

# Isolation of microflora in earthworms guts from different substrates

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**Abstract:** The fungi and bacteria were analysed in the gut of *Eudrilus eugeniae*, from different feed substrates like dry leaves, plastic waste, kitchen waste and waste paper. Certain species of fungi (*Saksenae vasiformis*, *Mucor plumbeus*, *Cladosporium carrionii*, *C. herbacium*, *Alternaria* sp., *Cunninghamella echinulata*, *Mycetia sterila*, *Syncephalostrum racemosum*, *Curvularia lunata*, *C. geniculata* and *Geotrichum candidum*) and bacteria (*Pseudomonas aeruginosa*, *Mima polymorpha*, *E. cloacae*, *Proteus vulgaris*, *P. mirabilis*, *P. rettgeri*, *Escherichia coli*, *Staphylococcus citreus*, *B. cereus*, *Enterococci* and *Micrococci*) were completely digested. Certain other species were not digested fungi like *Aspergillus fumigatus*, *A. flavus*. The microbial proliferation was more in the casts, due to the environment prevailing - rich in nutrient supply and large surface area available for growth and reproduction of the microbes that lead to enhanced microbial activity and humic acid contents in the casts.

**Keywords:** Microflora, Earthworm gut, Microbial Population, Dry Leaves, Plastic Waste, Kitchen Waste and Waste Paper

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## 1. Introduction

Earthworms are very important soil creatures as they make up a large portion of the total biomass of invertebrates of the soil. Though earthworms are well studied organisms all over the world. The work on their taxonomy is far beyond completion. More than 3000 species of earthworms are known. In one hectare of soil, rich in organic matter and good moisture content, one can expect a population of 50,000 to 400,000 earthworms which are able to produce 25-30 tones of castings. Earthworms are hermaphrodites and they can double their population in one month in ideal conditions of temperature, moisture and food i.e. organic matter.

The present study was aimed at studying the different aspects of the symbiotic relationship between the microbes found in the four different substrates such as dry leaves, plastic waste, kitchen waste and waste paper and species of earthworms – *Eudrilus eugeniae* (*Eudrilidae*), and to find whether some of these microbes are commonly found in these feed and whether there is a common requirement of

microbes as feed for earthworms at knowing gut microflora of earthworms in the fields of Sam Higgingbottom Institute of Agricultural Technology & Science (SHIATS), Allahabad, India.

## 2. Materials and Methods

### 2.1. Collection of Organic Wastes and Earth Worm Culture

Collection of earthworm culture on different substrates like dry leaves, plastic waste, kitchen waste and waste paper were made from the Department of Biological Science of SHIATS and were used as feed substrates for adult earthworms.

*Eudrilus eugeniae*, 50 worms were maintained in separate Brick bed containers (50 x 35 x 35 cm) containing 5kg of feed material at 28 ± 2°C, 75% moisture, 70% RH. The feed was not changed during the experiment lasting for 90 days. The worms were acclimatized for 2 days in the feed substrate before the commencement of the experiment. Microbial analysis was done on vermicasts collected

after 15 days of commencement of experiment.

Earthworms (*E. eugeniae*) collected from the vermicomposting unit of our college, were washed with sterilized tap water and placed on sterile Petri plate with moistened filter paper for 24 hours. They were then cleaned externally with 70% ethanol and dissected, weighed and homogenized for 15 minutes with vortex mixture in sterile 0.85% NaCl solution.

Isolation of microflora was done by dilution plate method. For this, the mid gut of the earthworm was excised and the gut content (1g) was suspended in 10ml of sterile 0.85% NaCl solution, serially diluted ( $10^{-1}$  to  $10^{-7}$ ). After serial dilution 0.1ml solution was taken using sterile micropipette and plated sugar as on nutrient agar medium. The plates were then incubated sample at 28°C for 24 hours. Three replicates were maintained from each dilution.

## 2.2. Isolation, Enumeration and Identification of Microflora

The population of fungi and bacteria, from the substrates (dry leaves, plastic waste, kitchen waste and waste paper) was determined by dilution plate techniques [1]. Each substrate of 1 gm was suspended in 1 ml sterile saline (1 g NaCl in 100 ml distilled H<sub>2</sub>O) in a sterile test tube and was shaken thoroughly in a mixer and used as inoculum for isolation and enumeration of fungi and bacteria from different substrates. Using micropipette, 0.01 ml of the inoculum was inoculated into Nutrient agar (NA) [2], plates and spread over each plate media by using platinum loop for bacterial growth, Potato dextrose agar (PDA) plates for fungi and incubated at 28°C for 48 hours for bacteria, and 28°C for 7 days for fungi, respectively. The different colony forming units (CFU) developing on the media were

estimated and expressed as CFU.

To identify the bacteria, Gram's staining tests were carried out as described by Mahon and Manuselis [3]. In addition to these, the current taxonomic sources were used in identification procedure. To identify the fungi, light microscopic examination was carried out in the Lactophenol cotton blue stained slides of each developed colonies in the Petri dish and also cultural characteristics such as colour of the fungal colony, number of days taken for the fungus to reach maximum diameter (9 cm) of the Petri dish, and the texture of fungal growth were noted. The morphological and cultural features of each fungus were compared with descriptions given by Kwon Chung and Bennett [4] for identification.

## 2.3. Statistical Analysis

The values were presented as means of different groups. Differences between the mean values were estimated using one way ANOVA. The results were considered statistically significant when  $p < 0.05$ .

## 3. Results and Discussion

The diversity of types and number of fungi and bacteria isolated from the gut of *E. eugeniae* as influenced by different feed substrates like dry leaves, plastic waste, kitchen waste and waste paper are tabulated in table 1, and figures 1 & 2. Of the four substrates, it was observed, that kitchen waste harbours the maximum variety and number of fungi and bacteria followed by dry leaves. The least diversity of microflora was found in control then plastic waste.

Table 1. Isolation of microbes in the gut of earth worms reared in different substrates.

Microbes	Substrate				
	Dry leaves	Plastic waste	kitchen waste	waste paper	Control
Fungi (CFU x 10 <sup>6</sup> g <sup>-1</sup> )					
<i>Aspergillus fumigatus</i>	32*	12	35*	28*	9
<i>Aspergillus flavus</i>	21*	17*	32*	22*	1
<i>Rhizopus</i> sp.	24*	10*	25*	11*	5
Total	77*	39*	92*	61*	15
Bacteria (CFU x 10 <sup>6</sup> g <sup>-1</sup> )					
G -ve					
<i>Pseudomonas aeruginosa</i>	2	1	5*	3	1
<i>Bacterium antitratum</i>	0	0	7*	2	2
<i>Enterobacteria erogenes</i>	28*	14	29*	17	17
G+ve					
<i>Bacillus subtilis</i>	3	1	5	1	1
Total	33*	16	46*	23	21

\*: Significant differences as compared with control group (  $P < 0.05$  ).

Earthworms, for their growth and reproduction, have been shown to meet their essential nutritional requirement by feeding on organic matter and microbes [5]. Edwards and Fletcher [6] concluded that fungi were the major sources of nutrients for earthworms and that bacteria were of minor nutritional importance and soil algae were of moderate importance.

A significant increase of microbial populations ( $p < 0.05$ ) in the guts of earthworm reared on different substrates was observed in the present study particularly fungi as well as bacteria - *A. fumigatus*, *A. flavus*, *Rhizopus* sp. *Pseudomonas aeruginosa*, *Bacterium antitratum*, *Enterobacter aerogenes*, *Bacillus subtilis*. Similarly increased population of fungi like *A. flavus*, *A. fumigatus*

[7,8], and bacteria like *B. idozus* and *B. cereus* [9] were reported in the casts.

In the present study, more amylolytic (*Aspergillus* sp., *Rhizopus* sp. and *Bacillus* sp.), cellulolytic (*Aspergillus* sp., *Bacillus* sp. and *Pseudomonas* sp.), proteolytic (*Aspergillus* sp. and *Bacillus* sp.), phosphate solubilizing (*Pseudomonas* sp., *Bacillus* sp. and *Aspergillus* sp.) and nitrifying (*Aspergillus flavus*, *Pseudomonas* sp., *Enterobacter* sp. and *Bacillus* sp.) microbes were found significantly in the gut of the earth worm.

The role of microbes and earthworms in decomposition of organic matter and particularly, in humification is well known [10,11]. Humification has been shown to be, predominantly, a microbial process [12-14] and recently earthworms, *E. eugeniae* have been shown to aid in humification [15]. In the present study, there is a direct correlation between the microbial population activity and humic acid content in the earthworm guts. With the increase in microbial population there is an increase of microbial activity and humic acid content.

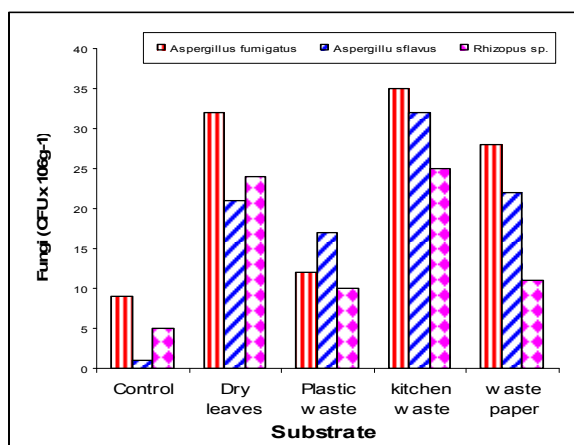


Figure 1. Isolation of fungi in the gut of earth worms reared in different substrates.

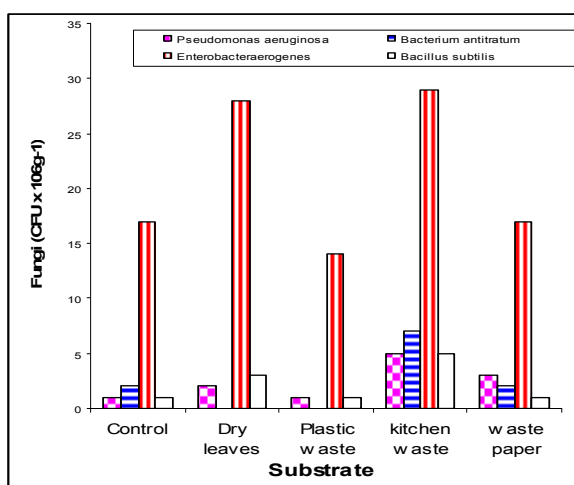


Figure 2. Isolation of bacteria in the gut of earth worms reared in different substrates.

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