Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(2): 1679-1683

Received: 12-01-2020
Accepted: 14-02-2020

Kondwani MsangoSoko
Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Rahul Chandel
Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Sakshi Gandotra
Care Pro Biosciences Pvt. Limited, Greater Noida, Uttar Pradesh, India

Kajal Yadav
Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Shubham Gambhir
Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Kirti Sharma
Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Sabtharishi Subramanian
Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Corresponding Author:
Kondwani MsangoSoko
Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Diversity of microbial groups associated with the gut of the eri silkworm, *Samia ricini*, (Lepidoptera: Saturniidae) and white grub, *Anamola dimidiata*, (Coleoptera: Scarabaeidae) larvae as revealed by phospholipid fatty acids analysis

Kondwani MsangoSoko, Rahul Chandel, Sakshi Gandotra, Kajal Yadav, Shubham Gambhir, Kirti Sharma and Sabtharishi Subramanian

Abstract
The phospholipid fatty acid (PLFA) technique was used to explore microbial groups that are associated with *S. ricini* and *A. dimidiata* larvae. A total of seven microbial groups were detected in the foregut, midgut and hindgut of *S. ricini*, respectively, as follows: Gram- bacteria (3.19%, 6.38%, 6.75%); Gram+ bacteria (15.3%, 15.5%, 13.5%); anaerobes (9.32%, 16.46%, 10.89%); Actinomycetes (1.13%. 2.0%, 0.62%); Methanotrophs (0.58%, 0.68%, 0.25%), micro-eukaryote (66.29%, 55.27%, 67.87%) and fungi (4.19%, 4.04%, 4.69%). Similar microbial groups detected in *S. ricini* were also found in the gut of *A. dimidiata* in varying proportions. This study has revealed that the gut of these two insects is associated with a diverse group of microbiota which may be critical to host physiology, and has generated a basic understanding of the diversity of microbial communities associated with these insects which will form a basis for further studies on the symbiotic status of these microbes.

Keywords: Phospholipid fatty acid analysis; microbial diversity; *S. ricini; A. dimidiata*

Introduction
Insects are the largest class of animals on earth found in almost all ecological niches, and thus encounter a wide range of challenges such as nutritionally poor and recalcitrant food diets, toxins, and threats from environmental extremes natural enemies [1, 2]. With their diverse environments and longtime co-evolution, they have developed amazing relationships with symbiotic microbes that are adapted specifically to insects as hosts. These microbes, which are particularly haboured in their gut are rich and complex with considerable metabolic activity that affect various developmental activities of the insect ranging from energy metabolism to being involved in providing immunity for the host [3, 4, 5]. Several insect orders including Coleoptera [6], Hemiptera [7], Hymenoptera [8], and Diptera [9, 10] have been reported to possess persistent bacterial symbionts. However, the gut microbiota of Lepidoptera one of the largest insect orders representing many economically important agricultural pests, pollinators, and biological models is sparsely described [11]. Out of the 157,424 recognized Lepidoptera species [12], <0.1% have been screened for bacterial symbionts, revealing the available limited knowledge on bacterial associates in Lepidoptera [13].
The eri silkworm, *Samia ricini*, is one of the five varieties of natural silkworms that is completely domesticated and commercially exploited in the Indian subcontinent. Eri culture (art and science of rearing eri silkworms and silk weaving) is an avowed agro- enterprise of North Eastern regions of India as it contributes to livelihood and nutritional security (as pupae of eri silkworm is consumed by ethnic communities of this region as a rich protein source) and to the manufacture of other medically important biomaterials [14, 15]. However, limited studies have been conducted to screen the beneficial gut-microbiota of *S. ricini* despite the significance of such in other organisms. Another insect Order, Coleoptera, contains the family Scarabaeidae with currently over 30,000 species [16]. The scarab beetles are economic pests of most agricultural crops in many parts of the world [17, 18, 19]. Scarabs are one of the major

edaphic invertebrates with an estimated biomass of 5-54 larvae m^{-2} reaching up to 600 larvae m^{-2} in severe outbreaks [20]. The guts of these saprophagous macro invertebrates also play a critical role in transforming soil organic matter and in carbon and nitrogen cycles of terrestrial environments. Many strategies are currently utilizing microbes based enzymes to degrade plant biomass for bioenergy production and scarab larvae have been suggested as a good model as they consume celluloses from multiple sources and have the ability to extract nutrients and energy from these sources with the help of endogenous enzymes complimented with proteinases and cellulolytic enzymes produced by symbiotic microbes. It is with this background that this study was conducted to explore the diversity of gut microbiome in *S. ricini* (Lepidoptera) and *A. dimidiate* (Coleoptera) by employing phospholipid fatty analysis (PLFA).

Materials and methods

Insect collection and rearing

Eri silkworm:

Eggs of eri silkworm were obtained from the Central Muga Eri Research and Training Institute, Jorhat, Assam and reared on caster leaves following the standard rearing method of [21] under a regime of 16:8 h light and dark period with rearing temperature of 26 ± 1ºC and 70% RH at the Division of Entomology, IARI, Pusa Campus; New Delhi, India, until hatching.

White grubs

The third instar *A. dimidiata* were collected from a potato field at the Indian Council for Agricultural Research (ICAR) regional station, the Central Potato Research Station (CPRS), Modipuram, UP, India. The insects were transferred to our laboratory in aerated plastic jars with field soil (with potato slices) and maintained under a regime of 16:8 h light and dark period with rearing temperature of 26 ± 1ºC and 70% RH at the Division of Entomology, IARI, Pusa Campus; New Delhi, India until they were dissected. The larvae were starved for 24 h to clear the gut prior to dissection.

Insect dissection and preparation of gut homogenates

White grubs

The larvae were rinsed in double distilled water for 30 s followed by 70% (v/v) ethanol for 60 s and again rinsed in double distilled water for 30 s to remove the disinfectant. The sterilized larvae were dissected using sterile microscissors under laminar flow to extract the gut. The extracted gut was separated into midgut (MG), anterior-hindgut (AHG), fermentation chamber (FC) and post-hindgut (PHG) and each compartment was briefly rinsed in sterilized 0.85% NaCl and placed in a sterile 1.5 ml eppendorf tube containing sterilized 0.85% NaCl and homogenized with a sterile homogenizer.

Eri silkworm

Third, fourth and fifth instar stages of *S. ricini* were used to study microbial groups using the PLFA technique. The larvae were rinsed in double distilled water for 30 s followed by 70% (v/v) ethanol for 60 s and again rinsed in double distilled water for 30 s to remove the disinfectant. The sterilized larvae were dissected using sterile microscissors under laminar flow to extract the gut. The extracted gut was separated into foregut (FG), Midgut (MG) and Hindgut (HG). Each gut compartment was briefly rinsed in sterilized 0.85% NaCl and placed in a sterile 1.5 ml Eppendorf tube containing 0.85% NaCl and homogenized with a sterile homogenizer.

Preparation of gut homogenates and PFLA analysis

Analysis of PLFAs provides a quantitative description of the microbial community in the particular environment sampled at a given time. A representative extraction of fatty acids from environmental samples is performed with organic solvents. In this study, analysis of PLFAs to determine microbial community in the gut of *S. ricini* and *A. dimidiata* was carried out as follows: In short, the homogenates of the individual gut compartments were prepared under laminar flow and inoculated into nutrient broth medium and incubated multiple times at 37ºC until an equivalent of about 5g of cell culture was attained. The Phospholipid fatty acids were extracted by using methods described by [22]. Briefly, 5 g of lyophilized broth of respective gut homogenate was extracted by Bligh-Dryer extraction and after evaporation; the lipids were separated on solid phase extraction column. Then phospholipids were eluted by 5 ml methanol and trans-esterified to fatty acid methyl ester and analyzed by Gas chromatograph Agilent Technologies, Wilmington, DE, USA) equipped with a 5972A mass selective detector (MSD II) using standard qualitative bacterial acid methyl ester mix and controlled with MIS Sherlock® (MIDI, Inc., Newark, DE, USA). The composition of microbial community was identified by microbial analysis software (Sherlock MIS 4.5 System, MIDI, USA) based on the spectrogram of specific PLFA. The microbial biomass was calculated by using the following formula:

$$Biomass = \frac{\text{Total Responce}}{\text{19: 0 C}} \times 25 \text{ η } moles = x : \frac{x}{\text{2 gms Of cell culture}}$$

Results and discussion

PFLA Analysis of gut samples from *S. ricini* and *A. dimidiata*

The results of PLFA signatures from the gut of *S. ricini* and *A. dimidiata* are provided in Tables 1 to 4 and Figure 1 (Chromatogram output). The rest of the chromatograms for individual gut compartments are provided in a supplementary material file (Figure S1 –S7). A total of nine microbial signatures were detected from the gut samples included: 10-methyl, Dimethylacetal (DMA), straight, 18:2ω6, 9c, branched, monounsaturated fatty acids (MUFA), 18:1ω9c, hydroxy, and polyunsaturated fatty acids (PUFA). These PLFA microbial biomarkers were found to be common in both insect samples in varying proportions (Tables 1 & 2). The PLFA biomarkers were translated into microbial groups; and the gut of *S. ricini* was found to be associated with the following microbial groups: Gram-negative bacteria [(3.19%, 6.38%, 6.75%); Gram positive bacteria (15.3%, 15.5%, 13.5%); anaerobes (9.32%, 16.46%, 10.89%); Actinomycetes (1.13%. 2.0%, 0.62%); Methanotrophs (0.58%, 0.68%, 0.25%), micro-eukaryote (66.29%, 55.27%, 67.87%) and fungi (4.19%, 4.04%, 4.69%)]. The results from *S. ricini* further indicated that Gram-negative bacteria were abundant in the midgut and hindgut; Gram-positive bacteria were abundant in the foregut and midgut; whereas anaerobes were abundant in the midgut and foregut. The microbial biomass in *S. ricini* was found to be higher in the hindgut, and least in the midgut (Table 3). Similar microbial groups were also present in the gut of *A. dimidiata* in the following proportions: the midgut, anterior hindgut, fermentation chamber and hindgut contained Gram-negative bacteria (7.64%, 15.94%, 2.74%,

5.95%); Gram positive bacteria (27.47%, 15.94%, 9.26%, 28.23%); anaerobes (14.38%, 7.79%, 14.17%, 8.68%); Actinomycetes (1.01%, 1.45%, 0.84%, 1.71%); Methanotrophs (0.38%, 0%, 0.37%, 0.37%), micro-eukaryote (43.72%, 56.77%, 69.27%, 46.5%) and fungi (3.42%, 10.66%, 3.37%, 8.93%). However, the PLFA results of the gut of *A. dimidiata* on the other hand revealed that both Gram- and Gram+ bacteria were abundant in the midgut, anterior hindgut and posterior hindgut; whereas anaerobes were abundant in the midgut and fermentation chamber. The results of microbial biomass for *A. dimidiata* indicated it was highest in the fermentation chamber and least in the anterior hindgut (Table 4). Apart from the above, the gut of both insects also registered PLFA signatures belonging to Actinomycetes, Methanotrophs, Micro-eukaryote and fungi in various proportions.

Table 1: Different groups of PLFA types of gut samples from *S. ricini*

	% Total fatty acids								
Gut compartment	**10-methyl**	**DMA**	**Straight**	**18:2ω6,9c**	**Branched**	**MUFA**	**18:1ω9c**	**Hydroxy**	**PUFA**
Foregut	0.98	9.53	16.67	16.3	8.45	2.14	3.13	0.64	53.35
Midgut	1.54	11.2	26.04	3.63	8.25	4.69	3.24	0.59	40.10
Hindgut	0.90	7.23	27.30	3.63	6.95	4.01	2.95	0.40	45.57
Mean	1.14	9.30	23.34	7.79	7.88	3.61	3.11	0.54	46.34

Table 2: Different groups of PLFA types of gut samples from midgut, anterior hindgut, fermentation chamber and posterior hindgut of *Anamola dimidiate*

	Total fatty acids (%)								
Section	**10-methyl**	**DMA**	**Straight**	**18:2ω6,9c**	**Branched**	**MUFA**	**18:1ω9c**	**Hydroxy**	**PUFA**
Midgut	1.48	13.14	15.87	2.61	24.1	6.49	2.41	0.33	34.13
Anterior Hindgut	2.67	6.62	10.9	9.11	6.38	5.8	7.39	0.51	50.12
Fermentation chamber	1.16	7.82	37.91	2.24	3.58	1.22	2.24	0.71	41.02
Posterior Hindgut	1.54	7.69	8.45	7.9	18.71	5.28		6	41.66
Mean	1.71	8.82	18.28	5.47	13.19	4.70	4.01	1.89	41.73

Table 3: Microbial groups from PLFA signatures obtained from the gut of *S. ricini*

	Microbial Groups (%)							
Gut compartment	**Gram Negative bacteria**	**Gram Positive bacteria**	**Anaerobe**	**Actinomycetes**	**Methanotrophs**	**Micro-eukaryote**	**Fungi**	**Biomass Content (η moles/gm)**
Foregut	3.19	15.30	9.32	1.13	0.58	66.29	4.19	1203.59
Midgut	6.38	15.50	16.46	2.00	0.68	55.27	4.04	1156.11
Hindgut	6.75	13.95	10.89	0.62	0.25	62.87	4.69	1654.53
Mean	5.44	14.91	12.22	1.25	0.50	61.48	4.31	1338.08

Table 4: Microbial groups from PLFA signatures obtained from the gut of *A. dimidiate*

	Microbial Groups (%)							
Gut compartment	**Gram Negative bacteria**	**Gram Positive bacteria**	**Anaerobe**	**Actinomycetes**	**Methanotrophs**	**Micro-eukaryote**	**Fungi**	**Biomass Content (η moles/gm)**
Midgut	7.64	29.47	14.38	1.01	0.38	43.72	3.42	1328.14
Anterior hindgut	7.41	15.94	7.79	1.45	-	56.77	10.66	663.56
Fermentation chamber	2.74	9.26	14.17	0.84	0.37	69.27	3.37	2136.26
Posterior Hindgut	5.95	28.23	8.68	1.71	-	46.50	8.93	1147.42
Mean	5.94	20.73	11.26	1.25	0.37	54.07	6.60	1318.85

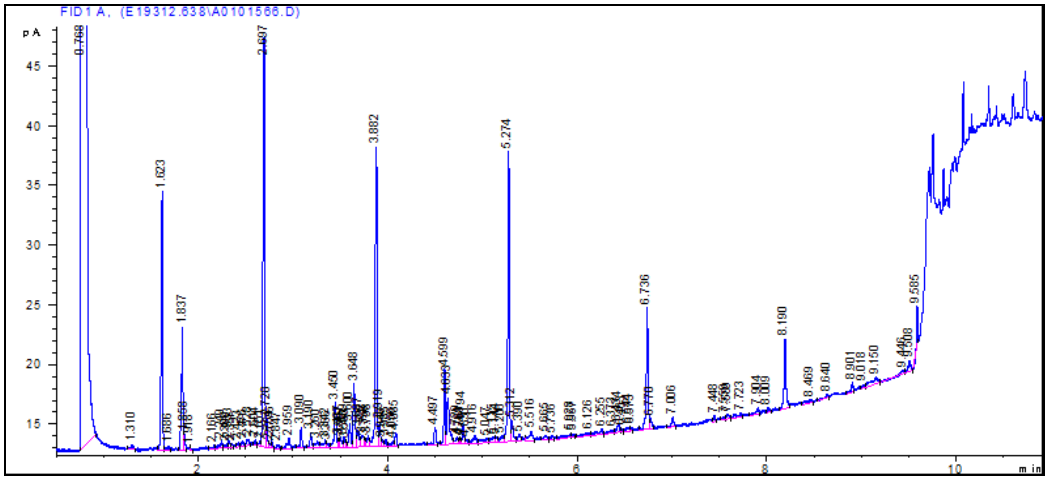

Fig 1: Gas Chromatography- Flame Ionization Detector (GC-FID) chromatogram of PLFA microbial signatures from the midgut of *S. ricini*

Conventional cultivation techniques alone are unable to characterize most microbes with 80-90% of microbial species not yet cultured. However, culture independent techniques such as PLFA analysis are able to provide a relatively unbiased view of the diversity of microbial communities [23] in environmental samples and have greatly improved the knowledge of microbes inhabiting insect guts [24]. PLFAs profiles have provided a quantitative description of the microbial community in a particular environment at the phenotypic level as different subsets of the microbial community have various PLFA patterns which are used as fingerprints i.e. micro-eukaryotes (polyunsaturated fatty acids), aerobic prokaryotes (monounsaturated fatty acids), gram-positive and anaerobic bacteria (saturated and branched fatty acids); C14 to C16 branched-chain fatty acids (iso and anteiso) are signatures for gram positive bacteria while gram negative bacteria contain unique hydroxyl fatty acids in the lipid portion of lipopolysaccharides in the cell wall. Linoleic acid (18:2ω6) is a good indicator of fungi and fungal biomass while fungal biomarker (16:1 5c) is an indicator for arbuscular mycorrhizal fungi [25, 26]. The results of PFLA signatures obtained from the gut of *S. ricini* and *A. dimidiata* revealed presence of nine microbial signatures which translated into seven microbial groups that colonized the guts of these insects. These results suggest that apart from symbiotic bacteria, the gut of these two insects is inhabited by a diverse community of other gut microbiota which may be complementing various biological functions to the host. The eri silkworm and white grubs belong to different insect orders, and have different diets and habitats. However, a comparative analysis of the microbial groups between the two insects revealed that they shared common PLFA biomarkers, thus suggesting that some symbiotic gut bacterial communities may be ubiquitous across insect orders. A study by [27] reported that the hindgut of scarab beetle larvae harbors a dense community of both obligate and facultative anaerobic bacteria and protozoa which are believed to play critical roles in food digestion. A similar study by [28] also indicated that for insects with complex microbial communities, it is their microbial based metabolism that shapes the conditions in the different gut compartments. The results of our study suggest that these microbial groups are enriched differently in the different compartments of the gut according to the physiological needs of the host as particular microbial types were found to be abundant in particular gut compartments. However, the observed variations between the different gut compartments could be due to the physicochemical conditions of the gut as reported by [29] who indicated that variation in physicochemical conditions in the different compartments of the insects' intestinal tract including pH and oxygen has a bearing on the kind of microbes associated with that insect host.

Conclusion

The use of PLFA analysis in the this study to explore the microbial diversity in the gut of *S. ricini* and *A. dimidiata* revealed that the intestinal tract of these insects is haboured by a complex community of gut microflora including gram-negative and gram-positive bacteria, Methanotrophs, anaerobes and fungi which could be influencing the host's physiology either singly or jointly. By profiling the gut bacteria of *S. ricini* and *A. dimidiata*, we have established a basis understanding of the presence of a diverse group of microbial community present in these insects which will inspire additional studies on how this diverse microbial community affects the host's physiology as well as studies on prospecting symbiotic microbes which could serve in probiotic formulations to boost Eri culturing as well as bio-prospecting for novel microbes with potential industrial use.

Acknowledgement

The authors gratefully acknowledge the help and support given by the ICAR-Indian Agricultural Research Institute, Indian Council of Agricultural Research, New Delhi and Indian Council for Cultural Relations, Government of India for their support for this research programme.

References

1. Chen B, Yu T, Xie S, Du K, Liang X, Lan Y *et al*. Comparative shotgun metagenomic data of the silkworm *Bombyx mori* gut microbiome. Scientific Data. 2018; 5(1):1-0.
2. Jones AG, Mason CJ, Felton GW, Hoover K. Host plant and population source drive diversity of microbial gut communities in two polyphagous insects. Scientific Reports. 2019; 9:2792.
3. Wong CN, Ng P, Douglas AE. Low-diversity bacterial community in the gut of the fruitfly *Drosophila melanogaster*. Environmental microbiology. 2011; 13(7):1889-900.
4. Berasategui A, Shukla S, Salem H, Kaltenpoth M. Potential applications of insect symbionts in biotechnology. Applied Microbiology and Biotechnology. 2016; 100(4):1567-77.
5. Shao Y, Chen B, Sun C, Ishida K, Hertweck C, Boland W. Symbiont-derived antimicrobials contribute to the control of the Lepidopteran gut microbiota. Cell Chemical Biology. 2017; 24(1):66-75.
6. Ceja-Navarro JA, Vega FE, Karaoz U, Hao Z, Jenkins S, Lim HC, Kosina P, Infante F, Northen TR, Brodie EL. Gut microbiota mediate caffeine detoxification in the primary insect pest of coffee. Nature Communications. 2015; 6:7618.
7. Salem H, Florez L, Gerardo N, Kaltenpoth M. An out-of-body experience: the extracellular dimension for the transmission of mutualistic bacteria in insects. Proceedings of the Royal Society B: Biological Sciences. 2015; 282(1804):20142957.
8. Sanders JG, Powell S, Kronauer DJ, Vasconcelos HL, Frederickson ME, Pierce NE. Stability and phylogenetic correlation in gut microbiota: lessons from ants and apes. Molecular Ecology. 2014; 23(6):1268-83.
9. Shin SC, Kim SH, You H, Kim B, Kim AC, Lee KA *et al*. Drosophila microbiome modulates host developmental and metabolic homeostasis via insulin signaling. Science. 2011; 334(6056):670-4.
10. Wang J, Weiss BL, Aksoy S. Tsetse fly microbiota: form and function. Frontiers in Cellular & Infection Microbiology. 2013; 3:69.
11. Whitaker MR, Salzman S, Sanders J, Kaltenpoth M, Pierce NE. Microbial communities of lycaenid butterflies do not correlate with larval diet. Frontiers in Microbiology. 2016; (7):1920.
12. Mitter C, Davis DR, Cummings MP. Phylogeny and evolution of Lepidoptera. Annual Review of Entomology. 2017; 62:265-83.
13. Paniagua Voirol LR, Frago E, Kaltenpoth M, Hilker M, Fatouros NE. Bacterial symbionts in Lepidoptera: Their

diversity, transmission, and impact on the host. Frontiers in Microbiology. 2018; 9:556.
14. Kim MY, Song HY, Kim JH, Kim BY, Park SW, Sung DK *et al*. Silkworm 30 K Protein inhibits Ecdysone-Induced apoptosis by blocking the binding of Ultraspiriacle to ecdysone receptor-B1 in cultured Bm5 cells. Archives of Insect Biochemistry and physiology. 2012; 81(3):136-47.
15. Meth T, Gogoi H. Original Research Article Rearing of Eri Silkworm (Samia cynthia ricini Boisd.) (Lepidoptera: Saturniidae) In Arunachal Pradesh: A Study In Papumpare District. Journal of Bioresources. 2016; 3(1):46-52.
16. Huang S, Sheng P, Zhang H. Isolation and identification of cellulolytic bacteria from the gut of Holotrichia parallela larvae (Coleoptera: Scarabaeidae). International Journal of Molecular Sciences. 2012; 13(3):2563-77.
17. Jackson TA, Klein MG. Scarabs as pests: a continuing problem. The Coleopterists Bulletin. 2006; 60(mo5):102-19.
18. Theurkar SV, Patil SB, Ghadage MK, Zaware YB, Madan SS. Distribution and Abundance of White grubs (Coleoptera: Scarabaeidae) in Khed Taluka, part of Northern Western Ghats, MS, India. International Research Journal of Biological Sciences. 2012; 1(7):1-6.
19. Bhattacharyya B, Bhagawati S, Mishra H, Gogoi D, Pathak K, Bhattacharjee S, Borkotoki S. Evaluation of some granular insecticides against white grub, *Lepidiota mansueta* B. in potato (Solanum tuberosum L.). Journal of Entomology and Zoology Studies, 2017; (5):1441-1444.
20. Majeed MZ, Miambi E, Barois I, Randriamanantsoa R, Blanchart E, Brauman A. Contribution of white grubs (Scarabaeidae: Coleoptera) to N2O emissions from tropical soils. Soil Biology and Biochemistry. 2014; 75:37-44.
21. Swathiga G, Umapathy G, Parthiban KT, Angappan K. Growth response of different eco races of ERI silkworm reared on various castor genotypes. Journal of Entomology and Zoology Studies, 2019; (7): 1406-1410.
22. Buyer JS, Teasdale JR, Roberts DP, Zasada IA, Maul JE. Factors affecting soil microbial community structure in tomato cropping systems. Soil Biology and Biochemistry. 2010; 42(5):831-41.
23. Zelles L. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biology and Fertility of Soils. 1999, 29(2):111-29.
24. Yun JH, Roh SW, Whon TW, Jung MJ, Kim MS, Park DS *et al*. Insect gut bacterial diversity determined by environmental habitat, diet, developmental stage, and phylogeny of host. Applied Environmental Microbiology. 2014; 80(17):5254-64.
25. Dickens SJ, Allen EB, Santiago LS, Crowley D. Exotic annuals reduce soil heterogeneity in coastal sage scrub soil chemical and biological characteristics. Soil Biology and Biochemistry. 2013; 1(58):70-81.
26. Kujur M, Patel AK. PLFA Profiling of soil microbial community structure and diversity in different dry tropical ecosystems of Jharkhand. Int. J. Curr. Microbiol. App. Sci. 2014; (3):556-75.
27. Zhang ZY, Yuan Y, Ali MW, Peng T, Peng W, Raza MF *et al*. Cultivable anaerobic and aerobic bacterial communities in the fermentation chambers of *Holotrichia parallela* (Coleoptera: Scarabaeidae) larvae. PloS One. 2018; 13(1).
28. Lemke T, Stingl U, Egert M, Friedrich MW, Brune A. Physicochemical conditions and microbial activities in the highly alkaline gut of the humus-feeding larva of Pachnoda ephippiata (Coleoptera: Scarabaeidae). Applied Environmental Microbiology. 2003; 69(11):6650-6658.
29. Engel P, Moran NA. The gut microbiota of insects–diversity in structure and function. FEMS Microbiology Reviews. 2013; 37(5):699-735.