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ORIGINAL ARTICLE

Comparative Study on Bacterial Population Dynamics of Foregut, Midgut, and Hindgut Content of *Perionyx excavatus* (Perrier) Isolated from Eco-friendly, Non-hazardous Vermicompost

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Abstract

The ideal condition of earthworm gut promotes growth and multiplication of beneficial soil microorganisms eliminating pathogens and converts organic wastes into nutrients rich compost. The present study has been carried out to determine the population dynamics of earthworm gut bacteria and to find out relative abundance of different functional bacterial groups in the foregut, midgut, and hindgut of earthworm *Perionyx excavatus*. To assess bacterial diversity, a viable plate count method was adopted. In the different gut region of earthworm, aerobic heterotrophic, amylolytic, *Bacillus*, Gram-negative, proteolytic, fat hydrolyzing, nitrate-reducing, nitrifying, asymbiotic nitrogen-fixing, *Azotobacter*, and phosphate solubilizing bacterial populations ranged from 22.2 to 241.6×10^6, 8.0 to 171.60×10^6, 1.83 to 2.79×10^6, 10.68 to 23.04×10^4, 3.70 to 5.52×10^4, 59.60 to 208.40×10^4, 1.86 to 7.34×10^4, 10.94 to 19.78×10^4, 0.80 to 3.42×10^4, 7.83 to 13.70×10^4, 1.31 to 2.67×10^4 cfu/ml gut suspension, respectively. The results of the one-way ANOVA revealed that the bacterial load of most of the bacterial groups was significantly higher ($p<0.05$) in the hindgut region, followed by midgut and foregut. Only the density of the proteolytic group was significantly higher ($p<0.05$) in the midgut region followed by foregut and hindgut. Starch hydrolyzing bacteria constitute the largest group of bacteria in the gut content. From principal component analysis, two components were extracted with the eigenvalues of 8.485 and 1.132. Agglomerative hierarchical cluster analysis revealed that the bacterial populations were clustered into four different groups. Quantitative variation among bacterial groups in earthworm's gut seems to determine the soil health and composting efficiency; from this point of view, the present study will provide a better understanding about different functional bacterial groups of earthworm's guts and might be helpful in sustainable agriculture and waste management.

Keywords Earthworm gut · *Perionyx excavatus* · Bacterial diversity · Population dynamics, Soil health

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Introduction

Earthworms are considered as ecosystem engineers as they play imperative responsibility in the modification of physicochemical and biological characteristics of the soil [1]. They break down bulky soil particles and organic wastes, making them accessible for microbial degradation and ultimately convert the debris to precious vermicompost with the help of microbes [2]. Indiscriminate use of chemical fertilizers and pesticides causes serious threats to natural water bodies and living organisms. In 2017, agrochemicals are projected at around 7.55 billion and by 2050 are anticipated to hit 9.8 billion to provide enough aliment for the global population [3]. The toxicity of chemical fertilizer and pesticides on physiology, oxidative stress biomarkers, development, and growth of different aquatic non-target organisms had been reported previously [4]. On the other hand, earthworm's involvement improves disintegration and biodegradation of organic wastes 60–80% by face lifting the growth and multiplication of beneficial decomposer bacteria [5]. Soil microorganisms, i.e., fungi, protozoa, algae, and bacteria are considered to be the major portion of diet for earthworms [6]. Earthworm gut is a tubular structure consisting of a mouth, muscular pharynx, gizzard, intestine, and associated digestive glands [7]. The intestine is further divided into foregut, midgut, and hindgut [8]. Earthworm gut provides ideal physico-chemical conditions like neutral pH, high moisture, optimum temperature, organic and mineral-rich mucus for the growth, and development of microorganisms [9, 10]. Earthworm gut is considered as a natural filter as it promotes growth and multiplication of beneficial soil microorganisms, elimination of soil pathogens, and conversion of organic wastes into nutrient rich compost [11, 12]. Microbial load in the earthworm gut is higher than that in the surrounding soil, and there is immense bacterial diversity in earthworm gut [13]. This bacterial community plays a crucial role in the degradation processes [14]. Several factors in the earthworm gut may regulate the bacterial community [15]. Enzymes like cellulose, amylase, lipase, chitinase, protease, lichenase, urease, nitrate reductase, invertase, acid phosphatase, and alkaline phosphatase have been reported from earthworm gut content [16, 17]. Some of them were produced by the earthworm itself [18, 19] while others were secreted by the ingested micro-organisms [20–22]. Those enzymes along with mucus and antibiotics in earthworm's gastrointestinal tract break down the organic macromolecules [17]. Thus, the microorganisms and earthworms act symbiotically and synergistically to speed up and improve the organic matter decomposition [23, 24].

Though earthworm gut bacteria play an important role in soil fertility, scanty literature is available on bacterial diversity in the gut region of earthworm [25]. In this context, the present study has been carried out to determine the population dynamics of earthworm gut bacteria and to find out their relative abundance in the foregut, midgut, and hindgut of earthworm *Perionyx excavatus* (Perrier, 1872).

Material and Methods

Collection of Earthworms

Live mature earthworms (*Perionyx excavatus*) were collected in a sterile plastic container from 30 days old composting beds of Kulti vermicomposting farm (23°12′ N, 88°30′ E) of Purba Bardhaman district of West Bengal, India. After collection, earthworms were

brought to the laboratory for further microbial analysis. Specimen was submitted to the Zoological Survey of India, Kolkata, and identified as *Perionyx excavatus* (Perrier) based on external morphological features such as number of segments, clitellum characteristics, and color.

Dissection of Earthworm and Collection of Gut Content

Live adult earthworms were superficially sterilized with 50% alcohol and rapidly transferred to the dissection tray. The digestive tract was cut open and the content from foregut, midgut, and hindgut was collected with a sterile loop and kept separately into sterile Eppendorf tubes.

Analysis of Earthworm Gut Content

To assess the bacterial diversity in the earthworm gut content, viable plate count method was adopted during the present study. Enumeration of the bacterial populations belonging to different groups was performed following standard methodologies [26, 27]. The gut content was diluted with sterile distilled water up to 10^{-4}. To determine the aerobic heterotrophic bacterial population, 50 µl gut suspension from 10^{-4} dilutions was added to 100 ml of nutrient agar (peptone 5.0, yeast extract 1.5, HM peptone 1.5, NaCl 5.0, agar 15 g l^{-1}, pH-7.4). Then the inoculated medium was distributed on five sterile petri plates and incubated at 30 ± 1 °C in the BOD incubator for 48 h. To determine other bacterial populations, 50 µl of gut suspension of different dilutions was added to 100 ml of each bacterial group specific culture medium and distributed in five plates and incubated at 30 ± 1 °C. The Gram-negative bacterial population was enumerated by incubating the gut suspension into MacConkey agar (peptone 3.0, pancreatic digest of gelatin 17.0, lactose monohydrate 10.0, crystal violet 0.001, NaCl 5.0, neutral red 0.030, agar 13.5, and bile salts 1.5 g l^{-1}, pH-7.1) for 24 h. To determine the amylolytic bacterial population, the sample was incubated in starch agar medium (soluble starch 2.0, meat extract 3.0, peptic digest 5.0, agar 15.0 g l^{-1}, pH 7.2 ± 0.1) for 24 h and only the colonies producing clear zone after flooding with Gram's iodine were counted as starch hydrolyzer. The total *Bacillus* population was recorded after 48 h incubation of gut suspension in the HiCrome *Bacillus* agar (peptone 10.0, HM extract 1.0, NaCl 10.0, D-mannitol 10.0, chromogenic mixture 3.2, Phenol red 0.025, agar 15 g l^{-1}, pH-7.1). To determine the nitrifying bacterial population, the sample was incubated in Winogradsky's medium (ammonium sulfate 1.0, dipotassium hydrogen phosphate 1.0, manganese sulfate heptahydrate 0.5, NaCl 2.0, ferrous sulfate heptahydrate trace, calcium chloride dihydrate 0.02, agar 10 g l^{-1}, pH-8.5), and the pink colonies were visualized by flooding the plates with α-naphthylamine and sulfanilic acid (1:1). The colony number of this particular group of bacteria was recorded at 5 days intervals from the date of incubation up to 30 days. But all other groups of bacterial populations were recorded after 72 h of incubation. Inorganic phosphate solubilizing bacterial population was determined by incubating the gut suspension on Pikovskaya's agar media (yeast extract 5.0, dextrose 10.0, calcium phosphate 5.0, ammonium sulfate 0.5, KCl 0.2, $MgSO_4$ 0.1, $MnSO_4$ trace, $FeSO_4$ trace, agar 15 g l^{-1}, pH-7.0) and counting the number of bacterial colonies producing a clear zone after 72 h incubation. Nitrate reducing bacterial population was recorded on nitrate reducing agar medium (potassium nitrate 1.0, peptone 5.0, HM peptone 3.0, agar powder 12.0, pH-6.8) by visualizing pink colonies after flooding the plates with α-naphthylamine and sulfanilic acid (1:1). The same procedure was

followed to count asymbiotic nitrogen-fixing bacterial population on nitrogen-free medium (mannitol 10.00, dipotassium hydrogen phosphate 0.50, magnesium sulfate 0.20, sodium chloride 0.20, manganese sulfate trace, ferric chloride trace, agar powder 18.00 g l^{-1}, pH 7.20 ± 0.2). Protein (gelatine) hydrolyzing bacterial population was determined by the presence of the halo zone around the colonies by flooding with $HgCl_2$ on nutrient agar medium with 2% gelatine. Spirit blue agar medium (casein enzymic hydrolysate 10.00, yeast extract 5.00, spirit blue 0.15, agar 17.00 g l^{-1}, pH 6.8 ± 0.2) with TWEEN 80 were used to determine fat hydrolyzing groups. *Azotobacter* population was enumerated by incubating the gut suspension on *Azotobacter* agar medium (mannitol 20.00, dipotassium phosphate 1.0, magnesium sulfate 0.20, sodium chloride 0.20, $FeSO_4$ trace, agar 15 g l^{-1}, pH-8.3) for 24 h. Incubation period varied among certain bacterial groups because off the growth rate of different bacterial groups varied in different culture media.

Statistical Analysis

The data obtained from the study were further subjected to statistical analysis using XLSTAT software to draw a more specific conclusion. After performing the normality check utilizing the Shapiro Wilk test, the difference in the abundance of bacterial groups in the foregut, midgut, and hindgut of earthworm was evaluated by one-way ANOVA followed by the Tukey test. The data obtained on the different groups of bacteria present in the earthworm gut were subjected to heat mapping and agglomerative hierarchical cluster analysis. Furthermore, the relation between various bacterial groups of earthworm gut was evaluated by principal component analysis (PCA) [28]. The principal components were chosen as per Gniazdowski [29].

Result

Bacterial population isolated from different gut regions of earthworm *Perionyx excavatus* showed a normal distribution in QQ plot (Fig. 1).

In the different gut regions of earthworm *P. excavatus*, bacterial loads of aerobic heterotrophic, starch-hydrolyzing, total *Bacillus*, Gram-negative, gelatin hydrolyzing, fat hydrolyzing, nitrate-reducing, nitrifying, asymbiotic nitrogen-fixing, *Azotobacter*, and phosphate solubilizing populations ranged from 22.2 to 241.6×10^6, 8.0 to 171.60×10^6, 1.83 to 2.79×10^6, 10.68 to 23.04×10^4, 3.70 to 5.52×10^4, 59.60 to 208.40×10^4, 1.86 to 7.34×10^4, 10.94 to 19.78×10^4, 0.80 to 3.42×10^4, 7.83 to 13.70×10^4, 1.31 to 2.67×10^4 cfu/ml gut suspension, respectively (Fig. 2). The results of the one-way ANOVA (Table 1) revealed that bacterial load of aerobic heterotrophic, starch-hydrolyzing, total *Bacillus*, Gram-negative, fat hydrolyzing, nitrate-reducing, nitrifying, asymbiotic nitrogen-fixing, *Azotobacter*, and phosphate solubilizing population were significantly higher ($p < 0.05$) in the hindgut region, followed by midgut and foregut. Only the density of the gelatin hydrolyzing group was significantly higher ($p < 0.05$) in the midgut region followed by foregut and hindgut.

The result from heatmap also confirms that the aerobic heterotrophic, starch-hydrolyzing, total *Bacillus*, Gram-negative, fat hydrolyzing, nitrate-reducing, nitrifying, asymbiotic nitrogen-fixing, *Azotobacter*, and phosphate solubilizing population exhibited higher density in the hindgut region and gelatin hydrolyzing bacterial population was higher in the midgut region (Fig. 3).

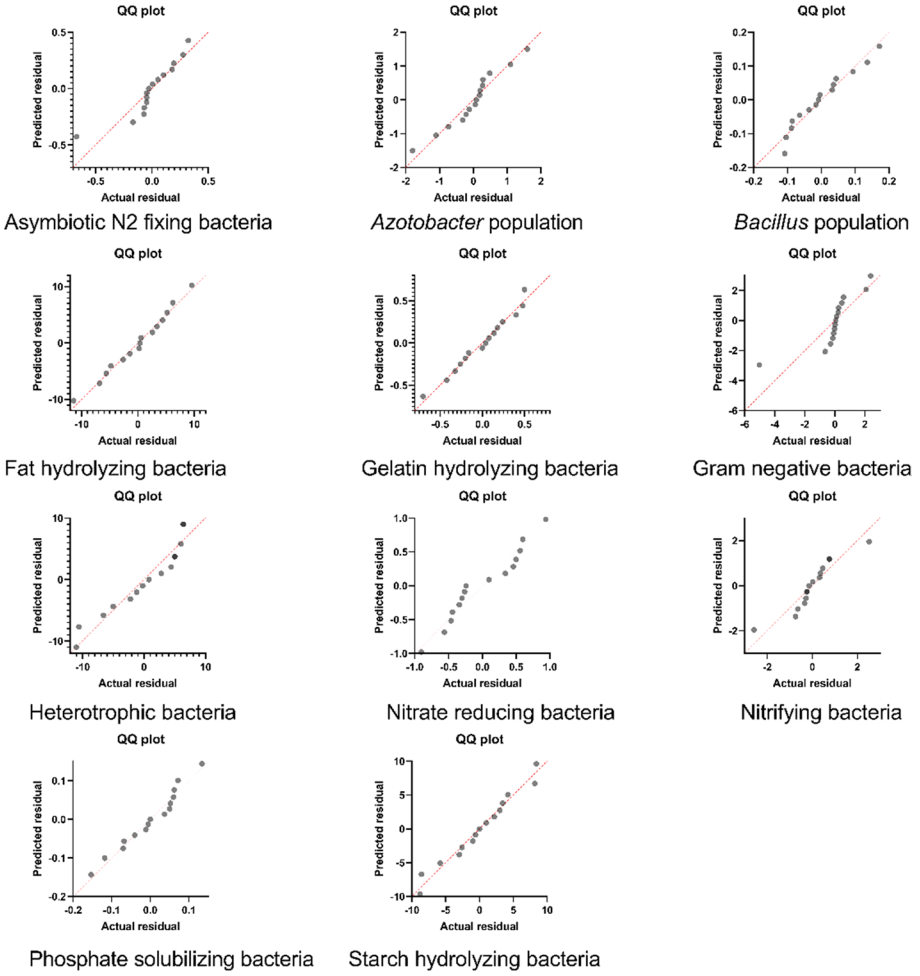

Fig. 1 QQ plot showing the normality of the obtained data of different bacterial groups isolated from gut of earthworm *Perionyx excavatus*

From principal component analysis (PCA), a total of 10-factor components were observed in the scree plot (Fig. 4c). Based on eigenvalues, factor 1 (8.485) and factor 2 (1.132) were considered that explained 96.171% (component 1: 84.847% and component 2: 11.324%) of the variance on the bacterial population in the different gut regions of the earthworm *Perionyx excavatus* (Table 2). The score plot of the observed variables (Fig. 4b) showed that component 1 successfully separated the bacterial population of hindgut from fore- and midgut whereas the component 2 separated the data obtained from bacterial population of midgut from fore- and hindgut regions.

The factor loading table showed that the 1st principal component is strongly correlated with aerobic heterotrophic, starch hydrolyzing, total *Bacillus*, Gram-negative, fat hydrolyzing, nitrate-reducing, nitrifying, asymbiotic nitrogen-fixing, *Azotobacter*, and phosphate solubilizing population and 2nd principal component is strongly correlated with gelatin hydrolyzing population (Table 3).

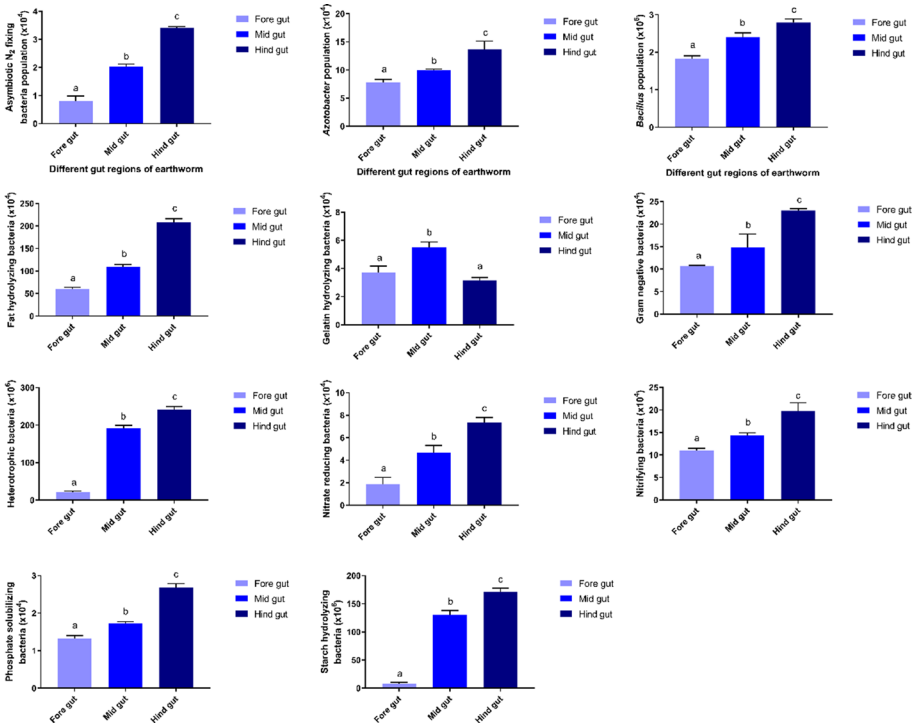

Fig. 2 Population dynamics (cfu ± SE) of different bacterial groups in the foregut, midgut, and hindgut of earthworm. Different alphabets (a–c) represent the significant difference between bacterial groups in earthworm gut (One way ANOVA followed by Tukey test, $p < 0.05$)

Table 1 One way ANOVA of population dynamics (cfu ± SE) of different bacterial groups in the fore, mid and hindgut of earthworm showing the DF, *F*, and *P* values

Bacterial population	One way ANOVA results		
	DF	*F* (DFn, DFd)	*P* value
Heterotrophic bacteria ($\times 10^6$)	2	$F(2, 12) = 1576$	$P < 0.0001$
Gram negative bacteria ($\times 10^4$)	2	$F(2, 12) = 65.26$	$P < 0.0001$
Starch hydrolyzing bacteria ($\times 10^6$)	2	$F(2, 12) = 1133$	$P < 0.0001$
Gelatin hydrolyzing bacteria ($\times 10^4$)	2	$F(2, 12) = 55.26$	$P < 0.0001$
Fat hydrolyzing bacteria ($\times 10^4$)	2	$F(2, 12) = 792.3$	$P < 0.0001$
Nitrate reducing bacteria ($\times 10^4$)	2	$F(2, 12) = 112.6$	$P < 0.0001$
Nitrifying bacteria ($\times 10^4$)	2	$F(2, 12) = 75.02$	$P < 0.0001$
Asymbiotic N_2 fixing bacteria ($\times 10^4$)	2	$F(2, 12) = 135.9$	$P < 0.0001$
Bacillus population ($\times 10^6$)	2	$F(2, 12) = 133.4$	$P < 0.0001$
Azotobacter population ($\times 10^4$)	2	$F(2, 12) = 56.14$	$P < 0.0001$
Phosphate solubilizing bacteria ($\times 10^4$)	2	$F(2, 12) = 346.1$	$P < 0.0001$

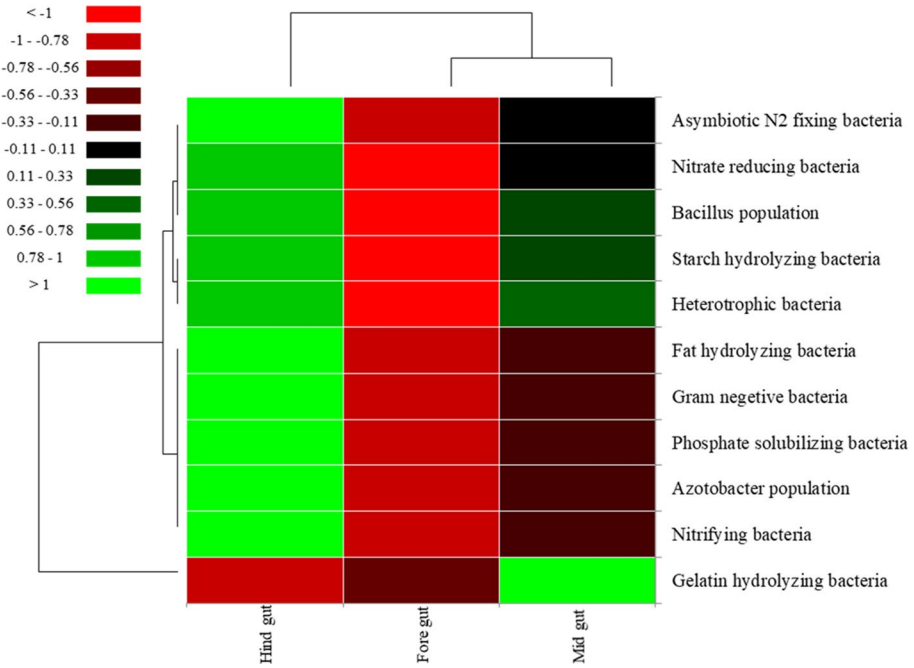

Fig. 3 Hierarchical clustering heatmap of different bacterial groups from earthworm gut indicating their distribution in foregut, midgut, and hindgut region

The bacterial groups were plotted on the quadrant plot, and it showed a projection of the initial variable in the four quadrants. The first quadrant was comprised of starch hydrolyzing, total *Bacillus*, nitrate-reducing, and asymbiotic nitrogen-fixing bacterial group; the second quadrant contained *Azotobacter*, nitrogen-fixing, fat hydrolyzing, Gram-negative and phosphate-solubilizing bacteria, and the fourth quadrant was comprised of a single group of gelatine hydrolyzing bacteria (Fig. 4a).

The result of the Pearson correlation matrix (Fig. 4d) revealed significant ($p < 0.05$) positive correlation among aerobic heterotrophic, starch-hydrolyzing, total *Bacillus*, Gram-negative, fat hydrolyzing, nitrate-reducing, nitrifying, asymbiotic nitrogen-fixing, *Azotobacter*, and phosphate solubilizing population, and all these bacterial groups were negatively correlated with the gelatin hydrolyzing bacterial group.

Agglomerative hierarchical cluster analysis (AHC) in respect of the number of various bacterial groups in different gut regions revealed that the bacterial populations were clustered into four different groups below the automatic truncation line. Phosphate solubilizing, fat hydrolyzing, Gram-negative, asymbiotic nitrogen-fixing, and nitrifying bacterial group created a single cluster in respect of their distribution pattern while the *Azotobacter* group diverged from them forming another cluster. *Bacillus* and starch-hydrolyzing and the nitrate-reducing bacterial group formed a different cluster and gelatine hydrolyzing bacteria further differed from these three groups forming single cluster (Fig. 5).

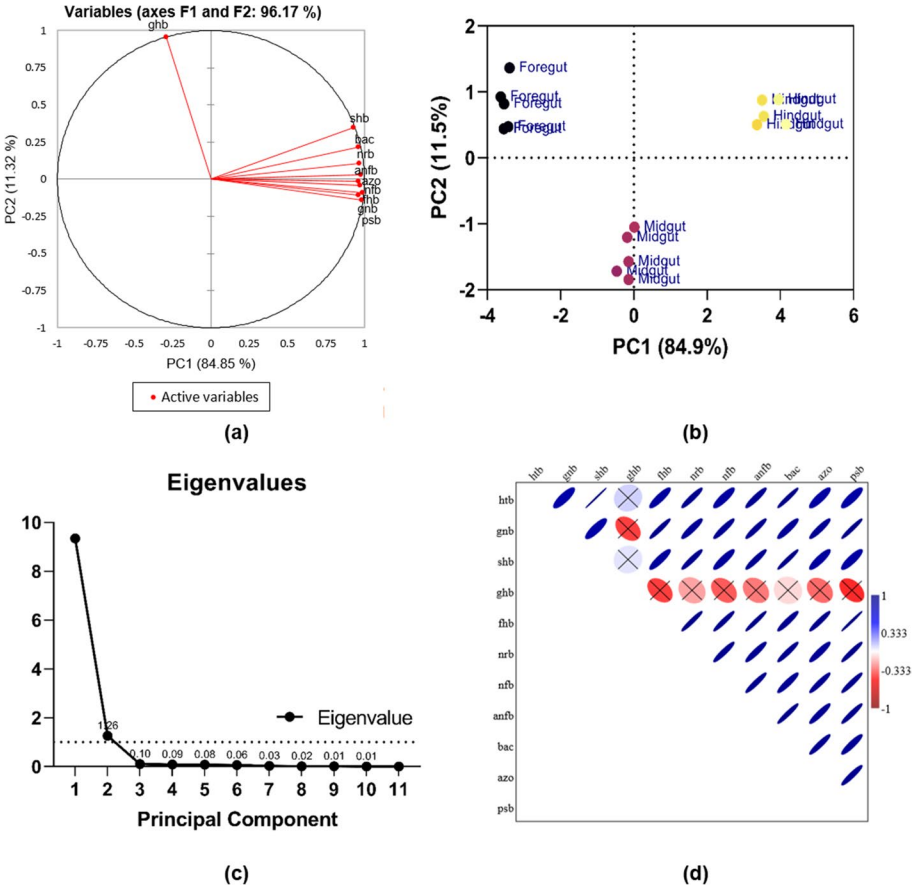

Fig. 4 **a** Ordination diagram of quadrant distribution of different bacterial groups from earthworm gut via PCA. **b** Score plot of observed variable showing the distribution and clustered of the obtaining data among two major components. **c** Scree plot showing the Eigenvalues, principal components and cumulative variability (%) derived from PCA regarding the bacterial groups from earthworm gut. **d** Pearson correlation matrix (*n*) between different bacterial groups of earthworm gut. Crossed mark represents insignificant correlation; blue color indicates positive and red color indicates negative correlation. (psb: phosphate solubilizing bacteria, fhb: fat hydrolyzing bacteria, gnb: Gram-negative bacteria, anfb: asymbiotic nitrogen-fixing bacteria, nfb: nitrifying bacteria, azo: *Azotobacter*, bac: total *Bacillus*, shb: starch-hydrolyzing bacteria, nrb: nitrate-reducing bacteria and ghb: gelatine hydrolyzing bacteria)

Table 2 Eigenvalues (values > 1) extracted from the principal component analysis

	F1	F2
Eigenvalue	8.485	1.132
Variability (%)	84.847	11.324
Cumulative %	84.847	96.171

Table 3 Squared cosines of the variables; values in bold correspond for each variable to the factor for which the squared cosine is the largest

	F1	F2
gnb	**0.925**	0.012
shb	**0.860**	0.119
ghb	0.085	**0.910**
fhb	**0.974**	0.009
nrb	**0.937**	0.012
nfb	**0.940**	0.002
anfb	**0.954**	0.001
bac	**0.927**	0.048
azo	**0.924**	0.000
psb	**0.961**	0.020

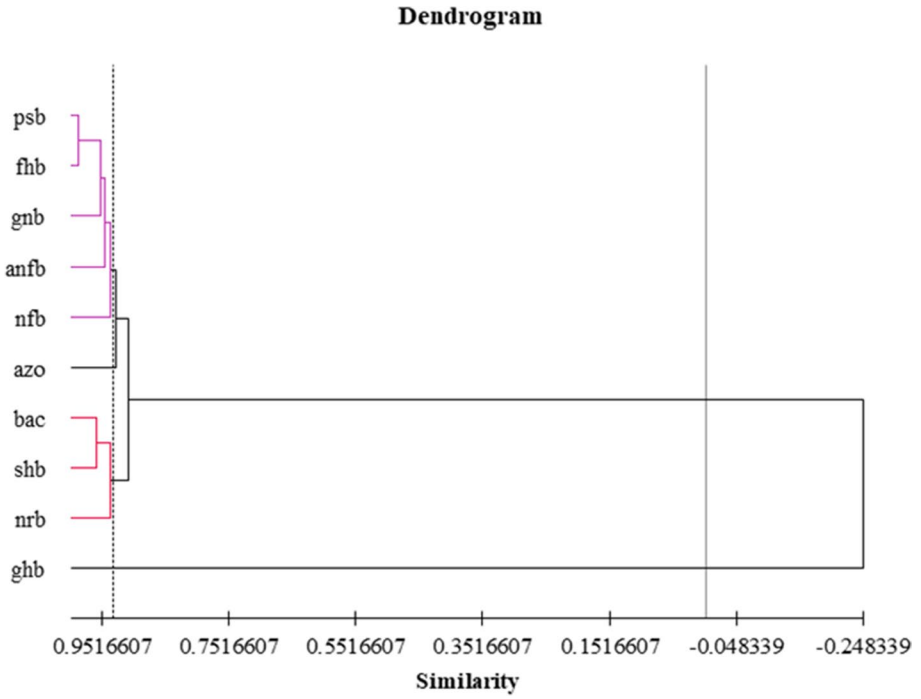

Fig. 5 Agglomerative hierarchical clustering (AHC) of different bacterial groups from earthworm gut; the dotted line indicates the automatic truncation, leading to four groups. (psb: phosphate solubilizing bacteria, fhb: fat hydrolyzing bacteria, gnb: Gram-negative bacteria, anfb: asymbiotic nitrogen-fixing bacteria, nfb: nitrifying bacteria, azo: *Azotobacter*, bac: total *Bacillus*, shb: starch-hydrolyzing bacteria, nrb: nitrate-reducing bacteria and ghb: gelatine hydrolyzing bacteria)

Discussion

The earthworm gut holds enormous bacterial diversity but only a little portion is explored [25]. Parle, one of the pioneer researchers in the field of on microbial presence in the earthworm gut, reported fungal, bacterial, and actinomycetes populations in three different

earthworm species in 1963 [18]. Thereafter, several studies have been made regarding the total aerobic bacterial population and changes in total bacterial loads while passing through the gut. But the detailed study on the qualitative and quantitative estimation of different bacterial groups from the different regions of the earthworm gut probably is not reported yet from these regions. Present study evinces that most of the bacterial groups exhibited higher density in the hindgut region of the earthworm whereas bacterial load was comparatively lower in the foregut region. Both the bacterial heat map and AHC analysis reflect the presence of a higher number of bacterial populations in the hindgut region. The highest aerobic heterotrophic bacterial load was found in the hindgut region followed by midgut and foregut. The gradual rise of the number of aerobic heterotrophs from the foregut to hind gut in *Perionyx excavatus* may be due to the epigeic nature of this earthworm species. When the organic debris passes through the digestive tract, it accumulates in hindgut which offers an ideal microclimatic environment for the profuse growth of the bacterial population. The earthworm gut acts like selective filter as well as fermentation vessel which provides favorable condition for the growth and activity of bacterial community. Several factors such as anoxic environment, neutral pH, high moisture content, ideal temperature conditions [30], and mucus-containing nutrients and easily metabolizable compounds collectively contribute making the earthworm gut ideal habitats for bacteria [31]. However, the total heterotrophic bacterial load may vary through the different regions of the earthworm digestive tract, and it may vary among different species of earthworms. Earlier reports support these findings as they stated that the population of soil microorganisms ingested by earthworm increases while passing through the gut [32]. The microbial population in the fresh vermicasts was higher than the midgut content of earthworms. Birundha et al. [33] reported if the bacterial population of midgut content was considered as 1, it increased to 1.20 in the fresh vermicastings of *Perionyx excavatus*. This result indicates that further bacterial replication occurs in the hindgut region, and the overall bacterial population increases. One of the earliest studies stated that the highest number of total bacterial population was found on hindgut followed by midgut and then foregut in *L. terrestris* [34]. Chowdhury et al. [35] studied total microbial communities in the gut content of *Perionyx excavatus* and found the highest abundance of bacteria-actinomycetes in hindgut followed by midgut and minimum in foregut. Though the maximum generic variation of bacteria-actinomycetes was detected in foregut followed by midgut and hindgut, Kristufek et al. [36] reported that cfu of total heterotrophic bacteria in the foregut region was 7×10^6/g gut content, but it amplified to 16×10^6 and 29×10^6 in the midgut and hindgut region, respectively in *Lumbricus rubellus*. But an opposite observation was also reported where bacterial population decrease towards the posterior region in the case of earthworm *Onycochaeta borincana* [37] and *Aporrectodea caliginosa* [36]. From another study, it was found that the midgut fluid taken from three earthworm species namely *Aporrectodea caliginosa*, *Lumbricus terrestris*, and *Eisenia fetida* could selectively suppress bacterial growth. But the hindgut fluid did not show such suppressive activity; moreover, the growth of most of the bacterial strains increased under the influence of hindgut fluid [6].

During the present study, most of the bacterial group exhibited higher density in the hindgut region but the proteolytic bacterial population was found maximum in the midgut region, followed by foregut and then hindgut. Another experiment was conducted by Mishra and Dash [38], and they reported higher proteolytic activity in the midgut region, followed by foregut and hindgut in *Lampito mauritii*. This enzymatic activity may be achieved by the proteolytic bacteria present in the midgut content.

From the present study, it was observed that the *Bacillus* population constituted a large portion of total bacterial load, and it was found higher in the posterior region. Kim et al.

[39] also described the genus *Bacillus* as the leading group in the gut region of earthworm. An increase in the number of *Bacillus cereus* was observed as the food shifts backward along the intestinal length of earthworm [40]. Among heterotrophos, starch-hydrolyzing bacteria constitute the largest group of bacteria in the gut content. They produce enzyme amylase which breaks down starch into simple sugars. Amylase-producing *Bacillus tequilensis* was isolated from the gut content of *Perionyx excavatus* [41]. There are many pieces of evidence of the presence of amylase in the different regions of the earthworm gut [38].

Microbial load in earthworms is an important parameter to determine the efficiency of composting. Benitez et al. [42] reported that earthworms and microorganisms act mutually in the degradation of organic matter. It was found that the bacterial load of 37×10^5 of cfu/g in the waste was amplified to 87×10^5 of cfu/g when treated with the earthworm *Eudrilus eugeniae*. Similarly, during the present study, it is observed that the total bacterial load significantly increases as the food passes through the different regions of the earthworm gut. The total bacterial load is increased from 22.2×10^6 to 241.6×10^6 while passing through the foregut to the hindgut. Other beneficial bacterial groups like nitrifying, denitrifying, phosphate solubilizing, lipolytic, asymbiotic N_2 fixing, but *Azotobacter* population were also significantly amplified during their journey in the earthworm gut.

Earthworm had been considered as ecological engineers for a long time; however, the importance of the earthworm gut microbial community in regulation of earthworm's metabolism and thereby in nutrient transformation had been studied in recent past years [43]. The anaerobic condition of the gut region of earthworm facilitates the colonization of different types of anaerobic bacteria [44]. This huge bacterial population in earthworm digestive tract facilitates the biotransformation of several soil pollutants including metals, microplastics, inorganic and organic chemicals, and antibiotics [45]. These bacterial groups are immensely important for soil fertility, plant growth, and recycling of nutrients, and the earthworms accelerate all these processes by facilitating growth of these bacterial groups [44].

Conclusion

Although the study of earthworm gut microbiota has been studied in the past few years, information regarding different functional bacterial groups remains limited. The present study shows the population dynamics of the different bacterial groups in the different gut regions of earthworm *Perionyx excavatus* for the very first time in these regions. From the observation of the present study, it may be concluded that most of the bacterial groups exhibited higher density in the hindgut region of the earthworm and bacterial load was comparatively lower in the foregut region. As bacterial load in earthworms seems to determine the soil health and efficiency of composting, the present piece of work will certainly illuminate the judicious and scientific exploitation of hindgut bacterial diversity of earthworm *Perionyx excavatus* as bioresource bacteria in sustainable agriculture and waste management program.

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Author contribution Sucharita Ghosh: conceptualization, validation, statistical analysis, data curation, methodology, and writing original draft. Soumendranath Chatterjee: study design, conceptualization, data curation, writing, review, editing, visualization, and supervision. Dipanwita Sarkar Paria: conceptualization, writing, review, editing, and visualization.

Data availability The data that support the findings of this study are available on request from the corresponding author.

Declarations

Ethics approval Proper approval from Institutional Biosafety Committee was obtained at starting of the research work.

Consent to participate Informed consent was obtained from all individual participants included in the study.

Consent for publication Consent was obtained from all individual participants included in the study.

Conflict of interest The authors declare no competing interests.

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