

16S rRNA gene based identification of gut bacteria from field collected larvae of *Helicoverpa armigera* (Lepidoptera: Noctuidae) from tomato ecosystem

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(Received: March, 2011; Accepted: April, 2011)

Tomato fruit borer *Helicoverpa armigera* (Hübner) is a polyphagous pest of different host plants and has developed resistance to most of the insecticide groups. In order to know the gut microbial flora of the insecticide resistant field collected larvae of *H. armigera*, whole gut was dissected from the fourth to fifth instar larvae of the *H. armigera* and the culturable bacterial species were identified by sequence analysis of 16S rRNA gene. Altogether eleven bacterial species of different genera were identified were *Stenotrophomonas* sp., *Enterococcus casseliflavus*, *Enterococcus* sp., *Enterococcus gallinarum*, *Enterococcus faecium*, *Bravundimonas diminuta*, *Staphylococcus* sp., *Pseudomonas aeruginosa*, *Acinetobacter calcoaceticus*, *Bacillus subtilis* and *Rhodococcus* sp., of which genera *Enterococcus* were found to be predominant. The nucleotide sequences of 11 isolates were submitted to NCBI-Gen Bank and accession numbers (HM446252, HM446253, HM446254, HM446256, HM446258, HM446260, HM446261, HM446263, HM446264, HM446265, and HM446266) were obtained.

Key words : *Helicoverpa armigera*, Gut bacteria, 16S rRNA, Nucleotide, Sequencing

Madhusudan, S., Jalali, S.K., Venkatesan, T., Lalitha, Y. and Srinivas, Prasanna (2011). 16S rRNA gene based identification of gut bacteria from field collected larvae of *Helicoverpa armigera* (Lepidoptera: Noctuidae) from tomato ecosystem. *Asian J. Bio. Sci.*, **6**(1): 106-113.

INTRODUCTION

Microorganisms play a key role in both host physiology and nutrition (Dillon and Charnley 1995; Nardi *et al.*, 2002). Bacteria and insects have evolved a diverse array of symbiotic interactions, which play a role in insect nutrition (Bernays and Klien, 2002; Bracho *et al.*, 1995; Douglas, 1988; Douglas and Prosser, 1992; Lal, *et al.*, 1994; Wicker, 1983), defence (Ferrari *et al.*, 2004; Kellner and Dettner, 1996; Oliver *et al.*, 2003; Piel, 2002), reproduction and development (Caspari and Watson 1959; Gherna *et al.*, 1991; Hurst *et al.*, 1999). Bacteria are associated with a number of different insect species across all major orders of the insects (Buchner, 1965; Dillon and Dillon 2004). The insect gut provides a suitable

habitat for bacteria (Bignell *et al.*, 1984). In many insect species the gut possess different types of bacteria, which are transient and do not remain in the gut during all life stages. However, in some cases, a variety of permanent microorganisms are present that supply essential nutrients to their host and some possess obligate microbial endosymbionts that benefit the insects (Bridges, 1981).

Although cultivation based biochemical techniques have been used for analysis of the specific groups of bacteria, several limitations are associated with such approaches, particularly for surveying intestinal bacterial ecosystem. The introduction of high resolution molecular techniques has improved the analysis of diverse microbial populations (Muyzer, 1999). The important advance has been the use of 16S rRNA as a molecular finger print to