Screening of Earthworm (*Eudrilus eugeniae*) Gut as a Transient Microbial Habitat

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**Abstract**

Earthworms are soil invertebrates which play a major role in recycling organic matter in soils. The unique microenvironment of the earthworm gut impacts on the catabolic activities of ingested soil microorganisms. The ingested microbial populations play an important role in earthworm nutrition by helping in the breakdown of organic matter. A study was carried out to find out the distribution of microorganisms in the gut of earthworm, *Eudrilus eugeniae*. The vermibeds were prepared with cow dung and pre-decomposed banyan leaf litter. The population of bacteria, fungi and actinomycetes were determined in the gut regions (foregut, midgut and hindgut) of earthworm *E. eugeniae*. The predominant microorganisms found in the foregut, midgut and hindgut were bacteria, actinomycetes and fungi respectively. It was observed that there are variations in the population of microorganisms in the foregut, midgut and hindgut. This report shows the distribution of various microorganisms in the gut regions and how it favors the animal to have a healthy life in the environment.

**Keywords**

Invertebrates, Gut, *Eudrilus eugeniae*, Leaf litter, Vermibeds

However, qualitative and quantitative surveys of microbial flora of various earthworms are still warranted. Biocomposting of solid wastes bring about stabilization of the organic matter and effectively reduces pathogen concentrations in sludge’s to very low levels. The absolute removal of pathogens becomes difficult to achieve and many survives the composting process [4].

2. Materials and Methods

2.1. Selection of Suitable Species

Selection of suitable species for vermiculturing was done according to the requirement, for composting, poultry and animal feed. In general, some species are ‘vermicultured’ that have other possible commercial utilization like *Eudrilus eugeniae* (Figure 1).

![Figure 1. Eudrilus eugeniae](image-url)
2.2. Earthworm Culture

*E. eugeniae* were collected from the Vermiculturing Unit maintained by the Department of Biosciences, NASC, Coimbatore, brought to the laboratory and mass cultured in a culture tank (1m×1m×1m) containing urine free cow dung. Cow dung was collected from nearby cattle shed, sun dried and powdered. Worms were acclimatized in cow dung and used for various experimental studies (Figure 2 & 3).

2.3. Vermibed Preparation

The pre-decomposed waste materials from the plastic tubes were taken for the vermibed preparation. The vermibeds were prepared with cow dung and pre-decomposed banyan leaf litter (CD and BL) in the ratio of 1:1 in plastic containers of 15cm×15cm×12cm size. Water was sprinkled over the vermibeds to hold the moisture content of 60% to 70% and kept for 24 hours. Ten healthy, matured *E. eugeniae* of 10 to 12cm length and 0.4 to 0.8g weight were taken from the culture tank and were introduced in to the plastic containers. The containers were covered with perforated lids for ventilation and for the prevention of predators (Figure 4).

2.4. Enumeration of Microbes

A healthy worm was selected for enumeration of microbes by pour plate method. It was dissected to three regions as foregut, midgut and hindgut. The serial dilutions were performed for each gut sections and were individually plated on to Nutrient agar, Actinomycetes isolation agar and Sabouraud’s dextrose agar for bacteria, actinomycetes and fungi respectively. Enumeration process was carried out at regular intervals up to 60th day.

3. Results

It was found that there was a gradual increase in their population from the initial day to final day. The maximum Bacterial population was found in the foregut region than in midgut and hindgut region. The fungal population was maximum in the hindgut region. The next highest population was noted in the foregut. Actinomycetes population was predominant in the midgut than foregut and hindgut (Table 1, Figure 5, 6 & 7).
Table 1. Microbial Population in the Gut of *E. Eugeniae* during the process of Vermicomposting

| Micro Organisms | Foregut (10⁵ x cfu/g) | Midgut (10⁵ x cfu/g) | Hindgut (10⁵ x cfu/g) |
|-----------------|-----------------------|----------------------|----------------------|
|                 | 0 15 30 45 60         | 0 15 30 45 60        | 0 15 30 45 60        |
| Bacteria        | 12* 50* 76* 90* 136*  | 30 41 52 55 80      | 43 52 65 81 96      |
| Actinomycetes   | 7 7 8 11 22           | 12* 14* 16* 18* 30*  | 10 9 6 9 14         |
| Fungi           | 15 18 25 25 32        | 11 17 20 23 31      | 19* 22* 31* 32* 43*  |

*p < 0.05 (n=3)

4. Discussion

In the present study, a significant number of microbial populations in the gut of earthworm were observed. It was increased from the initial day to final day of vermicomposting. Earthworms, for their growth and reproduction, have been shown to meet their nutritional requirement by feeding on organic matter and microbes [5]. Microorganisms constitute an important nutritional component of the earthworm diet [6]. Earthworms have been shown to be microbivorous [7-8]. The ingestion of microorganisms by earthworms is reported by a few authors but there is no agreed opinion as to what type of microorganism is eaten and digested by earthworms. Earthworm was shown to have minimal capacity to digest organic residues and obtain nutrition by digestion of microorganisms associated with ingested organic matter [9]. Differences in earthworm digestion and assimilation processes suggest the possible existence of ecological group-specific gut microbiota [10]. Although the microbial profile of the gut content is akin to that of soil and feed resources [11-14], it is not a coincidental combination of the microorganisms in soil [15]. Increase in microbial populations might be due to the environmental conditions.
prevailing and nutritional status in the gut of earthworm as reported by References [16-18]. It was observed that there are variations in the population of microorganisms in the foregut, midgut and hindgut of earthworm. The first documented studies on the effects of earthworms on microorganisms were those of Reference [19]. He isolated more than 60 species of microorganisms (less than 50 of them bacteria) from *L. terrestris* casts and gut contents.

5. Conclusion

The gut of the earthworm constitutes a unique microenvironment in soils. The selective digestion of microbes in the gut influences the type of nutrients that are available for subsequent assimilation by both the earthworm and members of the gut microflora. The variation in the microbial populations in the earthworm gut may be because of their nutritional needs and digesting ability of the earthworms. Indeed, the survival of microorganisms in the earthworm gut depends on their capacity to resist to digestive enzymes of microbial or earthworm origins, intestinal mucus, CaCO₃, or to bacteriostatic and microbial substances. The bacteria in the foregut helps to digest the food particles, actinomycetes in the midgut helps to destroy the pathogens by antagonistic activity, and the fungi help to bind the waste particles as castings in the hindgut. We look forward to future studies aimed at understanding the importance of this microniche.

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