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Research Article

Molecular identification of insecticide degradation by gut bacteria isolated from *Helicoverpa armigera* of Cotton plants

Madhusudan S*
Department of Biotechnology, Maharani Cluster University, Bangalore- 560001 (Karnataka), India
S.K. Jalali
Division of Molecular Entomology, National Bureau of Agriculturally Important Insect Resources (ICAR), Bangalore - 560043 (Karnataka), India
Sibi G
Department of Biotechnology, Indian Academy Degree College-Autonomous, Bangalore- 560043 (Karnataka), India

*Corresponding author. Email: mailmadhubiotech@gmail.com

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Abstract
The cotton bollworm *Helicoverpa armigera* occurs as a major pest in many economically important crops, including cotton, pigeon pea, chickpea, pea, cowpea, sunflower, tomato, sorghum, pearl millet and other crops. Intestinal microorganisms play important role in the degradation of diet components of insects. In order to know the role of gut bacteria in insecticide resistance five insecticides Chlorpyriphos (20% EC), Cypermethrin (25% EC), Malathion (50% EC), Quinalphos (25% EC), Triazophos (40% EC), were selected for the insecticide degradation studies. All the bacterial isolates from the gut of lab and field populations of *H. armigera* were identified using 16S rRNA gene-based identification and tested for their growth on minimal salt medium (MSM) along with the selected insecticides. A total of 11 bacterial isolates were tested and among them, isolate CL4 (*Rhodococcus* sp.) was found to grow on minimal salt medium (MSM) and with chlorpyriphos and isolate CL2 (*Enterococcus casseliflavus*) was able to grow in MSM with chloropyriphos ($C_{22}H_{19}C_{l2}NO_3$) and malathion ($C_{10}H_{19}O_6PS_2$) and no growth was seen in MSM without insecticide (control). Gas Chromatography analysis of the positive bacterial isolate cultures in MSM showed that the isolate CL4 (*Rhodococcus* sp.) was able to utilize 43.9% of chlorpyriphos and isolate CL2 (*E.casseliflavus*) was able to utilize 26% of chlorpyriphos and 57.1% of malathion in MSM broth cultures with comparison with the respective control cultures. Findings of the current work suggested that gut bacteria in the field populations of *H. armigera* plays a role in insecticide resistance.

Keywords: Cotton plants, Gut bacteria, *Helicoverpa armigera,* Insecticide degradation, Molecular identification

INTRODUCTION

Old-World bollworm (*Helicoverpa armigera*) has attained the status of national pest owing to its devastative nature on commercial crops in India. The cotton bollworm *H. armigera* occurs as a major pest in many economically important crops, including cotton, pigeon pea, chickpea, pea, cowpea, sunflower, tomato, sorghum, pearl millet and other crops. The cotton grown in over 5% of the total cultivated area has consumed more than 55% of the total amount of pesticide used in India (Kranthi *et al*. 2001)

A robust complement of prokaryotes is harbored in insects' alimentary canals, facilitating nutrient availability, utilization, and detoxification of environmental toxins. Intestinal microorganisms play important role in the degradation of diet components of insects (Hayashi *et al*., 2007). However, the indigenous gut bacteria also play a role in withstanding and colonization of the gut by non-indigenous species including pathogens (Dillon and Dillon, 2004). Precious little is known about the microbial community inhabiting the guts of various higher organisms, though it is acknowledged that microbes play an important role in metabolism, growth, development and protection of their host. Lepidoptera species feed on plants and the biomass digestion is

aided by gut associated microbes (Rajagopal *et al.,* 2002). However, the diversity of insect gut microflora is not revealed well and understanding the role of those microflora during the insect growth and development would help in effective pest management. *H. armigera* harbors diverse bacterial communities in its gut regions (Dar et al., 2021). Recently, *Bacillus* sp were isolated from gut region of *H. armigera* by culture dependent methods (Shinde *et al.,* 2019).

Detoxification of the environmental pollutants, pesticide detoxification in soil and other ecosystems by microorganisms have been reported earlier. Biological removal of chemo pollutants becomes the method of choice since microorganisms can use a variety of xenobiotic compounds including pesticide for their growth and mineralize and detoxify those (Kanekar *et al*., 2004). Microbial degradation, metabolites of degradation, enzymatic degradation and genetic basis of degradation of widely used organo phosphorous insecticides, like parathion, methyl parathion, malathion, monocrotophos and dimethoate are studied extensively. Insect gut microbes, especially insecticide-resistant pest, might play a role in detoxifying the insecticides. Microbial degradation of organo phosphorous insecticides have been reported in soil and consortia. Pure culture studies have shown cleavage of molecules and formation of different metabolites such as p-nitrophenol, diethylthiophosphoric acid, p-aminophenol, dimethyl phosphate.Verma *et al.* (2006) reported the degradation of endosulfan by a *Rhodococcus* strain isolated from gut of earthworm. Bacteria belonging to genera *Bacilli*, *Proteus*, *Micrococcus*, *Pseudomonas* and *Klebsiella* isolated from the gut of rice hispa, *Dicladispa armigera* (Olivier) were shown resistant to 1000 ppm endosulfan and to some extent chloropyriphos and quinalphos (Thakur *et al*., 2005). Carboxylesterases isolated from *H. armigera* play a role in pyrethroids detoxification (Bai *et al*., 2019). *H. armigera* larva fed with laccasses from *Yersinia enterocolitica* showed significant expression of enzymes and proteins for xenobiotic degradation (Ahlawat *et al.,* 2020).

Chemical insecticides are the most widely released xenobiotics by humankind into the environment, resulting in very high contamination of food and water resources with these compounds. Many insects and bacteria have developed resistance to these compounds by evolving enzymes to metabolize these xenobiotics. In-depth research is required to identify potential candidates with respect to gut microbes in the degradation of insecticides/xenobiotics. This study was carried out to determine the influence of gut microflora isolated from *H. armigera* in insecticidal resistance.

MATERIALS AND METHODS

Collection of field larvae

The culture of the *H. armigera* was initiated by collecting about 100 - 150 larvae on cotton (*Gossypium hirsutum* L., Kanchana variety) from the farmers' fields in Vikarabad (Andhra Pradesh-Lat-17º19'60N; Lon-77º55'0E), where insecticides were used frequently. On cotton, efforts were made to collect the larvae from non -Bt cotton, which was sprayed both for lepidopteran and sucking pests. Fourth- and fifth-instar larvae were collected in the plastic container with an artificial diet and brought to the laboratory and were used for the study. The insecticide use pattern was recorded at each place as per the protocols of the National Bureau of Agriculturally Important Resources, ICAR, Bangalore.

Rearing of field and laboratory populations of *H. armigera*

The collected larvae were brought to the National Bureau of Agriculturally Important Insects (NBAII) laboratory, Bangalore. The larvae were transferred onto a freshly prepared semi synthetic diet (Nagarkatti and Satyaprakash, 1974). After pupation, the pupae were collected, surface sterilized in sodium hypochlorite (0.1%) and washed in distilled water. The washed pupae were air-dried at room temperature and kept in plastic jars for the emergence of moth. From the emerged moths, 25 pairs were transferred to each oviposition cage lined with muslin cloth and covered with a black cotton cloth. Two balls of absorbent cotton, one with water and another one with 50% honey solution, were provided in the oviposition cage. The eggs were collected from the cages every 24 h were washed in sodium hypochlorite (0.1%) and formaldehyde (1%) solution, filtered through a black cloth and tagged onto the plastic container (7 cm dia., 4 cm height) for hatching at 27±2˚C and 70% RH.

The newly hatched neonates were transferred to 128 well diet trays containing solidified diet at 1 larva well^{-1} using a fine hairbrush and were covered with pull-n-peel tabs. Both diet trays and neonates transfer were carried out on a laminar airflow clean bench followed by maintenance in a BOD incubator maintained at 27±2˚C and 14:10 h light-dark. Fifty pupae of the susceptible strain of the *H. armigera* were also obtained from the Mass Production Unit of NBAIR, Bangalore, where it is reared on semi-synthetic diet for 10 years without replacing or adding any field population, hence was considered true susceptible populations. These pupae were also reared till the emergence of the adults and thus, the culture was further multiplied to be used in the bioassay studies with the same method as that of the field collected *H. armigera*. A homogenous stock of fifth instar larvae (10-11 days old) was for isolating the gut bacteria.

Isolation of gut bacteria from *H. armigera*

For the isolation of gut bacteria, ten larvae of *H. armi-*

gera were immobilized in chloroform and sterilized in sodium hypochlorite (0.1%) and ethanol (70%) solution for 5 seconds to remove the contaminants (Gebbardi *et al*., 2001). The sterilized larvae were dissected under aseptic conditions in a Laminar air flow hood to remove the entire gut. The gut was placed in a micro tube containing 500 µl of sterile peptone water, crushed mechanically and vortexed thoroughly. A hundred µl of the gut homogenate was placed on sterile nutrient agar and nutrient glucose agar plates in replicates and incubated at 30ºC for 48 h. The colonies obtained after 48 h incubation in the plates were further screened for colony morphology.

Bacterial DNA isolation

Bacterial genomic DNA was isolated as per the standard protocol (Hoffman and Winston 1987). A single colony from each of the bacterial cultures was inoculated in nutrient broth and grown for 48 h at 30ºC. The cells were harvested by centrifugation and added with 100 µl of lysozyme followed by incubation at room temperature for 30 mins to disrupt the cell membrane. The cells were lysed by the addition of lysis buffer (70 µl) containing guanidium isothiocyanate, SDS and tris-EDTA. The contents were mixed by inverting and 700 µl of isopropanol was added from the top. The two layers were gently mixed until the DNA strands were visible and the addition of ice cold ethanol precipitated the extracted DNA. The DNA was resuspended in 100 µl of TE buffer and the quality of DNA was checked in agarose gel electrophoresis. The purified genomic DNA was used as template for 16S rRNA gene amplification.

Oligonucleotide primers

16S rRNA gene primers were procured from Aristogene Biosciences (P) Ltd, Bangalore. The oligonucleotides were reconstituted to 100 ng/µl stocks in sterile TE buffer. The primers were used at a working concentration 100 ng/µl in sterile filtered distilled water. The sequences of the primers were as follows

Forward primer – 5'-ACTCCTACGGGAGGCAGCAG-3'
Reverse primer – 5'-ATTACCGCGGCTGCTGG-3'

Amplification of 16S rRNA gene by PCR

Gradient PCR was used to determine the optimum annealing temperature. The reaction mixture contained 100 ng of the extracted DNA, 1X PCR assay buffer (250 mM Tris-HCl, 10 mM KCl, 1.5 mM $MgCl_2$), 100 mM dNTP's, 100 ng/µL each of forward and reversed primers, 1 unit of *Taq* DNA polymerase (Sigma Aldrich, India). PCR performed with forward and reverse primers involved an initial denaturation for 2 mins at 94ºC, followed by 30 cycles of 94ºC denaturation for 1 min, 58ºC for annealing for 30 s and extension at 72 ºC for 1 min 30 s. Finally, the reactions were healed at 72ºC for 5 min. Specific and optimum amplification of the gene was seen at 58 ºC of annealing temperature. Subsequently, the gene was amplified at 58ºC and the amplified PCR product (1.5 Kb) was purified from low melting agarose gel as per the standard protocols (Sambrook *et al*. 2001) for further sequencing at Aristogene Biosciences (P) Ltd, Bangalore. The obtained sequences were analyzed through BLAST search and the bacterial species were identified.

Media preparation for insecticide degradation studies

The bacterial colonies isolated were tested for the selected eight commercial insecticides against chlorpyriphos, cypermethrin, malathion, quinalphos and triazophos, the most commonly used insecticides against *H. armigera* at a concentration range of 0.2, 0.4, 0.6, 0.8 and 1.0 ml/l, respectively. Initial studies were carried out on the bacterial isolates for their growth on Minimal Salt Medium (MSM) with and without insecticides. The composition of MSM included KH_2PO_4 1.0 g, K_2HPO_4 1.0 g, NH_4NO_3 1.0 g, $MgSO_4.7H_2O$ 0.2 g, $CaCO_3$ 0.02 g, $FeSO_4$ 0.01 g in 1 L of distilled water (pH 6.5). The isolated bacteria from the guts of lab and field larval populations *H. armigera* were purified from nutrient agar plated in duplicates and incubated at room temperature (28 ± 1ºC) for 48 h. Isolated colonies were picked and streaked onto nutrient agar plates to obtain pure cultures. All the pure bacterial isolates were tested for their growth on MSM with and without insecticides. Different concentration of selected insecticides as mentioned above was added into sterile MSM (agar-based) and poured into Petri plates in Laminar airflow. The Petri dishes were labelled according to the concentration of selected insecticides after solidification. Similarly,MSM plates were also prepared without insecticides. All the bacterial isolates were streaked onto these plates in aseptic conditions and were incubated at room temperature (28 ± 1ºC) for 48 h. Growth of the bacterial isolates on all the plates were recorded after 48 h. Bacterial isolates growing in a particular concentration of insecticide was isolated and subcultured on nutrient agar to obtain a pure culture. Positive cultures for growth on MSM with insecticide (0.2 ml/l) were further used for degradation studies. A series of 250 ml flask containing 50 ml of MSM (broth) supplemented with above mentioned insecticides at 0.2 ml/l concentration was incubated separately with 1 ml overnight grown positive cultures and incubated on a shaker 200 rpm at 30ºC for 10 days. Controls for each bacterium were maintained without insecticides and positive control of MSM with insecticides alone was also maintained. After 10 days incubation CFU count was recorded on nutrient agar plates. The cell mass in MSM with and without insecticides was separated by centrifugation at 8000 xg for 10 min and supernatant of 50 ml was collected separately in the fresh tubes. The super-

natant was further analyzed in GC to detect the concentration of insecticides at the GC-MS facility available at Bangalore Test House, Bangalore.

Gas Chromatography analysis of minimal salt media with insecticides

The samples containing insecticide and without insecticide were extracted with methylene chloride solvent, initially using 100 ml solution initially. Later the volume of 50 ml twice was used to partition the sample. The pooled extract was collected in the separate round bottom flask after passing through anhydrous sodium sulphate. The content was evaporated to dryness using a vacuum rotary evaporator and a volume of 2 ml of acetone was added to the residue. One µl of this solution was injected into GC-ECD.

GC Conditions

Instrument: Agilent 6890N GC with 975 inert MSD
Software: MSD chemstation D.02.00.275 with NIST Library Ver.2.0d
Column: DB-35 MSI, 30 m X 0.25 mm X 250 micron
Inlet Temperature: 250ºC
Injection Mode: Split less
Injection Volume: 1 µl
Carrier gas flow: Helium (constant flow) 1.0 ml/min
Oven program: 70ºC for 2 min, @ 4ºC/min, 160ºC for 5 min, 4ºC/min
Final temp.: 295ºC for 10 min.
Transfer line temp.: 280ºC
The concentration of insecticides in MSM broth cultures was calculated using the formula

$$\frac{\text{Sample peak area X Std. Conc. (µg/ml) X dilution}}{\text{Standard peak area}} \quad \text{.....Eq. 1}$$

Working std. conc. (µg/ml): 1.2 µg/ml
Dilution (ml): 13 µl of insecticide formulation was diluted to 25 ml using acetone and 1.0 µl was injected into GC-ECD instrument.

RESULTS AND DISCUSSION

Isolation of gut bacterial flora from the insecticide resistance larval populations collected from cotton and susceptible lab populations of *H. armigera*

Gut bacteria isolated from the gut of insecticide-resistant fourth and fifth instar larvae of *H. armigera* collected from the cotton fields of Vikarabad, Andhra Pradesh were coded as CL1, CL2, CL3, CL4, CL5, CL6 and CL7. Gram staining of the bacterial isolates showed that isolates CL1, CL3 were Gram +ve rods, isolates CL2, CL4, CL6 were Gram +ve cocci and isolates CL5, CL7 were Gram –ve rods. Gut bacteria was isolated from the gut of susceptible fourth and fifth instar larvae of *H. armigera* collected from the mass production unit of NBAII, Bangalore was coded as HL1, HL2, HL3, HL4, HL5 and HL6. Gram staining of the bacterial isolates showed that the isolates HL1, HL3, HL5, HL6 were Gram – ve rods and isolates HL2, HL4 were Gram +ve rods.

Isolation of DNA and 16s rRNA PCR amplification of gut bacteria of insecticide-resistant and susceptible larvae of *H. armigera*

DNA was isolated from eleven bacterial isolates from the gut of insecticide-resistant field larvae of *H. Armigera.* The presence of genomic DNA from all the seven isolates was confirmed on a 0.8% agarose gel stained with ethidium bromide (Fig. 1). An intense single band was seen in all seven wells along with the DNA marker. The extracted DNA was used as a template for amplification of 16S rRNA gene. The primers selected were specific. Initial standardisation by gradient PCR has facilitated the specific amplification as observed by high intense band. The optimum annealing temperature was found to be 58ºC and an intense single band of size approximately 1.5 kb was visible on 1% agarose gel stained with ethidium bromide (Fig. 2) in all the seven wells. Similarly, DNA was isolated from six bacterial isolates from the gut of susceptible larvae of *H. armigera*. The presence of genomic DNA from all the 6 isolates was confirmed on a 0.8% agarose gel stained with ethidium bromide (Fig. 3). An intense single band was seen in all six wells along with the DNA marker. The extracted DNA was used as the template for amplification of 16S rRNA gene. The primers selected were specific. The optimum annealing temperature was found to be 58ºC. An intense single band of size approximately 1.5 kb was visible on 1% agarose gel stained with ethidium bromide (Fig. 4) in all 6 wells.

Sequencing and sequence analysis of PCR amplified 16S rRNA gene of the eleven gut bacterial isolates

The partial sequence obtained from all the seven isolates ranged from 677, 660, 478, 459, 642, 514. 559 bp respectively in length and were analysed in BLASTn (www.ncbi.nlm.nih.gov) and the bacterial genera and species were determined. The partial 16S rRNAs sequence and the determined bacterial sp. along with the accession number (CL1-CL7) are shown in Table 1. The max identity of the sequence was 99-100%. The nucleotide sequences of seven isolates are submitted to NCBI-Gen Bank and the accession numbers were obtaind. The determined bacterial communities were found to be **CL1**-*Bacillus pumulis,* **CL2**-*Enterococcus casseliflavus,* **CL3**-*Bacillus subtilis,* **CL4**-*Rhodococcus* sp., ***CL5***-*Pseudomonas* sp., **CL6**-*Staphylococcus* sp.*,* **CL7**-*Pseudomonas aeruginosa.*

The partial sequence obtained from all the 6 isolates

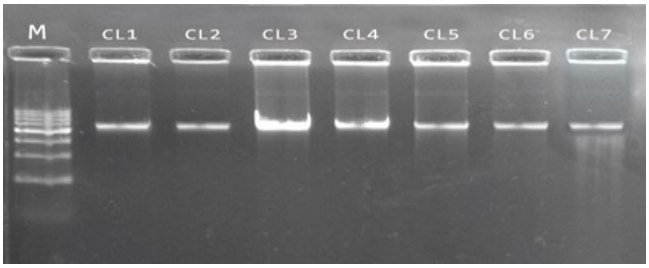


Fig 1. *Agarose gel electrophoresis of genomic DNA from seven bacterial isolates from gut of H. armigera from cotton crop. M- Standard DNA Marker, CL1 – CL7 – Genomic DNA of the bacterial isolates.*

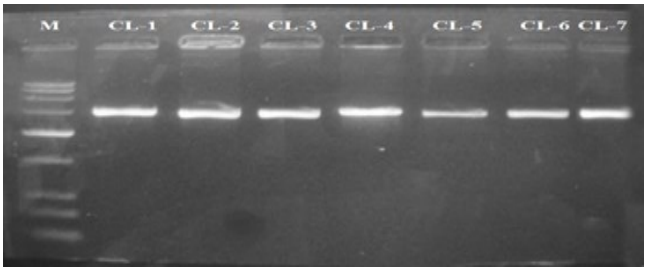


Fig. 2. *Agarose gel electrophoresis of 16S rRNA PCR amplicon from seven bacterial isolates from gut of H. armigera from cotton crop. M- 0.1-3 kb Low range marker: sizes- 100bp, 200bp, 300bp, 600bp, 1kb, 1.5kb, 2 kb, 2.5kb, 3 kb)., CL1 – CL7 – 16S rRNA PCR amplicon of the bacterial isolates.*

from the gut of the susceptible larvae of *H. armigera* ranged from 664, 861, 779, 623, 541, 693 bp. in length The partial 16S rRNAs sequence and the determined bacterial sp. along with the accession number (HL1-HL6) areshown in Table 2. The max identity of the sequence was 99-100%. The nucleotide sequences of 6 isolates were submitted to NCBI-Gen Bank and the accession numbers were obtained. The determined bacterial communities were found to be **HL1**-*Proteus vulgaris,* **HL2**-*Cellulosimicrobium cellulans,* **HL3**-*Klebsiella oxytoca,* **HL4**-*Bacillus subtilis,* **HL5** *Stenotrophomonas maltophilia,* and **HL6**-*Pseudomonas* sp.

Insecticide degradation studies by the gut microbial isolates from susceptible lab and field populations of *H. armigera*

All the five insecticides were selected for the insecticide degradation studies. Growth of the bacterial isolates recorded after 7 days of incubation at 30ºCis shown in Table 3. Among a total of 11 bacterial isolates tested, isolate CL4 (*Rhodococcus*sp.) was found to grow on minimal salt medium (MSM) and with chlorpyriphos and isolate CL2 (*Enterococcus casseliflavus*) was able to grow in MSM with chloropyriphos and malathion and no growth was seen in MSM without insecticide (control). The growth of isolates CL4 and CL2 on MSM without insecticide (control) and with insecticides is shown in Plate 1. Bacterial isolates CL4 and CL2 were further grown in MSM broth without and with insecticides and incubated in shaker at 30ºC for 7 days, after incubation 0.1 ml of the culture was plated on to sterile nutrient agar plates and were incubated at 30ºC for 48 h and the number of CFU was calculated along with control and data is shown in Table 4 GC graph results have been shown for MSM with insecticides, MSM with isolate CL4 without insecticide and with chlorpyiphos and MSM with isolate CL2 without insecticides and with chlorpyriphos and malathion in Figure 5, 6, and 7 respectively. The concentration of the insecticides in the MSM broth cultures along with the control are shown in Table 5. GC graph analysis showed that there was decrease in the concentrations of the insecticides in MSM broth cultures with insecticides when compared to that of MSM broth cultures without insecticides. The isolate CL4 (*Rhodococcus* sp.) was able to utilize 43.9% of chlorpyriphos and isolate CL2 (*Enterococcus casseliflavus*) was able to utilize 26% of chlorpyriphos and 57.1% of malathion in MSM broth cultures with comparison with the respective control cultures. Findings of the current results suggested that gut bacteria in the field populations of *H. armigera* is having a role in insecticide resistance.

Broderick *et al*., (2004) described the isolation and

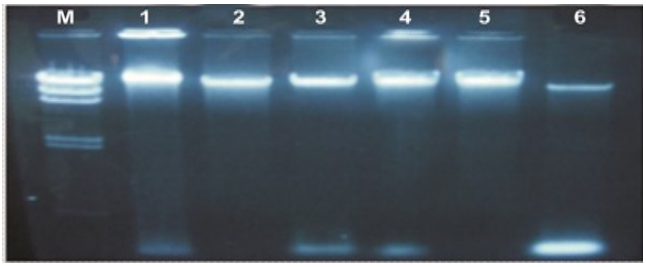


Fig. 3. *Agarose gel electrophoresis of genomic DNA from six bacterial isolates from the gut of larvae of laboratory populations of H. armigera. Lane M –Marker - Lambda/ HindIII digest (sizes-23130, 9416, 6557, 4361, 2322, 2027, 564). Lane 1 – 6 – Genomic DNA from 6 bacterial isolates.*

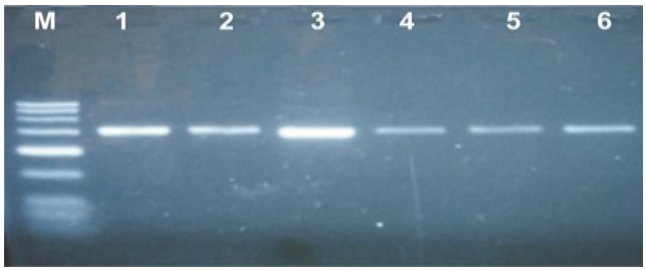


Fig. 4. *Agarose gel electrophoresis of 16S rRNA PCR amplicon from six bacteria isolates from gut of the larvae of laboratory populations of H. armigera. Lane M- Marker - 0.1-3 kb Low range marker: sizes- 100bp, 200bp, 300bp, 600bp, 1kb, 1.5kb, 2 kb, 2.5kb, 3 kb)., Lane - HL1 – HL6 – 16S rRNA PCR amplicon of the bacterial isolates, 1=HL1, 2=HL2, 3=HL3, 4=HL4, 5=HL5, 6=HL6.*

Table 1. Partial 16S rRNA sequence and identified bacterial species with GenBank accession number of the seven bacterial isolates from the gut of insecticide resistance field larval populations of *H. armigera* collected from cotton crop.

Isolate	Partial 16S rRNA gene sequence	Size (bp)	Identified Bacteria By BLASTn	GenBank Accession Number
CL1	GTGCATTGCGGGTGCTATACATGCAAGTCGAGCG- GACAGAAGGGAGCTTGCTCCCGGGTGTTAGCGGCGGACGGGT GAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCC GGGAAACCGGAGCTAATACCGGATAGTTCCTTGAACCGCATGG TTCAAGGATGAAAGACGGTTTCGGCTGTCACTTACAGATGGACC CGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGC GACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGG GACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGG GAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCG TGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGA AGAACAAGTGCAAGAGTAGCTGCTTGCACCTTGACGGTACCTAA CCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATA CGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGC TCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAA CCGGGGAGGGTCATTGGAAACTGGAAACTTGATTGCAGAAGAG GAGAGTGTAATTCCACGTGTAGCGGTGAAATGCGAAAAAA	677	*Bacillus pumulis*	HQ651050
CL2	GATTATGGCTCAGGACGAACGCTGGCGGCGTGCG- TAATACATGCAAG TCGAACGCTTTTTCTTTCACCG- GAGCNTGCTCCANCGNAAGAAAAAGAGTGGCGAACGGGTGAGT AACACGTGGGTAACCTGCCCATCAGAAGGGGATAACACTTGGA AACAGGTGCTAATACCGTATAACACTATTTTCCGCATGGAAGAA AGTTGAAAGGCGCTTTTGCGTCACTGATGGATGGACCCGCGGT GCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGAT GCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAG ACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTC GGCAATGGACGAAAGTCTGACCGAGCAACGCCGCGTGAGTGAA GAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAAG GATGAGAGTAAAATGTTCATCCCTTGACGGTATCTAACCAGAAA GCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGT GGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGG CGGTATCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGG AGGGTCATTGGAAACTGGGAGACTTGAGT	660	*Enterococcus cas-seliflavus*	HQ651051
CL3	TGCAGTCGAGCGGACAGATGGGAGCTT- GCTCCCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTA ACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAA TACCGGATGGTTGTTTGAACCGCATGGTTCAAACATAAAAGGTG GCTTCGGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTA GTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGA CCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCC AGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGA CGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTT CGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTACCGTTCG AATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGC TAACTACGTGCCAGCAGCCG	478	*Bacillus subtilis*	JF266592
CL5	GGGCCCGTGGGGGAGGACTACACATGCAAGTCGAGCG- GATGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGACGGGTGA GTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGG AAACGGGCGCTAATACCGCATACGTCCTGAGGGAGAAAGTGGG GGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGGATTA GCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAA CTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGG TCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAAT GGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGT CTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAG TTAATACCTTGCTGTTTTGACGTTACCAACAGAATAAGCACCGG CTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGC GTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCA GCAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCA TCCCAAAACTACTGAGCTAGAGTACGGTAGAGGGTGGTGGAAA T	642	*Pseudomonas* sp.	HQ651053

Contd……..

Table.1. Contd…...

CL6	GTCTGCGGCATGCTATACATGCAGTCGAGCGAACAGACGAGGAGCTT-GCTCCTTTGACGTTAGCGGCGGACGGGTGAGTAACACGTAGGTAACCT ACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGATAA TATTTCGAACCGCATGGTTCGATAGTGAAAGATGGCTTTGCTATCACTTA TAGATGGACCTGCGCCGTATTAGCTAGTTGGTAAGGTAACGGCTTACCA AGGCAACGATACGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGAA CTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTC CGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGATGAAG GTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACAAACGTGTAAGTA ACTGTGCACGTCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACG TGCCAGCAGCCGCGGTAATACGTAGGTGG	514	*Staphylococcus sp.*	HQ651054
CL7	CTGTTCTGTCGGCAGCTAGACATGCAAGTCGAGCG-GATGAAGGGAGCTTGCTCCTGGATTCAGCGGCGGACGGGTGAGTAATG CCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCT AATACCGCATACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCA CGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAAAGG CCTACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACA CTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGA ATATTGGACAATGGGCGAAGCCTTGATCCAGCCATGCCGCGTGTGTGAA GAAGTCTTCGGATTGTAAACACTTTAAGTTGGGAGAAGGCAGAAGTTAA TACCTTGGTTGTTTTGACGTACCACAGAATAAGCACCGGCTACTTCTGC CGCAGCCGCGTATACCAAGGTGCAGCGTAATTCGAATTACTGGCGTAAG CGCGCGAAGGGTTCACAGGTGGAAGGGAATCCCCGGCTC	559	*Pseudomonas aeruginosa*	HQ651055

characterization of gut microflora from Lepidoptera and the present study isolated the gut bacterial flora of *H. armigera* using culture dependent methods.Bacterial isolates were identified using 16S rRNA gene based molecular technique. Different gut bacteria belonging to different genera were isolated and identified from field and lab larval populations of *H. armigera*. Some of the similar gut bacterial isolates have been also isolated from *H. armigera* species in India (Mishra and Tandon, 2003; Priya *et al*., 2012) in China (Xiang *et al*., 2006). Actinobacteria, proteobacteria and firmicutes were the dominant bacteria in *H.armigera* fed on tomato plants (Ranjith et al., 2016). In another study, high prevalence of Gammaproteobacteria followed by Bacilli and Actinobacteria were detected in the *H. armigera* midgut (Paramasiva et al., 2014). Dar et al., (2018) isolated *Klebisiella* sp, cellulolytic bacteria, from gut of *H. armigera* with xylanase and β-glucosidase activities. Apart from bacterial population, a novel picorna-like virus, named *Helicoverpa armigera* Nora virus was found in the gut of *H. armigera* moths and larvae (Yang et al., 2019). Similarly, Ifla virus was isolated from *H. armigera* by Yuan *et al.* (2017). Higher animals like humans contain gut bacterial diversity in the range of 1500-2500. Further, the number of gut bacteria is ten times higher than the total number of host cells and the bacteria have more than ten times the metabolic diversity (Dillon and Dillon, 2004). It was also reported that sexual performance, mating preferences, and oviposition are influenced by the gut microflora (Gavriel *et al*., 2010; Sharon *et al*., 2010).

In order to decipher the non-pathogenic interaction between the bacteria and the host, we need first to study the gut flora of the host. In the present study, culture dependent studies showed that the field collected and the lab reared population showed significant differences in bacterial population, which is in agreement with the previous studies (Xiang *et al*., 2006). Rani *et al*., (2009) reported that the difference in lab reared and field insects is due to the fact that the laboratory raised insects are exposed to narrower range of food and environmental factors when compared to the field collect-

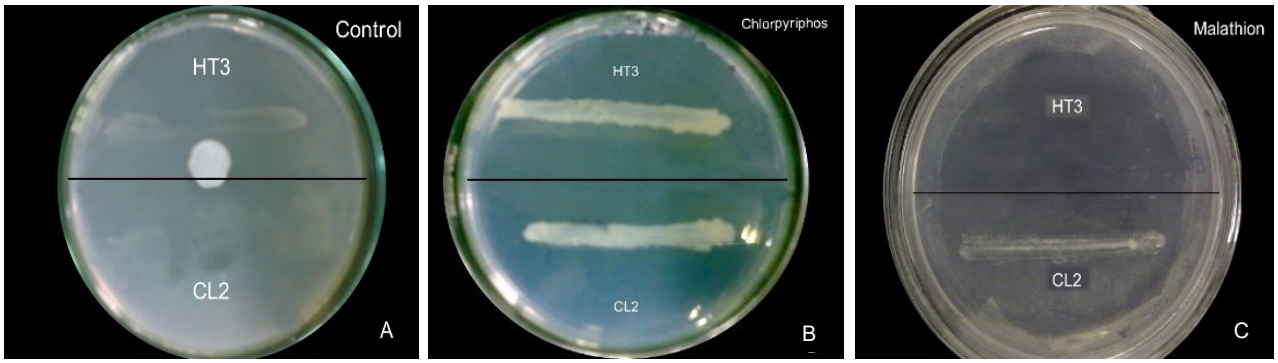


Plate 1. *Growth of bacterial isolate CL4 and CL2 on MSM with insecticides. CL4 – Rhodococcus sp. CL2 - Enterococcus casseliflavus. A – Control, B - MSM with Chlorpyriphos, C – MSM with Malathion.*

Table 2. Partial 16S rRNA sequence and identified bacterial species with GenBank accession number of the six bacterial isolates from the gut of susceptible laboratory larval populations of *H. armigera*.

Isolate	Partial 16S rRNA gene sequence	Size (bp)	Identified Bacteria By BLASTn	GenBank Accession Number
HL1	CATGCAGTCGAGCGGTAACAGGAGAAAGCTTGCTTTCTT-GCTGACGAGCGGCGGACGGGTGAGTAATGTATGGGGATCTGCC CGATAGAGGGGGATAACTACTGGAAACGGTGGCTAATACCGCAT GACGTCTACGGACCAAAGCAGGGGCTCTTCGGACCTTGCGCTAT CGGATGAACCCATATGGGATTAGCTAGTAGGTGAGGTAATGGCT CACCTAGGCAACGATCTCTAGCTGGTCTGAGAGGATGATCAGCC ACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGC AGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATG CCGCGTGTATGAAGAAGGCCTTAGGGTTGTAAAGTACTTTCAGC GGGGAGGAAGGTGATAAAGTTAATACCTTTGTCAATTGACGTTAC CCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGT AATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAG CGCACGCAGGCGGTCAATTAAGTCAGATGTGAAAGCCCCGAGCT TAACTTGGGAATTGCATCTGAAACTGGTTGGCTAGAGTCTTGTAG AGGGGGGTAGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATG TGAGGAATAC	664	*Proteus vulgaris*	JF266593
HL2	CATGCAGTCGAACGATGATGCCCAGCTTGCTGGGCGGATTAG-TGGCGAACGGGTGAGTAACACGTGAGTAACCTGCCCTTGACTTC GGGATAACTCCGGGAAACCGGGGCTAATACCGGATATGAGCCG CCTTCGCATGGGGGTGGTTGGAAAGTTTTTCGGTCAGGGATGGG CTCGCGGCCTATCAGCTTGTTGGTGGGGTGATGGCCTACCAAGG CGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTG GGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGG GAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGT GAGGGATGAAGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAA GAAGCGCAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTA CGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCC GGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCT GGTGTGAAAACTCGAGGCTCAACCTCGAGCTTGCATCGGGTACG GGCAGACTAGAGTGCGGTAGGGGAGACTGGATTTCCTGGTGTAG CGGTGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGG CAGGTCTCTGGGCCGCAACTGACGCTGAGGAGCGAAAGCATGG GGAGCGAACAGGATTAGATACCCTGGTTAGTCCATGCCGTAAAC GTTTGGCACTAGGTGTGGGGGC	861	*Cellulosimicrobium cellulans*	JF266594
HL3	ACGCTGGCGGCAGGCCCTAACACATGCAAGTCGAACGGTGAG-CACARAAGAGCTTGCTCTCGGGTGACGAGTGGCGGACGGGTGA GTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGA AACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGG ACCTTCGGGCCTCTTGCCATCAGATGTGCCCAGATGGGATTAGC TAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTG GTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCA GACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGC GCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCG GGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCGATAAGGTTAAT AACCTTGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAAC TCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTAAT CGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTC GGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAAC TGGCAGGCTGGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTA GCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAG GCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTG GGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCT	779	*Klebsiella oxytoca*	JF266595
HL4	TACATGCAGTCGAGCGGACAGATGGGAGCTT-GCTCCCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAA CCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATA CCGGATGGTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGCT TCGGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTG GTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGA GAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTC CTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGT CTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGT AAAGCTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGC GGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGT GCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGA ATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGAT GTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGG GGAACTTGAGTGCAGAAGAGGA	623	*Bacillus subtilis*	JF266596

Contd……..

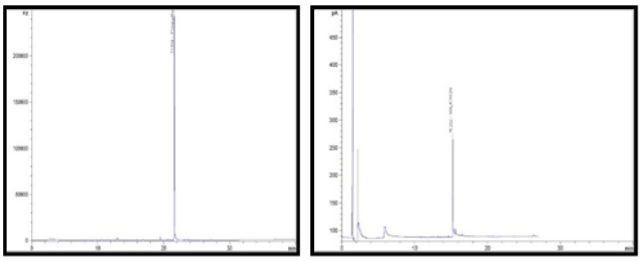
Table 2. Contd……

HL5	ACATGCAAGTCGAGCGGCAGCACAGGAGAGCTT- GCTCTCTGGGTGGCGAGTGGCGGACGGGTGAGGAATACATCGG AATCTACTCTGTCGTGGGGGATAACGTAGGGAAACTTACGCTAAT ACCGCATACGACCTACGGGTGAAAGCAGGGGACCTTCGGGCCT TGCGCGATTGAATGAGCCGATGTCGGATTATCTAGTTGGCGGGG TAAAGGCCCACCAAGGCGACGATCCGTAGCTGGTCTGAGAGGAT GATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGG AGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATC CAGCCATACCGCGTGGGTGAAGAAGGCCTTCGGGTTGTAAAGCC CTTTTGTTGGGAAAGAAATCCAGCTGGCTAATACCCGGTTGGGAT GACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAG CCGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGG CGTACAGCGTGCGTAGGTGGTCGTT	549	*Stenotropho-monas maltophilla*	JF266597
HL6	GTCGAGCGGCAGCACGGGTACTTGTAC- CTGGTGGCGAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTG CCTGGTAGTGGGGGATAACGCTCGGAAACGGACGCTAATACCG CATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTGCG CTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAAT GGCTCACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATC AGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGC AGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCC ATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAA GTTGGGAGGAAGGGCAGTTACCTAATACGTATCTGTTTTGACGTT ACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCG GTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAA AGCGCGCGTAGGTGGTTTGTTAAGTTGAATGTGAAATCCCCGGG CTCAACCTGGGAACTGCATCCAAAACTGGCAGGCTAGAGTATGG TAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGAT ATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATA CTGACACT	693	*Pseudomonas sp.*	JF266602

ed counterparts. However, a considerable difference in types of colonizing species was observed between the insects raised on artificial dietand the field collected ones.

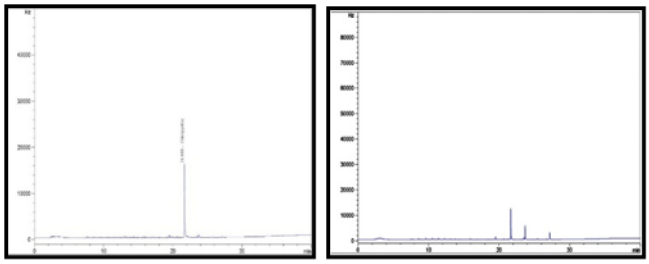
As previously described in other lepidopterans (Broderick *et al*., 2004; Xiang *et al*., 2006; Priya *et al*., 2012), the current study has also found *Enterococcus* in abundance when compared to rest of the phylotypes. The presence of *Enterococcus* in the gut benefits the host by reducing the gut pH thereby providing alkaline conditions that have a role in toxin effects (Wilson and Benoit, 1993). It was also identified that some of the gut microflora are involved in the degradation of polycyclic aromatic hydrocarbons (Lei et al., 2004) and insecticides (Hao et al., 2002). Other factors that influence the microbial diversity of the insect gut are host plant type and the host plant's geographical location. Therefore, artificial diet can therefore be considered a poor diet compared to field insects' diet which ultimately influences the gut microflora diversity. As mentioned earlier, crop leaf surface influences the gut bacterial flora of Helicoverpa larvae. The phyllosphere bacteria offers functional resistance to its host plant and influences the gut bacterial community of the insect larva feeding on it (Rajendran *et al*. 2007). Further analysis of these systems may identify as to what other factors contribute to the significant variation in gut bacteria among larvae from different crops from different locations.

In the present study,eleven gut bacterial isolates from the gut of larvae of the insecticide resistant field and susceptible lab populations of *H. armigera* were tested for their growth on minimal salt media with chlorpyriphos, cypermethrin, malathion, quinalphos, and triazophos along with the control MSM without insecticides. The result analysis reveals that the bacterial isolates *Rhodococcussp.* and *Enterococcus casseliflavus* from gut of the field populations of *H. armigera* were able grow on an MSM medium with chlorpyriphos



A B

Fig. 5. *GC analysis of MSM with Chloropyriphos and Malathion, A- Chlorpyriphos B- Malathion*



A B

Fig. 6. *GC analysis of MSM with Chloropyriphos and bacterial isolate HT3, A- Chlorpyriphos B- Control.*

Table 3. Growth of bacterial isolates on MSM medium with insecticides from the gut of *Helicoverpa armigera.*

Bacterial Isolates	Isolate Name	MSM	MSM + Chlorpyriphos	MSM + Cypermethrin	MSM + Malathion	MSM + Quinalphos	MSM + Triazophos
CL1	*Bacillus pumillis*	–	–	–	–	–	–
CL2	*Enterococcus casseliflavus*	--	++	–	+	--	–
CL3	*Bacillus subtilis*	–	–	–	–	–	–
CL4	*Rhodococcus* sp.	–	++	–	–	–	–
CL5	*Pseudomonas* sp.	–	–	–	–	–	–
CL6	*Staphylococcus* sp.	–	–	–	–	–	–
CL7	*Pseudomonas aeruginosa*	–	–	–	–	–	–
HL1	*Proteus vulgaris*	–	–	–	–	–	–
HL2	*Cellulosimicrobium cellulans*	–	–	–	–	–	–
HL3	*Klebsiella oxytoca*	–	–	–	–	–	–
HL4	*Bacillus subtilis*	–	–	–	–	–	–
HL5	*Stenotrophomonas maltophilla*	–	–	–	–	–	–
HL6	*Pseudomonas* sp.	–	–	–	–	–	–

MSM: Minimum Salt Medium, +: Good growth, ++: Very good growth, −: No growth
CL1-CL2: Bacterial isolates from the larval gut of insecticide resistant field larvae of *H. armigera* from cotton. HL1-HL2: Bacterial isolates from the larval gut of lab populations of *H. armigera.*

($C_{22}H_{19}Cl_2NO_3$) and malathion ($C_{10}H_{19}O_6PS_2$). From the analysis of the Gas Chromatography, the isolate CL4 (*Enterococcus* sp.) was able to utilize 43.9% of chlorpyriphos and isolate CL2 (*Enterococcus casseliflavus*) was able to utilize 26% of chlorpyriphos and 57.1% of malathion in MSM broth cultures with comparison with the respective control cultures. This indicates that these bacteria can utilize the insecticides as carbon source and why these two bacteria can utilize chlorpyriphos and malathion and why not other insecticides is not clear until now and further studies have to be done in this line of research. Earlier reports have indicated the role of different species of *Enterobacteriacae* in degradation of organophophorous insecticides like chlorpyriphos (Singh *et al*., 2004), phosphates (Lee *et al*., 1992) and glyphosates (Dick and Quinn, 1995). Carboxylesterases isolated from *H. armigera* play a role in pyrethroids detoxification (Bai et al., 2019). H. armigera larva fed with laccasses from *Yersinia enterocolitica* showed significant expression of enzymes and proteins for xenobiotic degradation (Ahlawat et al., 2020). Thakur *et al*. (2005) showed that the bacterial isolates from the whole gut of the rice hispa, *Dicladispa armigera* (Olivler), belonging to genera *Bacillus, Proteus, Micrococcus, Pseudomonas and Klebsiella* were resistant to 1000 ppm endosulfan and to some extent chlorpyriphos and quinalphos. The GLC analysis showed that the isolated gut bacteria had the potential to degrade endosulfan. This work probably can be a support to the current research to show that insect gut bacteria are playing a role in insecticide resistance. The study shows the potential of symbiotic bacteria in imparting insecticide resistance to insect hosts. As organic phosphorous compounds are used so widely in agriculture for pest control, symbiont detoxification could represent a rapid and previously unappreciated mechanism for insecticide resistance in insects (Whalon *et al*., 2008). Given the general detoxification ability of microbes and their ability to evolve quickly, they could provide a potent means for rapid acquisition of insecticide resistance in hosts. Many insects harbor a robust complement of prokaryotes in their alimentary canals,

Table 4. Colony forming units of the bacterial isolates (CL4 and CL2) on nutrient agar.

MSM broth culture	CFU/ml
Control	00
MSM + CL4	3×10^{-4}
MSM + CL4 + Chlorpyriphos	7×10^{-4}
MSM + CL2	1×10^{-4}
MSM + CL2 + Chlorpyriphos	3×10^{-4}
MSM + CL2 + Malathion	2×10^{-4}

Table 5. Concentration of insecticides in MSM broth cultures with bacterial isolates CL4 and CL2 analysed by GC-ECD.

MSM broth culture	Chlorpyriphos Conc. (µg/ml)	Malathion Conc. (µg/ml)
Control	00	00
MSM	38.29	112.27
MSM + CL4	16.81	---
MSM + CL2	10.05	64.13

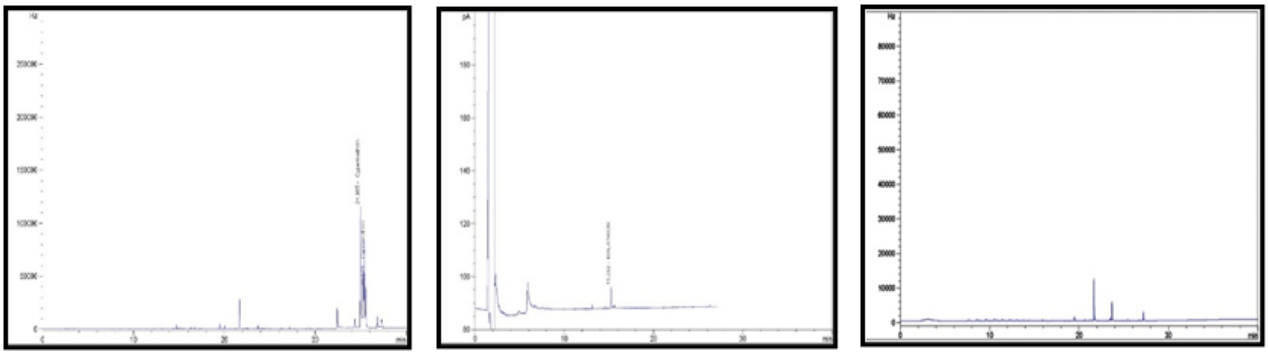


Fig. 7. *GC analysis of MSM with chloropyriphos, malathion with bacterial isolate CL2 A- Chlorpyriphos B- Malathion C - Control.*

which facilitate nutrient availability, utilization and detoxification of environmental toxins, degradation of insect diet components (Williams and Roans, 2006; Hayashi *et al.,* 2007). Earlier reports have also shown that the gut bacterial isolates of other insect species play an important role in insect growth and development. Jing *et al.,* (2020) described the roles of gut bacteria as essential nutrient provisioning, digestion and detoxification.*Bacillus thuringiensis* isolated from the gut region of *H. armigera* play a major role in inducing pathogenicity of Bt toxin in the host (Regode *et al.,* 2016). Carol *et al*., (2003) reported that the gut bacterial isolate *Enterobacter agglomerans* from the apple maggot fly was able to degrade and detoxifiy Dihydrochalcone Phloridzin, a plant derived compound toxic to *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae). Vesta *et al*.,(2006) reported that bark beetles *Ips typographus* (Coleoptera: Scolytidae) fed on conifers which produce myrcene (MR), among some other defensive compounds, six bacterial isolates from the gut of the bark beetles were most resistant to the bactericidal compound. 16S rRNA analysis of these bacteria showed that they are related to *Enterobacteriaceae.*Studies conducted by Genta *et al.* (2006) on antibiotic-treated and non-treated larvae of *Tenebrio molitor* suggested that microbial products play subtle roles in the life of the insect, digestion of refractory food, detoxification of secondary plant compounds and modify the volatile profiles of the insect host. Visotto *et al.,* (2009) treated the bacterial colonies isolated from the gut homogenate of velvetbean caterpillar, *Anticarsia gemmatalis* with tetracycline. They found that the antibiotic was sensitive to the bacteria and inhibited the bacterial proteases. Their results suggested that gut bacteria may significantly contribute to lipid- and mainly protein-digestion in velvetbean caterpillars. Anand *et al*. (2010) reported that the bacterial species *Proteus vulgaris, Klebsiella pneumoniae, Escherichia coli, Citrobacter freundii, Serratia liquefaciens, Enterobacter* sp., *Pseudomonas fluorescens, P. aeruginosa, Aeromonas* sp., and *Erwinia* sp. isolated from the gut of *Bombyx mori* L. were able to produce digestive enzymes that degrade the carbohydrates present in the mulberry leaves. In the current study, gut bacteria from laboratory populations of *H. armigera* and laboratory and field population of *T. chilonis* could not utilize the insecticides. It can be justified that they are not exposed to insecticides, whereas in the field populations one of the total isolated bacteria could utilize the insecticides for their growth. This might be because field populations are under constant stress of insecticides, which might induce the bacteria to produce enzymes to degrade and detoxify them. In field populations, only one per cent of the total bacterial isolates were able to utilize the insecticides and a possible explanation may be that microorganisms need an adaptation period to produce the necessary degradative enzymes (Jilani and Khan, 2004).

Conclusion

Enterococcus casseliflavus and Rhodococcus sp isolated from the gut region of *H. armigera* were able to grow in the presence of selected insecticides. Concluding from the current study results, it can be stated that gut bacteria in insecticide-resistant field populations of *H. armigera* play a significant role in insecticide resistance of the host. Understanding the insect gut microbial diversity, their functional roles in insect growth and development would offer crucial information for designing future pest management strategies.

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Conflict of interest

The authors declare that they have no conflict of interest.

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