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Microbial Diversity from the Gut of Earthworms Involved in The Process of Vermicomposting Using Vegetable and Temple Waste

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ABSTRACT: Vermicomposting is an eco-friendly and economical technique for managing various organic wastes, including agricultural, temple, and vegetable. As crucial soil invertebrates, Earthworms exhibit beneficial effects on the soil environment, influencing both physical properties and organic matter. They play a key role in recycling organic matter. Within the earthworm's gut, numerous aerobic and anaerobic bacteria, as well as fungi, are present. These microflorae establish a mutual symbiotic relationship with the earthworm's digestive tract. The bacterial community functions as plant growth promoters, free-living nitrogen fixers, biocides, or phosphate solubilizers. Recognizing the immense importance of these gut microflora, a present investigation was conducted to study the microbial diversity in the gut of earthworms involved in vermicompost preparation using vegetable and temple waste. The selected earthworm species for this study was *Eisenia fetida*. Cow dung, vegetable, and temple waste were used in a ratio of 1:1 for vermicompost preparation. The experiment spanned approximately 60 days. The microbial diversity isolated from the gut of earthworms involved in the process of vermicomposting of vegetable and temple waste included various species of bacteria and fungi such as *Escherichia, Staphylococcus, Proteus, Pseudomonas, Enterococcus, Mucor, Rhizopus, Aspergillus,* etc.

KEYWORDS: Bacteria, Earthworms, Fungi, Gut Microflora, Vermicomposting.

1. INTRODUCTION

Waste materials developed by industrialization, agriculture and domestic activities are a serious problem nowadays and the modern technologies used for its management have ill effects on the environment and health (Kashmiri, 2020). Vermicomposting technology is globally popular for managing solid waste (Gomez-Brandon et al. 2012; Manyuchi et al. 2013). Vermicomposting is a natural process that uses earthworms to break down organic waste into nutrient-rich vermicompost. The vermicompost is used an organic fertilizer to nourish the plants and improve soil health (Prajapati et al. 2023). It is obtained from a wide variety of organic waste including residual sludge; when sewage sludge is managed with vermicomposting techniques, the resulting product supplies nutrients, and more stable organic matter and works as a soil conditioner (Del Aguila Juárez et al. 2011).

Earthworms are indicators of soil health (Bhadauria and Saxena, 2010). They play a major role of decomposers, aerators, crushers, mixers, chemical degraders, and biological stimulators in the soil vicinity. They also play a role in soil structure modification, water infiltration, acceleration of organic matter decomposition, nutrient recycling, and bioremediation (Brown and Doube, 2004; Dominguez et al. 2009). They effectively harness the beneficial soil microflora, destroy soil pathogens, and convert organic wastes into vitamins, enzymes, antibiotics, growth hormones, and protein-rich casts (Govindarajan and Prabaharan, 2014). 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. The gut of many soil organisms contains microbial communities that are usually helpful in digestion. These microbial-animal relationships create mutualisms. Earthworms also have a mutualistic relationship with soil microorganisms (Bacteria, Fungi, etc.) passing through their digestive tract, but the nature and role of the microbiota inhabiting their gut are virtually unknown (Lavelle and Spain, 2001). The microorganisms in the worms' gut were found in the soil environment too. earthworm gut provides suitable conditions for the development of bacterial colonies. In this piece of research work isolation and characterization of gut microflora from the gut of earthworms involved in the process of vermicomposting using vegetable and temple waste were performed and analyzed.

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2. MATERIALS AND METHODS

2.1 Selection of Earthworm Species

The species of earthworm was selected according to the experiment's requirements. For the present study, the *Eisenia fetida* species was selected. The earthworms were brought from Mukul Mushroom Farm and Vermicomposting Projects, Bharatwada, Nagpur (MS), India.

Fig. 1: *Eisenia fetida*

2.2 Maintenance of Earthworm

E. fetida was cultured and maintained in a tank of 1m X 1m X 1m tank at the Department of Zoology, Dada Ramchand Bakhru Sindhu Mahavidyalaya, Nagpur. They were acclimatized in cow dung and used for various experimental setups.

Fig.2: Earthworm in vermibed

2.3 Collection of the Waste

The vegetable waste was collected from the kitchen and the temple waste from nearby temples. The vegetable and temple waste collected were segregated and unwanted materials were removed. The cleaned waste was chopped into small pieces and then used for experiments.

2.4 Experimental Set-up of Vermicompost

For the present study, three different bedding sets for vermicomposting were prepared. The first bedding container comprises pre-decomposed cow dung, the second vermibed was prepared with cow dung and pre-decomposed vegetable waste in a ratio of 1:1 and the third container contains cow dung and pre-decomposed temple waste in a ratio of 1:1. The containers were kept in shed covered with gunny bag. They were constantly monitored throughout the study (Table 1). Water was regularly sprinkled over the vermibeds to hold the moisture content of 60 to 70%. Fifty healthy, matured *E. fetida* of 10- 12cm length and 4.49-5.86g weight were introduced in experimental containers.

Table 1: Experimental Set of Vermicomposting

Container	Vermicomposting Bed	Period of vermicomposting
1	Cow dung	60 Days
2	Cow Dung + Vegetable Waste	60 Days
3	Cow Dung + Temple Waste	60 Days

2.5 Isolation and Enumeration of Gut Microbes

After the completion of the experiment, a few earthworms, *Eisenia fetida* were collected from all the containers and dissected to gut for isolation of microbes. The enumeration of microbes was carried out by the pour plate method. The serial dilutions were performed for gut sections. They were individually plated into Nutrient agar, Eosin Methylene Blue agar, Pseudomonas isolation agar, Cystine-Lactose-Electrolyte-Deficient agar, Potato Dextrose agar, Sabouraud Dextrose agar, Endo agar, Baired Parker agar and Congo Red Yeast Extract Mannitol Agar for bacterial and fungal analysis. Inoculated plates of selective media were incubated at 37°C for 24 hrs. The bacterial and fungal growth was observed after incubation. The appearance of the colony and its characterization were observed. Gram staining was performed and gram-positive and gram-negative bacteria were differentiated. Lactophenol cotton blue staining for fungal species was performed and morphological characteristics were examined.

3. RESULTS

After the completion of 60 days, it was observed that the population of earthworms gradually increased in all three containers. In the present study, it has been found that gut microflora of earthworms consists of different kinds of microorganisms which include bacterial species such *as Escherichia, Staphylococcus, Proteus, Pseudomonas, Enterococcus, Enterobacter, Bacillus, Micrococcus, Rhizobium* and fungal species such as *Rhizopus, Mucor, Penicillium, Candida, Aspergillus*. The microflora in the gut of earthworms involved in the process of vermicomposting using cow dung, cow dung and vegetable waste, and cow dung and temple waste are shown in Table 2.

Table 2: Gut Microbes of earthworms after vermicomposting

Sr. No.	Bacteria/Fungus	Cow Dung	Cow Dung + Vegetable Waste	Cow Dung + Temple Waste
1	*Escherichia coli*	+	+	+
2	*Staphylococcus spp.*	+	+	+
3	*Proteus spp.*	+	+	+
4	*Pseudomonas spp.*	+	+	+
5	*Enterococcus spp.*	+	+	+
6	*Enterobacter spp.*	+	+	+
7	*Bacillus spp.*	+	+	+
8	*Micrococcus spp.*	-	-	+
9	*Rhizobium spp.*	+	+	+
10	*Rhizopus spp.*	-	-	+
11	*Mucor spp.*	+	+	+
12	*Aspergillus spp.*	+	+	+
13	*Penicillium spp.*	-	+	+
14	*Candida spp.*	-	-	+

4. DISCUSSION

Earthworms play a beneficial role in improving the fertility of the soil through continuous plowing and manuring (Govindarajan and Prabaharan, 2015). They also help to aerate and quickly absorb water in the soil. The subsoil they bring to the surface is finer and rich in organic matter. Their excretory wastes and secretions are rich in nitrate, calcium, magnesium, potassium, and phosphorous which form important nutrients for plants. Vermicomposting is a biotechnological process that enables the recycling of organic waste materials into manure through the combined action of earthworms and mesophilic microorganisms. (Yadav and Garg, 2013). It is globally popular for managing solid waste obtained from a wide variety of organic waste including residual sludge; when sewage sludge is managed with vermicomposting techniques, the resulting product supplies nutrients, more stable organic matter and works as a soil conditioner (Del Aguila Juárez et al. 2011). Microorganisms such as bacteria and fungi are important food components of soil invertebrates including earthworms. The earthworm's gut is an ideal habitat for microorganisms such as bacteria and fungi (Wolter and Scheu, 1999) because earthworms can directly regulate the microbial population of their gut by consuming large amounts of soil. Several studies showed that increased microbial numbers in the guts of earthworms versus the soil, in which earthworms were living. The present research work focuses on the microbial diversity of earthworms' guts involved in the vermicomposting process using vegetable waste and temple waste.

Interactions between earthworms and soil microorganisms are key characteristics for soil processes such as the decomposition and transformation of plant residue, humus formation, and the formation of the pool of nutrients (Govindarajan and Prabaharan, 2015). However, the interaction between earthworm activity on soil and microorganisms in the vicinity is not clearly understood. The previous studies reveal that the earthworm gut microbial community is qualitatively not much different from the microbial community in the surrounding soil vicinity.

Pérez-Pérez et al. 2018 acknowledged the following species of bacteria, *Bacillus, Paenibacillus, Solibacillus, Staphylococcus, Arthrobacter, Pantoea, Stenotrophomonas, Acinetobacter* and *Aeromonas* from the digestive tract of *Eisenia foetida*. All these species are typical soil bacteria. We observed *Escherichia coli, Staphylococcus spp., and Proteus spp. Pseudomonas spp., Enterococcus spp., Enterobacter spp., Bacillus spp., Micrococcus spp., and Rhizobium spp.* of bacteria in the gut of earthworms involved in the vermicomposting process using vegetable waste and temple waste.

Likewise, fungi are also found in the gut of earthworms. In the present study, *Rhizopus spp., Rhizopus spp., Mucor spp., Aspergillus spp., Penicillium spp., and Candida spp.* species of fungi were obtained in the gut of earthworms involved in producing vermicompost using temple and vegetable waste. Vaclav and Novakova, 2003 observed *Aspergillus fumigates, Mucor circinelloides, F. circinelloides*, and *Penicillium expansum* in the intestine of earthworms.

Earthworms and gut microflora act symbiotically to accelerate and enhance the process of decomposition of temple and vegetable waste. Microorganisms break down the cellulose in the food waste and help earthworms convert the waste into valuable compost.

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