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Isolation and Characterization of *Bacillus thuringiensis* Strains from the Ecosystem of Central India

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The importance of *Bacillus thuringiensis* (*Bt*) in crop pest management is now well established. Acting as an environmentally safe manner, *Bt* is an alternative to many chemical insecticides and for the control of chemically resistant insect pests. *Bt* is ubiquitous soil bacteria can be isolated readily from soil, insects, stored product material, sericulture environment, the phylloplane etc. The aim of this study was the isolation of *Bt* from agroecology of central India. Physiological, morphological and biochemical characters were studied for isolation and identification of different Bacillus thuringiensis strains in terms of their protein toxicity to the crop pests.

Key words: *Bacillus thuringiensis* (Bt), Isolation, Ecosystem.

There have been many efforts by various researchers and companies to isolate new, more virulent strains of *Bt* with increased toxicity to target insect pest. It acts as an environmentally safe alternative as compared to chemical insecticides and also for management of insecticide resistant insect pests. *Bt* is ubiquitous soil bacteria. *Bt* can be isolated readily from the soil, insects, stored product material, sericulture environments, the phylloplane etc. (Donovan *et. al*., 1988). New strains have been characterized by unusual crystal morphology or protein profile, reactivity with crystal antisera and insecticidal activity. (Chilcott *et.al*., 1994)

Initially, *Bt* was isolated from a flour moth collected in the German Province of thuringien and described by Berliner in 1915. It is Gram +ve, aerobic, endospore forming, bacterium crystalliferous belonging to morphological Group I along with *B. cereus, B. anthrasis* and *B. laterosporus* (Parry *et. al.,* 1983). It is recognized by its parasporal body known as crystal having toxic nature against wide range of the insect pests belonging to Lepidoptera, Coleoptera and Diptera (Hofte and Whitely, 1989).

Classified into different serotypes based on their antigens and also on crystal protein serology, these are either based on difference in pathogenicity or difference in biochemical reaction. The Novel strain of *Bt* can be identified on the basis of toxicity to crop pests. In the present study, *Bt* was isolated from soils and characterize by various culture and biochemical characters. The best isolate was screened by antibiotic sensitivity test and finally confirmed by insect bioassay.

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MATERIAL AND METHODS

Chemicals and media materials used for present study were obtained from Himedia Ltd. as well as from Qualigen chemicals.

Locations

Soil samples were collected from 10 different locations from Vidarbh region; those are Akola (I), Yeotmal (II), Buldhana (III), Khamgaon (IV), Amravati (V), Malkapur (VI), Chandrapur (VII), Washim (IX) and Balapur (X). These isolates were further subjected to various tests in comparison with standard isolate *Bt k* HD-I, which was obtained from Dr. Donald Dean, Ohio State University, US.

Isolation of *Bacillus* spp.

Bt was isolated by serial dilution with heat treatment at 80°C for 18 Min. on standard Luria agar. These isolates were maintained on Luria slants (Travers *et. al*., 1987).

Identification

Identification of *Bt* was done by morphological character by Gram staining and cultural characteristic by observing typical colonies on Luria agar.

Characterization

Bt characterization was done by biochemical character and insect bioassay. Biochemical characters comprises IMVIC test, Sugar fermentation by using Glucose, Lactose, Arabinose and Sucrose with acid and gas production. Enzymes like Catalase, Oxidase, Urease, Gelatinase, Amylase, Caesinase, Lecithinase, Deaminase, Cellulase, Lipase, β-Galactosidase, Nitrate reeducates were studied. The results obtained of these biochemical tests were matched with standard results from Bergy's manual. Antibiotic sensitivity test was performed by using Octadisc OD-040.

Crude protein were separated by Acetone preparation method and powder was stored at 4°C (Dulmage, 1970). The protein estimation was done by Bradford method (Bradford 1976). Crude protein content was not found matching with toxicity while protein content had direct relation with the toxicity. The insect bioassays were performed on *Helicoverpa armigera* a major polyphagous pest infesting all major crop pests in India. Relative toxicity of *Bt* was calculated by taking mortality data.

RESULT AND DISCUSSION

In present study, organism was isolated from soils of Vidarbha region. These isolates were compared with standard *Bt* K HD-I. The isolate showed identical colony characters as that of standard except few morphological variations as mentioned in Table 1.

The Biochemical character has importance in confirmation of *Bt*. These isolates showed similar results and were in line with Bergy's manual. Results are summarized in Table 2.

The *Bt* isolates were subjected to antibiotics sensitivity test using octadisc OD-040. The sensitivity was recorded on the basis of diameter of the clear zone of inhibition. The data

Table 1. Cultural colony characteristic of isolates along with standard strain HD-1

Isolates	Shape	Surface	Color	Edge	Elevation	Opacity	Gram character
Bt K HD-1	circular	Rough	Off white	Irregular	Flat	Opaque	Gm + ve
I	circular	Rough	white	Irregular	Flat	Opaque	Gm + ve
II	circular	Rough	white	Regular	Flat	Opaque	Gm + ve
III	circular	Rough	Off white	Irregular	Flat	Opaque	Gm + ve
IV	circular	Rough	Off white	Irregular	Flat		Gm + ve
V	circular	Rough	Off white	Irregular	Flat	Opaque	Gm + ve
VI	circular	Rough	white	Regular	Flat	Opaque	Gm + ve
VII	circular	Rough	Off white	Regular	Flat	Opaque	Gm + ve
VIII	circular	Rough	Off white	Irregular	Flat	Opaque	Gm + ve
IX	circular	Rough	Off white	Irregular	Flat	Opaque	Gm + ve
X	circular	Rough	white	Irregular	Flat	Opaque	Gm + ve

recorded is given in Table 3. It is clearly seen that these isolates were very much sensitive to ciprofloxacin, cephalexin. The test isolates showed either no or poor sensitivity against trimethoprim and ampicillin.The isolates showed moderate sensitivity against rest of antibiotic.

Table 2. Biochemical characteristic of isolate (n=10) with standard strain *Bt k* HD-1

Characteristics	*Bt K* HD-1	*Bt* isolates(n=10)
IMViC		
Indole production	+	+
Methyl Red	+	+
Vogus-proskauer	-	-
Citrate utilization	-	-
Sugar Fermentation		
Glucose	A/-	A/-
Lactose	-/-	-/-
Mannitol	-/-	-/-
Arabinose	-/-	-/-
Sucrose	A/-	A/-
Enzyme study		
Catalase	+	+
Oxidase	+	+
Urease	+	+
Gelatinase	+	+
Amylase	-	-/+
Caesinase	-	-
Lecithinase	+	+
Deaminase	+	+
Cellulase	-	-
Lipase	-	-
β-Galactosidase	-	-
Nitrate Reductase	-	-

A/- : Acid +ve /no gas, -/- : No Acid/No Gas.

Table 3. Antibiotic sensitivity test of isolates

Antibiotic	Bt k HD-1	Bt isolates (n=10)
Ampicillin	+	+
Nitrofurantion	++	++
Augmentin	++	++
Ciprofloxacin	++++	++++
Nalidixic acid	++	++
Trimethoprim	-	-
Cephalexin	++++	++++
Gentamycin	+++	++

- : No sensitivity; +: poor; ++: good; +++: moderate; ++++: High sensitivity

A range of antibody techniques has been used to distinguish *Bt* strains containing protein having different insecticidal activities. Antibodies prepared against solubilized crystal proteins showed that most crystals contained common antigenic determinants (Krywienczyk and Angus, 1967).

All the *Bt* isolates were white colored except pinkish ting in V and IX. Protein content of isolates estimated by Bradford method was in the range of 2.145 to 3.635 µg/mg of acetone powder. The isolate has got the highest protein amongst others as well as compared to HD-1.

All the above approaches can contribute to the characterization of new *Bt* strains. However, screening for insecticidal activity is still the confirmative method to identify strains with increased activity against particular pest like *H. armigera,* which found to cause crop losses in India (Kranthi, *et. al.,* 2000). As the locally isolated *Bt* showed toxicity against *H.armigera* it can be very well integrated for the management of dreaded lepidepteran pest of India especially *H.armigera*. Insect bioassays were conducted against *H.armigera* to see the toxicity and were compared with standard HD-1. The results revealed that all the ten isolates showed toxicity and was compared with standard HD-1.

Table 4. Protein estimation of isolates by Bradford method and Dose (0.1 mg Acetone powder /ml) mortality response of *H.armigera*

Isolates	Protein in µg/mg of Acetone powder	Percent mortality in 0.1mg A.P./ml
Bt K HD-1	3.36	90%
I	3.63	50%
II	2.14	30%
III	3.23	55%
IV	3.03	45%
V	2.62	55%
VI	3.04	65%
VII	3.29	40%
VIII	3.11	80%
IX	2.73	35%
X	2.27	60%

A.P.: Acetone Powder

The results also revealed that all the ten isolates showed toxicity against *H.armigera*, but were less effective than HD-1. However among the ten isolate tested no. VI and VIII were found most effective which confirm insecticidal activity of these isolates. In present investigation, as regards to cultural and biochemical characters, the test isolates were more or less similar to the reaction given by standard isolate HD-1. The test isolates VIII has been identified as a promising biocide against *H. armigera*. Further studies on their toxins need to be undertaken.

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