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# Selection, Characterization and Potency Determination of a *Bacillus thuringiensis kurstaki* Isolate Toxic to Major Lepidopteran Pests

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**KEYWORDS**

*Bacillus thuringiensis*

Potency

*Spodoptera litura*

*Helicoverpa armigera*

*Plutella xylostella*

*Achaea janata*

**ABSTRACT** Screening 50 isolates of *Bacillus thuringiensis* (*Bt*) *kurstaki* from soil samples of Rajasthan and Telangana states, India led to identification of 10 isolates promising against the polyphagous pests *Spodoptera litura* (Fabricius) and *Helicoverpa armigera* (Hübner). Rep-PCR revealed distinctness of one isolate DOR *Bt*-127 with cry1Ac, 1Ae, 1C, 1E, 2Aa, 2Ab genes coding for toxins effective against a wide range of insect pests. Spore-crystal mixture of *Bt*-127 had a high spore count of $9 \times 10^{22}$/g and delta endotoxin content 18.28%, possessed bipyramidal crystals (0.64–2.12 µm) and ellipsoidal spores (1.92–2.2 µm). SDS-PAGE resulted in a 62 kDa band corresponding to the Cry1 toxin. Potencies against the less susceptible pests *S. litura* and *S. exigua* (Hübner) were 71309 and 46205 SU/mg, respectively while potencies against the susceptible pests *H. armigera*, *Plutella xylostella* (Linnaeus) and *Achaea janata* (Linnaeus) were 35844, 26571 and 51667 IU/mg, respectively. DOR *Bt*-127 holds great promise for management of these important pests overcoming the need for using different *Bt* varieties.

## INTRODUCTION

The lepidopteran pests *Spodoptera litura*, *S. exigua*, *Helicoverpa armigera*, *Plutella xylostella* and *Achaea janata* are economically important polyphagous pests of global importance. In India, *S. litura* is widespread in almost all the states and inflicts significant losses to crops of economic importance like soybean (Punithavalli *et al*., 2014), cotton (Gedia, 2008), groundnut (Patil *et al*., 1996), ladies finger and pulses, etc. (Armes *et al*., 1997). Host species for *H. armigera* come from a broad spectrum of families and include important agricultural crops such as tomato, maize, chickpea, pigeonpea, sorghum, sunflower, soybean and groundnut (Fitt, 1989). *S. exigua* is known to feed on more than 50 plant species *viz*., corn, alfalfa, peas, tomatoes, potatoes, legumes, soybeans, sunflower, onions, lettuce, cotton, tobacco and several ornamental plants *(Natwick et al.,* 2012*). A. janata* causes excessive defoliation of castor crop thereby affecting photosynthesis. During later stages of the crop, larvae feed on the tender capsules of primary and secondary spikes. It is estimated that castor yields are reduced by 30–50% due to *A. janata* alone

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(Rao *et al*., 2012). *P. xylostella* is a major pest on mustard, cabbage (Badenes *et al*., 2004) and cauliflower (Saeed *et al*., 2010). These pests have developed resistance to insecticides such as pyrethriods and carbamates, resulting in the failure of effective control (Aydin and Gurkan, 2006).

Among the alternative control measures available to decrease environmental impact problems is the insecticidal bacterium, *Bacillus. thuringiensis* (*Bt*) which is safe to the non-target insects and the environment. *Bt* toxins have been employed globally as topical pesticides to protect crops from major insect pests like *H. armigera*, *P. xylostella*, *Ostrinia nubilalis*, *Agrotis ipsilon*, *S. exigua*, etc. (George and Crickmore, 2012). Commercial use of *Bt* pesticides in a given crop environment is limited because a given δ-endotoxin shows toxicity to a narrow range of target pests. *Bt* var. *kurstaki* (Btk) HD-1 produces several δ-endotoxins and is, therefore, toxic to a relatively broad range of lepidopteran insects. However, formulations based on the known δ-endotoxins, including *Btk* HD-1 are not effective against some of the important crop pests like *Spodoptera* sp. that also belong to order Lepidoptera (Tuli, 2006). We report here results of studies carried out for the selection of a local *Btk* isolate DOR *Bt*-127 effective against five major lepidopteran pests *viz*., *S. litura*, *S. exigua, H. armigera, P. xylostella* and *A. janata* and characterization of the isolate and determination of its potency to enable its utilization as a reference standard.

## MATERIALS AND METHODS

### Isolation and Multiplication of *B. thuringiensis*

Overall, 34 soil samples were collected from the topsoil at 5 cm soil depth from various farmers' fields both cultivated and fallows covering different areas of Telangana and Rajasthan, India (Table 1) that had no history of treatment with *Bt* formulations. *Bt* isolations from soil samples were carried out selectively by the sodium acetate method (Travers *et al*., 1987). Colonies with *Bt* spores and crystals were further sub cultured on nutrient agar (NA) slopes and later multiplied in nutrient broth (NB) at 30°C and 200 rpm for 72 h in an incubator shaker (Lab companion, SIF 5000R). The broth was then centrifuged at 10000 rpm for 10 min, resultant pellet was air dried, powdered and used for further studies.

### Larval Bioassays

*Bt* powders were suspended in phosphate buffered saline with Tween 80 (PBST) to get the required concentrations. Initial screening of isolates was carried out through larval bioassays against 5-day-old larvae of *S. litura* and *H. armigera* at doses 1.5 and 1 mg/mL, respectively. Bioassays were carried out by the leaf disc technique for *S. litura*. Fresh leaves of castor were washed, shade dried and cut into discs of 50.24 $cm^2$. Leaf discs were treated on both surfaces with 500 µL each of the test suspensions of *Bt* powders, allowed to air dry and placed on moist cotton in Petri plates; 10 larvae of *S. litura* were released on each leaf disc with three leaf discs/treatment. A total of 30 larvae were used per treatment. For control, leaf discs were treated with PBST buffer and diet surface treatment technique against *H. armigera* larvae was done according to Murthy *et al*. (2014).

### $LC_{50}$ and Potency Determination

For the purpose of $LC_{50}$ determination, bioassays were conducted with spore-crystal mixtures of DOR *Bt*-127 (MTCC 5976) and *Btk* reference standard (potency 31000 IU/mg against *H. armigera*) supplied by M/s Wockhardt Life Sciences Ltd., Aurangabad, India at doses ranging 0.25–1.25 mg/mL against *H. armigera* by the diet surface treatment technique. Since the *Btk* standard was not found effective against *S. litura*, a commercial *Btk* formulation Delfin of known potency (53000 SU/mg against *S. exigua*) was used as the reference standard in bioassays by the leaf-disc technique with *Bt*-127 against *S. litura* and *A. janata* at doses ranging 0.5–2.5 and 0.05–0.25 mg/mL, respectively. Sunflower and cabbage leaf discs were used for bioassays against *S. exigua* (0.25–2.0 mg/mL) and *P. xylostella* (0.02–0.2 mg/mL). Potency of *Bt*-127 against the test insects was calculated using the formula:

Potency (test material) =
($LC_{50}$ of standard/$LC_{50}$ of *Bt*-127) × potency of standard

### Statistical Analysis

Observations of larval mortality in the bioassays were recorded at 24, 48 and 72 h after treatment (HAT). Data was subjected to analysis of variance (ANOVA) and probit analysis using the statistical package SAS 9.3. Mortality data were angular transformed before analysis. ANOVA and Tukey's HSD test were used to

test for differences among *Bt* treatments.

**Scanning Electron Microscopy**

One drop of *Bt* suspension was taken on a metal die coated with graphite tape followed by vacuum drying for 24 h. The die was then coated with Au-Pd for 20 s through a sputter coating system (JEOL JFC-1600) for about 2 min to get a coating of thickness around 2 nm. Electron microscopy was performed with a scanning electron microscope (Model: JOEL-JSM 5600) at an accelerating voltage of 5 kV at RUSKA Lab, Rajendranagr, Hyderabad, India.

**Heat Viable Spore Count, Protein Extraction, Estimation and δ-Endotoxin Quantification**

10 mg of *Bt*-127 powder was suspended in 1mL of sterile deionised water and given heat shock at 80°C for 10 min. Number of colonies per plate were recorded at 48 h and the heat viable spore count/g was calculated. Protein was extracted according to Murthy *et al*. (2014) and estimated by Lowry *et al*. (1951) followed by ELISA.

**SDS-PAGE**

Alkali solubilised protein sample from *Bt*-127 was run on 10% SDS-PAGE by the procedure of Laemmli (1970). Using 5 µg of the sample and unstained protein molecular weight marker (7–175 kDa), samples were loaded into separate wells. The gel was run at constant voltage of 50 V for about 1 h and stained with coomassie brilliant blue overnight followed by destaining (26 mL methanol + 6 mL acetic acid made with 50 mL with dist. water) for 30 min.

**Molecular Characterization**

**Cry gene profiling**

Cultures of *Btk* isolates were grown overnight in Luria-Bertani broth in an incubator shaker at 200 rpm and DNA was extracted. Total genomic DNA isolated from *Bt* isolates was used for cry gene profiling through PCR. PCR products were separated using agarose gel electrophoresis in 1% Tris-EDTA (TE) buffer and stained with 0.2 mg/mL ethidium bromide (Ausubel, 1994). Forward and reverse primers for cry1Aa, 1Ab, 1Ae, 1Ac, 1B, 1C, 1D, 1Da, 1F, 2Aa, 2Ab and 2Ac genes were designed from the Gene bank database according to the Pinto and Furiza (2003) synthesized by Bioserve Biotechnologies, India Pvt Ltd.

**Repetitive element palindromic PCR (rep-PCR)**

Genomic finger printing was carried out independently with two sets of primers BOX and ERIC. These Primers were synthesized based on the published primer sequences (Rademaker and Bruijin, 1997). Gels were scored for presence with score of '1' and absence with '0' score of the corresponding band among the standards as well as the local isolates. Binary data generated was analyzed for genetic similarity using unweighted pair group arithmetic mean (UPGMA) program of NTSYSpc2.0 software.

**RESULTS**

**Isolation, Multiplication and Screening of *B. thuringiensis* Isolates**

Isolations from 35 soil samples resulted in 50 isolates of *Btk*. Colonies of *B. thuringiensis* isolates were rough and galvanized in appearance. Microscopic examination of the stained preparations revealed different crystal shapes ranging from bi-pyramidal, cuboidal, rhomboidal, flat, rectangular and irregular.

Through selective bioassays, only 10 *Btk* isolates *Bt*-127, 143, 145, 147, 151, 154, 157, 165, 171, 172 caused varying levels of mortality of 5-day-old *S. litura at* 1.5 mg/mL. Highest mortality of 73.3 and 83.3% was observed with isolate 127 at 48 and 72 HAT, respectively. Isolates 172, 165, 151, 143 caused moderate larval mortalities ranging 46.7–60.0%, while isolates 145, 147, 154, 157 and 171 caused low larval mortalities ranging 26.7–36.7% at 48 HAT although feeding cessation was observed with all isolates. Larval mortality increased to 60.0–70.0% by 72 h for all isolates except isolate 154 (43.3%). DOR Bt-1 did not cause any mortality at 48 and 72 HAT.

In larval bioassays against 5-day-old *H. armigera* at 1.0 mg/mL, highest larval mortality of 80.0 and 73.3% was observed with *Bt*-127 and DOR *Bt*-1, respectively at 48 HAT and complete mortality by 72 HAT. Moderate mortalities ranging 46.7-56.7% were observed for isolates 165, 171 and 172 while rest of the isolates caused low mortalities ranging 10.0–30.0% at 48 HAT. However, at 72 HAT these isolates caused near complete larval mortality 83.3–96.7%. (Table 2)

**Table 1. Profile of *Bacillus thuringiensis* var. *kurstaki* isolates from Rajasthan and Telangana**

| S. no | Region | Latitude | Longitude | Crop | Isolate no. | Protein content (mg/g) | ELISA (%) |
|---|---|---|---|---|---|---|---|
| 1. | | N26.51587 | E070.59796 | Pearl millet | 143 | 63.6 | 5.60 |
| 2. | | | | | 145 | 90.5 | 9.20 |
| 3. | | N26.15633 | E071.11967 | Fallow | 147 | 138.8 | 13.30 |
| 4. | | | | | 151 | 92.8 | 9.48 |
| 5. | Rajasthan | N26.25558 | E071.13232 | Pearl millet harvested | 154 | 77.2 | 7.43 |
| 6. | | N26.06697 | E072.52568 | Sesame | 157 | 110.3 | 12.30 |
| 7. | | N26.6883 | E.071.18189 | Pearl millet | 165 | 94.9 | 10.25 |
| 8. | | N25.55724 | E072.16192 | Sesame | 171 | 71.7 | 6.15 |
| 9. | | | | | 172 | 119.4 | 15.64 |
| 10. | Telangana | N18.8492 | E 78.6261 | Chilly and cotton | 127 | 168.0 | 18.28 |

**Table 2. Efficacy of *Bacillus thuringiensis* var. *kurstaki* isolates against 5-day-old *S. litura* and *H. armigera* larvae**

| S. no | Isolate no. | Larval mortality (%)* against *S. litura* larvae at 1.5 mg/mL | | Larval mortality (%)* against *H. armigera* larvae at 1.0 mg/mL | |
|---|---|---|---|---|---|
| | | 48 HAT | 72 HAT | 48 HAT | 72 HAT |
| 1. | 143 | 43.08 (46.7)$^{ab}$ | 57.3 (70.0)$^{a}$ | 19.39 (16.7)$^{e}$ | 77.55 (93.3)$^{a}$ |
| 2. | 145 | 30.99 (26.7)$^{bc}$ | 50.7 (60.0)$^{a}$ | 25.37 (20.0)$^{de}$ | 71.57 (90.0)$^{a}$ |
| 3. | 147 | 30.78 (26.7)$^{bc}$ | 44.71(50.0)$^{a}$ | 18.43 (10.0)$^{e}$ | 83.53 (96.7)$^{a}$ |
| 4. | 151 | 48.85 (56.7)$^{ab}$ | 56.79 (70.0)$^{a}$ | 33.21 (30.0)$^{cde}$ | 74.84 (90.0)$^{a}$ |
| 5. | 154 | 34.22 (33.3)$^{abc}$ | 41.16 (43.3)$^{ab}$ | 23.86 (16.7)$^{e}$ | 78.59 (90.0)$^{a}$ |
| 6. | 157 | 36.93 (36.7)$^{abc}$ | 51.15 (60.0)$^{a}$ | 26.57 (20.0)$^{de}$ | 83.53 (96.7)$^{a}$ |
| 7. | 165 | 46.92 (53.3)$^{ab}$ | 54.99 (66.7)$^{a}$ | 43.07 (46.7)$^{bcde}$ | 71.57 (90.0)$^{a}$ |
| 8. | 171 | 36.84 (36.7)$^{abc}$ | 50.94 (60.0)$^{a}$ | 44.92 (50.0)$^{abcd}$ | 68.86 (86.7)$^{a}$ |
| 9. | 172 | 50.77 (60.0)$^{ab}$ | 52.77 (63.3)$^{a}$ | 48.93 (56.7)$^{ab}$ | $^{c}$66.15 (83.3)$^{a}$ |
| 10. | 127 | 60.0 (73.3)$^{a}$ | 70.61 (83.3)$^{a}$ | 63.44 (80.0)$^{a}$ | 89.50 (100.0)$^{a}$ |
| 11. | DOR Bt-1 | 0.499 (0)$^{c}$ | 0.49 (0)$^{b}$ | 59.0 (73.3)$^{ab}$ | 89.50 (100.0)$^{a}$ |
| | SE Mean ± | 7.25 | 7.93 | 6.59 | 7.38 |
| | CD ($P$ = 0.05) | 15.027 | 16.45 | 13.67 | 15.32 |
| | CV (%) | 23.25 | 20 | 21.86 | 11.64 |

*Values are angular transformed, values in parenthesis are original means; Means followed by the same lower-case letter in a column did not differ significantly according to Tukey's HSD ($P$ = 0.05).

**Table 3. Cry gene profile of promising isolates of *Bacillus thuringiensis* var. *kurstaki***

| S. no | Isolate no. | cry gene profile |
|---|---|---|
| 1. | 143 | cry1Aa,1Ab, 1C, 1F, 2Aa and 2Ab |
| 2. | 145 | cry1Aa, 1Ab, 1B, 1C, 2Aa and 2Ab |
| 3. | 147 | cry1Aa, 1Ab, 1B, 1C, 2Aa and 2Ab |
| 4. | 151 | cry1Aa, 1Ab, 1B, 1C, 1F and 2Aa |
| 5. | 154 | cry 1C, 2Aa and 2Ab |
| 6. | 157 | cry1Aa, 1Ab, 1B, 1C, 1F and 2Aa |
| 7. | 165 | cry1Aa, 1Ab, 1C, 1F, 2Aa and 2Ab |
| 8. | 171 | cry1Aa, 1Ab, 1B, 1C, 2A and 2Ab |
| 9. | 172 | cry1Aa, 1Ab, 1C, 2Aa and 2Ab |
| 10. | 127 | cry1Ac, 1Ae, 1C, 1E, 2Aa and 2Ab |

**Table 4. Determination of $LC_{50}$ and potency of DOR *Bt*-127 isolate against five major lepidopteran pests**

| Insect | Hours after treatment (HAT) | Sample | $LC_{50}$ value mg/ mL (confidence limits) | Regression equation (Y) | Potency (IU or SU/mg) |
|---|---|---|---|---|---|
| *S. litura* | 72 | *Bt*-127 | 0.55 (-0.51–0.97) | -0.330 + 0.006X | 71,309 SU** |
| | | Delfin | 0.74 (0.12–1.04) | -0.624 + 0.008X | - |
| *S. exigua* | 48 | *Bt*-127 | 0.39 (0.12–0.57) | 0.058 + 0.015X | 46,205 SU** |
| | | Delfin | 0.34 (0.06–0.51) | -0.54 + 0.016X | 53,000 SU |
| *H. armigera* | 48 | *Bt*-127 | 0.32 (0.003–0.5) | -0.459 + 0.014X | 35,844 IU* |
| | | *Btk* standard | 0.37 (0.13–0.54) | -0.47 + 0.01X | 31,000 IU |
| *A. janata* | 48 | *Bt*-127 | 0.12 (-0.23–0.23) | -1.32 + 0.108X | 51,667 IU* |
| | | Btk standard | 0.20 (0.08–0.28) | -0.57 + 0.028X | - |
| *P. xylostella* | 24 | *Bt*-127 | 0.14 (0.12–0.18) | -0.995 + 0.067X | 26,571 IU |
| | | *Btk* standard | 0.12 (0.09–0.14) | -1.13 + 0.092X | - |

$LC_{50}$ denotes the concentration of spore and crystal mixture causing mortality of 50% of the test insects;* calculated using *Btk* standard (31000 IU/mg from Wockhardt);**Calculated using commercial *Btk* formulation Delfin of 53000 SU/mg

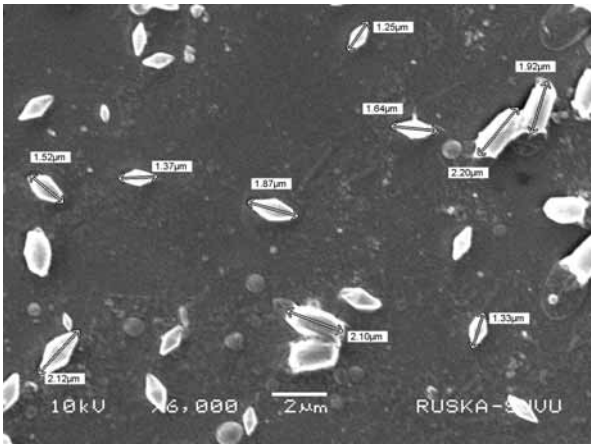


**Fig. 1. Scanning electron micrograph of DOR *Bt*-127 revealing bipyramidal crystals and ellipsoidal spores.**

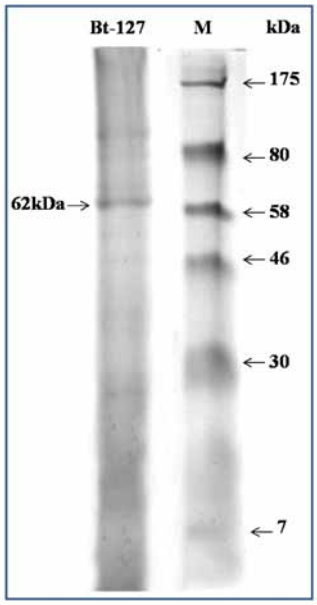


**Fig. 2. SDS PAGE of *Bt*-127.**

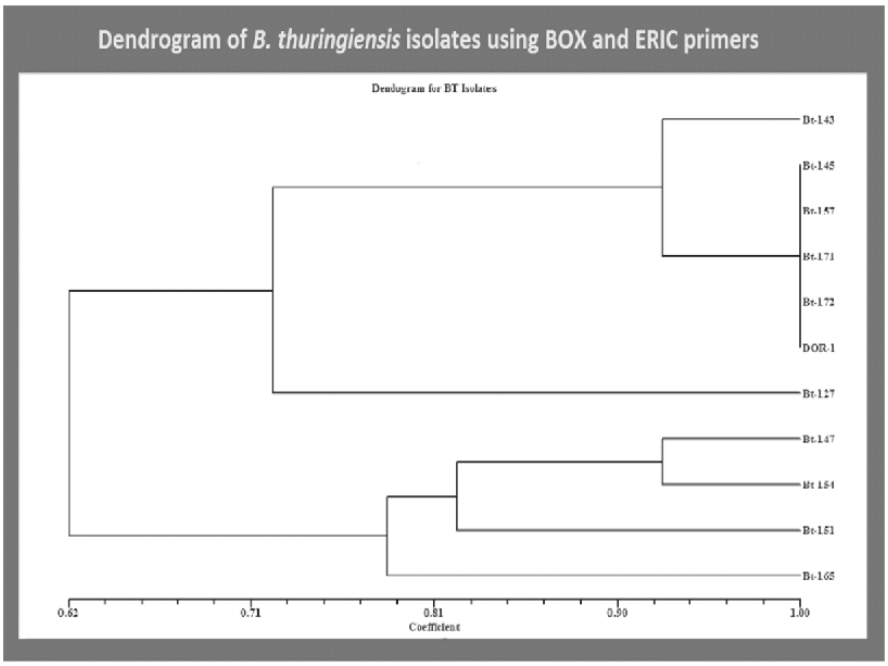


**Fig. 3. Dendrogram produced by cluster analysis (UPGMA) based on Jaccard coefficient by using molecular fingerprint of 11 isolates of *B. thuringiensis* var. *kurstaki.***

**Heat Viable Spore Count, Protein Extraction, Estimation and δ-Endotoxin Quantification**

Total protein in spore-crystal mixtures of the *Bt* isolates varied widely from 63.6 to 168 mg/g. Highest protein content of 168 mg/g was observed with *Bt*-127 isolate followed by isolates 147, 172 and 157 containing 138.8, 119.4 and 110.3 mg/g, respectively. Rest of the isolates had a protein content lower than 100 mg/g. A similar trend was observed for the δ-endotoxin contents with the highest value of 18.28% for *Bt*-127 isolate followed by 15.6, 13.3 and 12.3% for isolates 172, 147 and 157, respectively. Spore-crystal mixture of *Bt*-127 contained a high heat viable spore count of $9 \times 10^{22}$/g in addition to the high δ-endotoxin content of 18.28% (Table 1). Scanning electron microscopy of *Bt*-127 revealed the presence of bipyramidal crystals (0.64–2.12

µm) and ellipsoidal spores (1.92–2.2 µm) (Fig. 1). SDS-PAGE of alkali soluble protein from *Bt*-127 powder revealed the presence of a 62 kDa band thus confirming the presence of Cry1 toxin (Fig. 2).

**Molecular Characterization**

**Cry gene profiling**

PCR analysis with cry gene specific primers was carried out to identify specific classes of cry1 and cry2 genes present in the 10 promising isolates identified through selective larval bioassays. Results revealed that 8 isolates harboured cry1Aa and 1Ab genes, 1 isolate harboured cry1Ae gene, one isolate harboured cry1Ac gene, 5 isolates harboured cry1B gene, 10 isolates harboured cry1C gene and 4 isolates harboured cry1F gene. Analysis for the subclasses of cry2 gene, revealed that the majority of the isolates carried cry2Aa (10/10) and cry2Ab (8/10) genes whereas none possessed cry2Ac gene. (Table 3).

**Repetitive element palindromic PCR (rep-PCR)**

The phylogenetic tree was constructed by using NTSYS ver.2 software with UPGMA programme. The plotted tree was divided into two major clusters, cluster-I and cluster-II. Cluster-I was further divided into two sub-clusters, IA and IB. In sub-cluster IA, *Bt*-143 formed a separate lane with similarity of 92% with other isolates present in the cluster, whereas in the same cluster five isolates *Bt*-145, 157, 171, 172 and DOR *Bt*-1 show 100% similarity in the coefficient scale. *Bt*-127 isolate formed a separate sub-cluster IB showing only 71.5% similarity with other isolates.

Cluster-II was further divided into two sub-clusters IIA and IIB. Cluster IIA was further divided into sub-clusters $IIA_1$ and $IIA_2$. In cluster $IIA_1$, two *Bt* isolates *Bt*-147 and *Bt*-154 showed the same similarity, *i.e.,* 93% in coefficient scale. In sub-cluster $IIA_2$, isolate *Bt*-151 only showed 81.5% similarity with *Bt*-147 and *Bt*-154. Cluster IIB had only one isolate *Bt*-165 with 77% similarity in coefficient scale. Hence, the dendrogram reveals the five isolates were similar in the genome sequence and formed in the same cluster. Whereas two isolates, *Bt*-127 and *Bt*-165 gave complete diversity in genome as they formed different lanes in their respective clusters on the coefficient scale. More or less other isolates were above 80% similar according to the dendrogram coefficient scale. (Fig. 3).

**Potency Determination of *Bt*-127**

Bioassays revealed a high susceptibility of *S. litura*, *S. exigua*, *H. armigera*, *P. xylostella* and *A. janata* to *Bt*-127 with low initial feeding followed by feeding cessation. $LC_{50}$ values of *Bt*-127 against *H. armigera and A. janata* were 0.32 mg/mL and 0.12 mg/mL with corresponding values of 0.37 mg/mL and 0.20 mg/mL for the *Btk* standard 48 HAT. $LC_{50}$ values against *P. xylostella* were 0.14 mg/mL and 0.12 mg/mL for *Bt*-127 and standard, respectively at 24 HAT while $LC_{50}$ values against *S. exigua* and *S. litura* were 0.39 and 0.55 mg/mL for *Bt*-127 with corresponding values of 0.34 and 0.74 for Delfin, respectively 72 HAT. Spore-crystal mixture of *Bt*-127 was highly toxic to the less susceptible pests *S. exigua* and *S. litura* with potencies of 46205 and 71309 SU/mg, respectively. The potency against susceptible *H. armigera* was 35833 IU/mg and for highly susceptible pests *P. xylostella* and *A. janata* it was 26571 and 51667 IU/mg, respectively (Table 4).

## DISCUSSION

Preliminary screening of 50 *Btk* isolates resulted in only 10 isolates causing varying degrees of mortality against *S. litura* larvae although highly effective against *H. armigera* larvae. Hence, these ten isolates *Bt*-127, 143, 145, 147, 151, 154, 157, 165, 171, 172 along with another isolate DOR *Bt*-1 effective against *H. armigera* and *A. janata* (Vimala Devi and Vineela, 2015) were evaluated through larval bioassays against *S. litura* and *H. armigera*. All the isolates were effective against *H. armigera* while only *Bt*-127 isolate caused significant mortality of *S. litura* larvae. Isolates 143 and 155 induced 70% mortality of *S. litura* larvae 72 HAT. DOR *Bt*-1 did not cause any mortality of *S. litura* larvae revealing that it is ineffective against this pest. Endotoxin content of this spore-crystal mixture was high at 18.28% coupled with a high heat viable spore count of $9 \times 10^{22}$/g. Effectiveness of *Bt* depends on the quality and type of proteins present in the crystal coupled with the insect's ability to digest the crystal and release the active toxic fraction in the midgut. Quicker kill of larvae is due to the action of the crystal alone and the spore is at best only as secondary factor in killing insects. In the less susceptible insect species, effectiveness also depends on the ability of the ingested spores to invade the hemolymph, germinate, multiply and cause a lethal septicemia indicating that both the spore and crystal are necessary for optimal kill (Dulmage 1993).

rep-PCR revealed that *Bt*-127 and *Bt*-165 were diverse from the other isolates forming distinct sub clusters in the 2 major clusters I and II, respectively while remaining isolates were more or less similar to each other. Both the isolates carry genes coding for toxins reported effective against *S. litura* and *H. armigera*. However *Bt*-127 carries two genes cry1C and 1E coding for toxins reported effective against *S. litura* while *Bt*-165 carries only the cry1C gene. This could be the reason for the higher mortality of *S. litura* larvae caused by *Bt*-127 over *Bt*-165.

A wide range of lepidopteran larvae are susceptible to CrylAa, lAb or lAc δ-endotoxins, whereas *S. littoralis and S. exigua* larvae are almost insensitive to these δ-endotoxins but susceptible to CrylC and lE toxins (Chang *et al*., 1998). *Spodoptera* species presented low susceptibility to the *Btk* based products (Bohorova *et al*., 1997). CrylC toxin is produced by *B. thuringiensis* vars. *entomocidus* and *aizawai* (Sanchis *et al*., 1988) while CrylE is produced by *B. thuringiensis* vars. *kenyae* and *dendrolimus* (Van Rie *et al*., 1990). Cry1E genes encode highly specific crystal proteins against *S. exigua* and *S. littoralis* (Visser *et al*., 1990, Whitlock *et al*. 1991). Cry1Ac gene is effective against *S. exigua* (Konecka *et al*., 2013). Although *Bt*-127 belongs to var. *kurstaki*, it possesses the genes coding for Cry1C and 1E toxins along with Cry1Ac toxin which explains its effectiveness against both *S. litura* and *S. exigua*.

Majority of the *Btk* isolates studied were highly effective against *H. armigera* and possessed either cry1Aa, 1Ab or 1Ac genes. Cry1 δ-endotoxins of *Bt* are a family of 130 to 140 kDa proteins that exhibit specific toxicities to numerous lepidopteran insects. Cry1Aa, 1Ab, 1Ac, 2Aa, and 2Ab toxins have been reported to be toxic for *H. armigera* although larvae of *H. armigera* are particularly susceptible to Cry1Ac (Brevault *et al*., 2009) while Cry toxins 1Aa, 1Ab, 1E and 2A have been reported to be highly effective against *A. janata* (Lakshminarayana and Sujatha, 2005). Earlier studies have indicated that Cry2Aa and 2Ab proteins are very effective against *H. armigera* (Liao *et al*., 2002).

Cry 1Ab and 1C toxins were reported to be highly toxic to field collected larvae of *P. xylostella* from different locations of India with $LC_{50}$ values 5.8–485.5 ng/mL and 12.2–86.4 ng/mL, respectively (Mittal *et al*., 2007). Cry1C was reported highly toxic to *Spodoptera* spp and *P. xylostella* (Liu *et al*., 1996). Gonzalez *et al*., (2001) reported that Cry1Ab, 1Ac and 1Aa are most toxic to *P. xylostella* 3rd instars with $LC_{50}$ values of 0.14, 0.17 and 2.72 mg/L at 5 DAT, respectively. Effectiveness of *Bt*-127 against *P. xylostella, S. litura* and *S. exigua* can be attributed to the presence of both cry1Ac and 1C genes coding for the respective toxins.

*Bt*-127 possesses genes coding for toxins reported were effective against lepidopteran insects *S. litura*, *S. exigua*, *H. armigera*, *P. xylostella* and *A. janata*. Our bio-efficacy studies with DOR *Bt*-127 strain are in conformity with its *cry* gene profile. Of late, *S. litura* has emerged as a devastating pest on soybean in India (Fand *et al*., 2015). Since the International standard HD-1S-1980 is no longer available, there is a need to identify a *Btk* isolate with a broad host range to be used as a standard for identifying new indigenous strains with high potencies against the major lepidopteran pests so as to promote use of *Bt* in pest management in India. Hence *Bt*-127 can be used as a reference standard and can contribute effectively for management of all these major pests by overcoming the need for use of different *Bt* varieties for their management.

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