to polyhedrosis virus. It also aimed to prove the possibility of *Bacillus thuringiensis* being and intermediate host for polyhedrosis virus.

MATERIALS AND METHODS

Strains

a. Three *Bacillus thuringiensis* strains; *B.t.* var *aizawai* (HD-134), *B.t.* var. *galleriae* (HD-234), and *B.t.* var. *kurstaki* (HD-341), were used throughout this investigation.

b. Polyhedrosis virus prepared from diseased cotton leaf worm *Spodoptera littoralis*.

Media

a. H-broth top layer and bottom layer media for the detection of bacteriophages (4) were used.

b. Fodder yeast medium (5).

All media were sterilized at 120°C and 1.5 atmosphere.

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Lysis induction and phage preparation

The three *Bacillus thuringiensis* varieties were grown in H broth for 18 hr at 30°C on a shaker. Cell suspensions were (4) exposed to UV lamp (20 W) at a distance of 15 cm in open petri dishes for 30 min. Samples of 0.1 ml were added to top layer tubes of Edgar after melting and cooling to 45°C. The tubes were mixed vigorously and each was poured to a bottom layer plate of Edgar. The plates were incubated at 30°C for three days. The plaques were counted and their viruses were harvested in 0.1% ammonium acetate, mixed with chloroform and centrifuged. Phage preparations were isolated and assayed for pest control.

Insect rearing and assay

A standard colony of *Spodoptera littoralis* has been maintained in the laboratory at 27±2°C on a semi-artificial diet (10). Assay of the pathogen preparations was carried out according to Dulmage *et al.* (3)

RESULTS AND DISCUSSION

The mean number of plaques obtained following lysis induction with UV of the three *Bacillus thuringiensis* strains were determined and correlated with the efficiency of each strain against *Spodoptera littoralis* second instar larvae.

Table 1 gives the mean numbers of plaques/ml following lysis induction with UV for 30 min for each *Bacillus thuringiensis* strain and the relationship between the number of plaques and potency of each strain against *Spdoptera littoralis*.

Table 1: Mean numbers of plaques/ml following lysis induction with UV for 30 min for each *Bacillus thuringiensis* strain and the percentages of *Spodoptera littoralis* larval mortality.

Strain	Mean numer of plaxues/ml	% Mortality
B.t. var aizawai (HD-134) original	230	70
UV resistant 10% NaCl resistant	120	80
-Colony No. 1	75	40
-Colony No. 2	50	10
B.t. var. galleriae (HD-234) original	40	15
UV resistant	50	20
10% NaCl resistant	40	20
B.t. var. kurstaki (HD-341) original	700	65
UV resistant	300	90
UV, 10% NaCl resistant	430	80
10% NaCl resistant	500	35

Results in Table 1 proved that each strain contains different frequencies of phage particles which appeared as number of plaques following UV lysis induction. Although the high frequencies of plaque formation might correlate with the mortality percentages of second larval instar of *S. littoralis*, higher mortality percentages might appear following the selection of UV resistant cells which might be lysogenic and resistant to lysis induction. On the other hand, NaCl resistant strains, i.e., tolerant to high temperatures, which showed lower mortality with high frequency of plaques might be due to its instability and the existence of lower frequencies of the lysogenic cells.

The virus of each strain was harvested in 0.1% ammonium acetate, mixed with chloroform and centrifuged. Phage preparations were isolated and assayed for their activity against *S. littoralis*. They gave mortality that ranged between 20 and 25% with exact symptoms of polyhedrosis virus. These results indicate that *Bacillus thuringiensis* can be intermediate host for polyhedrosis virus. Its existence in the temperate or vegetative state in the lysogenic bacteria increased the efficiency of *Bacillus thuringiensis* against the target insect.

The isolation of virus free *B. thuringiensis* var. kurstaki (HD-341) cells

The *Bacillus thuringiensis* var. kurstaki (HD-341) was grown in fodder yeast medium (5) on a shaker for three days at 30°C. The bacterial cells were exposed to UV lamp (20 W) at a distance of 15 cm in open petri-dishes for 30 min. Samples were tested for lysis Edgar's method (4).

The plates were incubated at 30°C for three days and scored for plaques. Cells far from any plaques were isolated. They were grown in fodder yeast medium for another three days on a shaker at 30°C and rechecked, using the same method, for the developing of any new plaques.

No plaques were obtained indicating that the cells are virus free.

***B. thuringiensis* infection with polyhedrosis virus**

B. thuringiensis cells, grown in fodder yeast medium for three days on a shaker at 30°C, were mixed well with polyhedrosis virus preparation and 0.3% CaCl was added to the mixture. The mixture was then freezed for half an hour followed by incubation at 30°C for an hour.

Samples were tested for the existence of virus using Edgar's method (4) and UV exposure for 30 min.

Large number of plaques appeared after the plates incubation at 30°C for three days indicating polyhedrosis virus infection of the bacterial cells.

The plaques were harvested in 0.1% ammonium acetate, mixed well with chloroform and centrifuged at 4000 rpm for 20 min. The virus preparation was tested

for its activity against the second larval instar of *Spodoptera littoralis*. It gave 30% mortality with exact symptoms of polyhedrosis virus.

Moreover, when virus preparations were used to infect *B. thuringiensis* var. *galleriae* strain HD-234, the isolated lysogenic strains showed 35% mortality more than the strain HD-234 without virus.

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