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Feeding Biology of Earthworms (*Perionyx Excavatus and Eudrilus Eugeniae*) and Bacteria Associated with their Guts and Vermicompost

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Abstract

The feeding behaviours of *Perionyx excavatus* and *Eudrilus eugeniae* were carried out. *P. excavatus* and *E. eugeniae* took an average of 3h. and 3h. 30 mins respectively to fill their guts. So, in *P. excavatus* 8(eight) gut fillings could occur in a day whereas in *E. eugeniae* 7(seven) gut fillings could occur in a day. Analysis of gut contents and vermicompost bacteria revealed the presence of *Bacillus* sp. (A), *Bacillus* sp. (B) and *Klebsiella* sp. *Micrococcus* sp. was isolated only from the gut of *Perionyx excavatus.* However, it was absent in the vermicompost. Further, *Proteus* sp. was isolated only from the gut of *Eudrilus eugeniae* but it was absent in the vermicompost. Further, *Proteus* sp. was isolated only from the gut of *Eudrilus eugeniae* but it was absent in the vermicompost. The study emphasizes importance of the rate of organic waste materials movement through the intestine of the worm and time taken during passing through the intestine and microbial changes in the worm casts ageing in the plastic tub.

Keywords: Feeding Biology; *Perionyx excavatus*; *Eudrilus Eugeniae*; *G*ut Contents; Bacteria; Vermicomposts

Introduction

An understanding of the feeding biology and digestive capability of earthworms is essential in their role in the complex decomposition process. It has also been known that environmental factors greatly influence the various life activities of many earthworms [1,2]. The epigeic (Surface-dwelling) species *Eisenia foetida*, *Eudrilus eugeniae* and *Perionyx excavatus* had gained popularity throughout the world for their potentials as decomposer subsystem and because of their importance various studies have been made on their different biological aspects.

The growth and reproduction of *Eisenia foetida* and *Eudrilus eugeniae* are greatly influenced by seasonal environmental factors [3-9]. Further, the growth and cocoon production pattern of *Perionyx excavatus* and *Eudrilus eugeniae* have also been studied [10,11]. The temperature tolerance of *Eisenia foetida*, *Eudrilus eugeniae* and *Perionyx excavatus* has been investigated and found that *Eudrilus eugeniae* and *Perionyx excavatus* could be utilized in vermiculture [12]. The life cycles of *Eudrilus eugeniae* and *Perionyx excavatus* have been well documented and both the species were proved to be potential vermicomposting agents [12-16]. Further, the growth and reproduction of

Eisenia foetida is also influenced by temperature [17,18]. The digestive system of earthworms consists of a tubular alimentary canal extending from mouth to anus. The system can be differentiated as buccal chamber/cavity, pharynx, oesophagus, crop, gizzard, intestine and rectum. Feeding process of earthworms is subjected to the enzymatic actions within the gut. The capacity of mixing and turning over of substrate by earthworms varies with species to species. Data on substrate turnover for *Eisenia foetida* and *Drawida calebi* are available [19,20].

Earthworms graze over a wide variety of organic matters. Influence of feeding pattern on growth and reproduction of *Eisenia foetida* is known [21,22]. Preference for certain food materials by earthworms have also been reported [23-28]. The effects of different organic wastes on vermiculture and vermicomposting has also been known [29-31]. Earthworms affect soil microfloral and faunal population directly or indirectly like the joint action of earthworms and microflora in forest soils [32]. The effects of earthworms on soil microflora leading to changes in soil properties, processes, micro floral and faunal community structure and plant and soil health have been known [33]. The earthworms also graze over soil microflora and that they have requisite enzymes to degrade complex plant substances [24,34]. Selective grazing by earthworms may reduce the number of some organisms and increase others that could be beneficial [35].

The role of earthworms in the dispersal of soil organisms had been known [36,37]. The bacterial feeding by earthworms [38-40]. The gut of earthworm constitutes a mobile anoxic microzone to which the microorganisms of aerated soils are subjected [41]. It provides an optimum incubation temperature, near neutral pH and abundant soluble organic carbon creating an ideal condition for high microbial activity. The cast in turn contains high assimilable carbon, favourable water regime and higher concentrations of many nutrients [42] providing an excellent condition for microfloral and faunal proliferation. The intestine of earthworms is rich in microorganisms [43].

Our previous study revealed 4 bacteria types 2 Actinomycetes types, 15 fungal types, 1-protozoa group, and 2 Nematodes types. For these 2 (two) types of worms, the richest microorganism were fungal types. 15 fungal types were isolated from the previous study [44]. The changes in the microbial status of earthworm cast varied based on their age [45]. The microbial composition of vermicompost are derived from various organic sources [46,47]. A detailed microbe of the gut of *Eudrilus eugeniae* and *Perionyx excavatus* have also been made [48,49]. The effects of gut passage, the age of cast material and the type of ingested substrate on microorganisms in *Lumbricus terrestris* faces are known [50]. The rate that material moves through the intestine depends on whether the worm is feeding, food takes about 20 hours to pass, but when burrows are being formed materials passes in about 12 hours [43]. Further, changes in the microbial status of the casts are noticed as they age under field condition [45]. The present study deals with organic wastes feeding behaviours of *Perionyx excavatus* and *Eudrilus eugeniae* in regards to their ingestion, gut load, transit time and egestion. And to examine further bacteria isolated from the gut content and vermicompost (Vermicast) produced by the worms.

Materials and Methods

Study on the Feeding Biology of Earthworms (*Perionyx Excavatus* and *Eudrilus Eugeniae*)

Experimental rearing of two earthworms (*Perionyx excavatus* and *Eudrilus eugeniae*) (Figures 1A & 1B) were conducted in the Life Sciences Department, Manipur University for a period of three years.

Figure 1A: A view of *Perionyx excavatus*.

Figure 1B: A view of *Eudrilus Eugeniae*.

The organic wastes like cowdung, Kitchen wastes, Litter, and agricultural wastes were mixed prior to vermicosting (Figure 2A). For studies on the feeding biology of *Perionyx excavatus* and *Eudrilus eugeniae* both species were reared separately in plastic tubs (30 cm diameter and 8 cm height) in vermicomposting shed under natural conditions (Figure 2B). Observations on the feeding behaviours like ingestion, gut load, transit time and egestion were made (Figure 2C).

Figure 2A: Organic wastes prior to vermicomposting.

Figure 2B: Culture tubs containing cowdung and different organic wastes.

Figure 2C: A view of harvested vermicompost.

Rate of Gut Loading and Transit Time

Five (5) worms each of *Perionyx excavatus* and *Eudrilus eugeniae* were randomly collected from the respective culture tubs and brought to be laboratory. The gut of each individual worm was cleared by keeping it in half covered Petri dishes containing distilled water. To study the rate of gut loading and transit time of the feed materials, one gut cleared worm of each species were introduced to each culture tub containing 1kg of air dried, powdered and sieved (pore size: 2mm) cowdung. Two sets of experiments (one with *Perionyx excavatus* and the other with *Eudrilus eugeniae*) each with three replications were maintained. Worms were taken out at hourly intervals from the three replications of a set and their guts were cleared. The dry mass of the gut content was measured. The time periods at which the worms started casting were also noted.

Ingestion, Egestion and Assimilation

Five (5) earthworms of each species were introduced into plastic tubs (30x8cm) separately containing 1kg of air dried, powdered and sieved cowdung. Additional powdered cowdung was also spread thinly and uniformly in the tub. The surface of the cowdung was carefully smoothened so that the freshly laid cast was easily distinguished. The Cast were collected at 24hrs interval for a week and the dry mass of each sample was determined. Further, percentage egestion and assimilation of the ingested materials were also calculated on weekly basis. The intestinal loading rate and transit time of cowdung manure of two worms were applied following [51,52].

Isolation and Identification of Bacteria associated with the Gut of Earthworms and Vermicompost

Earthworm Gut Bacteria: Mature worms of *Perionyx excavatus* and *Eudrilus eugeniae* were collected separately in sterile containers from the vermicomposting plastic tubs and brought to the laboratory for investigation of the gut microflora. Five (5) adult worms each of *Perionyx excavatus* and *Eudrilus eugeniae* were collected separately in sterile containers at random from the culture tubs. Representative worms were fixed in 70% alcohol for 10 mins and then washed repeatedly with sterilized distilled water. Each worm was cut into 3 pieces: **anterior**, **middle** and **posterior** with sterilized scissors. The gut contents of the different regions (anterior, middle and posterior) were collected in sterilized petri dishes, containing 2 ml of sterilized water. Gut contents of each region of individual worm were inoculated into petri dishes containing nutrient agar for bacteria in an inverted position at 30±1°C. Colonies so developed were counted for every 24 hrs interval. Morphologically dissimilar colonies were separated and subcultured pure were obtained [44].

Vermicompost Bacteria

Vermicompost obtained from feed mixture of cowdung + kitchen wastes + litters + paddy waste in 1:1:1:1 ratio was collected in sterilized Petri dishes using sterilized spatula and brought to the laboratory for investigations of bacterial (Figure 2B). Five (5) samples each of l gram vermicompost was randomly collected from the vermicomposting tubs. Each sample was mixed with 5ml of sterilised distilled water separately and later subjected to serial dilution up to 10^{-6} dilutions. For the isolation of bacterial components, 1 ml each of the suspension was inoculated in nutrient agar plates (in triplicate) and incubated in an inverted position at 30±1°C. Bacterial colonies so developed were counted for every 24h interval. Suitable controls were maintained. Morphologically dissimilar colonies were separated and transferred as subcultures. Pure cultures were obtained following [53].

Results and Discussion

Different regions and number of segments found in two species of earthworms are provided (Table 1A).

Regions and Segments	*Eudrilus Eugeniae (cm)*	*Perionyx Excavatus (cm)*
Contracted size	7	9
Length of anterior region	2	2.5
Length of middle region	2	2.5
Length of posterior region	3	4
No. of segments in anterior region	3	40
No. of segments in middle region	30	40
No. of segments in Posterior region	40	50

Table 1A: Size and Length (in cm) of different regions and number of segments found in two species of earthworms.

A comparative account of various biological aspect of *Perionyx excavatus* and *Eudrilus eugeniae* are provided (Table 1B).

Biological Characteristics	*Eudrilus Eugeniae*	*Perionyx Excavatus*
Duration of life cycle (days)	± 60	± 46
Growth rate (mg worm^{-1} day^{-1})	12	3.5
Max body mass (mg)	4294	600
Maturation obtained at age (days)	± 40	± 21
Start of cocoon production (days)	± 46	± 24
Coccon production (worm^{-1} day^{-1})	1.3	1.1
Incubation period (days)	± 16.6	± 18.7
No. of hatchings from one worm	1-5	1-3

Table 1B: A Comparative account of various biological aspects of *Perionyx excavatus* and *Eudrilus eugeniae*
Source: Gunathilagraj, K. and Sahaya Alfred, cited by Devi KB [49].

Gut Load and Transit Time

Transit time and rate of gut loading of cow dung and other organic wastes by the two earthworms varied with each other. *Perionyx excavatus* and *Eudrilus eugeniae* could consume 9.7 mg (dry mass) and 20.96 mg (dry mass) of cowdung respectively in one hour and 22.46 mg (dry mass) and 47.32 mg (dry mass) respectively in two hours. In three hours, *Perionyx excavatus* showed casting activity while *Eudrilus eugeniae* continued gut loading. By around three and half hours *Eudrilus eugeniae* started casting (Table 2A).

Duration	Earthworm Species	
	Perionyx Excavatus	***Eudrilus Eugeniae***
1h	9.7 ± 0.36	20.96 ± 0.52
2h	22.46 ± 0.48	47.32 ± 0.64
2h and 30 min	Gut loading continued	Gut loading continued
3h	Casting	Gut loading continued
3h and 30 min	–	Casting

Table 2A: Transit time and rate of Gut loading of cowdung (in mg dry mass) for two earthworm species.
Hence, in one day 8(eight) gut fillings could take place in *Perionyx excavatus* while 7(seven) gut fillings could take place in *Eudrilus eugeniae*.

Ingestion, Egestion and Assimilation

In the experimental tubs with *Perionyx excavatus*, the worms consumed about 98% of the cowdung and other organic wastes in a week but 69.44% of the ingested material was excreted while in the tubs with *Eudrilus eugeniae*, 95% was ingested in a week and 60.58% of the ingested material was excreted. Percentage assimilation of ingested material per week was higher in *Eudrilus eugeniae* than *Perionyx excavatus* by recording a value of 39.42% and 30.56% respectively. The ingestion, egestion and assimilation rate is provided (Table 2B).

Biological Activities	Earthworm Species	
	Perionyx Excavatus	***Eudrilus Eugeniae***
Food ingested % week^{-1}	98	95
Food egestion % ingested week^{-1}	69.44 ± 0.86	60.58 ± 0.85
Assimilation % ingested week^{-1}	30.56 ± 0.86	39.42 ± 0.85

Table 2B: Ingestion, Egestion and Metabolic rate of *Perionyx excavatus* and *Eudrilus eugeniae* in cowdung.

Bacteria associated with the Gut of Earthworms and Vermicompost

The investigations showed a number of bacterial colonies being developed on nutrient agar plates (Figure 3A). The bacterial colonies so developed were isolated and identified. The results of the various tests conducted were recorded and compared with the key to taxonomic bacteria from standard literatures [54,55]. The bacterial types isolated from the gut of *Perionyx excavatus* and *Eudrilus eugeniae* and vermicompost (Figure 2C) are also presented (Table 3). Altogether four genera of bacteria were isolated and identified. Two species of *Bacillus* (Figures 3B & 3C) and one species of *Klebsiella* were isolated from both *Perionyx excavatus* and *Eudrilus eugeniae*. However, *Micrococcus* sp. was isolated from *Perionyx excavatus* alone and *Proteus* sp. was also isolated from *Eudrilus eugeniae* alone.

1. Bacillus sp. [A]: Rod shaped, single, short/long chain, Gram +ve, Acid Fast – Not tested, spore +ve, Capsule/Cyst – Not tested, Flagella – Not tested, and Motality +ve. (Figure 3B)
2. Bacillus sp. [B]: Rod, single or chain (2-3**µ**mL), spore cylindrical, Gram +ve, Acid Fast – Not tested, spore +ve, Capsule/Cyst – Not tested, Flagella – Not tested, and Motality +ve. (Figure 3C)
3. Klebseilla sp.: Straight rod, upto 5.0**µmL, singly, rarely chain**, Gram -ve, Acid Fast -ve, spore -ve, Capsule /Cyst +ve, Flagella -ve, and Motality -ve.
4. Micrococcus sp.: Spherical, single, pair & in clusters, Gram +ve, Acid Fast -ve, spore -ve, Capsule/Cyst -ve, Flagella = Not tested, and Motality -ve.
5. Proteus sp.: Straight, rod about 3**µ**mL pair or chain, Gram -ve, Acid Fast -ve, spore -ve, Capsule/Cyst -ve, Flagella +ve, and Motality +ve.

Figure 3A: Bacterial colonies developed on Nutrients agar medium.

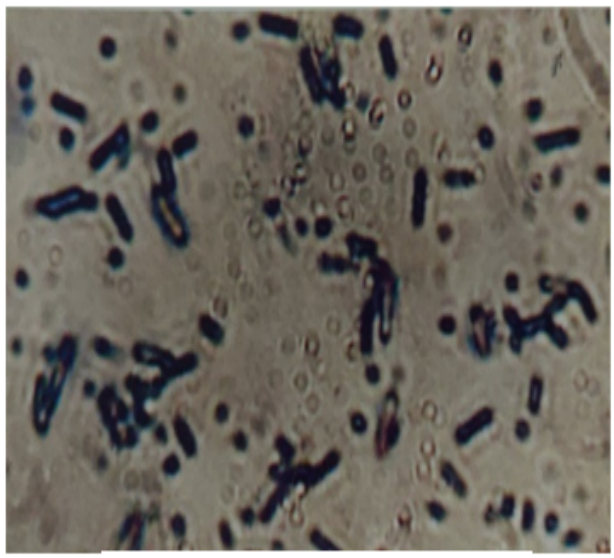

Figure 3B: Bacillus sp.

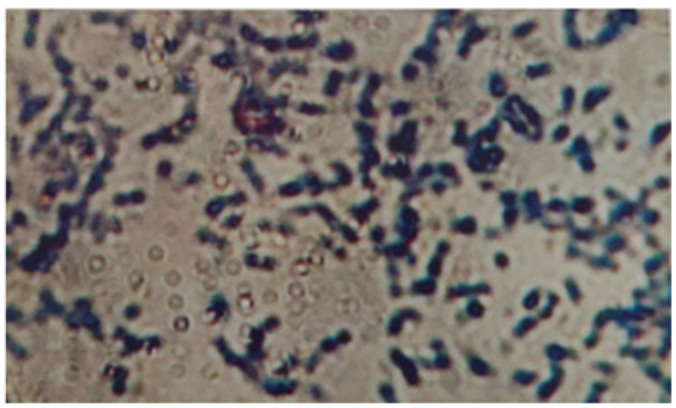

Figure 3C: Bacillus sp.

Different region and number of segments found in two species of earthworm is provided (Table 3).

Types of Becteria	Gut Content		Vermicompost	
	Eudrilus Eugeniae	***Perionyx Excavatus***	***Eudrilus Eugeniae***	***Perionyx Excavatus***
Bacillus sp. A	Isolated	Isolated	Isolated	Isolated
Bacillus sp. B	Isolated	Isolated	Isolated	Isolated
Klebsiella sp.	Isolated	Isolated	Isolated	Isolated
Micrococcus sp.	Not Isolated	Isolated	Not Isolated	Not Isolated
Proteus sp.	Isolated	Not Isolated	Not Isolated	Not Isolated

Table 3: Bacteria isolated from the gut content and vermicompost of *Eudrilus eugeniae* and *Perionyx excavatus.*

From the vermicompost (Figure 2C) produced by using *Perionyx excavatus* and *Eudrilus eugeniae*, one species each of *Bacillus* and *Klebsiella* could be isolated as the common bacterial flora. However, *Bacillus* sp. (B), *Micrococcus* Sp. and *Proteus* sp. could not be isolated from the vermicompost produced by using both the earthworms. In the present study, both *Perionyx excavatus* and *Eudrilus eugeniae* grow well and actively performed their various feeding and casting activities in cowdung and other organic wastes. In fact, cowdung can be considered as the most suitable medium for rearing of these earthworm species. These findings agreed with the previous findings [4] for *Eudrilus eugeniae* and [56] for *Perionyx excavatus*. Transit time and the rate of gut loading of *Perionyx excavatus* showed similar results with *Octochaetona surensis* [57] and that of *Eudrilus eugeniae* with *Allobophora rosea* [58] and *L. mauritti* [57]. The results of the ingestion, egestion and assimilation rates agreed previous workers [56]. In nature, it is observed that organisms ingesting smaller amount of cellulose and other plant materials exhibit higher assimilation efficiency, whereas organisms which have a lower assimilation capability always ingest larger amounts of food materials to meet their need. Hence, the lower ingestion and higher assimilation efficiency in *Eudrilus eugeniae* and higher ingestion and lower assimilation efficiency in *Perionyx excavatus* confirmed the above hypothesis.

Vermicomposting is one of the most efficient methods for converting organic wastes into valuable plant nutrients [59]. *Eudrilus eugeniae* and *Perionyx excavatus* are nowadays widely used in vermicomposting as they are prolific breeders and can adapt easily to various organic waste materials [56,57]. Production of vermicompost depends on the consumability of the worms and also on the nutritional status and texture of organic wastes. The rate of breakdown of waste depends on the types of litter used [27]. In the present study, *Perionyx excavatus* showed better production of vermicompost than *Eudrilus eugeniae*. These results agreed with the previous findings [7,60].

Earlier study revealed influence of environmental factors on survival and growth of Earthworms. *P. excavatus* does not grow much at low temperatures although it can survive them 4°C (39.2°F) but it is less susceptible to high temperature over 30°C (86°F) than *E. eugeniae*. Even in tropical areas, *P. excavatus* does not grow during low-winter temperatures but can survive the high-summer temperatures, whereas *E. eugeniae* has a much narrower tolerance range for temperature and cannot survive either the extreme low winter or the high summer temperatures [61]. In the present study too, earthworms move to more suitable areas in the culture tub if the environmental limits are greatly exceeded. Bacteria in the gut flora increased greatly in number [43] but the result suggest that changes in the microbial population during passage through the worm gut tended to be logarithmic, indicating that increases were by bacteria growth and not by the worm selecting food material with high bacteria count. The results of the present investigation revealed the presence of a number of bacteria in the gut of the earthworms. However, in the vermicompost, some of the microbes present in the gut were not isolated. Among the bacterial flora, one species of *Bacillus* and one species of *Klebsiella* were isolated from the vermicompost. The outcome of the present study shows the presence of bacteria like *Bacillus* and *Klebsielal* in the vermicompost indicating that they were not digested. These may be due to the production of antibiotic and/or presence of strong outer coat and/or production of phytotoxic metabolites. Both *Perionyx excavatus* and *Eudrilus eugeniae* could thrive well in organic waste materials and they can be used as potential vermicomposting agents. Through their decomposing activities, they help in the recycling of organic wastes.

Conflict of Interest

The authors have no financial conflict of interest to declare.

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