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Characterization of lepidopteran specific *Bacillus thuringiensis* isolates using RAPD-PCR*

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Bacillus thuringiensis is an important microbial agent used in pest management. This gram positive, aerobic, spore-forming bacterium is characterized by the production of Insecticidal Crystal Proteins (ICPs) during sporulation. Several *B. thuringiensis* isolates having lepidopteran toxicity were characterized. The chance of developing resistance to *B. thuringiensis* based bioinsecticides increased the need for isolating new *B. thuringiensis* strains, with higher virulence and novel activity against additional group of insects. The toxicity of *B. thuringiensis* can be related to the *cry* gene content (Padidam 1992). Different molecular tools are being used to understand the distribution of cry genes in different strains and to study the genetic diversity between the stains.

This paper deals with the characterization of novel native isolates of *B. thuringiensis* pathogenic to *Spodoptera litura* by RAPD-PCR technique for estimating their genetic diversity. These isolates were collected from the western Ghats of Kerala.

A preliminary screening was conducted with 20 native isolates of *Bacillus thuringiensis* to find out the lepidopteran toxic ones. These isolates and the reference strain, HD 1 were obtained from Centre for Plant Biotechnology and Molecular Biology, College of Horticulture, Vellanikkara. Of the 20 isolates screened, 3 isolates were found to cause mortalities ranging from 76.7 to 86.7% against *S. litura*. The reference strain, HD–1 (*B. thuringiensis* subsp. *kurstaki*) caused 96.7% mortality to the larvae. A standardized bioassay was conducted with the selected isolates. The bacterial isolates were grown in LBA medium (Miller 1972) and T3 medium (Travers *et al.* 1987) and 5 different concentrations (1×10^7, 1×10^8, 1×10^9, 1×10^{10} and 1×10^{11} spores/ml) of the selected isolates were prepared for the bioassay. A 100 ml of the spore suspension was applied uniformly on the upper and lower surface of the leaf discs (3 cm diameter) and larvae were released after the leaf discs get dried. Larvae feed on the leaf discs were observed for mortality for 7 days. All the isolates were equal in effectiveness with the standard strain, HD 1 (Jacob *et al.* 2009). Staining with Comassie brilliant blue was done (Sharif and Alaeddinoglu 1988) for examining the presence of crystal protein inclusions. The smear was observed under 100× objective of a compound binocular microscope. Molecular characterization of these isolates was performed by RAPD-PCR technique. The DNA of the selected isolates were extracted by following the protocol of Sambrook and Russel (2001). The quality of the isolated DNA was assessed by agarose gel electrophoresis (Sambrook *et al.* 1989). The DNA bands separated by electrophoresis were viewed under UV light using UVP Gel Doc It™ Imaging System.

Twenty randomly selected decamer primers were screened to identify the best primers for RAPD analysis. The genomic DNA extracted from the *B. thuringiensis* isolate, KAU 166 was used for the screening. RAPD-PCR was conducted with 10 primers (Table 1) that gave good amplification. The total number of amplicons, per cent polymorphism and

Table 1 Selected primers for RAPD analysis of *Bacillus thuringiensis* isolates

Primers	Nucleotide sequence (5′–3′)
OPF 3	CCT GAT CAC C
OPF 5	CCG AAT TCC C
N 6	GAG ACG CAC A
S 01	GTT TCG CTC C
S 05	TGC GCC CTT C
S 07	GGT GAC GCA G
S 22	TGC CGA GCT G
S 23	AGT CAG CCA C
S 30	GTG ATC CTG G
S 103	AGA CGT CCA C

*Short note

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Table 2 Details of RAPD profiles of selected isolates and HD 1

Primersused	Total number of amplicons	Number of polymorphic bands	Number of monomorphic bands	Per cent polymorphism	Differentiating power (%)
OPF 3	9	9	0	100	100
OPF 5	14	14	0	100	100
N 6	15	15	0	100	100
S 01	12	12	0	100	100
S 05	8	8	0	100	100
S 07	11	10	1	90.9	100
S 22	8	8	0	100	100
S 23	5	5	0	100	100
S 30	13	13	0	100	100
S 103	11	11	0	100	100

discrimination power of primers were calculated using the following formulae (Table 2).

$$\text{Per cent} = \frac{\text{Total number of polymorphicbands}}{\text{Total number of bands}} \times 100$$

$$\text{Discrimination power of a primer} = \frac{\text{Number of unique RAPD phenotypes}}{\text{Total number of phenotypes}} \times 100$$

RAPD profiles were compared and scored as one or zero based on the presence or absence of bands. The data was analysed for estimating genetic diversity by computing DICE coefficient using the Numerical Taxonomy System of Multivariate Statistical Programme (NTSyS) software package. The clustering was done and dendrogram was drawn using Unweighed Pair Group Method of Arithematic Averages (UPGMA) (Sneath and Sokal 1973) using the above NTSyS programme.

The quality of isolated DNA was found to be good when subjected to agarose gel electrophoresis. Nine primers produced 100% polymorphism while the primer, S 07 produced 90.9% polymorphism (Table 2). The high differentiating power (100%) of the primers shows their ability to expose the genetic diversity of these isolates (Table 2). Dissimilar RAPD profiles produced by different primers showed that these primers could be used for conducting RAPD-PCR technique for *B. thuringiensis*.

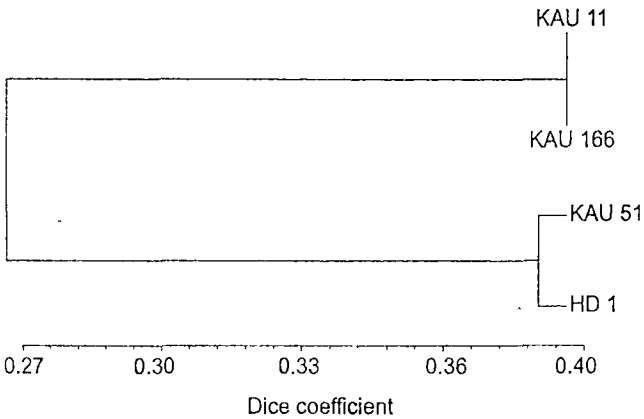

Fig 1 Dendrogram showing the similarity of *B. thuringiensis* isolates

In the dendrogram, the three *B. thuringiensis* isolates and HD 1 were divided into two distinct major clusters '1' and '2' at 27% similarity. The first major cluster comprised 2 isolates, KAU 11 and KAU 166 which showed maximum similarity between the isolates (Fig 1). The similarity coefficient between the isolates was 0.39. These 2 isolates were collected from two different districts of Kerala namely Calicut and Palakkad. Similarity between the clones of genomically similar or highly related strains of *B. thuringiensis* from geographically distinct locations was reported as due to the limited exchange of chromosomal DNA (Rivera and Priest 2003). The second major cluster formed a sub-cluster in which KAU 51 and HD 1 were grouped together with a similarity coefficient of 0.38. Clusters produced could give an indication on the toxicity of *B. thuringiensis* isolates belonging to clusters in terms of insecticidal similarities within the same cluster (Sadder *et al.* 2006). Thus the similarity coefficients between *B. thuringiensis* isolates ranged from 0.24 to 0.39. Minimum similarity was observed between the isolates, KAU 11 collected from Thusharagiri (Calicut) and KAU 51 collected from Kolahalamedu (Idukki).

Cry toxins present in the standard strain, HD 1 were *cry* 1, *cry* 6, *cry* 7, *cry* 8, *cry* 12, *cry* 18 and *cry* 23. The isolates KAU 11 contained *cry* 1 and *cry* 10 toxins and KAU 51 contained *cry* 1 toxin. The cry toxins present in the isolate KAU 166 were *cry* 1, *cry* 3, *cry* 4, *cry* 6, *cry* 12 and *cry* 13. The identification of cry toxins should be carried out before the large-scale multiplication and application of these biocontrol agents in the field.

RAPD-PCR is used in this work to evaluate the molecular variability of the *B. thuringiensis* isolates. Seventy isolates of *B. thuringiensis* isolated from soils of cotton fields were characterized by randomly amplified poylmorphic DNA (RAPD) markers to determine their genetic diversity pattern based on their source of origin (Kumar *et al.* 2008). The discrimination of different *B. thuringiensis* isolates and

serotypes using randomly amplified polymorphic DNA was also reported by Rai *et al.* (2009). This technique helps to obtain preliminary informations about the genetic variability between the isolates. It was not advisable to use RAPD analysis alone to characterize different strains of *B. thuringiensis*. It should be performed along with other biochemical tests to differentiate the isolates. There is a necessity to isolate, identify and characterize more *B. thuringiensis* isolates to overcome the problem of insect resistance and also to study about the noval host range.

SUMMARY

A study was conducted during 2006–08 to characterize *Bacillus thuringiensis* isolates from Kerala, pathogenic to *Spodoptera litura* were characterized by RAPD-PCR technique using 10 decamer primers. The similarity coefficients of the isolates ranged from 0.24 to 0.39. Isolates, KAU 11 and KAU 166 showed maximum similarity (0.39). Minimum similarity was observed between KAU 51 and KAU 11 (0.24). The isolates, KAU 51 and the standard reference strain, HD 1 showed a similarity coefficient of 0.38.

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