

Characterization of native *Bacillus thuringiensis* from the Western Ghats of Kerala

P.M. NEEMA, D. GIRIJA, C.APARNA AND P.M. FIROZ

Centre for Plant Biotechnology and Molecular Biology, Kerala Agricultural University, THRISSUR (KERALA) INDIA

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Eleven *Bacillus thuringiensis* isolates were obtained from the soils of Western Ghat region of Kerala. Morphology of the isolates on LBA media revealed similarity with *Bacillus thuringiensis* HDI. All the isolates produced creamy white, puffy colonies. Staining with Coomassie brilliant blue revealed the presence of dark blue crystal proteins having spherical, irregular, bipyramidal and triangular shapes. Biochemical characterization of the isolates showed variable response to hydrolysis of urea, starch and esculin. Profiling of cry1 and cry4 genes from *B. thuringiensis* isolates was done using universal cry1 and cry4 primers for detection of cry genes and prediction of their insecticidal activities. Amplification with universal cry1 gene primer was obtained for eight isolates KY2, KY3, KY5, KY6, KK7 KY8, KY9 and EM11 along with the reference strain HD1. Presence of cry 4 gene, specific to dipteran larvae was detected in isolates KY1 and EM10.

Key words : *Bacillus thuringiensis*, ecological niche, parasporal inclusions, biochemical tests

INTRODUCTION

The continuous and prolonged use of synthetic pesticides has led to the emergence of resistance in agricultural pests and to environmental degradation. This has created an urgent need for the development of environment friendly pesticides to reduce contamination and the likelihood of insect resistance (Sheltan *et al.*, 2002). Recently, there has been a renewed interest in the development of biological alternatives to chemical pesticides.

Bacillus thuringiensis Berliner is considered as one of the most versatile microbial insecticides. It has been used as a successful biological insecticide for more than 40 years and is a uniquely specific, safe and effective tool for the control of a wide variety of insect pests (Nester *et al.*, 2002). The use of *Bacillus thuringiensis* as a microbial insecticide has several advantages over the use of chemical control agents, as they are highly specific for certain hosts and are not toxic to other insects, plants and vertebrates.

Bacillus thuringiensis is a rod shaped, gram positive, facultative anaerobic, spore forming bacterium. The insecticidal activity is based on the ability of the bacterium to produce large quantities of larvicidal proteins known as delta-endotoxins. These delta-endotoxins are toxic to more than 150 insects belonging to the orders Lepidoptera, Diptera, Coleoptera and many others.

As a result of the increased selection pressure and continuous exposure to a single kind of toxin, insect pests

have evolved varying levels of resistance. In order to face the certainty of wide spread *Bacillus thuringiensis* resistance, wise management strategies have to be adopted. Intensive screening programmes are going on worldwide to isolate large number of *B. thuringiensis*, in order to identify new strains with increased levels of insecticidal activity against a broader spectrum of insect pests. The natural habitat of *Bacillus thuringiensis* are plant surfaces, trees, soils, stored products and insect hosts. It is a wide spread bacterium detected in different habitats including soil, grain dust, diseased insect larvae, deciduous and coniferous leaves and sericultural farms (Dulmage and Aizawa, 1982, Obeidat *et al.*, 2004). Soil is the natural reservoir for insecticidal *Bacillus thuringiensis* with moderate to low toxicities (Ishi and Ohba, 1993).

In the present study, *B. thuringiensis* isolates were obtained from the Western Ghats of Kerala. The Western Ghats of India is one among the eighteen hotspots of biodiversity of the world, which is relatively undisturbed and is expected to harbour novel isolates of *B. thuringiensis*. Morphological and biochemical characterization of the isolates was also done.

MATERIALS AND METHODS

The experiment was carried out in the Molecular Biology Laboratory of the Centre for Plant Biotechnology and Molecular Biology, College of Horticulture, Vellanikkara, Thrissur, Kerala during the period 2005-2007. The chemicals used for the study were obtained from different