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# Microbial Analysis of The Gut of *Eudrilus eugineae.*

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## ABSTRACT

*Eudrilus eugineae* possesses an immense bacterial diversity within their digestive tracts and is very little explored mainly because of the non-cultivatable character of a large quantity of microorganisms which mainly come from soil. All these organisms establish relationships among themselves in highly varied and complex ways which contribute to soil characteristics because of their role in the modification of solid, liquid and gaseous stages.

The aim of this study was, therefore, to screen the microbiology in the gut of *Eudrilus eugineae* which are used in management of biowaste

It can be concluded from this study that the composting ability of *Eudrilus eugineae* is enhanced by the microbial activities in the gut and can be used in the management of number of organic waste generated in agriculture, horticulture, rural industries including household section creating environmental population and problem.

**Keywords-** *Eudrilus eugineae*, Vermicomposting, Biowaste Earthworms.

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## INTRODUCTION

Vermicomposting is the process by which earthworms are used to convert organic materials, usually wastes, into a vermicompost. Vermicomposting is a non-thermophilic biological oxidation process in which organic materials are converted into vermicompost which is a peat like material, exhibiting high porosity, aeration, drainage, water holding capacity and rich microbial activities, through the interactions between earthworms and associated microbes [1]. Vermiculture is a cost-effective tool for environmentally sound waste management [3]. Earthworms such as *Eudrilus eugineae* play an important role in carbon turnover, soil formation, participates in cellulose degradation and humus accumulation. They actively profoundly affect the physical, chemical and biological properties of soil. Earthworms (such as *Eudrilus eugineae)* intestine's contains a wide range of microorganisms, enzymes and hormones which aid in rapid decomposition of half-digested material transforming them into vermicompost in a short time (nearly 4-8 weeks) [22] compared to traditional composting process which takes the advantage of microbes alone and thereby requires a prolonged period (nearly 20 weeks) for compost production. [20] Earthworms are classified into epigeic, anecic and endogeic species based on definite ecological and trophic functions. Epigeic earthworms are smaller in size, with uniformly pigmented body, short life cycle, high reproduction rate and regeneration. They dwell in superficial soil surface within litters, feeds on surface litter and mineralize them. They contain an active gizzard which aids in rapid conversion of organic matter into vermicompost's. Epigeic earthworm includes *Eisenia fetida*, *Lumbricus rubellus*, *Bimastus minusculus*, *Dendrodrilus rubidus*, etc. Endogeic earthworms are small to large sized worms, with weakly pigmented body, life cycle of medium duration, moderately tolerant to disturbance, forms extensive horizontal burrows and they are geophagous feeding on particulate organic matter and soil. They can efficiently utilize energy from poor soils, hence can be used for soil improvements. Endogeics include *Aporrectodea caliginosa*, *Octolasion cyaneum*, *Dontoscolex corethrurus*, etc. Aneceic earthworms are larger dorsally pigmented worms with low reproductive rate, sensitive to disturbance, nocturnal, phytogeophagous, bury the surface litter, forms middens and extensive deep, permanent vertical burrows and live in them. *Lumbricus terrestris*, *Lumbricus polyphemus* and *Aporrectodea longa* are examples of aneceic earthworms.[16] Epigeics and aneceics are harnessed largely for vermicomposting [3]. Epigeics have been used in converting organic wastes into vermicompost. Earthworms thus act as natural bioreactors, altering the nature of the organic waste by fragmenting them.[21] [24]

Earthworm's gut is a straight tube starting from mouth followed by a muscular pharynx, oesophagus, thin walled crop, muscular gizzard, foregut, midgut, hindgut, associated digestive glands and ending with anus. The gut is an effective tubular bioreactor, which maintains a stable temperature regulatory mechanism, thus accelerating the rates of the bioprocess and preventing enzyme inactivation caused by high temperatures. The gut consisted of mucus containing protein and polysaccharides, organic and mineral matters, amino acids and microbial symbionts viz., bacteria, protozoa and micro-fungi. The increased organic carbon, total organic carbon and nitrogen moisture content in the earthworm gut provide an optimal environment for the activation of dormant microbes and germination of endospores, etc. A wide array of digestive enzymes such as amylase, cellulase, protease,

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lipase, chitinase and urease were reported from earthworm's alimentary canal.

Enzyme activity in earthworms is regionally specialized and influenced by physiological state, age and microorganisms. Digestive enzymes like cellulase, xylanase, acid phosphatase and alkaline phosphates were found to be present in the gut of *Eudrilus eugineae*. Amylase, cellulose, acid phoshatase, alkaline phosphates and nitrate redutase were secreted in the gut of the earthworms due to increase in presence of microorganisms in it. Amylase, cellulase, xylanase, endoglucanase, cellobiase, acid phosphatase, alkaline phosphate and nitrate reductase produced jointly by earthworms and gut microflora are supposed to play a central role in the process of digestion and humification of soil organic matter. Amylase, cellulase, xylanase, endoglucanase and cellobiase act upon the complex biomolecules such as starch, cellulose, xylan and cellodextrins. Acid phosphatase and alkaline phosphates and nitrate reductase are involved in the metabolism of phosphates and nitrogen. The gut microbes were found to be responsible for cellulose and mannose activities. [21] As the organic matter passes through the gizzard of the earthworm it is grounded into a fine powder after which the digestive enzymes, microorganisms and other fermenting substances act on them further aiding their breakdown within the gut, and finally passes out in the form of "casts" which are later acted upon by earthworm gut associated microbes converting them into manure product, the "vermicompost". [11] When the organic matter passes through the gut of the earthworm, it gets mixed up with the digestive enzymes and finally leaves the gut in partially digested form as "casts" after which the microbes takes up the process of decomposition contributing to the maturation phase. [17] 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 population in the earthworm's gut maybe because of their nutritional needs and digesting ability of the earthworms. 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 helps to bind the waste particles as casting in the hindgut. We look forward to work on the types of microbes present in the gut and the enzymes produced by them extracellularly that in turn helps in the digestion of the waste products into valuable castings or vermicompost.

## EXPERIMENTAL METHODS

### 2.1. Collection of Earthworms:

The specimens selected for the present investigation were the adult earthworms namely *Eudrilus eugineae.* The worms were collected from Koonpura- The house of mushrooms, Trivandrum and from Vivekananda centre, Kanyakumari. Species identification was confirmed using the general characters of the worms presented in Result section 3.1, table 1 and the taxonomical classification is presented in Results section 3.1 table 2.

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**Figure 1 – *Eudrilus eugineae***

## 2.2 Dissection of *Eudrilus eugineae.*

Healthy adults from each type were collected and allowed to starve for 24 hours. They were then disinfected with 50% ethanol. A sterile surgical blade was used for dissection. Bell pins was inserted into the ventral surface of the clitellar region and with the body slightly raised up. With the sterile surgical blade an incision was made longitudinally along the worm. The gut was then freed from the surrounding blood vessels. With a flamed forceps the gut section was removed. This was then transferred to saline solution (0.85% NaCl solution) and homogenized for 5 minutes in a vortex. This serves as the samples for further analysis.

**Figure 2 – Dissecting of earthworm**

## 2.3 Microbial analysis of the gut of *Eudrilus eugineae*

The gut isolated from *Eudrilus eugineae* was further tested for the number of total heterotrophic and fungi. The biochemical identification of the microbes observed during the enumeration of heterotrophic counts were also performed.

# RESULTS

## 3.1 Physical and Taxonomical classification of *Eudrilus eugineae*

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**Table 1- General Characteristic's**

| **Features** | ***Eudrilus eugineae*** |
|---|---|
| Size | 115 to 165 mm × 4 mm |
| Segments | 160-203 |
| Colour | Reddish brown dorsally and pale sandy yellow ventrally |
| Behaviour | Very active |
| Prostomium | Small, open epilobous |
| Setae | 8 per segment |
| Clitellum | In 14-18; incomplete ventrally |
| Spermathecal pores | One pair in segment 14 |
| Intersegmental septa | Septa between segments 4/5, 7/8/9 and 14/15 thickened |
| Male pores | In segment 17 |
| Female pores | In segment 14; combined with spermathecal pores |
| Genitial markings | Large central raised pad in segment 17 |
| Spermathecae | One pair in segment 14 |
| Nephridia | Holoic |
| Cocoons | Dark coloured with tapered lemon shape |

**Table 2. Taxonomical classification of *Eudrilus eugineae***

| | |
|---|---|
| Kingdom | Animalia |
| Phylum | Annelida |
| Class | Clitellata |
| Subclass | Oligochaeta |
| Order | Haplotaxida |
| Family | Eudrilidae |
| Genus | Eudrilus |
| Species | E.eugeniae |

### 3.2 Results for the number of heterotrophic bacteria.

In nutrient agar plates colony count was determined and the colony count (CFU/ml) found is tabulated in Table 3 below.

**Table 3 – Heterotrophic plate counts**

| Dilution Factor | Number of Colonies (CFU/ml) | | |
|---|---|---|---|
| | Original | Duplicate | Average |
| $10^{-4}$ | 191 | 169 | 180 |
| $10^{-5}$ | 55 | 69 | 65 |
| $10^{-6}$ | 33 | 21 | 27 |

### 3.3 Results for the number of fungi.

The fungal count was determined by spread plate technique onto Rose Bengal agar plates. The colony count is tabulated in the table 4 below.

**Table 4 – Fungi Counts**

| Dilution Factor | Number of Colonies (CFU/ml) | | |
|---|---|---|---|
| | Original | Duplicate | Average |
| $10^{-4}$ | 199 | 179 | 189 |
| $10^{-5}$ | 98 | 61 | 79.5 |
| $10^{-6}$ | 23 | 11 | 17 |

### 3.4 Identification of heterotrophic bacteria.

The bacterial isolates were identified by various morphological and biochemical characterizations and the results are presented in Table 5.

**Table 5 - Results for biochemical identification**

| Identification tests | $EB_1$ | $EB_2$ |
|---|---|---|
| Gram staining | Gram negative rods | Gram negative rods |
| Motility | Non motile | Non motile |
| Indole test | positive | positive |
| Methyl red test | positive | positive |
| Voges proskauer test | negative | negative |
| Citrate utilization test | positive | positive |
| Triple Sugar Iron agar test | Alkaline slant acid butt | Alkaline slant acid butt |
| Catalase test | positive | positive |

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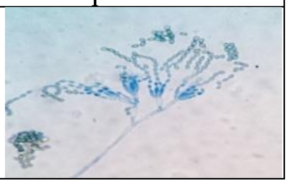
| | | |
|---|---|---|
| Oxidase test | negative | negative |
| Starch hydrolysis test | negative | negative |

### 3.5 Identification of fungi

The fungal isolates were identified by microscopic examination by LCB mounting technique and by colony morphology and results are presented in table 6

**Table 6 – Results for fungi isolates**

| Isolate | Colony morphology | Microscopic examination by LCB mounting technique |
|---|---|---|
| FF1 | Blue Green color colony |  |

## DISCUSSION

In the present study, the microbial analysis of the gut of *Eudrilus eugineae*, was studied. The worms' gut was dissected and screened for the microbial analysis. The vermicomposting ability of the worm is enhanced by the gut microbes.

Earthworms possesses an immense bacterial diversity within their digestive tracts and is very little explored mainly because of the non-cultivatable character of a large quantity of microorganisms which mainly come from soil. Earthworms activity engineers the soil by forming extensive burrows which loosen the soil and makes it porous. Earthworm feeding reduces the survival of plant pathogens such as *Fusarium* sp. *and Verticillium dahliae* and increases the densities of antagonistic fluorescent pseudomonads and filamentous actinomycetes while population density of *Bacilli* and *Trichoderma* spp. remains unaltered. [14]

The gut microbes of earthworm were found to be responsible for the cellulase and mannose activities. [21

In the present study Heterotropic bacteria namely *Klebsiella* sp were identified in the gut of *Eudrilus eugineae* by Morphological and biochemical characterizations. Among the two isolates all showed same biochemical results and was found to be *Klebiella* sp. The bacteria namely *Pseudomonas* sp, *Enterobacter* sp, *Bacillus* sp, *Klebsiella* sp, were found significantly in the gut of earthworm *Eudrilus eugineae*. [30] In the present study fungal isolate obtained from the gut of *Eudrilus eugineae* was identified by morphological examination. It was identified as *Mucor* sp.

Thus the microorganisms present in the gut of earthworms are the major cause for casting. These organisms produce enzymes which are responsible for the breakdown of the waste products into valuable casting or vermicompost.

## CONCLUSION

In the present study *Eudrilus eugineae was* collected for the microbial analysis of their gut microflora. It was concluded that the earthworm composting ability is enhanced by the microbial activities in the gut. The earthworms can be used in the management of number of organic waste generated in agriculture, horticulture, rural industries including household section creating environmental population and problem.

Vermicomposting seems to be a natural tool for waste management since it convert the waste into wealth in form of compost and the environmental population will be mitigated. Thus the way of management of organic waste seems to be vermicomposting technique. Vermicomposting is an efficient eco-friendly method of waste management.

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