EXCELLENT PUBLISHERS

International Journal of Current Trends in
Pharmacobiology and Medical Sciences

Volume 1 • Number 2 (July-2016) • ISSN: 2456-2432

Journal homepage: www.ijctpms.com

Original Research Article

A Microcosm Study of Cast and Gut of an Epigeic Earthworm *Perioynx ceylanensis* Reared on Different Substrates

P. K. Nagalakshmi[1] and M. Prakash[2]*

[1]*Research Scholar, Department of Microbiology, Kanchi Shri Krishna College of Arts and Science, Kanchipuram-631 551, Tamil Nadu, India*
[2] *HOD & Associate Professor, Department of Microbiology, Kanchi Shri Krishna College of Arts and Science, Kanchipuram-631 551, Tamil Nadu, India*

Corresponding author.

Abstract

This study focused on the effect of vermicmposting using *Perionyx ceylanensis* on different wastes, teak leaf litter (TLL), paper mill sludge (PMS) and vegetable waste (VW) and analysed the gut and cast of *Perionyx ceylanensis* for the microflora. The organic wastes such as TLL, PMS and VW as substrates for the earthworm *Perionyx ceylanensis* were collected. Microbial analysis was done on vermicasts collected after 10, 15, 20 and 60 days interval. The Population of microbial flora was determined by serial dilution plate technique. Each substrate of 1 g was suspended in 1ml sterile saline and plated on respective media. The gut contents of *Perionyx ceylansis* reared from TLL, PMS and VW were dissected out using sterile scissor and contents were transferred to 1ml sterile saline in sterile test tubes. The dilutions were plated on Nutrient agar, Blood Agar, MacConkey agar, SDA and Actinomycetes agar plates and incubated for their growth. The diversity of types and number of fungi, bacteria, yeast and protozoa were isolated from the gut and cast of *Perionyx ceylanensis* influenced by different substrates. The microbial population was found to be numerous in the cast and gut of the worm in vegetable waste. Earthworms for their growth and reproduction have been shown to meet their nutritional requirement by feeding organic matter and microbes. Microorganisms constitute an important component of earthworm. The variation in microbial population in the earthworm gut may be because of their nutritional needs and digesting ability of earthworm. Thus the role of microbes-earthworm throws light on the flux of nutrients, particularly trace elements between microbes and earthworm.

Article Info

Accepted: 14 July 2016
Available Online: 25 July 2016

Keywords

Microflora
Organic substrates
Perionyx ceylanensis
Vermicasts

Introduction

Environmental degradation is a major threat confronting the world, and the rampant use of chemical fertilizers contributes largely to the deterioration of the environment through depletion of fossil fuels, generation of carbon dioxide (CO_2) and contamination of water resources. It leads to loss of soil fertility due to imbalanced use of fertilizers that has adversely impacted agricultural productivity and causes soil degradation. Now there is a growing realization that the adoption of ecological and sustainable farming practices can only reverse the declining trend in the global productivity and environment protection (Nagavallemma et al., 2006). Earthworms have been scientifically studied by man right from the time of Darwin (1881) and though different aspects such as development, physiology and ecology are studied, attention has been paid to the understanding of the relationship between earthworm and microbe only in the last two decades.

Soil, the major reservoir of microbes, meets the food requirement of earthworms and this has necessitated the establishment of different kinds of relationship between earthworms and microbes. They are: (1) microbes form a part of food for earthworm, (2) microbes are proliferated in the gut and vermicomposts, (3) earthworm help in the distribution of microbes and (4) together with earthworm microbes mineralise, humifies organic matter and facilitates chelation of some metal ions (Parthasarathi et al., 2007). Earthworms have the capacity to utilize soil microbes as their food. Growth and reproduction in earthworms require C and N and these were obtained from litter, grit and microbes (Edwards and Bohlen, 1996).

The earthworm gut varies depending on the species of earthworm studied, season and feeding regime of the earthworm (Kristufek et al., 1992). Esakkiammal and Lakshmibai (2013) showed that the number of microorganisms present in the gut of earthworm depended on the substrate that the earthworm fed on soil, and either no changes or higher numbers in earthworms fed on decomposed leaves, than in earthworms fed on inert substrate.

Vermicomposts contain in nutrients in forms that are readily taken up by the plants. *Perionyx ceylanensis* is an epigeic earthworm species with a short life cycle, recently explored for its potential in vermicomposting and the effect of vermicompost on plant growth and yield (Karmegam and Daniel, 2008). *Perionyx ceylanensis* is a purple red coloured epigeic earthworm, mainly found in biogas slurry, dung pats, composting heaps and decomposing leaf litter heaps. In view of this, the present study was conducted to estimate the quantitative and qualitative microbial association of earthworm *(Perionyx ceylanensis*) gut content, cast and adjacent soil.

Materials and methods

Source of earthworm

The earthworm, *Perionyx ceylanensis* for the study, originally collected from culture bank of the Department of Biology, Gandhigram Rural Institute- Deemed University, Tamil Nadu, India was mass multiplied in cow dung and used for vermicomposting studies.

Vermibed formulation and treatment design

Teak leaf litter (TLL) was collected from an agrofarm near KSK College, Kanchipuram. The paper mill sludge (PMS) was procured from a private mill near Kanchipuram. Vegetable wastes (VW) were collected from market shop of Kanchipuram, Tamil Nadu and subjected to initial decomposition in rectangular raining cement tanks of 75cm×60cm×45cm size by sprinkling water, regular mixing and turning of the substrates for 15 days. The cowdung (CD) was collected from nearby cattle sheds in fresh form and each predecomposed substrates were mixed with cow-dung in 1:1 ratio (50:50) on dry weight basis in separate plastic trays.

The moisture level of vermibeds was maintained at 65–70%, 60% RH and 12 L/12 D photoperiod throughout the study period by periodic sprinkling of adequate quantity of tap water. Periodic turning of vermibeds were also carried out. Each tray containing vermibed substrate was introduced with 60 adult Epigeic species of earthworm *Perionyx ceylanensis* were inoculated manually in selected bedding materials in plastic tubs (Prakash and Karmegam, 2010). The culture tubs were placed indoor in the laboratory. The bedding material upper surface was covered with wire mesh to avoid entry of predators. Microbial analysis was done on vermicasts collected after 15 days, 30 days, 45 days and lastly, after 60 days. That is, a time interval of fifteen days was taken as the standard for taking out the samples. These samples were analyzed for chemical parameters (organic carbon, total nitrogen, available phosphorus, and exchangeable potassium).

Isolation of microflora

The population of fungi, bacteria, actinomycetes and yeasts from the substrates (TLL, PMS and VW and their respective casts (C) of worm) was determined by dilution plate. Each substrate of 1 gram was suspended in 9 ml sterile saline (1 g NaCl in 100 ml distilled H_2O)

in a sterile test tube and was shaken thoroughly in a vortex mixer. From this stock, various dilutions were prepared from 10^{-1} to 10^{-7} with sterile distilled water and used as inoculum for isolation and enumeration of fungi, bacteria, actinomycetes and yeast from different substrates.

Using micropipette, 0.01 ml of the inoculum was inoculated into Blood agar (BA), Nutrient agar (NA) and MacConkey agar (MA) plates and spread over each plate media by using platinum loop for bacterial growth, Sabouraud's dextrose agar (SDA) plates for fungal and yeast growth and Actinomycetes agar (AA) and SDA plates for actinomycetes growth and incubated at 30ºC and 37ºC for 18-24 hrs for bacteria, 25ºC and 28ºC for 4-7 days for fungi, 30ºC and 37ºC for 10-12 days for actinomycetes and 25ºC and 37ºC for 12-14 days for yeast, respectively. The different colony forming units (CFU) developing on the media were estimated and expressed as CFU × 10^4 g^{-1} (for fungi), CFU × 10^6 g^{-1} (for bacteria) and CFU × 10^5 g^{-1} (for actinomycetes and yeast), respectively according to the method of Baron et al. (1994).

Enumeration and identification of microflora

The gut contents (G) [3-3.5 cm of gut ranging from 18-145 segments in *Perionyx ceylanensis*] of earthworm reared from TLL, PMS and VW were dissected out using sterile scissors and the contents were transferred to 1 ml sterile saline into a sterile test tube. It was vortexed and dilution plated on Nutrient Agar, Blood Agar, MacConkey agar, SDA and Actinomycetes agar plates. The casts were collected after 15 days of feeding and 1g was transferred to 1ml sterile saline, shaken well and 0.01 ml taken as inoculum to spread on NA, BA, MA, SDA and AA plates. The plates were incubated and observed for fungi, bacteria, actinomycetes and yeast colonies as stated above for total counts and identification (Parthasarathi et al., 2007).

A wet mount was prepared from each of the substrate, gut contents and casts of each earthworm species for microscopical observation of protozoa. For species identification a smear was prepared and stained with Gram's stain and haematoxylin and eosin, adopted a standard manual as described by Wenyon (1926). The number of cells (protozoa) were counted using haemocytometer and by using the formula, the observed protozoans were expressed in number of cells/cu.mm.

$$\frac{\text{Number of cells counted} \times \text{depth factor} \times \text{dilution factor}}{\text{Area counted}}$$

Results and discussion

The diversity of types and number of fungi, bacteria, actinomycetes, yeast and protozoa isolated from the gut and casts of *Perionyx ceylanensis* influenced by different feed substrates like TLL, PMS and VW are tabulated in Tables 1-4 and Figs. 1-2. The present study was conducted to evaluate the efficiency of different substrates for preparing vermicompost and the physico-chemical characteristics of vermicasts produced by *Perionyx ceylanensis* which showed an increase in electrical conductivity, total nitrogen, total phosphorus and total potassium where as organic carbon, C/N and C/P ratio showed high in vegetable waste as in Table 1.

Table 1. Physico-chemical characteristic of composting period using the *Perionyx ceylanensis* on comparison with control sample with different substrates.

S. No.	Parameter	TLL	PMS	VW	Control
1.	pH (at 25ºC)	7.21	7.30	7.80	6.75
2.	E.C. (mS/cm)	3.20	4.30	5.10	4.25
3.	Ca (%)	1.20	1.50	2.00	1.30
4.	Na (%)	0.22	0.18	0.54	0.20
5.	K (%)	0.95	0.87	1.05	1.00
6.	Mg (%)	0.34	0.45	0.50	0.40
7.	Org. carbon (%)	26.50	27.00	28.00	25.20
8.	N (%)	1.16	1.30	1.60	1.10
9.	P (%)	1.29	0.98	1.30	1.15
10.	C/N Ratio	22.14	21.54	22.29	21.10

The total bacterial population in foregut, midgut, and hindgut of *Perionyx ceylanensis* was enumerated in cowdung with Teak leaf litter, Papermill sludge and vegetable wastes for vermicompost. An important observation in the physico-chemical characteristics of vegetable waste with CD (1:1) substrates subjected to vermicomposting with *Perionyx ceylanensis* for 60 days was the changes in nutrient contents. Similiar changes were also observed by Prakash and Hemalatha (2013) in earthworm species – *Eisenia fetida.* The parallel results have also been reported by Prakash et al. (2008), Prakash and Karmegam (2010) and Ndegwa and Thompson (2000). Similiar results were reported by composting different substrates with different epigeic worms by Arjun Singh et al. (2014). Therefore, biological treatment methods have received much attention and considered as efficient, low cost treatment for organic waste.

Table 2. Total Microbial population count during the process of vermicomposting with reference to the control.

S. No.	Substrates	Microbes	Interval (in days)				
			0	15	30	45	60
1.	TLL	Bacterial count	45	65	78	125	145
		Fungal Count	42	57	67	89	123
		Actinomycetes count	24	35	46	53	67
2.	PMS	Bacterial count	42	55	78	122	143
		Fungal Count	39	57	69	75	109
		Actinomycetes count	24	32	37	39	40
3.	VW	Bacterial count	46	67	120	135	156
		Fungal Count	50	68	75	87	112
		Actinomycetes count	29	43	54	67	78
4.	Control	Bacterial count	39	45	55	65	66
		Fungal Count	31	42	45	52	57
		Actinomycetes count	25	30	35	38	40

Table 3. Number of microorganisms in different regions of gut of *Perionyx ceylanensis* from different substrates.

S. No.	Substrates	Microbes	Gut of *Perionyx ceylanensis*		
			Fore gut	Mid gut	Hind gut
1.	TLL	Bacterial count	57	79	82
		Fungal Count	10	12	8
		Actinomycetes count	20	24	18
2.	PMS	Bacterial count	45	67	57
		Fungal Count	9	12	10
		Actinomycetes count	17	24	12
3.	VW	Bacterial count	67	89	61
		Fungal Count	27	41	32
		Actinomycetes count	21	42	36
4.	Control	Bacterial count	24	42	56
		Fungal Count	13	19	12
		Actinomycetes count	13	16	10

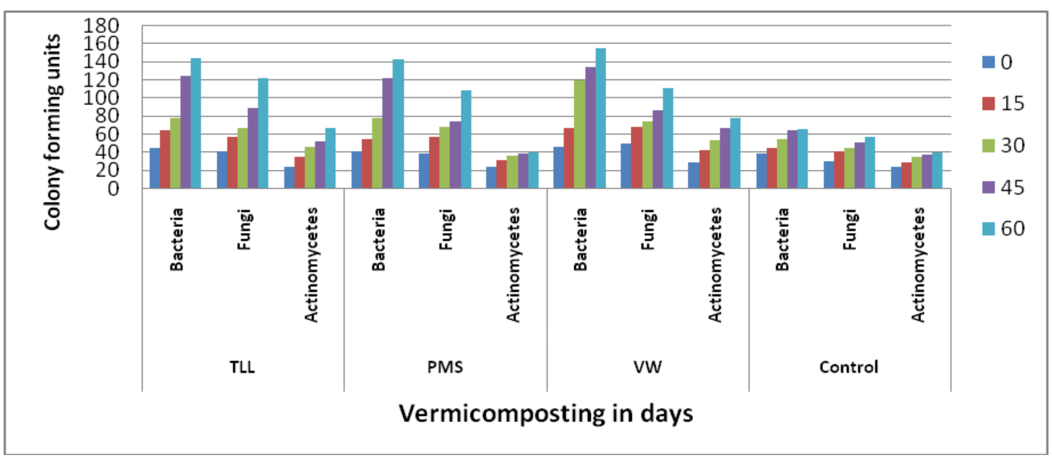

Fig. 1: Total Microbial population count during the process of vermicomposting with reference to the control.

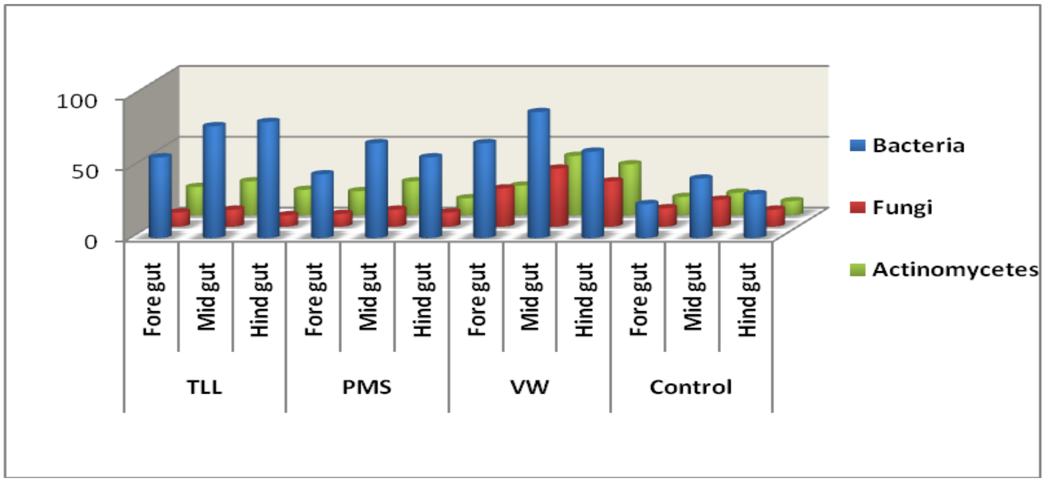

Fig. 2: Number of microorganisms in different regions of gut of *Perionyx ceylanensis* from different substrates.

Table 4. List of bacteria-actinomycetes and fungi obtained from different regions of gut of *Perionyx ceylanensis* in all substrates.

Sl. No.	Bacteria-actinomycetes	Fore gut	Mid gut	Hind gut
1.	*Bacillus subtilis*	+	+	+
2.	*Micrococcus sp.*	+	+	+
3.	*Arthrobacter sp.*	+	-	-
4.	*Azotobacter sp.*	+	+	-
5.	*Pseudomonas aeruginosa*	+	+	+
6.	*Escherichia coli*	+	+	-
7.	*Enterobacter aerogenes*	+	+	-
8.	*Flavobacterium sp.*	+	+	+
9.	*Serratia sp.*	+	+	+
10.	*Rhizobium sp.*	+	+	+
11.	*Rhizobacter sp.*	+	+	+
12.	*Streptomyces sp.*	+	+	+
	Fungi			
13.	*Penicillium rubrum*	+	+	+
14.	*Aspergilus niger*	+	+	+
15.	*Fusarium sp.*	+	+	+
16.	*Trichoderma viride*	+	-	-
17.	*Rhizopus nigricans*	+	+	-
18.	*Cephalosporium sps*	-	-	-
19.	*Mucor sp.*	+	-	-

(+ isolated ; - not isolated)

The bacterial count increased by the end of the experiment (Table 2). Of the three substrates, it was observed, that VM harbours the maximum variety and number of fungi (7 species), bacteria (11 species), actinomycetes (5 species), yeast (2 species) and protozoa (2 species), followed by TLL (6 species fungi, 8 species bacteria, 2 species actinomycetes and 3 species protozoa). The least diversity of microflora was found in PMS (3 species fungi, 5 species bacteria, 2 species actinomycetes and 1 species protozoa). The bacterial populations were higher in the midgut region than in the foregut and hindgut region. The selective activity of the gut fluid of earthworms could be a significant factor for the animals nutrition as well as for regulating the steady state of the intestinal microbial community, and modification of microbial communities in soil (Byzov et al., 2007).

Vermicasts are highly valuable organic manure hold micro and macronutrients, microorganisms and a variety of enzymes (Parle, 1963). In the present study vermicasts collected from three different substrates of *Perionyx ceylanensis* showed increased nutrients, microbial population and enzyme activities. The compost characteristics of vermicasts, such as pH, electrical conductivity, organic carbon, NPK, C/N and C/P ratios showed significant variation with VM. The physic chemical characteristics of vermicasts and worm-unworked composts showed notable changes in their composition. The vermicomposting studies carried out with various organic materials using *Eudrilus eugeniae* and *Eisenia fetida* also gave similar results (Karmegam and Daniel, 2000). The nutrient level of vermicompost depends on the nature of the organic waste used as food source for earthworms (Chowdhury et al., 2007). Similar results was explained by Saikrithika et al . (2015). It has been reported that mineral aggregates are more stable in the presence of organic particles. Studies on the microbial colony forming units have established the superiority of the vermicast over the worm-unworked compost by their presence in higher counts indicating their symbiotic association with the earthworms, which is essential for the biodegradation of organic wastes. This observation reveals the fact that the physical property of the substrates is more feasible for consumption by earth worms (Shobith et al., 2015). The inter-relationship of microorganisms with macroorganisms by their presence inside or outside the body of macroorganism and in their environment has been well established (Manyuchi and Whingiri, 2014).

Conclusion

Earthworms are important in turnover of soil, contribute most to soil invertebrate biomass contain the highest microflora. In present study our findings report that vegetable waste, a wealthy organic resource may serve as good feeding material for the earthworm *Perionyx ceylanensis*, comparing the physico-chemical, microbiological activities in the vermicasts. Thus the role of microbes-earthworms throws light on the flux of nutrients, particularly trace elements, between microbes and earthworms.

Conflict of interest statement

Authors declare that they have no conflict of interest.

References

Arjun Singh, Singh, R. V., Saxena, A. K., Shivay, Y. S., Nain, L., 2014. Comparative studies on composting efficiency of *Eisenia foetida* (Savigny) and *Perionyx excavatus* (Perrier). J. Exp. Biol. Agric. Sci. 2(5), 508-517.

Baron, J.E., Peterson, R.L., Finegold, M.S., 1994. Cultivation and isolation of viable pathogen. In: Diagnostic Microbiology, 9th Edn. Mosby, London. pp.79-96.

Byzov, B.A., Khomyakov, N.V., Kharin, S.A., Kurakov, A.V., 2007. Fate of soil bacteria and fungi in the gut of earthworms. Eur. J. Soil. Biol., 43, 149-156.

Chowdhury, A., Hazra, A.K., Mahajan, S., Choudhury, J., 2007. Microbial communities of earthworm (*Perionyx excavatus* Perrier) gut, cast and adjacent Soil in two different fields of west Bengal. Rec. Zoo. Surv. India. l07(Part-4), 101-111.

Darwin, C., 1881.The Formation of Vegetable Mould through the Action of Worms with Observation on their Habits. Murray, London.

Edwards, C.A., Bohlen, P.J., 1996. Biology and Ecology of Earthworms. Chapman and Hall, London.

Esakkiammal, B., Lakshmibai, L., 2013. Enumeration of bacterial population in the gut region of *Eudrilus eugeniae*. Int. J. Curr. Microbiol. Appl. Sci. 2(5), 267-270.

Karmegam, N., Daniel, T., 2000. Decomposition of leaf litters using the compost worm, *Eisenia fetida*. Ind. J. Environ. Ecoplan. 3(1), 111-116.

Kristufek, V., Ravasz, K., Pizl, V., 1992. Changes in densities of bacteria and microfungi during gut transit in *Lumbricus rubellus* and *Aporrectodea caliginosa* (Oligochaeta: Lumbricidae). Soil Biol. Biochem. 12, 1499-1500.

Manyuchi, M.M., Whingiri, E., 2014. Effect of vermicomposting period, substrate quantity, cow dung composition and their interactions on *Eisenia fetida* during vermicomposting. Int. J. Curr. Microbiol. Appl. Sci. 3(8), 1021-1028.

Nagavallemma, K. P., Wani, S.P., Stephane Lacroix, P.V.V., Vineela, C., Baburao, M., Sahrawat, K.L., 2006. Vermicomposting: Recycling wastes into valuable organic fertilizer. Global Theme on Agrecosystems Report. International Crops Research Institute for the Semi-Arid Tropics.2(1):1-16.

Ndegwa, P. M., Thompson, S.A., 2000. Effects of C-to-N ratio on vermicomposting of biosolids. Bioresour. Technol. 75, 7-12.

Parle, I.N., 1963. Microorganisms in the intestines of earthworms. J. Cell. Microbiol. 31, 1-11.

Parthasarathi, K., Ranganathan, L. S., Anandi, A., Zeyer, J., 2007. Diversity of microflora in the gut and casts of tropical composting earthworms reared on different substrates. J. Environ. Biol. 28(1), 87-97.

Prakash, M., Hemalatha, N., 2013 Dynamics of microorganisms during vermistabilization of organic substrates and enhances performance of plant growth promoting Rhizobacteria on black gram. Int. J. Curr. Microbiol. Appl. Sci. 2(7), 171-187.

Prakash, M., Jayakumar, M., Karmegam, N., 2008. Physico-chemical characteristics and fungal flora in the casts of the earthworm, *Perionyx ceylanensis* Mich. reared in *Polyalthea longifolia* leaf litter. J. Appl. Sci. Res. 4(1), 53-57.

Prakash, M., Karmegam, N., 2010. Vermistabilization of pressmud using *Perionyx ceylanensis* Mich. Bioresour. Technol. 101, 8464-8468.

Saikrithika, S., Santhiya, K.R., Veena Gayathri, K., 2015. Effects of different substrates on vermicomposting using *Eudrilus eugeniae* on the growth of *Vinca rosea*. Int. J. Scient. Res. Publ. 5(9), 1-11.

Shobith, G.M., Pallavi, P., Rajpurohith, Patel, S. J., 2015. Effect of different substrates and effluent on vermicomposting by surface feeder species. Int. J. Sci. Res. 4(2), 951-953.

Wenyon, C.M., 1926. Protozoology: A Manual for Medical Men, Veterinarians and Zoologists. Vol II. Tindall and Cox Publishers, London.

How to cite this article:

Nagalakshmi, P. K., Prakash, M., 2016 A microcosm study of cast and gut of an epigeic earthworm *Perioynx ceylanensis* reared on different substrates. Int. J. Curr. Trend. Pharmacobiol. Med. Sci. 1(2), 45-51.