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Research Article

MIDGUT MICROBIOTA OF TWO EARTHWORMS *PHERETIMA POSTHUMA* AND *EISENIA FETIDA*-A METAGENOMIC APPROACH

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A R T I C L E I N F O

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A B S T R A C T

Earthworms are friends to mankind from time immemorial and are said to be farmers with friends. Among the soil invertebrates, earthworms play an essential role in carbon turnover, nitrogen mineralization, soil formation, cellulose degradation and humus accumulation etc. Nowadays, it is well known, that earthworms and other soil macrofauna modify the soil physical properties and affect soil organic matter decomposition and regulate carbon fluxes and nitrogen cycling. The present study was aimed to study the bacterial diversity of two earthworm species *Pheretima posthuma* and *Eisenia fetida* using metagenomic sequencing tools. Metagenomic DNA was isolated from the supplied earthworm gut sample by using c-TAB and Phenol: Chloroform Extraction method followed by AMPure XP bead purification. 1µl of sample was loaded in NanoDrop for determining A260/280 ratio. QC pass DNA samples were processed for first Amplicon generation followed by NGS Library Preparation Using Nextera XT Index Kit (Illumina inc.). The mean of the library fragment size distribution are 594bp and 592bp for sample 1A and sample 2A for *Pheretima posthuma* and *Eisenia fetida* respectively. Proteobacteria is predominant in both the earthworm species followed by Cyanobacteria, Actinobacteria, Firmucutes, Planctomycetes, TM7, Verrucomicrobia species. The earthworm gut metagenomes exhibited varied functional attributes like cellulose degradation, aromatics remediation, nitrogen fixation, denitrification, sulphur oxidation and reduction. Hence from the present study it can be concluded that bacterial species plays a vital role in the gut of earthworm and is a natural selection process and earthworm can be used for various process of degradation, pollution control and bioremediation process and the determinant of the species is in the order of group-habitat-species.

INTRODUCTION

Earthworms are regarded as friends of the farmer (Darwin, 1881) due to their role in soil formation and organic matter decomposition. As their activity leads to the formation of physical distractions such as burrows, castings in the soil, they are often termed as "ecological engineers". Soil bio resources have been recognized as the foundation for sustainable livelihood and food security. The importance of earthworms cannot be ignored because they have enormous potential to improve soil condition on a sustainable basis. Earthworms are ancient organism since they have been on our planet for 600 million years. They have survived through the mass extinction, and helped life to sustain on the earth and human civilization by ploughing and fertilizing the soil.

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In India, 418 species and subspecies belonging to 69 genera have been reorganized on the basis of morphological characteristics (Julka and Paliwal, 2005). This number is expected to rise to about 800 with extensive surveys of large unexplored areas. High earthworm diversity in India is primarily due to its geographical location with a wide latitudinal range (between 8.4°N and 37.6°N and longitudinal range 68.7˚E and 97.25˚E), complex topography, varied climate (ranging from temperate to arctic in the Himalaya to tropical in the peninsular India) and past geological history that is linked to ancient super continent of Gondwana land from which it separated in the late Jurassic and drifted to collide with the Asian mainland in the Eocene (Julka J.M., *et al*., 2009). Microbial ecology studies have provided a better understanding of the structure of microbial communities and the evolution of these communities under various climatic, biotic and xenobiotic conditions and the activities of microorganisms. For several years, investigations of microbial diversity were mainly based on isolation and laboratory

cultivation of bacteria which lead to underestimation of the true diversity (Amann *et. al.,* 1995).

Vermiculture technology is emerging as an "environmentally sustainable", "economically viable" and "socially acceptable" technology all over the world. Vermicomposting is the term given to the process of conversion of biodegradable matter by earthworms into vermicast. In this process, the unavailable nutrients contained in the organic matter are partly converted to more bioavailable forms. The use of earthworms was known for ages as "waste managers" for efficient "composting of food and farm wastes" and as "soil managers" for "fertility improvement" for "farm production". It is now being more scientifically and also commercially revived. Vermicast is also believed to contain hormones and enzymes which are acquired during the passage of the organic matter through the earthworm gut(Tamizhazhagan *et al*.,2016). The hormones and enzymes are believed to stimulate plant growth and discourage plant pathogens. All- in- all, the vermicast is believed to be an excellent organic fertilizer and soil conditioner. Experiments conducted by Gajalakshmi and Abbasi (2002, 2004) confirm the earlier reports that vermicompost has a more beneficial impact on plants than the compost.

To better understand the role of earthworms in nature and their potential use, the mechanisms of earthworm intestinal microorganisms must be studied. It is known that earthworms cannot exist on pure microbial cultures but they need mixed cultures of microbial species (Edwards and Bohlen, 1996). As food, bacteria are of minor importance; algae are of moderate importance, fungi and to a lesser extent protozoa, are of major importance (Edwards and Fletcher, 1988). It is generally accepted that earthworms do not commonly consume bacteria and actinomycetes but they may proliferate in the process of passage through gut (Kristufek *et al.*, 1992).

Molecular analysis of 16S rRNA is now central to studies examining the diversity of microorganisms in the environment. Methodologies for the analyses of a DNA-based phylogeny (using the 16S rRNA gene) are now well established but the direct targeting of 16S rRNA, as a potential indicator of activity (Felske *et. al.* 1998), has received comparatively less attention, due primarily to the lack of suitable protocols for extraction from natural environments.

MATERIALS AND METHODS

Earthworm sampling, preservation and dissection

Two earthworm species *Pheretima posthuma and Eisenia fetida* (Lumbricidae, Annelida) were used in this investigation. Earthworms were collected using a spade and soil hand-sorting. Adult earthworms used for the evaluation of DNA extraction methods were collected from vermicomposts of Doomdooma College, Doomdooma, Tinsukia Assam, India (27° 35'48.2" N 95° 33' 29.9" E, 114m) in June 2017. The mean body weight of both the earthworm species were as 30±0.8 g, n=20 for *Pheretima posthuma* and 8-10±0.5 g, n=20 for *Eisenia fetida*. The samples were named as 1A and 2A for *Pheretima posthuma* and *Eisenia fetida* respectively for the further study.

Earthworms along with native soil were carried to the laboratory in sterile polythene bags containing a chloroform soaked cotton ball kept in ice filled sterile box and stored (Thakuria *et al*., 2009). Prior to dissection (to harvest the gut content, earthworms were first washed with distilled water to remove the soil and other particles and prior to that before dissection surface sterilization was done with 30% ethanol) after that dissection was carried out. Earthworm gut contents were harvested by dissecting the earthworms, posterior to the clitellium part up to the hind gut (Giraddi *et al.,* 2009). The dissection was carried out in both the species in sterile lab condition. Prior to the dissection, all lab instrument and dissection tray was surface sterilized with 100% ethanol. After that the dissection and extraction procedure was carried out. The washed intestine soil sample of each individual earthworm used for DNA extraction is hereafter referred as gut sample of earthworm. For the DNA extraction experiment, mean fresh weight of gut contents was 100±10 ml of both the earthworm species, respectively.

DNA extraction

DNA was extracted from gut content of both the earthworm samples by using c-TAB and Phenol: Chloroform Extraction method followed by AMPure XP bead purification. From each earthworm species 20-20 earthworms were selected and gut content was dissected out for DNA extraction procedure. 1µl of the sample was loaded in Nanodrop for determining 260/280 ratio and 260/230.

DNA quality and quantity

The absorption spectrum of DNA extracts (230–280 nm and 260-230nm) was determined using Nano-drop(R) ND-1000 spectrophotometer (Eurofins Genomics Bioinformatics Lab) according to the manufacturer's instructions. Pure DNA is known to produce 260/230 and 260/280nm ratios 1.80 (Sambrook *et. al*., 1989). DNA was visualized by electrophoresis of 5-µl aliquots through 1.2% (w v^{-1}) agarose gels containing 0.5 µg ml^{-1} ethidium bromide, and DNA was quantified (µg DNA $0.1g^{-1}$ fresh gut content) as previously described (Thakuria *et. al*., 2008).

Preparation of 2×300 MiSeq library

The amplicon libraries were prepared using Nextera XT Index Kit (Illumina inc.) as per the 16S Metagenomic Sequencing Library preparation protocol (Part # 15044223 Rev. B). Primers for the amplication of the 16S rDNA gene specific for bacteria were designed at Eurofins Genomics Bioinformatics Lab and Synthesized at Eurofins Genomics Lab facility. Amplication of the 16S rDNA gene targeting bacteria was carried out. 3µl of PCR product was resolved on 1.2% Agarose gel at 120V for approximately 60min or till the samples reached 3/ 4 of the gel.

Primers used in the present study:-
16S rRNA F: GCCTACGGGNGGCWGCAG
16S rRNA R: ACTACHVGGGTATCTAATCC

Applicants with the illumina adaptors were amplified by using i5 and i7 primers that add multiplexing index sequences as well as common adapters required for cluster generation (P5 and P7) as per the standard illumina protocol. The amplicon library was purified by 1X AMpureXP beads and quantified using Qubit fluorometer.

Quantity and quality check of library on Agilent 4200 Tape Station

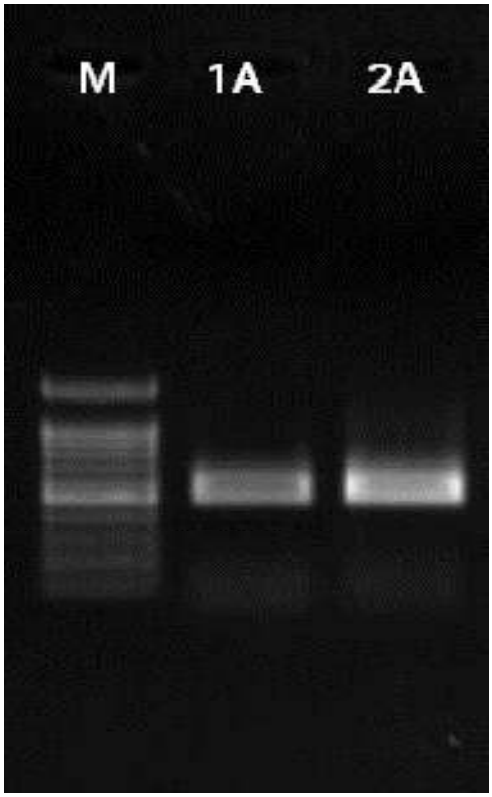
The amplified libraries were analyzed in 4200 Tape Station systems (Agilent Technologies) using D1000 Screen tape as per manufacturer instructions.

Cluster generation and Sequencing

After obtaining the peak size from Tape Station profile, libraries were loaded onto MiSeq at an appropriate concentration (10-20pM) for cluster generation and sequencing. Paired-End sequencing allows the template fragments to be sequences in both the forward and reverse direction on MiSeq. The kit reagents were used in the binding of samples to complementary adapter oligos on paired-end flow cell. The adapters were designed to allow selective cleavage of the forward strands after re-synthesis of the reverse strand during sequencing. The copied reverse strand was then used to sequence from the opposite end of the fragment.

RESULT

QC on Agarose Gel



Figures 1 QC of first Amplicon on 1.2% Agarose gel

NanoDrop reading of gDNA

Table 1 Table showing the nanodrop reading of both the samples

Sr.No.	Sample ID	NanoDrop Readings (ng/µl)	NanoDrop OD $A_{260/280}$	NanoDrop OD $A_{260/230}$	Remarks
1	1A	88.7	1.61	1.52	QC Pass
2	2A	121.7	1.75	1.32	QC Pass

Tape-Station Profile of libraries of Sample 1A and 2A

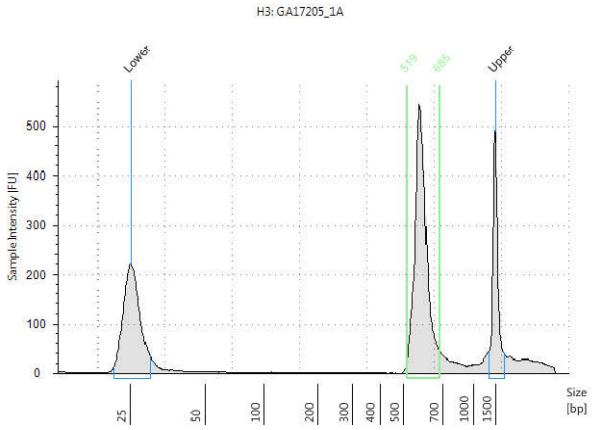


Figure 2 Library Profile of sample-1A on Agilent Tape Station using D1000 Screen Tape

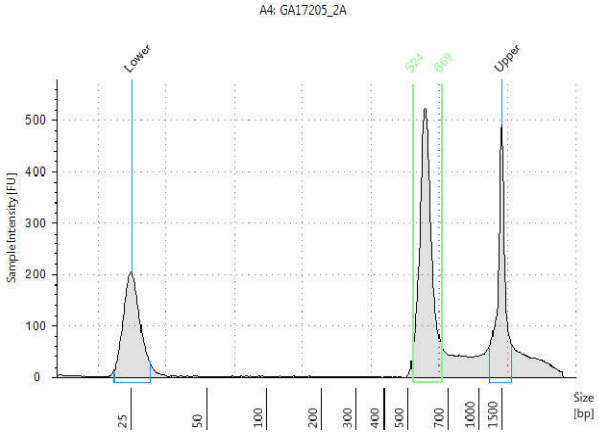


Figure 3 Library Profile of sample 2A on Agilent Tape Station using D1000 Screen Tape

QC pass DNA samples were processed for first Amplicon generation followed by NGS library preparation using Nextera XT Index Kit (Illumina inc.). The mean of the library fragment size distribution is 594bp and 592bp for sample 1A and Sample 2A respectively. Libraries were sequence of MiSeq using 2×300bp chemistry.

Table 2 Table showing the total bases read in both the samples

Sr. No.	Sample	#Reads	Total Bases	Data in MB
1	1A	676,774	346,507,919	~346
2	2A	762,725	391,653,372	~391

Taxonomic distribution of sample 1A at species level

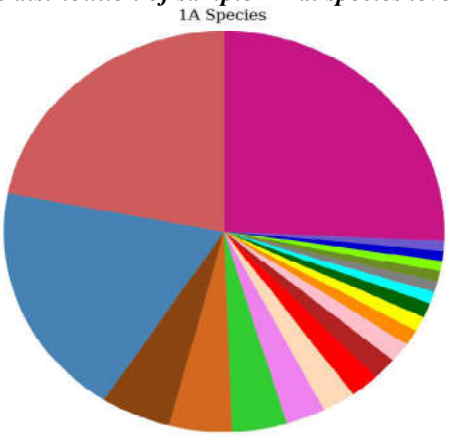


Figure 4 Pie-chart showing the absolute abundance of each species within each microbial community in sample 1A

Table 3 Table showing the species level abundance in respect to the pie-chart for the sample 1

1A Species legend

Legends	Taxonomy	Abundance
	k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__Streptophyta;f__Unclassified;g__Unclassified;s__Unclassified	21.9%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__mitochondria;g__Nelumbo;s__nucifera	18.93%
	k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Helicobacteraceae;g__Unclassified;s__Unclassified	5.16%
	k__Bacteria;p__Verrucomicrobia;c__[Spartobacteria];o__[Chthoniobacterales];f__[Chthoniobacteraceae];g__DA101;s__Unclassified	4.59%
	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Unclassified	4.02%
	k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Helicobacteraceae;g__Helicobacter;s__pullorum	2.92%
	k__Bacteria;p__Verrucomicrobia;c__[Spartobacteria];o__[Chthoniobacterales];f__[Chthoniobacteraceae];g__Candidatus Xiphinematobacter;s__Unclassifi	2.43%
	k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Campylobacteraceae;g__Campylobacter;s__Unclassified	2.16%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylocystaceae;g__Unclassified;s__Unclassified	1.81%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Rhodoplanes;s__Unclassified	1.61%
	k__Bacteria:p__Proteobacteria:c__Alphaproteobacteria:o__Rhizobiales:f__Bradyrhizobiaceae:g__Bradyrhizobium:s__Unclassified	1.27%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__mitochondria;g__Spirodela;s__polyrhiza	1.19%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Unclassified;g__Unclassified;s__Unclassified	1.18%
	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Unclassified;s__Unclassified	0.93%
	k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Gemmatales;f__Isosphaeraceae;g__Unclassified;s__Unclassified	0.88%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylocystaceae;g__Rhodoblastus;s__acidophilus	0.88%
	k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Gemmatales;f__Gemmataceae;g__Unclassified;s__Unclassified	0.84%
	k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Unclassified;s__Unclassified	0.77%
	k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Pirellulales;f__Pirellulaceae;g__Unclassified;s__Unclassified	0.77%
	Others	25.76%

Taxonomic distribution of sample 2A at species level

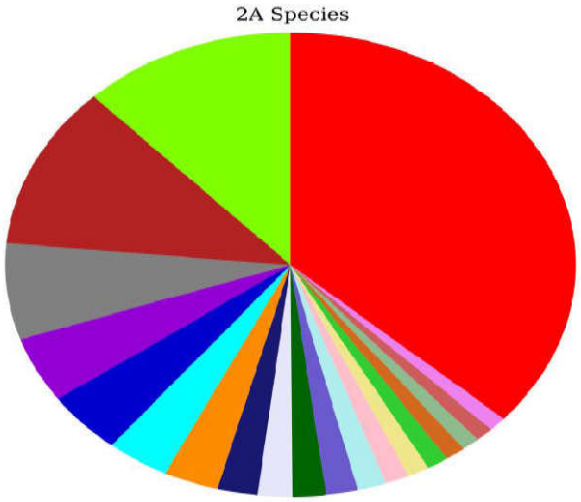


Figure-5 Pie-chart showing the absolute abundance of each species within each microbial community in sample 2A

Table-4: Table showing the species level abundance in respect to the pie-chart for the sample 2A

2A Species legend

Legends	Taxonomy	Abundance
	k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__Streptophyta;f__Unclassified;g__Unclassified;s__Unclassified	12.12%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__mitochondria;g__Nelumbo;s__nucifera	11.42%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae;g__Unclassified;s__Unclassified	6.66%
	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Sinobacteraceae;g__Unclassified;s__Unclassified	4.64%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Rhodoplanes;s__Unclassified	4.37%
	k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__Unclassified;g__Unclassified;s__Unclassified	3.56%
	k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Pirellulales;f__Pirellulaceae;g__Unclassified;s__Unclassified	3.1%
	k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus;s__Unclassified	2.79%
	k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Helicobacteraceae;g__Unclassified;s__Unclassified	1.95%
	k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__Unclassified;g__Unclassified;s__Unclassified	1.89%
	k__Bacteria;p__TM7;c__TM7-1;o__Unclassified;f__Unclassified;g__Unclassified;s__Unclassified	1.8%
	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Rhodanobacter;s__Unclassified	1.64%
	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__MND1;f__Unclassified;g__Unclassified;s__Unclassified	1.36%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Devosia;s__Unclassified	1.23%
	k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Planctomycetales;f__Planctomycetaceae;g__Planctomyces;s__Unclassified	1.21%
	k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Helicobacteraceae;g__Helicobacter;s__pullorum	1.15%
	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Butyrivibrio;s__Unclassified	1.02%
	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Sinobacteraceae;g__Steroidobacter;s__Unclassified	1.01%
	k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__EB1017;g__Unclassified;s__Unclassified	1.01%
	Others	36.57%

A-diversity of both the sample

α-diversity summarizes the diversity of the organisms in a sample with a single number. The alpha diversity of the annotated sample can be estimated from the distribution of the species-level annotations.

Table 5 Table showing alpha diversity of both the samples with respect to Shannon alpha diversity

Sample	Observed species	Shannon alpha diversity
1A	4,256	6.35
2A	4,686	7.84

Comparative analysis of Sample 1A and 2A

The comparative analysis between the samples has been done at different taxonomic levels taking 0.5% threshold.

Table 6 Table showing comparative analysis taxonomy distribution for both the samples

Taxonomy	1A%	2A%
k- Bacteria; p- Proteobacteria	51.6	49.0
k-Bacteria; p- Cyanobacteria	12.2	22.2
k- Bacteria; p- Actinobacteria	12.0	5.4
k- Bacteria; p- Firmicutes	8.3	7.1
k- Bacteria; p- Planctomycetes	6.7	3.8
k- Bacteria; p- TM7	2.3	0.1
k- Bacteria; p-Verrucomicrobia	1.4	7.5
k- Bacteria; p- Chloroflexi	1.3	2.3
k- Bacteria; p-Bacteroidetes	1.3	0.7
k- Bacteria; p- Acidobacteria	1.2	0.8
k- Bacteria; p- Gemmatimonadetes	0.6	0.1
k- Bacteria; p- Teniricutes	0.2	0.5

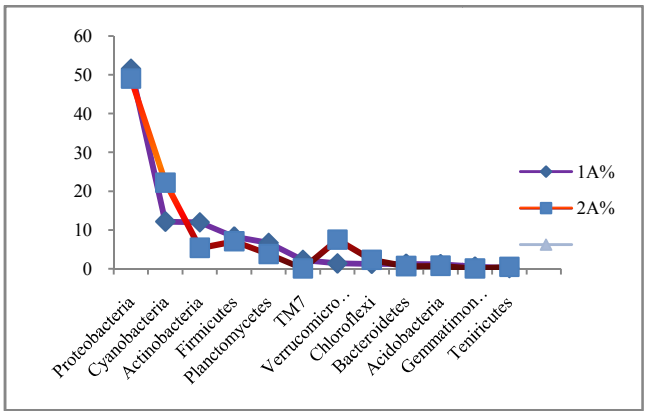


Figure 6 Graphical representation of taxonomy distribution for both the samples

Rarefaction analysis

Rarefaction allows the calculation of species richness for a given number of individual samples, based on the so-called rarefaction curves. The curve is a plot of the number of species as a function of the number of sample. On the left, the steep slope indicates that a large faction of the species diversity remains to be discovered. The vertical axis displays the diversity of the community, while the horizontal axis displays the number of sequences considered in the diversity calculation.

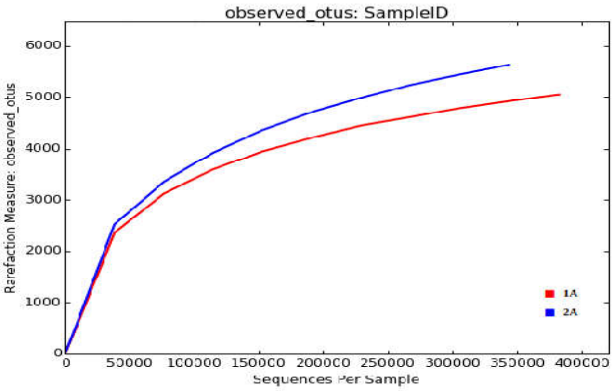


Figure 6 Rarefaction curve of sample 1A and 2A

Heatmap

Heatmap are generated to visualize the OTU table at different levels where each row corresponds to an OTU and each column corresponds to a sample. The higher the relative abundance of a OTU in a sample, the more intense the color at the corresponding position in the heatmap. Red contributes Low percentage of OTUs to sample while purple contributes high percentage of OTUs.

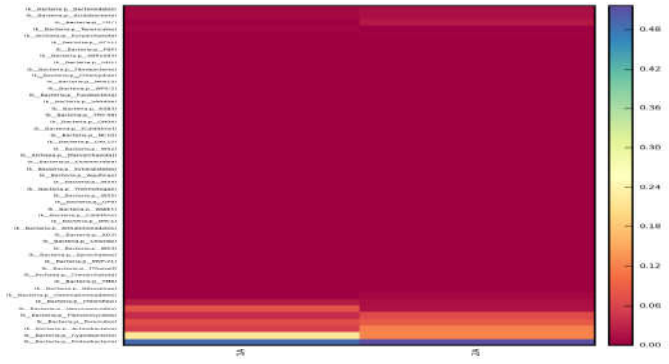


Figure 7 Heatmap of both the samples at phylum level bacterial diversity abundance

Krona chart preparation of both the samples at species level for bacterial diversity abundance

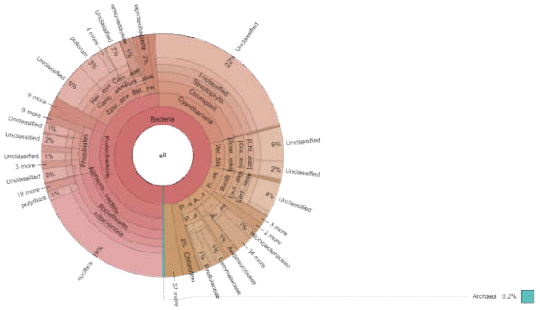


Figure-8: Krona chart showing species level bacterial diversity for Sample 1A

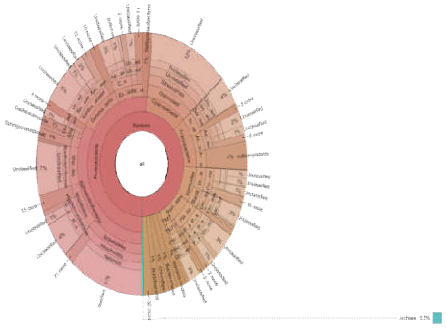


Figure-9: Krona chart showing species level bacterial diversity for Sample 2A

DISCUSSION

The present study was undertaken to study the bacterial diversity both culturable and unculturable of the selected two earthworm species *Pheretima posthuma* and *Eisenia fetida* guts sample using Illumina Miseq studies. The two earthworm species were named as 1A for *P. posthuma* and 2A for *E. fetida* for the further study. Metagenomic DNA was isolated from the supplied earthworm gut samples by using c-TAB and Phenol: Chloroform Extraction method.

The absorption spectrum of DNA extracts (230–280nm and 260-230nm) was determined using Nano-drop(R) ND-1000 spectrophotometer (Eurofins Genomics Bioinformatics Lab) according to the manufacturer's instructions. Sample 1A showed nanodrop reading for 1.61 and 1.52 for 230–280nm and 260-230nm respectively. Sample 2A showed 1.75 and 1.32 for 230–280nm and 260-230nm namedrop reading respectively.

The amplicon libraries were prepared using Nextera XT Index Kit (Illumina inc) As per the 16S Metagenomic Sequencing Library preparation protocol. Primers for the amplication of the 16S rDNA gene specific for bacteria were designed at Eurofins Genomics Bioinformatics Lab and Synthesized at Eurofins Genomics Lab facility.The total bases read of bacterial diversity for the sample 1A named after earthworm species *Pheretima posthuma* is 346,507,919 and for sample 2A named after *Eisenia fetida* is 391,653,372.

In the present study it is seen that Proteobacteria is predominant with highest count in both earthworm species followed by Cyanobacteria, Actinobacteria, Fermicutes, Planctomycetes, TM7, Verrucomicrobia, Chloroflexi,

Bacteroidetes, Acidobacteria and so on.In Sample 1A in abundance taxonomy it is found that Proteobacteria count for 48.98% at phylum level, Alphaproteobacteria count for 32.9% at class level and Streptophyta count for 21.9% at order, family, genus and species level.In Sample 2A in abundance taxonomy it is found that Proteobacteria count for 51.55% at phylum level, Alphaproteobacteria count for 33.08% at class level, Rickettsiales count for 12.91% at order level, Mitochondria count for 12.87% at family level, and Unclassified family from Streptophyta Order count for 12.12% at genus and species level.

In Sample 1A Streptophyta order with unclassified family genus and species counted the highest with 21.9% followed by *Nelumbo nucifera* from family mitochondria counted for 18.93% in total count. *Spirodela polyrhiza* from family mitochondria count for 1.19% *Rhodoblastus acidhophilus* for family Methylocystaceae count for 0.88%, unclassified species from different order and family counted 53.24% of which phylum Proteobacteria, Verrucomicrobia, Firmicutes, were counted dominant and others with unknown classification counted for nearly 25.76%.In sample 2A Streptophyta order with unclassified family, genus and species counted the highest with 12.12%, *Nelumbo nucifera* from family mitochondria counted for 11.42% in total count. *Helicobacter pullorum* from family helicobacteraceae counted 1.15%, unclassified species from different order and family counted nearly 50.86% of which phylum Cyanobacteria, Proteobacteria, Actinobacteria, Planctomycetes, Firmicutes were dominant and others with unknown classification counted for nearly 36.57%.

Several novel species were identified in both the samples. *Nelumbo nucifera, Helicobacter pullorum, Rhodoblastus acidophilus* and *Spirodela polyrhiza* were isolated from Sample 1A and *Nelumbo nucifera* and *Helicobacter pullorum* from Sample 2A. However the importance in the gut microbiota of the mentioned bacterial species is still in dark as no selective reference being published.The multidimensional soil habitat is composed of an immensely heterogeneous distribution of habitat types and food resources (Ritz *et al*., 2004). Species are known to adapt themselves according to habitat type to reduce the limiting effects of biotic and abiotic factors of existence (Odum, 1971). In doing so, earthworms adjust themselves in terms of their diets, and diet quality and availability are habitat specific.

Earthworms of different functional groups, or even different species within the same functional group, have a particular mode of food selection, ingestion, digestion, assimilation and movement, thus their importance in mixing, decomposition or nutrient release, as well as in the structure and activity of microbial communities will vary both qualitatively and quantitatively (Curry & Schmidt, 2007).

In general neutral to slightly alkaline conditions are considered more favorable for bacterial growth than acidic conditions (Alexander, 1977; Sylvia *et al*., 2005). The selection of bacteria associated with the gut wall of anecic and endogeic species of earthworms was a natural selection process, with the major determinants being ecological group, followed by habitat and species (Thakuria *et al*. 2010:Ma *et al.,* (1995) found that presence of earthworms in soil enhances the degradation of organic contaminants like phthalate, phenanthrene and fluoranthene. Contaminants are degraded by enzymatic activity called 'Cytochrome P 450' system working in earthworms. This enzymatic activity has been found to operate particularly in *Elsenia fetida* which survive the benzopyrene concentration of 1008 mg/kg of soil (Achazi *et al*., 1998).

Actinobacteria, especially *Streptomyces* spp., are recognized as the producers of many bioactive metabolites that are useful to humans in medicine, such as antibacterials (Mahajan, 2012), antifungals (Gupte *et. al.,* 2002), antivirals, antithrombotics, immunomodifiers, antitumor drugs, and enzyme inhibitors; and in agriculture, including insecticides, herbicides, fungicides, and growth-promoting substances for plants and animals (Bressan, 2003, Atta, 2009).

Actinobacteria-derived antibiotics that are important in medicine include aminoglycosides, anthracyclines, chloramphenicol, macrolide, tetracyclines, etc. Actinobacteria have high guanine and cytosine content in their DNA (Ventura *et. al.,* 2007). Stable isotope probing studies have found that some members of the phylum TM7 can degrade toluene (Luo *et. al*., 2009). Despite the associated toxins which many of the members of this phylum cyanobacteria produce, some microalgae also contain substances of high biological value, such as polyunsaturated fatty acids,amino acids (proteins), pigments, antioxidants, vitamins, and minerals (Christaki *et. al.,* 2011).Degradation of PAHs occurs when microorganisms break the aromatic rings and produce aliphatic compounds that readily enter the tricarboxylic acid cycle (metabolic activity) operating in living cells. *Cunniughamela elegans* and *Candida tropicalis* have been reported to degrade PAHs (Kanaly and Harayama 2000).

Members of the Firmicutes were found in the intestinal tissues of earthworm species *L. terrestris, Octolasion cyaneum, Lumbricus rubellus* and *Onychochaeta borincana* (Jolly *et al*., 1993, Singleton *et al*., 2003, Valle-Molinares *et al.*, 2007). Pheretima worms are administered as a medicine in China. The worm contains biological agents beneficial in rat models of stroke. In clinical practice, it has been recognized for its curative effects in the treatment of epilepsy (Liu *et. al.,* 2012). It contains hypoxanthine, lumbrofebrin, and lumbritin.
A few of the gut wall-associated bacteria that were relatively abundant in both the endogeic and anecic earthworm species were closely related to Bradyrhizobium, Mycobacterium, Acidovorax and Streptomyces strains. The presence of these species may be functionally significant in terms of both C and N metabolism. Mycobacteria are known to use humic and fulvic acids in soils (Kirschner *et al.,* 1999), and *Mycobacterium avium* and *M. gastri* strain were previously isolated from *L. rubellus* guts (Fisher *et al.,* 2003).

Streptomyces are believed to be involved in the assimilation of hemicellulose, xylans and xylose present in ingested crop residues, because the majority of Streptomyces found in soil possess glucose isomerase activities (Killham and Prosser, 2007). By facilitating the formation of an appropriate gut wall-associated bacterial community, we will maximize our ability to exploit benefits of earthworms for sustain ability of soil ecosystem at local, regional and global scales.

CONCLUSION

Understanding the composition and function of the earthworm gut wall-associated bacterial community will help designing in

management practices for sustainable agriculture and other land uses along with biodegradation, bioremediation and pollution control aspects. More findings and research is required for better understanding their aspects in animal world.

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