

# A COMPARATIVE ANALYSIS ON THE GUT MICROFLORA OF EARTHWORMS *EUDRILUS EUGENIAE* AND *GLOSSOCOLEX PAULISTUS*

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**Abstract:** The present study investigated the bacterial population (Cfu/g) in the gut of *Eudrilus eugeniae* and *Glossocolex paulistus* used in the composting of *Eichornia crassipes* grown in textile effluent and dyes (Reactive Green 3G and Purple vat). Total bacterial count (Cfu/g) was analysed by standard plate counting method. The results showed that the total bacterial count (Cfu/g) in the vermicompost of *E. eugeniae* and *G. paulistus* when compared, was found to be higher in the vermicompost of *G. paulistus* in all treatments and control. The isolates of bacteria from the gut of *E. eugeniae* contained six species such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Escherichia coli*, *Enterococcus faecium*, *Bacillus subtilis* and from the gut of *G. paulistus* five species such as *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Escherichia coli*, *Enterococcus faecium*, *Bacillus subtilis* were isolated.

**Index Terms-** *Eudrilus eugeniae*, *Glossocolex paulistus*, *Eichornia crassipes*, *Bacillus subtilis*, vermicompost, *Pseudomonas aeruginosa* and total bacterial count.

## I. INTRODUCTION

Earthworms are referred to as the farmer's friend. This is because they help in the breakdown of complex organic matter as well as in loosening of the soil. In the words of Rombke *et al.*, (2005) earthworms are the most important soil invertebrates in the soil ecosystem in terms of biomass and activity. It is also supported by Lavelle (1988), who states that they are often considered as ecosystem engineers. Breakdown of complex organic matter into inorganic substances like carbon, water and nutrients is done by microorganisms called decomposers. The important steps in the process of decomposition are fragmentation, leaching, catabolism, humification and mineralisation. Few recent studies have shown that earthworms too have a role in humification (Manivannan *et al.*, 2004; Ranganathan and Parthasarathi, 2005). Parthasarathi *et al.*, (2007) showed that the count of microorganisms present in the gut of earthworm depended on the substrate that the earthworm feeds. Complex nature of interactions is found between earthworms and microorganisms in the degradation process. Earthworms are reported to have association with such free living soil bacteria and are the part of the drilosphere (Ismail 1995). In this way, it is known that microbial biomass and activity are usually enhanced in the drilosphere, with greater numbers of microbial colony forming units (CFUs) in the burrow walls and earthworm casts than in the parent soil (Aira *et al.*, 2007).

The earthworm gut is a natural bioreactor and the gizzard is a novel colloidal mill in which the feed is ground into particles smaller than one micron giving enhanced surface area for the microbial processing (Dkhar and Dkhar, 2004). The earthworm's alimentary canal provides a suitable environment for the growth of bacterial colonies and this is the evidence for the fact that earthworm castings contain significant number of bacteria that is present in the surrounding soil. The present study was carried out to find out the microbial flora in the gut of *Eudrilus eugeniae* and *Glossocolex paulistus* and also to identify different strains of bacterial population present in their gut.

## II. MATERIALS AND METHOD

Two species of earthworms *Eudrilus eugeniae* and *Glossocolex paulistus* were used to vermicompost *Eichornia crassipes* grown in textile effluent and dyes (Reactive Green 3G and Purple vat). After 60 days of vermicomposting few worms were taken out, washed. The worms were dissected and the gut contents were subjected for culturing using agar nutrient medium. Favourable laboratory conditions were maintained. After the colonies had appeared in the culture plates, counting and analysis of species were carried out. Total Bacterial Count (Cfu/g) in the gut of *Eudrilus eugeniae* and *Glossocolex paulistus* used in the composting of were analysed by standard plate counting method (Nagarathinam *et al.*, 2000). Agar was used as the nutrient medium. Triplicates were maintained.

### Statistical Analysis

Experimental data were expressed as mean  $\pm$  S.E of triplicates and all these data were analyzed using SPSS statistical package. The significant difference  $P < 0.05$  was estimated.

## III. RESULTS AND DISCUSSION

The earthworm's gut is a rich reservoir of microbial colonies which helps in breakdown of organic matters. The total bacterial count (Cfu/g) of vermicompost of *E. eugeniae* and *G. paulistus* ranged from  $32 \pm 2.00$  to  $35 \pm 2.00$  in control, from  $30 \pm 2.00$  to  $34 \pm 2.00$  in reactive dye Green 3G, from  $28 \pm 2.51$  to  $36 \pm 2.51$  in purple vat dye and from  $35 \pm 2.00$  to  $38 \pm 2.00$  in industrial dye effluent. When both the vermicompost of *E. eugeniae* and *G. paulistus* were compared, the total bacterial count was found to be higher in the vermicompost of *G. paulistus* in all treatments and control (Table: 1). In the present study the result is in accordance with the report of Ankaram *et al.*, (2012) who also reported an increase in bacterial count in vermicompost treatments when compared with its decomposed treatment. This also clearly indicates that the vermicompost produced by the combination of water hyacinth and cow dung (50% + 50%) showed enhanced bacterial flora. The combined effect is more suited for increased number of bacteria. When the total count of bacteria in control and different treatments of earthworms were made individually the highest count was observed in industrial dye effluent *E. eugeniae* ( $35 \pm 2.00$ ) and *G. paulistus* ( $38 \pm 2.00$ ) followed by in control vermicompost of *E. eugeniae* ( $32 \pm 2.00$ ) and in the vermicompost of *E. crassipes* grown in purple vat dye in the case of *G. paulistus* ( $36 \pm 2.51$ ). Statistically significant difference ( $P < 0.05$ ) was found between control and other treatments in the gut bacterial count of *E. eugeniae* whereas no significant difference was found in *G. paulistus*. Harithadevi *et al.*, (2009) observed an increased microbial count in vermicompost produced from agricultural waste and correlated this increase with enhanced enzyme activities in vermicompost. Higher amount of bacterial count in the gut of the earthworm species could be due to the nutrient rich organic feed and could have acted as a good substrate for their growth. Earthworm gut provides a good microenvironment for multiplication and activates growth of nitrous oxide producing bacteria because earthworm's gut is anoxic and it is the site of nitrous oxide production produced by soil bacteria ingested active in favourable physicochemical environment of earthworm gut (Horn *et al.*, 2003). Nagarathnam *et al.*, (2000) observed an increase in the bacterial population by 114 to 136 % over the control in five different vermibeds containing the biomass of different weed plants in 60 days of study. Similar increases in microbial population were observed and stated in other vermicomposting systems also (Suthar, 2010). Karmegam and Daniel (2000-b) also have reported that the alimentary canal of the earthworm carries large number of bacteria.

Table 1 Total Bacterial Count (Cfu/g) in the gut of *Eudrilus eugeniae* and *Glossocolex paulistus* used in composting of *Eichornia crassipes* grown in textile effluent and dyes (Reactive Green 3G and Purple vat).

Total Bacterial Count (cfu/g)			
S.No.	Treatments	<i>E. eugeniae</i>	<i>G. paulistus</i>
1	Control	32±2.00*	35±2.00
2	Reactive Green 3G	30±2.00*	34±2.00
3	Purple vat	28±2.51*	36±2.51
4	Industrial dye effluent	35±2.00*	38±2.00

\*The mean difference is significant at  $P < 0.05$  level.

#### Bacterial Isolates from the gut of *E. eugeniae* and *G.paulistus*

The isolates of bacteria from the gut of *E. eugeniae* contained six species such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Escherichia coli*, *Enterococcus faecium*, *Bacillus subtilis* and from the gut of *G.paulistus* five species such as *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Escherichia coli*, *Enterococcus faecium*, *Bacillus subtilis* were isolated (Table: 2). The findings of the present study coincided with the results obtained by (Emperor and Kumar, 2015) in their study done in vermicompost of *E. eugeniae*. As earthworms digest organic matter, their digestive system disintegrates, grinds, and degrades this material. These activities can be affected by the activity and concentration of beneficial and pathogenic microbes. The selective activity of the gut fluid of earthworms could be an important factor for the animal's nutrition as well as for regulating the steady state of the intestinal microbial community (Byzov *et al.*, 2007). The study conducted by Sivasankari and Anandharaj (2016) also supported the observation made in the present study.

Table 2 Bacterial species isolated from the gut of *Eudrilus eugeniae* and *Glossocolex paulistus* used in composting of *Eichornia crassipes* grown in textile effluent and dyes (Reactive Green 3G and Purple vat).

S.NO.	Bacterial species	Vermicompost	
		<i>E. eugeniae</i>	<i>G.paulistus</i>
1.	<i>Klebsiella pneumoniae</i>	+	-
2.	<i>Pseudomonas aeruginosa</i>	+	+
3.	<i>Enterobacter aerogenes</i>	+	+
4.	<i>Escherichia coli</i>	+	+
5.	<i>Enterococcus faecium</i>	+	+
6.	<i>Bacillus subtilis</i>	+	+

\*The mean difference is significant at  $P < 0.05$  level.

#### IV. CONCLUSION

The increased levels of bacterial count were observed in two different species *Glossoscolex paulistus* and *Eudrilus euginae* gut analysis. It reflects on the effective nature and efficiency of decomposition when the organic materials pass through the gut of earthworms. The microbial flora improves the decomposition capacity of organic matter and other enzymatic reactions and thus the vermicompost is converted into a good soil supplement.

#### V. REFERENCES

- [1] Aira, M. Fernando Monroy and Jorge Domnguez. 2007. *Eisenia fetida* (Oligochaeta: Lumbricidae) Modifies the Structure and Physiological Capabilities of Microbial Communities Improving Carbon Mineralization during Vermicomposting of Pig Manure. *Microbial Ecology*, 54: 662-671.
- [2] Ankaram, S. R. Mushan, L.C. and Rao, K. R. 2012. Management of Water Hyacinth (*Eichhornia crassipes*), an Aquatic Weed Waste, by Vermicomposting Technology. *International Journal of Environmental Technology and Management*, 15(3/4/5/6):195–207.
- [3] Byzov, B. A. Khomyakov, N.V. Kharin S.A. and Kurakov, A.V. 2007. Fate of Soil Bacteria and Fungi in the Gut of Earthworms. *European Journal of Soil Biology*, 43:14-156.
- [4] Dkhar, D. N. and Dkhar, M. S. 2004. Decomposition of Pine Needles by the Earthworm *Drawida papillifer* in laboratory condition. *Asian Journal of Microbiological, Biotechnological and Environmental Sciences*, 6:521-524.
- [5] Emperor, G. N. and Kumar, K. 2015. Microbial population and activity on vermicompost of *Eudrilus eugeniae*. *International Journal of Current Microbiological Applications and Sciences*, 4(10): 496-507.
- [6] Haritha Devi, S. Vijayalakshmi, K. 2009. Pavana Jyotsna, K. Shaheen, S.K. Jyothi, K. Surekha Rani , M. Comparative Assessment in Enzyme Activities and Microbial Populations During Normal and Vermicomposting. *Journal of Environmental Biology*, 30:1013–1017.
- [7] Horn, M.A. Schramm, A. and Drake, H.L. 2003. The Earthworm Gut: An Ideal Habitat for Ingested N<sub>2</sub>O-Producing Microorganisms. *Applications of Environmental Microbiology*, 69: 1662-1669.
- [8] Ismail, S.A. 1995. Earthworms in Soil Fertility Management In: Thampan PK (ed). *Organic Agriculture*, 77-100.
- [9] Karmegam, N. and Daniel, T. 2000-b. Selected Physico-chemical Characteristics and Microbial Population of the Casts of Earthworm, *Pontosiolus corethrurus* (Muller) and Surrounding Soil on Undisturbed Forest Floor in Sirumalai Hills South India. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences*, 2(3/4):231-234.
- [10] Lavelle P.1988. Earthworms and the Soil System. *Biol. Fertil. Soil.*, 6:237-251.
- [11] Manivannan, S. P. Ramamoorthy, K. Parthasarathi and Ranganathan, L.S. 2004. Effect of Sugar Industrial Wastes on the Growth and Reproduction of Earthworms. *Indian Journal of Experimental Zoology*, 7(1):29-37.
- [12] Nagarathinam, B. Karmegam, N. and Daniel, T. 2000. Microbial Changes in Some Organic Materials Subjected to Earthworm Action. *Journal of Ecobiology*, 12(1):45-48.
- [13] Parthasarathi, K. Ranganathan, L.S. Anandi, V. and Zeyer, J. 2007. Diversity of Microflora in the Gut and Casts of Tropical Composting Earthworms Reared on Different Substrates. *Journal of Environmental Biology*, 28: 87-97.
- [14] Ranganathan, L.S. and Parthasarathi, K. 2005. Humification of Cane Sugar Mill Wastes by *Eudrilus eugeniae* (Kinberg). *Journal of Ann. Uni.*, 41: 1-8.

- [15] Römbke, J.S. Jänsch and Didden, W. 2005. The Use of Earthworms in Ecological Soil Classification and Assessment Concepts. *Ecotoxicological Environmental Safety*, 62: 249-265.
- [16] Sivasankari, B. and Anandharaj, M. 2016. A Comparative Study on Gut Microflora of Earthworms *Eudrilus eugeniae* and *Eisenia fetida*. *International Journal of Advanced Research*, Volume 4(5):1402-1407.
- [17] Suthar, S. 2010. Evidence of Plant Hormone like Substances in Vermiwash: An Ecologically Safe Option of Synthetic Chemicals for Sustainable Farming. *Journal of Ecological Engineering*, 6:1089-1092.

