

# Culturable and Unculturable Gut Microflora of *Spodoptera Litura* (Fab) and Associated Host Fitness Attributes

Sanjay R. Yalashetti<sup>1</sup>, Mahesh S. Yandigeri<sup>2</sup>, G. Sivakumar<sup>3</sup> and M. Mohan<sup>4</sup>

<sup>1,2,3,4</sup>ICAR-National Bureau of Agricultural Insect Resources, P.B No. 2491, HA Farm Post,  
Bellary Road, Bengaluru-560024, Karnataka, India

E-mail: <sup>1</sup>[sanjuhrg@gmail.com](mailto:sanjuhrg@gmail.com), <sup>2</sup>[micromahesh@gmail.com](mailto:micromahesh@gmail.com), <sup>3</sup>[spicessiva@yahoo.co.in](mailto:spicessiva@yahoo.co.in), <sup>4</sup>[mohan\\_iari@yahoo.com](mailto:mohan_iari@yahoo.com)

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**Abstract**—Insects are the most successful organisms on the earth and accounted for more than 80% of the all living organisms. Insect pests are one of the limiting factors in increasing productivity of many agricultural and horticultural crops in the world. *Spodoptera litura* is a polyphagous insect pest, causes appreciable damage on cotton, legumes, crucifer vegetables, chilli and in many other crops. Insecticides are the main mode of controlling this pest. Due to which the pest has developed resistance to many insecticide chemistries. In many cases, the insect gut microfloras are responsible for the host insect's resistance to the insecticides. The current investigation isolated culturable gut microflora from field as well as laboratory reared 4<sup>th</sup> instar larvae of *Spodoptera litura*. The field population was originally collected from the heavily insecticide sprayed cabbage fields of Battalahalli village, Karnataka. These organisms were classified using Gram's staining and other biochemical tests like amylase test, protease test, gelatin hydrolysis test were studied. Based on these tests, five microflora were selected. These microflora were identified using universal 16S rRNA primers pA-AGAGTTTGATCCTGGCTCAG and pH-AAGGAGGTGATCCAGCCGCA. Unculturable microbial diversity was studied using 16S rRNA cloning by pGEMT vector and *E. coli* XLI blue chemical competent cells following metagenomic approaches. The ligated transformed bacterial clones were amplified using primer 27F-AGAGTTTGATCMTGGCTCAG and primer: 806r- GGACTACHVGGGTWTCTAAT. Amplified DNA of culturable microorganisms were sequenced and identified as *Lysinibacillus macroides*, *Pseudomonas stutzeri*, *Staphylococcus sciuri*, *Staphylococcus saprophyticus* and *Enterobacter cloacae*. Unculturable microflora from the field collected *S. litura* were identified as *Gilliamella apicola*, *Enterococcus termitis*, *Enterococcus rotai*, *Enterococcus moraviensis*, *Parvimonas micra*, *Erysipelatoclostridium ramosum*, *Dysgonomonas* sp., *Gilliamella* sp. and *Enterococcus* sp. Sequences were submitted to GenBank database obtained accession numbers for culturable microflora as KT818800-KT818804 and unculturable as KU960901-KU960910. All culturable microflora were subjected to in vitro degradation of insecticide acephate, which was incorporated with minimal agar medium. *Pseudomonas stutzeri* and *Lysinibacillus macroides* were found potent in degradation of acephate up to 500 ppm concentration based on their luxurious growth, indicating their involvement in acephate degradation.

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