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Isolation and Identification of gut bacteria from white grubs *Anomala dorsalis* and *Lepidiota mansueta* Larvae (Coleoptera: Scarabaeidae)

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ABSTRACT

The guts of most insects harbor nonpathogenic microorganisms. Recent work suggests that gut microbiota not only provide nutrients, but also involve in the development and maintenance of the host immune system. However, the complexity, dynamics and types of interactions between the insect hosts and their gut microbiota are far from being well understood. White grub is a polyphagous and nefarious pest of specific significance as it adversely affects the economic status of the farmers. This study aimed to explore the aerobic and anaerobic gut bacterial flora of the larvae of two white grub pests, *Anomala dorsalis* Fabricius and *Lepidiota mansueta* Burmeister (Coleoptera: Scarabaeidae) of different insect gut Segments through cultivation-dependent approaches coupled with 16S rRNA gene analysis. The results revealed that twenty one out of 23 gut bacterial isolates from the guts of larvae of *A. dorsalis,* cultured under aerobic condition belonged to Firmicutes and only two types *viz, Vibrio rumoiensis and Enterobacter xiangfangensis* which were detected from rectum belonged to Proteobacteria. The highest percentage (35%) isolates were from *Bacillus sp* followed by *Bacillus subtilis* and *Bacillus aerophilus* with 22 percentages of occurrences. Whereas, Intestinal bacteria of *L. mansueta* larvae cultured under anaerobic condition were classified into two groups: Bacteroidetes and Proteobacteria. Two bacterial phylotypes belonging to Bacteroidetes were Chryseobacterium sp *and Dysgonomonas termitidis* which were found in midgut and rectum while the other remaining two isolates viz., *Enterobacter sp* from midgut *and Comamonas sp* from fermentation chamber belonged to Proteobacteria. Furthermore, the colony count was higher on Nutrient Agar media with a mean of (225.56 $x10^{10}$cfu/ml) followed by Luria Bertani Agar and Actinomyces Agar irrespective of gut segments. On Nutrient agar, the fermentation chamber had the highest mean count of 298.00$x10^{10}$cfu/ml followed by hindgut (242.40$x10^{10}$cfu/ml) and rectum (224.80 $x10^{10}$cfu/ml), Therefore, the highest colony count was recorded on the fourth day and increased incubation time increased colony count on the growth medium. The results demonstrated that a core microbial community of this insect species. Understanding the functional role of these gut bacterial isolates may pave the way for developing novel pest control strategies.

Keywords: Gut bacteria, diversity, white grub, *Anomala dorsalis*, *Lepidiota mansueta*, 16s ribosomal RNA and gene.

1. Introduction

There is a continuing need to increase food production, particularly in developing countries, in context of continuous growth of human population. One of the practical means of maximizing food production is to minimize the losses associated with crop pest insects, notably insects. Coleopteran insect pests cause a major damage to the agricultural products worldwide. The white grubs are larvae belonging to Scaraeboidea super family. White grubs are the soil inhabiting and root feeding immature

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stages of scarab beetles, of which larval stage is destructive in nature (Theurkar, 2013). Scarabaeidae is one of the largest groups of beetles with over 30,000 species (Khanal *et al.,* 2012).

White grubs, the most destructive and troublesome of soil insects have become a challenging subject for control in various parts of the world. Many species of the white grubs are widely distributed around the world. *Heteronychus licas* Klug is mainly distributed in east, west and South Africa. Many authors reported this species as a serious pest of sugarcane, grasses, yam, swamp rice, maize, wheat, barley and natural pasture (Vercambre, 1997). In Northern Tanzania white grubs had been observed to reduce yield from 125 tons/ha of plant cane and 60 tons/ha of ratoon crop, to an average of 50 tons/ha in both plant cane and ratoon crop (Waiyaki, 1980). In the Sudan, white grubs were reported during the last century as pests of crops other than sugarcane by Pollard (1957) and Schmutterer (1969). In Kenana, two species of white grubs, *viz*, *Heteronychus licas* Klug and *Adoritus emarginatus* Ohaus were first recorded from sugarcane by Williams (1975) and considered as most serious pests of sugarcane. Abdalla (2007) reported that two species of *Schizonycha spp*. beside *H. licas* and *Anomala plebeja* to occur in the agro ecosystem of sugarcane at Kenana. In Nepal three main genera *viz., Phylophaga sp., Holotrichia spp*. and *Anomala sp.* were reported as major pest (Khanal *et al.,* 2012). In India, nearly about 300 species of white grub have been recorded from the states of Rajasthan, Uttar Pradesh, Gujarat, Maharashtra and Karnataka (Bhawane *et al.,* 2012). The damage caused by white grub larvae is estimated to reduce the crop yield by about 12-60 per cent.

The *Anomala dorsalis* Fabricius is a species of white grub belonging to the Scarabaeidae family (Sub family: Rutelinae:) distributed in all over the India (Andaman and Nicobar Island, Assam, Bihar, Chhattisgarh, Haryana, Jammu and Kashmir, Karnataka, Madhya Pradesh, Maharashtra, Sikkim, Tamil Nadu, Uttar Pradesh, Uttarakhand and West Bengal) (Gupta *et al.,* 2014). The species *viz.*, *A. dorsalis*, *A. elata*, *A. biharensis* and *A. bengalensis* are of common occurrence in various states.

The *Lepidiota mansueta* Burmeister is a species of white grub belonging to the Scarabaeidae family (Sub family: Melolonthinae). Recently, this species has been recorded as a key pest of many field crops in Majuli, the largest midriver deltaic island of the world located in Jorhat district of Assam, India and also in the sugarcane growing areas of Haridwar and the adjacent areas of Uttarakhand (Anon., 2009). In Assam, the severely affected crops are potato, sugarcane, colocasia and green gram and the extent of damage is found to vary from 15- 40 per cent in different crops. The *L. mansueta* has a biennial life cycle, the first of its kind in North East India, and the duration of egg, grub, and pupal stages varied from 12 to 17, 635 to 671, and 28 to 35 days, respectively. Third instar grubs cause heavy damage to crops and have a prolonged developmental period ranging from 545 to 563 days. *L. mansueta* can be regarded as a rare species, because it spends its entire life cycle under the ground except for a short period during which adults emerge from the ground for mating. There was no evidence that the adults fed on plants either in the field or in the laboratory and hence this species has the unique distinction of being the first Indian phytophagous white grub species with non feeding adults (Bhattacharyya *et al*., 2015).

Every year billions of dollars are spent worldwide on insect control in agriculture (Krattiger, 1996). Despite this expenditure, up to 40% of a crop can be lost to insect damage, particularly in developing countries (Oerke, 2006). Broad spectrum chemical insecticides have been the primary control agent for agricultural pests. Over the years, the widespread use of pesticides has led to pesticide resistant insects, a reduction in beneficial insect populations and harmful effects to humans and the environment (Fitt,1994). The control of white grubs by chemicals has been tried by several workers in the world. Effective control is possible only if chemical is applied, when the grubs are tiny or young. Control failure of effective insecticides led researchers to develop alternative and environmentally safe strategies to combat the white grub pests.

Many bacteria contribute to various physiological functions, including nutrition, development, reproduction, resistance to pathogens, production of pheromones, and immunity (Dillon and Dillon, 2004). Some symbionts can play essential roles in the insect gut, compensating for diets deficient in certain nutrients or containing recalcitrant organic compounds. For instance, in xylophagous termites, the gutmicroflora enable the host to digest cellulose and fix atmospheric nitrogen (Ohkuma, 2003) and in phytophagous aphids the endocellular symbiont *Buchnera aphidicola* synthesizes the essential amino acids that are absent in phloem sap (Shigenobu, 2000). The intestinal microbial bacteria in insects also have been shown to play important roles, such as providing vitamins, aiding in fat and carbohydrate metabolism, preventing the invasion of external bacteria, and promoting the function of the immune

system (Eutick *et al*., 1978; Abe *et al.,* 2000; Suchodolski and Ruaux, 2004). These gut-microbe interactions are diverse and include antagonism, commensalism, and mutualism and range from obligate to facultative (Douglas, 2009). Obligate symbiotic microorganisms are typically vertically transmitted during early stages of oogenesis or embryogenesis, whereas facultative symbionts can colonize native hosts through horizontal transmission between individuals or acquisition from the diet or the environment (Koga *et al.,* 2012). Some symbionts produce compounds that are used as aggregation pheromones by bark beetles (Brand *et al.,* 1975), whereas others produce volatile metabolites that can be exploited by natural enemies of bark beetles (Sullivan and Berisford, 2004). All these properties and the important roles that symbionts have in host biology can be exploited for devising novel control strategies for controlling the insect pests (Crotti, *at al.,* 2012).

The present work aimed to explore the aerobic and anaerobic gut bacterial flora of larvae of two white grub pests, *Anomala dorsalis* Fabricius and *Lepidiota mansueta* Burmeister (Coleoptera : Scarabaeidae) of different Insect gut Segments through cultivation-dependent approaches coupled with 16S rRNA gene analysis and the information generated may be of practical utility in exploiting the gut microbes for management of these pests.

2. Materials and Methods

2.1. Insect collection

The *Anomala dorsalis* and *Lepidiota mansueta* larvae were collected from a sugarcane field, Amroha district (28o 54'N; 78o 31'E) Utter Pradesh, India. The 3rd larval instar was dominant in sampling. Samples of larvae were collected in sterilized plastic containers. The larvae were kept in the laboratory for one week prior to dissection to avoid possible infestations from the field and to reduce any potential insecticide residual effects. The collected larvae were maintained individually in containers filled with soil (Plate 1). All the larvae were fed with potato, surface sterilized with 70% ethanol, and the diets were replaced every 3 days plastic containers half-filled with soil. The larvae were surface sterilized in 70% ethanol for 1 min and kept in -20 °C for 15 min prior to dissection. The larvae were dissected in dissection trays containing 0.85% saline and the guts were aseptically removed. The gut segments were transferred into micro centrifuge tubes (1.5 ml) and homogenized with the help of a hand-held homogenizer in a sterile micro centrifuge tubes containing of 50 µl 0.85% saline, final volume was made up to 500 µL and storage at 4 °C.

2.2. Isolation and characterization of gut bacterial isolates

Gut bacterial isolates from *Anomala dorsalis* larvae were characterized using both the culture-dependent and culture-independent methods.

2.2.1. Culture-dependent methods

2.2.1.1. Isolation and maintenance of gut bacterial isolates

The gut homogenate was serially diluted (Eight serial ten-fold dilutions) with 0.85% NaCl aseptically. Bacterial cultures from gut homogenates were isolated by following spread plate technique on a range of microbiological media viz., Nutrient Agar (NA), Luria Bertani (LB) and Actinomycetes (Ac) media (plate 2).

2.2.1.2. Isolation of bacterial culture

About 50 µL of 10^{-4} and 10^{-8} dilutions of the homogenate was spread on the previously prepared different agar plates and incubated at 37±1°C. Plates were examined every 24 h for development of new colonies (plate 2.A). The colony differentiation was done on the basis of size, colour and morphology. Later on, a single representative of each morpho type was transferred to new agar by quadrate streaking (plate 2.B). Purity of culture was ascertained by microscopic examination.

2.2.1.3. Purification of bacterial cultures

Cultures were purified by re-streaking them on freshly prepared agar plates to get single isolated colony.

2.2.1.4. Maintenance of bacterial cultures

Isolated colonies were streaked onto different agar slants which were then stored in refrigerator at 4°C for future use.

2.2.1.5. DNA extraction

Single colony of each isolate was separately inoculated in 25 mL nutrient, Luria Bertani and Actinomycetes broth in falcon tubes (plate 2.**C**) and incubated overnight at 37°C and 100 rpm. DNA was extracted by Cetyltrimethyl Ammonium Bromide (CTAB) method (Moore *et al.,* 2004).

2.2.1.6. Generic characterization using 16S rRNA probes:

Amplification of 16S rRNA gene was carried out using the genomic DNA extracted from different bacterial cultures of the gut of two white grub species as template. Two universal primers, forward primer 27 F (5' AGAGTTTGATCCTGGCTCAG 3') and reverse primer 1492 R (5' AAGGAGGTGATCCAGCCGCA 3') were used for the amplification of 1492 bp region of 16S rRNA gene. A 50µl of reaction mixture was prepared on cool pack containing:

Components	Amount (µL)
Master mix	25
27F (10 µM)	3
1492R (10 µM)	3
Bacterial DNA as template	5
Nuclease free water	14

The amplification of the target sequence was performed in thermal cycler with the reaction conditions as follows:

1. Pre-denaturation of 5 min at 95oC
2. Followed by 35 cycles of denaturation at 95oC for1 min, annealing at 58oC for 1 min and extension at 72oC for1 min
3. The final extension of 10 min at 72oC

Then, the PCR products were run in 2% agarose gel for ensuring amplification.

After assurance, PCR products were sent for sequencing. The nucleotide sequences obtained were submitted to the National Centre for Biotechnology Information (NCBI) database.

2.2.2. Culture-in dependent methods

The gut homogenate was used for inoculation of 15 mL nutrient, Luria Bertani and Actinomycetes broth separately in falcon tubes and incubated overnight at 37°C and 100 rpm. . All three broths pooled and sent for PLFA analysis.

2.2.3. Data Analysis

Observation of Bacterial Colonies with different morphological appearances was observed on bacteriological media and the colonies were counted. The colony count technique had been routinely used. The mean number of colony forming units (cfu) of bacteria was calculated using the colony forming unit formula (Chikere and Udochukwu, 2014).

$$\textbf{CFU/G} = \frac{\text{Average count}}{\text{Dilution x volume plated}}$$

Phylogenetic and molecular evolutionary analyses with the sequences of 27 (23 from *A. dorsalis* and 4 from *L. mansueta*) isolates were conducted by using MEGA5 software. All sequences of 16S rDNA of bacteria were aligned using multiple sequence alignment program MUSCLE. Sequences were examined and edited using BioEdit Sequence Alignment Editor and compared with 16S rRNA sequences available in NCBI by BLAST search.

2.3. Isolation of bacterial culture under anaerobic condition

Gut bacterial isolated from *L. mnsueta* larvae was cultured under anaerobic condition (plate 3). About 50 µL of the homogenate was spread on the R- 2A agar plates and incubated on desiccator flushed with argon gas for 5min). Plates were examined every 24 h for development of new colonies. The colony differentiation was done on the basis of size, colour and morphology. Later on, a single representative of each morpho type was transferred to new agar plates by quadrate streaking. Purity of culture was ascertained by microscopic examination and again incubated on desiccator flushed with argon gas for 5min. Single colony of each isolate was separately inoculated in 25 mL broth in culture vessels flushed with argon gas for 5min, tightly closed with butyl rubber septa secured in place by aluminum caps and incubated overnight at room temperature .DNA was extracted by Cetyltrimethyl Ammonium Bromide (CTAB) method (Moore *et al.,* 2004).

Plate 1: Collection and maintenance of Scarabaeid larvae

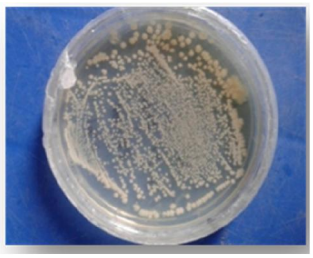

Plate 2: A Bacteria colonies

Plate 2: **B** Pure culture (single isolated colony)

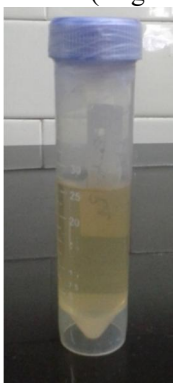

Plate 2: C Falcon tube with broth inoculated with pure culture

Plate 2: Isolation and maintenance of gut bacterial isolates (aerobic conditions)

Desiccator used for anaerobic incubation

A

B

Gas supply equipments (a) A gas regulator attached to a compressed gas cylinder, (b) Oxygen purging while putting the nozzle tip into the liquid directly.

A **B** **C** **D**

Plate 3: Isolation and maintenance of gut bacterial isolates (anaerobic conditions)

Culture vessels and tools for anaerobic cultivation (A) The vessels culturing anoxygenic phototrophic bacteria; (B) Caps used for anaerobic cultivation. Butyl rubber septa and aluminum caps; (C) Hand crimper for crimping aluminum caps on vials, and (D) decapper for removing aluminum caps from the vials.

2.4. Statistical analysis

Data of bacterial enumeration was analyzed by Variance ANOVA. Means were separated for significance by Duncan's Multiple Range Test (Gomez and Gomez, 1984).

3. Results and Discussion

3.1. Enumeration of culturable gut bacteria from the larvae of *Anomla dorsalis*

Enumeration of gut bacterial isolates was done from different gut regions of *A. dorsalis* larvae. The total bacterial count on insect gut segments samples varied significantly (Table 1). The colony count was higher on Nutrient Agar media with a mean of (225.56 $x10^{10}$cfu/ml) followed by Luria Bertani Agar and Actinomyces Agar irrespective of gut segments. On Nutrient agar, the fermentation chamber had the highest mean count of 298.00$x10^{10}$cfu/ml followed by hindgut (242.40$x10^{10}$cfu/ml) and rectum (224.80 $x10^{10}$cfu/ml), while, the lowest colony count was recorded from midgut (175.40 $x10^{10}$cfu/ml), which was statistically on par with foregut (187.20 $x10^{10}$cfu/ml). On Luria Bertani Agar, gut homogenate from rectum recorded the highest mean colony count of 49.60$x10^{10}$cfu/ml which was statistically on par with that of midgut (48.00 $x10^{10}$cfu/ml) and fermentation chamber (47.60$x10^{10}$cfu/ml), While the lowest colony count was recorded from hindgut (2.80$x10^{10}$cfu/ml). On actinomycesagar, midgut homogenate recorded relatively higher colony count (56.00$x10^{10}$ cfu /ml) followed by rectum (32.00$x10^{10}$cfu/ml).

Table 1: Number of bacterial Colonies on three different growth media of Insect Gut Segments

S.N	Insect gut segments	Number of bacterial Colonies (CFU/ML 10^{10})*		
		Nutrient agar	Luria bertani agar	Actinomyces agar
1	**Foregut**	187.20^{c}	41.60^{b}	56.00^{a}
2	**Midgut**	175.40^{c}	48.00^{a}	56.00^{a}
3	**Hindgut**	242.40^{b}	2.80^{c}	6.40^{c}
4	**Fermentation Chamber**	298.00^{a}	47.60^{a}	8.00^{c}
5	**Rectum**	224.80^{b}	49.60^{a}	32.00^{b}
Mean		**225.56**	**37.92**	**31.68**
SEd		**11.94**	**2.176**	**2.00**
CD (P= 0.05)		**25.45**	**4.64**	**4.26**
CV%		**7.48**	**8.12**	**8.92**

*Mean of four replications.CFU (Colony Forming Unit) Means with same superscripts in columns are not significantly different by DMRT (P = 0.05)

The effect of incubation time on the cultivation of insect gut bacteria is presented in Fig. 1. On the first day of incubation, there were visible colony formation on the Nutrient agar and the Luria Bertani Agar plates. As incubation time increased, there was also a corresponding increase in colony count. The highest colony count was recorded on the fourth day. In Actinomyces agar plates, there was no visible colony from the first day of incubation but, after the second day, visible colonies appeared on the Actinomyces Agar plates. Therefore, increased incubation time increased colony count on the growth medium. Increase incubation time improved the cultivation of bacteria (Eichorst *et al.*, 2007). The successful isolation of members of bacteria genus that are widely distributed and common in insect gut seems to be a result of using appropriate media and extended incubation time. This investigation demonstrates the significance of medium choice and incubation time on the successful isolation of insect gut bacteria.

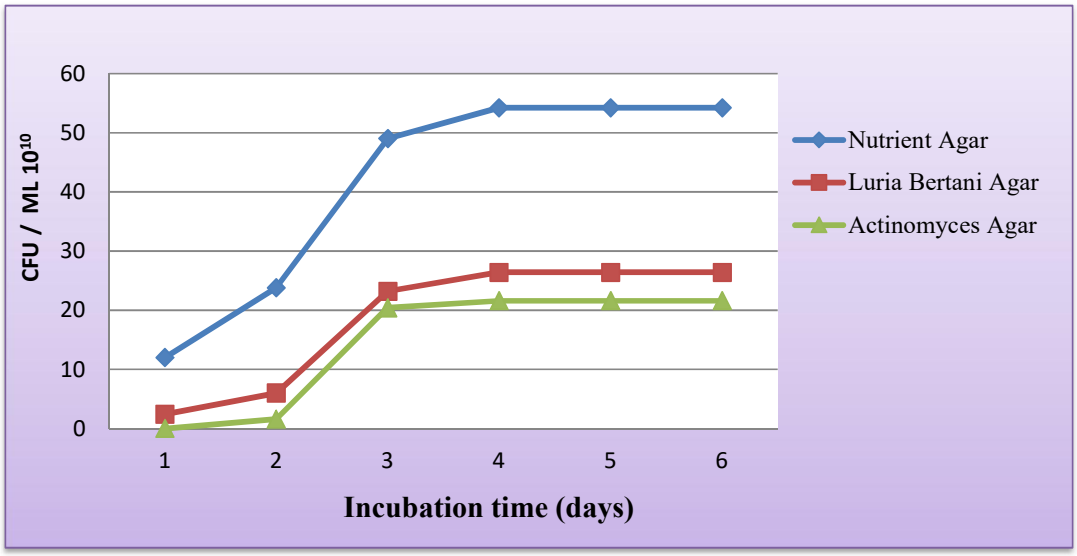

Fig. 1: Effect of extended incubation time on the number of bacterial Colonies with respect to three different growth media

3.2. Isolation and identification of gut bacteria from *Anomala dorsalis* and *Lepidiota mansueta*

Microbial diversity is defined as the number of elements indicated by species or genes within a system (Avidano *et al.,* 2005). Most of the microbiome in the gut regions remains unexplored due to the existence of many uncultured bacteria species. The analysis of intestinal bacteria was performed by culture dependent and molecular methods. The culture- dependent technique defined the gut microbes by phenotypic characterization (morphology, immunology, and physiological- biochemical reaction), but unculturable bacteria were largely ignored (Lysenko, 1985). Molecular biology methods allowed extracting the total genomic DNA of bacteria directly from samples and then sequencing and analyzing the DNA to characterize the bacteria species composition and abundance (Yu *et al*., 2008). Molecular-based approaches are useful for determining diversity of various bacterial populations (Hugenholtz*et al.,* 1998). Therefore, we used molecular methods based on DNA analyses in this study to investigate bacterial populations .in the gut of *A. dorsalis* and *L. mansueta* larvae. Intestinal bacteria of *Anomala dorsalis* larvae identified by using 16S rRNA gene presented in Table 2. After analysis of the sequences, the intestinal bacteria of the larvae were classified into two groups: Firmicutes and Proteobacteria. Twenty one out of 23 gut bacterial isolates from the guts of larvae of *A. dorsalis,* belonged to Firmicutes and only two types *viz ,Vibrio rumoiensis and Enterobacter xiangfangensis* which were detected from rectum belonged to Proteobacteria. The *Bacillus sp*was the predominantgut bacterial isolate. The *Bacillus aerophilus* was found in all of the gut segments except the fermentation chamber. The percentage of occurrence ofisolated bacterial strains was calculated and represented in Fig 2. The highest percentage (35%) isolates were from *Bacillus sp* followed by *Bacillus subtilis* and *Bacillus aerophilus* with 22 percentages of occurrence. In this present work, many sequences showed > 99% similaritywith those of the phylotypes from GenBank database.

Table 2: Gen bank accession details of Intestinal bacteria of *Anomala dorsalis* larvae identified by using 16S rRNA

Gut section	Sample	Organism	Gen bank accession number	Similarity (%)
Fore gut	FGR.1	*Bacillus aerophilus*strain MER_79.1	KT719654.1	99%
	LB.FG	*Bacillus sp.* M22 (2010) strain M22	GQ340462.1	99%
Mid gut	MGI.R1	*Bacillus pumilus*strain KD3	EU500930.1	100%
	MGI.R2	*Bacillus licheniformis*strain 2C	KF993663.1	99%
	MGII.R1	*Bacillus aerophilus*strain MER_79.1	KX245016.1	100%
	MGII.R2	*Bacillus aerophilus*strain HQB216	KT758415.1	95%
	AC.MGI	*Bacillus sp.* M95(2010) strain M95	GQ340487.1	99%
	LB.MGI	*Bacillus sp.* B18(2008)	EU362164.1	99%
	LB.MGII	*Bacillus subtilis*strain BS16045	CP017112.1	99%
Hind gut	HGI.R1	*Bacillus sp.* strain 251.1	KX454023.1	99%
	HGI.R2	*Bacillus sp.* strain 251.1	KX454023.1	100%
	HGII.R1	*Bacterium* strain CDSHDTYG-*6*	KU743240.1	100%
	HGII.R2	*Bacillus aerophilus*strain MER_79.1	KT719654.1	99%
	AC.HGII	*Bacillus subtilis*strain LLP-4	*KU821697.1*	99%
	LB.HGI.	*Bacillus sp.* BAB-5102	KR998246.1	99%
Rectum	RECT.R1	*Vibrio rumoiensis*strain 0201	KP236329.1	100%
	RECT.R2	*Bacillus aerophilus* strain MER_79.1	KT719654.1	99%
	AC.RECT	*Bacillus subtilis*strain R2A	KJ580523.1	100%
	LB.RECT	*Enterobacter xiangfangensis*strain LMG27195	CP017183.1	99%
Fermentation chamber	FCHP.R1	*Bacillus subtilis*strain LLP-4	KU821697.1	99%
	FCHP.R2	*Bacillus sp.* strain TS3	KX710327.1	99%
	LB.FCHA	*Bacillus subtilis*strain GSC-3	GQ144705.1	99%

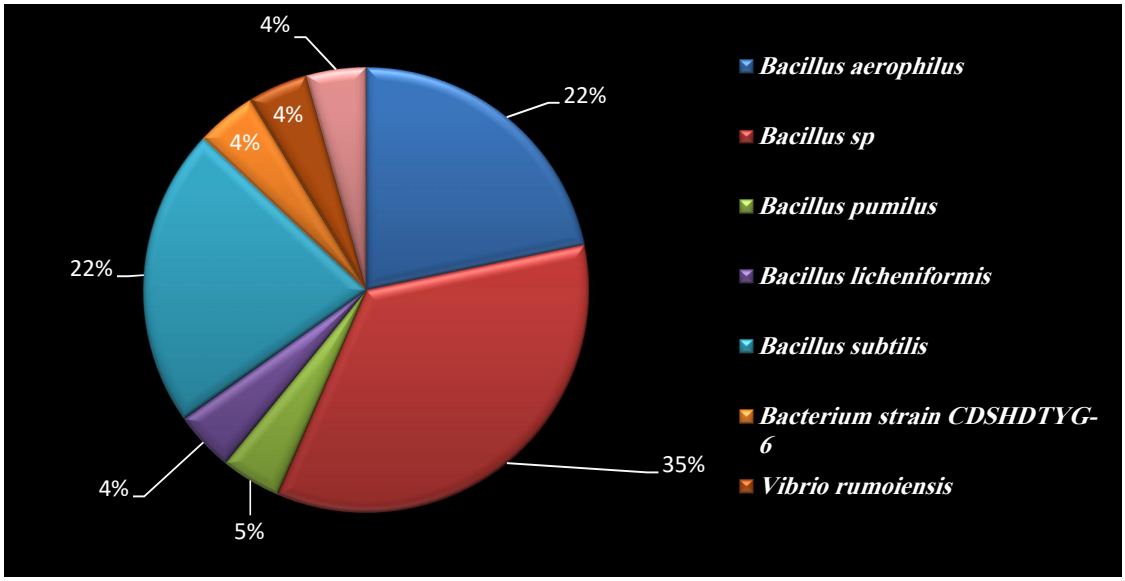

Fig. 2: Proportion of bacterial strains isolated from the t gut of *Anomala dorsalis*

However, a significant proportion of the *Bacillus aerophilus* sequences showed only 95% homology identity with the bacterial *16S rRNA* sequences database of Gen bank. Members of the Proteobacteria family and Enterobacteriaceae were commonly found in the gut communities of a wide range of animals, including humans and insects, and can aid in vitamin biosynthesis, pheromone production and degradation of plant compounds (Breznak, 1982; Xu and Gordon, 2003). *Bacillus subtilis* has the ability to produce and secrete the hydrolyzing carbohydrate enzyme, amylase (Panneerselvam and Elavarasi, 2015). Species of the genus *bacillus,* which are Gram-positive bacteria

and mostly non-pathogenic, often can be found in the guts of many insects. Yuan *et al*. (2006) discovered that the main bacteria in the intestinal tract of the silkworm *B. mori*were *Arthrobacter* (Actinomycetales: Micrococcaceae), *Lactobacillus* (Lactobacillales: Lactobacillaceae), *Escherichia* (Enterobacteriales Enterobacteriaceae), *Pseudomonas* (Pseudomonadales: Pseudomonadaceae), *Bacillus*, and *Staphylococcus*. Yang *et al*., (2006) analyzed the different bacteria in termites and found that they were *Streptococcus* (Lactobacillales: Streptococcaceae), *Bacteroides* (Bacteroidales: Bacteroidaceae), *Bacillus*, *Staphylococcus,* and *Enterobacter*. Wang (2008) obtained 28 different DGGE bands of bacteria from the guts of larvae of the mosquito *A. sinensis*, and Gammaproteobacteria, Flavobacteria, Actinobacteria, Betaproteobacteria, and Firmicutes were observed.

Table 3: Intestinal bacteria identified from the gut of *Lepidiota mansueta* larvae under anaerobic condition

S.N	Gut Section	Sample	Organism	Accession number	Identity (%)
1	Mid gut	MG_3	*Chryseobacterium sp* **Family:** Flavobacteriaceae	LC034275.1	98%
		MG_4	*Enterobacter sp* **Family:**Enterobacteriaceae	KT248046.1	98%
2	Fermentation chamber	Fch_1	*Comamonas sp* **Family:** Comamonadaceae	JN873188.1	99%
3	Rectum	$Rect_1$	*Dysgonomonastermitidis* **Family***:* Porphyromonadaceae	AB971823.1	99%

4. Conclusion

This study demonstrates that the larvae of white grubs *Anomala dorsalis* and *Lepidiota mansueta* harbor a dense and diverse community of bacteria in their midgut, hindgut and rectum and that bacteria in the hindgut have an important role in the degradation of the roots and other organic matter consumed by scarab larvae. The present study outlines a detailed investigation of the composition of common gut symbionts of these white grubs and these gut bacteria may help in developing novel methods of biological control of these pests. Moreover, the effect of growth media and incubation time were investigated. Some media that are traditionally used for soil microbiological studies returned low viable counts and did not result in the isolation of members of rarely isolated groups. Furthermore, the colony count was higher on Nutrient Agar media compared with Luria Bertani Agar and Actinomyces Agar irrespective of gut segments.

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