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Research Note

Toxicity of different isolates of *Bacillus thuringiensis* Berliner to the larvae of diamondback moth, *Plutella xylostella* (Linnaeus)

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ABSTRACT: ***Bacillus thuringiensis*** **Berliner (Bt) has been successfully employed in pest management programmes both in agriculture and public health. Efficient isolation methods and intensive screening have yielded novel isolates of *Bt*, more potent than the available ones. In the present study, LC_{50} values for 18 isolates of *Bt* obtained from different sources, *viz.* soil, leaf, seed dust and insect cadavers were determined on five-day-old larvae of *P. xylostella*. Among the 18 isolates (five, two and one from soil, insect cadavers and seed dust, respectively), were more toxic to the larvae of *P. xylostella*. The relative toxicity values for the above isolates ranged from 1.0 to 5.5 times as compared to the International standard, HD-1-S-1980. The more potent isolates could be used in developing sprayable formulations and also a source of novel crystal toxin genes.**

KEY WORDS: *Bacillus thuringiensis*, bioassay studies, more toxic isolates

The crystal toxins (Cry toxins) produced by *Bacillus thuringiensis* (*Bt*) are successfully employed in pest management programmes, both in agriculture and public health (Crickmore *et al.*, 1998). Development of efficient methods of isolation of *Bt* and intensive screening programme has resulted in the isolation of more potent strains for e.g. HD-1 and NRD-12 (Beegle and Yamamoto, 1992). *Bt* formulations based mainly on the subspecies *kurstaki* were used for the successful management of many lepidopterans including the diamondback moth, *Plutella xylostella* (Linnaeus). However, intensive application of *Bt* formulations has resulted in the development of resistance in the field populations of *P. xylostella* (Tabashnik *et al.*, 1990). Further advances in molecular techniques have stirred a renewed interest on *Bt* for the isolation of more potent crystal protein genes (*Cry* genes), both from the characterized and uncharacterized isolates. In this scenario, there is a need to look for more potent isolates of *Bt* active on different agriculturally important pests including *P. xylostella* and to work out their LC_{50} values.

Larvae of *P. xylostella* were collected from cabbage crop, with no previous application of any *Bt* formulations, from the experimental farm of Indian Institute of Horticultural Research, Bangalore, India

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Table 1. List of *Bt* isolates toxic to *P. xylostella*

Habitat	*Bt* isolates
Soil	BDR-3, RCR-1, RCR-6, BRY-4, TMR-10, BLRF-2, MDA-5, MYR-5, HSN-3, CRGC-2, CMLR-8, CMLR-9, KWR-2
Seed dust	DVu-1, DLa-1
Insect cadaver	KPx-1, IPd-1

and the stock culture was maintained at the Department of Entomology, University of Agricultural Sciences, GKVK, Bangalore, on potted cabbage plants in wooden cages (45 x 45 x 45 cm). Culturing was carried out at temperatures ranging from 25 to 32°C and relative humidity of 72 to 90% and five-day-old larvae were used for bioassay studies. In a preliminary study, out of 33 isolates, 18 isolates were found to be toxic to the five-day-old larvae of *P. xylostella* (Table 1). The International standard for *Bt* subspecies *kurstaki*, HD-1-S-1980 was obtained from Institute Pasteur, France, for the purpose of determining the relative toxicity of the above isolates. The various *Bt* isolates and HD-1-S-1980 were cultured on nutrient agar and incubated at 30°C for 72 hours for complete lysis. Colonies were scraped using sterile plastic cell scraper and homogenized in 50 ml sterile distilled water containing Triton X-100 (0.01%). The spore-crystal mixture was washed twice with 0.5 M NaCl and sterile distilled water containing 1mM phenyl methyl sulfonyl fluoride (PMSF) at 9000 *g* for 10 minutes at 4°C and stored at -20°C until further use.

Bioassay was carried out using the spore-crystal mixture from the 18 *Bt* isolates and HD-1-S-1980. The spore-crystal mixture was solubilized in 2 N NaOH and incubated at 37°C for one hour and centrifuged at 9000 *g* for 10 minutes at 4°C. An aliquot of the supernatant was used for protein estimation (Lowry *et al.*, 1951). Serial dilutions were made using sterile distilled water containing Triton X-100 (0.01%) and applied on adaxial and abaxial surface of the cabbage leaf discs (66 cm^2) @ 100 μl/side. A single leaf disc was placed in Petri plates (10 x 10 cm) and ten numbers of five-day-old larvae of *P. xylostella* were released in Petri plates. Three replications for each treatment and also an untreated control (only sterile water) were maintained. Mortality of the treated larvae was observed at 24 hours interval for 72 hours. The data on mortality were subjected to dose-mortality analysis (LC_{50}) using Abbott's formula (Abbott, 1925) and expressed as ng/cm^2 of leaf. The LC_{50} value calculated for each local isolate of *Bt* was divided by the LC_{50} value obtained for the International standard, HD-1-S-1980 in order to arrive at the relative toxicity. The type of insecticidal crystals produced by the above 18 local isolates of *Bt* were observed by transmission electron microscopy (TEM) to attribute possible role in contributing to the variation in toxicity. For carrying out TEM, spore-crystal mixture of each isolate was applied to the farmvar coated copper grids (300 mesh), stained with uranyl acetate (2%) and vacuum dried for five hours. The samples were observed in a transmission electron microscope (Joel, Japan) at an accelerating voltage of 80 kVA.

Bioassay studies showed that eight out of the 18 isolates of *Bt* were more toxic to the larvae of *P. xylostella* than HD-1-S-1980. Of the above eight isolates, six isolates that were obtained from soil, *viz.* BDR-3, RCR-1, RCR-6, BRY-4, CRGC-2 and KWR-2, had a relative toxicity value of 1.2, 3.2, 1.2, 2.0, 1.0 and 2.0, respectively. The two *Bt* isolates that were obtained from the insect cadaver, *viz.* KPx-1 and IPd-1, had higher level of toxicity (5.5 and 3.0) than HD-1-S-1980. In terms of LC_{50}, lowest value of 0.37 ng/cm^2 was obtained for KPx-1 and the highest value of 7.76 ng/cm^2 was obtained for TMR-10, which was isolated from the soil (Table 2). There was no mortality in the untreated control.

Table 2. Bioassay of *Bt* isolates against five-day-old larvae of *P. xylostella*

Bt isolate	LC_{50} (ng/cm^2)	Fiducial limit		Relative toxicity as compared to HD-1-S-1980
		Lower	Upper	
BDR-3	1.71	1.65	1.77	1.18
RCR-1	0.62	0.57	0.68	3.24
RCR-6	1.65	1.59	1.71	1.22
BRY-4	1.00	0.94	1.05	2.01
TMR-10	7.76	7.62	7.89	0.26
BLRF-2	4.24	4.12	4.36	0.47
MDA-5	2.96	2.85	3.07	0.68
MYR-5	2.41	2.35	2.47	0.83
HSN-3	2.80	2.70	2.90	0.91
DWD-1	2.81	2.74	2.88	0.72
CRGC-2	1.95	1.89	2.01	1.03
CMLR-8	3.16	3.05	3.27	0.64
CMLR-9	2.16	2.05	2.27	0.93
KWR-2	0.99	0.93	1.05	2.03
KPx-1	0.37	0.31	0.43	5.51
lPd-1	0.68	0.62	0.74	2.96
DVu-1	3.16	3.06	3.27	0.64
DLa-1	3.64	3.60	3.70	0.55
HD-1-S-1980	2.01	1.89	2.13	-

The transmission electron microscopic studies showed that all the 18 isolates of *Bt* tested in the present investigation produced bipyramidal crystals. Generally the lepidopteran active isolates of *Bt* produce bipyramidal crystals with some exceptions of spherical crystals produced (Wasano *et al.*, 2000). Even though all the 18 isolates of *Bt* produced bipyramidal crystals, the variation in their toxicity could be attributed to the composition of different crystal toxins in each of the above isolates, e.g., different isolates of *Bt* subspecies *kurstaki* produce varying levels of Cry1 Aa, Cry1 Ab, Cry1Ac and Cry2A that are found in the bipyramidal crystals. Even some isolates of *Bt* subspecies *kurstaki* produce only one type of crystal proteins and also form bipyramidal crystals. Bravo *et al.* (1998) showed a direct correlation between the crystal protein content of the Mexican isolates of *Bt* and their activity against *Spodoptera exempta* (Walker). Similarly, Travers *et al.* (1987) reported that the lepidopteran active *Bt* isolates, *viz.* HD-1 and NRD-12, produce bipyramidal crystals but differed in contents of *Cry*1Aa2 and *Cry*1Ab1 genes and hence their differential toxicity to *Choristoneura fumiferana* Clemens. It is also likely that new *Cry* genes could be present in some of the 18 isolates contributing to the differential toxicity. Further work on molecular characterization would throw more light on the types of crystal protein genes present in the above isolates. The more

potent isolates, *viz.*, KPx-1 and IPd-1, could be used for the management of *P. xylostella*.

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