

Original Research Article

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Gut Enzyme Activities of the Earthworm, *Perionyx ceylanensis* Collected from Different Vermibed Substrates

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ABSTRACT

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Enzymes in the gut of three different stages (juvenile, pre-clitellate, clitellate) of the earthworm species *Perionyx ceylanensis*, reared in the vermibed substrates namely, teak leaf litter (TLL), paper mill sludge (PMS) and pressmud (PM) in combination with cowdung were analysed. The enzymes, amylase (EC 3.2.1.1), cellulase (EC 3.2.1.4.), protease (EC 3.4.0), phosphatase (EC 3.1.3.1, 2) and invertase (EC 3.2.1.20) were analysed from gut contents of the earthworm reared in TLL, PMS and PM on 60th day. The activity of the enzymes amylase, cellulase, protease, phosphatase and invertase were invariably observed to be more in clitellate adults followed by pre-clitellates and juveniles. The results also showed variation in enzyme activities with respect to different vermibed substrates.

Introduction

Disposal of solid waste has become a major problem recently due to shortage of dumping sites and strict environmental laws. As a result emphasis is now on aerobic composting, defined as a microbiological process that converts waste into organic manure rich in plant nutrients and humus (Sharma *et al* 1999). Even though recycling organic waste has been known since biblical times, there are many aspects that should be improved. One of these areas includes reduction in overall time required for composting, especially during the winter

season. Various studies have shown that vermicomposting of organic waste accelerates organic matter stabilization (Neuhauser *et al* 1988; Frederickson *et al* 1997) and gives a product rich in chelating and phytohormonal elements (Tomati and Galli, 1995) which has a high content of microbial agents and stabilized humic substances (Ferruzi, 1986).

Vermiculture has been receiving considerable attention in recent years internationally for its potential role in organic farming and sustainable development (Kale, 1998).

Species identified as potentially useful to break down organic wastes were *Eisenia fetida*, *Dendrobaena veneta* and *Lumbricus rubellus* from temperate areas and *Eudrilus eugeniae* and *Perionyx excavatus* from the tropics (Edwards, 1998). The survival, growth, mortality and reproduction of these species have been studied thoroughly in the laboratory, using a wide range of organic wastes (Edwards, 1998). Frederickson *et al* (1997) combined vermiculture with traditional green waste composting systems. Kale and Bano (1986) employed an African earthworm, *Eudrilus eugeniae*, for degradation of organic wastes. The cast thus obtained was used as bio-organic fertilizer. Goswami and Kalita (2000) showed the efficiency of some indigenous earthworm species of Assam in vermiculture. In India vermiculture is being tested for treatment of agricultural, sugar, food processing wastes (Kale, 2000).

Knowledge about the food requirement and digestive capability of the earthworms is essential to understand the decomposition process during vermicomposting. The enzymes and the enzyme activity in the gut of earthworms and in casts are extremely important since they are responsible for the degradation of organic materials and to make the nutrients in available form for plants. Lee (1985) reported increased enzyme activities and microbial populations in worm casts as compared with the underlying soil. Microorganisms and soil enzymes are closely associated with soil fertility; information on biological characteristics of worm casts can define the importance of worm casting activity in soil fertility. Application of vermicompost to the soil has multitude of advantages such as conservation of energy, increasing the fertilizer use efficiency, improvement of soil properties and enhancement of soil microbial activity. Devi and Prakash (2015) studied the activities of

enzymes, amylase, cellulase, invertase, phosphatase and protease in vermicomposts of three different vermibed substrates. All the enzymes analysed in the study showed higher levels in vermicompost than in composts (control sets without earthworms).

In the present study, the enzymes amylase, cellulase, protease, phosphatase and invertase were analysed from gut contents of three different stages (juvenile, pre-clitellate, clitellate) *Perionyx ceylanensis*, reared in the vermibed substrates namely, teak leaf litter, paper mill sludge and pressmud in combination with cowdung on 60th day to analyse the different stages of the earthworm and different substrates show any relation.

Materials and Methods

Teak leaf litter (TLL) was collected from an agroform near Kanchipuram. The paper mill sludge (PMS) was procured from a private mill near Kanchipuram. The filter mud or pressmud (PM) was collected from Cheyyar Co-operative Sugar Mills Ltd., located in Thenthandalam, Anakkayur, Thiruvannamalai District, Tamil Nadu. The cowdung was collected from nearby cattle sheds in fresh form and allowed to stabilize for one week and used for the study. The organic substrates, TLL, PMS and PM were subjected to initial decomposition in rectangular draining cement tanks of 75cm×60cm×45cm size by sprinkling water, regular mixing and turning of the substrates for 20 days. The earthworm, *Perionyx ceylanensis* Mich. 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. Based on the studies reported by Karmegam and Daniel (2009a and 2009b) and Prakash and Karmegam (2010) on vermicomposting of different organic substrates using *P.*

ceylanensis, the ratio of organic substrate mix, i.e., 1:1 (50:50) proportion on dry weight basis was used in the present study. Accordingly, the pre-decomposed organic substrates were mixed with cowdung in 1:1 ratio on dry weight basis, transferred to vermibeds and moistened to hold 60-70% moisture content. The vermicomposting studies were carried out for 60 days using *P. ceylanensis* in three replicates twice under controlled conditions. The enzymes, amylase (EC 3.2.1.1), cellulase (EC 3.2.1.4.), protease (EC 3.4.0), phosphatase (EC 3.1.3.1, 2) and invertase (EC 3.2.1.20) in gut of three different stages (juvenile, pre-clitellate, clitellate) of the earthworm species *Perionyx ceylanensis* reared in three different substrates were analysed after 60th day. For the enzymatic analysis of worm guts, gut content cleared tissue was used. The activity of amylase, cellulase and invertase was done according to method described by Galstyan (1965). Protease and phosphatase activities were analysed adopting the methods of Sarath et al (1989) and Jannossy (1963), respectively.

Results and Discussion

The activity of the enzymes, amylase, cellulase, invertase, phosphatase and protease in the gut of different stages of *P. ceylanensis* is given in Tables 1, 2 and 3. As observed

from the results of three different substrates, higher activity was shown by clitellate stage of *P. ceylanensis*.

The activity of amylase, 1.11, 3.22 and 7.19 mg glucose/gram of oven dry samples incubated for 24 hours were recorded for juvenile, preclitellate and clitellate *P. ceylanensis* collected from TLL+CD substrate respectively. Similar trend was observed for cellulase, invertase, phosphatase and protease enzymes also (Tables 2 and 3).

In all the stages, invertase showed maximum activity when compared with other enzymes studied. The activity of invertase, 4.00, 8.05 and 13.40 mg glucose/gram of oven dry samples incubated for 24 hours were recorded for juvenile, preclitellate and clitellate *P. ceylanensis* collected from PM+CD substrate respectively (Table 3).

Invariably activity of all the enzymes in preclitellate worms were significantly higher than juveniles ($p < 0.05$), whereas, the activity of enzymes in clitellate worms showed significantly higher activity than juveniles and preclitellates. The clitellate *P. ceylanensis* collected from PM+CD showed 9.00, 10.83, 13.40, 10.33 and 14.67 mg/g activity of amylase, cellulase, invertase, phosphatase and protease respectively (Table 3).

Table.1 Enzyme activities in gut contents of the earthworm, *Peryionyx ceylanensis* reared in teak leaf litter + cowdung (1:1)

Enzymes analysed	Juvenile	Pre-clitellate	Clitellate
Amylase	1.11 ^a	3.22 ^b	7.19 ^c
Cellulase	1.98 ^a	4.63 ^b	8.80 ^c
Invertase	2.62 ^a	5.25 ^b	12.63 ^c
Phosphatase	1.30 ^a	4.17 ^b	9.91 ^b
Protease	2.70 ^a	5.61 ^b	11.33 ^c

(Activities of amylase, cellulase and invertase are expressed as mg of glucose/g of oven dry samples for 24 hrs of incubation; Protease activity: mg of glutamic acid/g of oven dry substrates for 24 hrs of incubation; Phosphatase activity: mg/phenol/g of oven dry substrates for 24 hrs of incubation).

Table.2 Enzyme activities in gut contents of the earthworm, *Peryionyx ceylanensis* reared in paper mill sludge + cowdung (1:1)

Enzymes analysed	Juvenile	Pre-clitellate	Clitellate
Amylase	1.12 ^a	1.98 ^b	8.33 ^c
Cellulase	1.06 ^a	4.33 ^b	9.16 ^c
Invertase	2.13 ^a	5.76 ^b	12.46 ^c
Phosphatase	2.75 ^a	4.40 ^b	9.70 ^b
Protease	2.63 ^a	4.53 ^b	12.10 ^c

(Activities of amylase, cellulase and invertase are expressed as mg of glucose/g of oven dry samples for 24 hrs of incubation; Protease activity: mg of glutamic acid/g of oven dry substrates for 24 hrs of incubation; Phosphatase activity: mg/phenol/g of oven dry substrates for 24 hrs of incubation).

Table.3 Enzyme activities in gut contents of the earthworm, *Peryionyx ceylanensis* reared in press mud + cowdung (1:1)

Enzymes analysed	Juvenile	Pre-clitellate	Clitellate
Amylase	0.71 ^a	4.45 ^b	9.00 ^c
Cellulase	3.16 ^a	6.67 ^b	10.83 ^c
Invertase	4.00 ^a	8.05 ^b	13.40 ^c
Phosphatase	2.36 ^a	4.93 ^b	10.33 ^b
Protease	3.68 ^a	6.32 ^b	14.67 ^c

(Activities of amylase, cellulase and invertase are expressed as mg of glucose/g of oven dry samples for 24 hrs of incubation; Protease activity: mg of glutamic acid/g of oven dry substrates for 24 hrs of incubation; Phosphatase activity: mg/phenol/g of oven dry substrates for 24 hrs of incubation).

Comparing the substrates, PM+CD showed high activity of the enzymes analysed in the gut. Pressmud (filter cake), a major-by product of sugarcane processing is produced 12 million tones annually (Parthasarathi, 2007). Pressmud is the residue obtained from sedimentation of the suspended materials such as fiber, wax, ash, soil and other particles from the cane juice. Pressmud (filter cake), a major-by product of sugarcane processing has previously been shown to be a substrate suitable for vermicomposting (Parthasarathi and Ranganathan, 2002). The interaction of the microbes and the earthworms contributes to breaking the complex organic compounds in pressmud into simpler forms (Ahmad and Bhargava, 2005).

Earthworms are important in turnover of soil, contribute most to soil invertebrate biomass, contain the highest international units of cellulase and are believed to be capable of

producing cellulases and chitinases other than through intestinal microflora (Parle, 1963; Hartenstein, 1981). Cellulases are a group of enzymes that catalyze the degradation of cellulose – a polysaccharide built of β -1,4 linked glucose units. This group consists of endo 1,4 – glucanase, exo 1,4- glucanase and β -D-glucosidase. The products of cellulose degradation are glucose, cellobiose and high molecular weight oligosaccharides.

The reasons for more enzyme activities in the casts of *E. eugeniae* than *P. excavatus* are greater consumption rate, enhanced gut microbial population (Parthasarathi, 1997; Parthasarathi *et al* 1997; Parthasarathi and Ranganathan, 2002), enhanced microbial population in the casts (Parthasarathi *et al* 1997) and more moisture. From the results of the study, it is concluded that the enzyme activity in the gut of *Perionyx ceylanensis* is developmental stage and substrate dependent.

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