ISOLATION OF SCARABID SPECIFIC *BACILLUS THURINGIENSIS* AND SCREENING OF *CRY 8* GENE FROM SUGARCANE ECOSYSTEM.

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ABSTRACT

A study was undertaken to isolate *B.thuringiensis* from sugarcane ecosystem in Tamil Nadu, India and to identify isolates containing crystalline protein of Bt that are toxic to Coleopteran insects.. The isolation of *B.thuringiensis* was carried out on Traver's media following heat treatment. Isolates were identified based on the ability of the isolate to produce crystal toxin which can be detected under phase contrast microscope. The *cry* gene content of 58 *B.thuringiensis* isolated from this study was identified by Polymerase Chain Reaction Method. The universal primers of *cry* 8 gene which is specific against the members of *scarabaeidae* family of the order Coleoptera was used to detect environmental isolates that would be positive for the gene. Isolate Bt 378 which was found *cry8* positive revealed that the amplified nucleotide sequence when analyzed with ClustalW2 showed a maximum identity of 88.92 % with other *cry* 8 genes. Further confirmation on its toxicity is being confirmed on the coleopteran beetle *Holotrichia serrata* (Fabricius).

INTRODUCTION

Bacillus thuringiensis strains have insecticidal activity against certain insect species among the orders Lepidoptera, Coleoptera, and Diptera. They can be isolated from the soil. Genetic diversity and distribution of cry genes in *B. thuringiensis* strains vary based on geographical location from which they are isolated. Each habitat may contain novel *B. thuringiensis* isolate that have more toxic effects on target spectra of insects.

AIM

The aim of this study was to isolate *B. thuringiensis* strains active against coleopteran species from different soil samples and to identify the crystalline protein gene type of the isolates.

The main objectives of this study were:

1) To isolate *B. thuringiensis* strains active from soil samples of Sugarcane ecosystem.

2) To identify crystalline toxin protein gene of the isolates by PCR analysis with universal primers of *cry 8* genes which are active against Coleopteran insects.

METHODOLOGY

ISOLATION OF *B. THURINGIENSIS* STRAINS

Weigh 10 gm dry soil sample in 100 ml 0.85% NaCl in a conical flask

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Agitate the flasks in Orbital shaker - 5 min/ 200rpm

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Heat shock in water bath at 80^{0}C for 15 minutes

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Transfer 1 ml to 50 ml Luria broth and incubate at 30°c overnight with 200 rpm shaking

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Serially dilute 1 ml of the broth in 9 ml of saline

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Plate 100µl, 200µl, and 300µl dilution each on T3 medium

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Incubate the plates at 30°C for 48 hours

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Pick single *Bacillus* like colonies and streak on T 3 medium (incubate at 30^{0}C, 72 hrs)

↓

Phase contrast microscopic observation

↓

After conformation, Streak the selected colonies to make Glycerol stocks

IDENTIFICATION OF cry 8 GENE BY PCR ANALYSIS

The PCR conditions for screening the isolates were as follows:

A step cycle program -35 cycles ,single denaturation - 5 min at 94°C, denaturation 1min at 94°C , annealing 30 secs for at 51°C, and extension 1min at 72°C .Finally an extra step of extension given at 72°C for 7 min . Simultaneously, each PCR sample was electrophoresed on 1 % agarose-ethidium bromide gel in Tris-Actate EDTA (TAE) electrophoresis buffer [pH 8]) at 75V for 40 min. Low range DNA ruler plus was used as marker for comparison of DNA bands.

RESULTS

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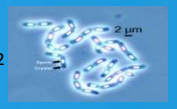
2 

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1. Isolated B. thuringiensis strains 2.Spores with crystal image 3. Gel Electrophoresis of Bt 378 isolate.

CONCLUSION : In this study, 20 different soil samples were used and had yielded 35 isolates.. Spherical crystals were found in all the isolates.PCR screening was carried out on 35 isolates for *cry8* gene. The PCR analysis revealed that one isolate *Bt378* was found positive for *cry 8* gene among the 35 isolates screened.

REFERENCES : Baig, D.N. and Mehnaz,S. (2010). Determination and distribution of cry-type genes in halophilc *Bacillus thuringiensis* isolates of Arabian Sea sedimentary rocks, Microbiological Research 165 : 376 -383

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