Vermicultural and molecular characterization of composting endemic earthworms

M. Biruntha, J.A. John Paul[a,*] and P. Mariappan

PG and Research Department of Zoology, Raja Serfoji Government College,
Thanjavur–613 005, Tamil Nadu, India
[a] DST-PURSE Scheme, Faculty of Agriculture, Annamalai University, Chidambaram – 608 002,
Tamil Nadu, India
* Corresponding Author: jajpaul@gmail.com.

Abstract

Vermiculture efficiency of two endemic earthworm species i.e., *Perionyx ceylanensis* and *Perionyx excavates* was compared in different organic wastes such as municipal solid waste (MSW), sugarcane trash (ST), paddy straw (PS) and farm yard manure (FYM) to check the suitable substrate. The percentage decomposition, total biomass of the worms recovered, the total number of worms and the cocoons recovered were high in the treatment, which contained FYM and cowdung (CD) (1:1). The worm meal of *P.ceylanensis* cultured in various substrates was subjected to molecular characterization, in which the isolated DNA did not show any descriptive changes in the bands obtained. The molecular weight of the band was found to be 13400 bp in all the substrates. The RNA showed some changes in the bands, the molecular weight was found to be 2190 bp, 2260 bp, 2275 bp and 2210 bp. RAPD analysis was performed to identify the level of genetic variation and polymorphism in *P.excavates* collected from four different locations. SDS-PAGE of vermiprotein of *P.ceylanensis*, which were grown in various substrates, showed several bands ranged from 37 to 250 kDa.

Key words: *Perionyx ceylanensis, Perionyx excavates*, vermiculture, SDS-PAGE, RAPD

{**Citation:** M. Biruntha, J.A. John Paul, P. Mariappan. Vermicultural and molecular characterization of composting endemic earthworms. American Journal of Research Communication, 2013, 1(3): 168-180} www.usa-journals.com, ISSN: 2325-4076.

Introduction

Rearing of earthworms in a controlled environment is vermiculture. Earthworms have been successfully used in the vermistabilization of urban, industrial and agricultural wastes in order to produce organic fertilizers and obtain protein for animal feed. Although many species could be used for these ends, given their nutritional requirements and reproductive biology, only *P. excavatus* and *Lampito mauritii* are widely used. Various agricultural wastes like post-harvest stubbles, sugar cane trash, coir waste and paper pulp, and faecal matter of cow, sheep, horse, and biogas sludge of poultry droppings have been tried as food source for these worms. An endemic compost worm, *P. excavatus*, with a wide range of distribution in the subcontinent, is an excellent waste decomposer. Its potentiality in developing indigenous vermitechnology for waste control and protein production could be exploited under natural conditions. Another similar endemic earthworm species *P.ceylanensis* has been identified and utilized for vermitechnology (Karmegam *et al.*, 2003 and Paul *et al.*, 2011) possesses enough potentiality for its use in vermicomposting in our country. Molecular characterization of *P. ceylanensis* in organic substrates has not yet been fully described, although some specific aspects of their biology have been investigated (Karmegam *et al.*, 2003; Paul, 2005 and Paul *et al.*, 2011).

Kale and Bano (1994) showed that the earthworms preferring nitrogen rich diet grow faster and produce more cocoons than those feeding on mineralised soil. The knowledge on the biology, food habits and habitat selections of worms are important factors for encouraging culturing of worms for degradation of animal waste, plant residues and wastes from the food processing units.

The main role of DNA molecules is the long-term storage of information and DNA is often compared to a set of blueprints, since it contains the instructions needed to construct other

components of cells, such as proteins and RNA molecules. The DNA segments that carry this genetic information are called genes, but other DNA sequences have structural purposes, or are involved in regulating the use of this genetic information (Alberts *et al*., 2002). RNA is a nucleic acid polymer consisting of nucleotide monomers that plays several important roles in the processes that translate genetic information from DNA into protein products; RNA acts as an essential carrier molecule for amino acids to be used in protein synthesis (Fiers *et al*.,1976). This is the first report on molecular characterization of newly identified endemic vermicomposting earthworm, *P.ceylanensis.*

Morphologically earthworm species can be distinguished on the basis of several characteristics like growth, number of segments, length, and position of clitellum. Although, morphological characters of these organism depends on environmental conditions and organic matter which forms part of their diet (Curry and Schmidt, 2007). Molecular markers are essential to understand its population biology as well as the underlying selective processes such as those imposed by temperature gradients or change, migration rates, population isolation due to habitat fragmentation, historical events and also mating behavior (Avise, 1994).

The RAPD is a PCR- based assay that has been shown to be useful in generating molecular markers for species or strain identification in a variety of organisms (Welsh *et al.*, 1990). Earthworms are being used as bio-indicators to assess terrestrial pollution. However, it is often not known whether their populations possess a uniform genetic structure, which would allow comparison of residues or biological properties of earthworms from different sampling locations. In order to investigate this point, RAPD variation is surveyed in endemic earthworm, *P.exccavatus*.

Materials and Methods

Vermiculture

A new endemic earthworm species *P. ceylanensis* has been identified and utilized for vermitechnology from the Department of Biology, Gandhigram Rural Institute- Deemed University, possesses enough potentiality for its use in vermicomposting in developing countries. Another endemic compost worm, *P. excavatus*, with wide range of distribution in the subcontinent, is an excellent waste decomposer. Its potentiality in developing indigenous vermitechnology for waste control and protein production could be exploited under natural conditions. Vermiculture efficiency of these two endemic earthworm species was compared in different organic wastes such as municipal solid waste (MSW), sugarcane trash (ST), paddy straw (PS) and farm yard manure (FYM) to check the suitable substrate.

Plastic containers of 45 x 35 x 15 cm size were filled with 2 kg of pre-decomposed organic wastes and 2 kg cowdung (w/w) mixed with water. The vermibed was allowed 24 h for stabilization. A total of 50 pre-weighed clitellate of *P.ceylanensis* and *P.excavatus* of uniform size were separately introduced in the respective troughs. The troughs without earthworms served as the control. Vermibed was maintained in a controlled environment for 50 days. Three replicates were maintained for each substrate. Samples were collected at the initial and after 50 days, the vermicompost were removed and sieved through a 2 mm sieve to remove the worms. The percentage decomposition of organic wastes, biomass of earthworms, total number of worms recovered and cocoon produced in various substrates by these two earthworm species were recorded at the end of the study.

Percentage decomposition

The percentage decomposition was calculated as follows (Goswami and Kalita, 2000):

$$\text{Percentage decomposition} = \frac{A - B}{A} \times 100$$

where,

A=Total weight of organic substrate in the vermibed

B=Weight of decomposed material

(sieved through material)

The percentage decomposition of the various organic wastes by both species was calculated.

Molecular Characterization

Isolation of earthworm DNA

Genomic DNA from adult clitellate earthworms of *P. ceylanensis* cultured in four different substrates and *P. excavatus* collected from four different regions was extracted by using the method of Sambrook *et al.* (1989). The DNA was characterized using agarose gel electrophoresis.

RAPD analysis

The DNA isolated from *P. excavatus* of four different regions was further characterized for RAPD analysis.

Primers used in RAPD analysis

S.No	Primer code	Primer sequence(5'-3')
1	EP-A	5' GTG CTG CAG GTG TAA ACT TGT ACC AG 3'
2	EP-B	5' ACA TTA CTA ACC CGT CCG GC 3'
3	EP-C	5' GTA GCA TTC CAC TTT ATT CCA GGC C 3'
4	EP-D	5' CAC GGA TCC GGT AGC AGC GGT AGA GT 3'

Temperature and time Profile (35 cycles)

Initial denaturation - 94 for 5min

Denaturation - 94 for 30sec

Annealing - 55 for 30sec

Extension - 72 for 1 min

Final extension - 72 for 15min

MgCl2 - 1 .5mM final concentration

Isolation of earthworm RNA

RNA from adult clitellate earthworms of *P. ceylanensis* cultured in four different substrates was extracted by using the method of Sambrook *et al.* (1989). The RNA was characterized using agarose gel electrophoresis

Separation of earthworm protein

Protein extracted from adult clitellate earthworms of *P. ceylanensis* cultured in four different substrates was done by SDS–PAGE (Pingod *et al.* 2002).

Results and Discussion

The percentage decomposition of the various organic wastes treated with *P.ceylanensis* and *P.excavatus* is given in Table 1. The percentage decomposition was high in the treatment, which contained FYM i.e., 69.42% followed by 68.30% in MSW for *P.ceylanensis*. The percentage decomposition of organic wastes treated with *P.excavatus* showed insignificant difference. The composts (without worms) of all substrates showed low rate of decomposition which ranged from 23.70±1.03 to 28.92±1.34%. Similar studies suggest that the decomposition rate depends on the efficiency of earthworm species and the nature of organic material mix used for vermicomposting (Karmegam and Daniel, 2009a; Prakash and Karmegam, 2010).

Table 1. Percentage decomposition of various organic substrates by *P.ceylanensis* and *P.*excavatus at the termination of experiment (50 d)

	MSW	ST	PS	FYM
Control (WUW)	27.05±1.83	26.15±1.20	23.70±1.03	28.92±1.34
P.ceylanensis				
Vermicompost (WOW)	85.33±4.90	75.47±3.30	74.02±3.46	94.58±5.24
% Increase over control	68.30±2.67	65.35±1.90	67.98±2.25	69.42±2.84
*P.*excavatus				
Vermicompost (WOW)	77.25±3.81	73.13±2.04	69.50±2.94	84.19±4.33
% Increase over control	64.98±1.71	64.07±1.56	65.90±2.93	65.65±2.75

The total biomass of the worms recovered from the various substrates after 50 days of vermicomposting is given in Table 2. The total biomass of worms recovered was high in the treatment, which contained FYM i.e., 75.6±0.8 for *P.ceylanensis* and 68.9±0.9 for *P.excavatus*. The total number of worms and the cocoons recovered from the vermicompost of various substrates after 50 days of vermicomposting is given in Table 2. The total number of worms and the cocoons recovered were high in the treatment, which contained FYM. The total number of worms recovered in each substrate significantly differed at 0.5% level except ST and PS for both the species. The total number of cocoons recovered in each substrate significantly differed at 0.5% in all the four substrates for *P.ceylanensis*. Paul *et al.* (2011) reported the worm biomass, total number of worms recovered and cocoons recovered in different ratio of MSW:CD.

Kaur *et al.* (2010) reported that cattle dung increased suitability of paper mill sludge as feed for both microbes and the earthworm (*E. fetida*) which is evident from higher nutrient

content and better population buildup in the mixtures over 100% paper mill sludge. Singh *et al.* (2010) reported that minimum mortality and maximum population buildup of *E.fetida* were observed in 50:50 mixture of biosludge and cattle dung. The recovery of a high number of earthworms in the treatment inoculated with *E.fetida* showed the short life cycle of this earthworm species which is an essential characteristic of vermicomposting species (Karmegam and Daniel, 2009b).

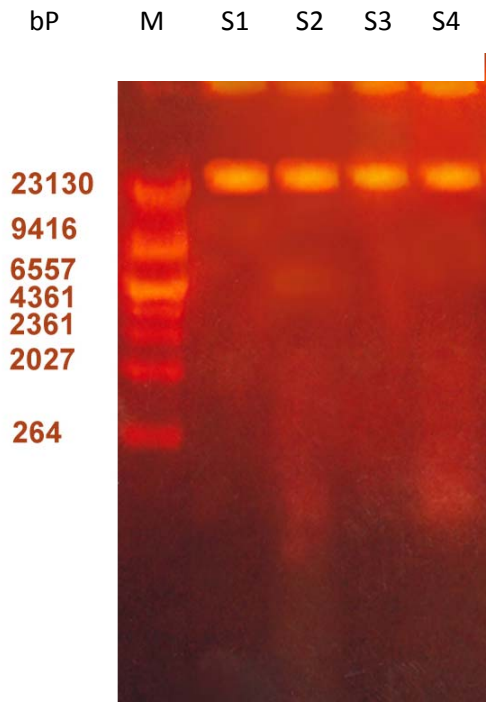
Table 2. Number of worms, biomass and cocoons of *P.ceylanensis* and *P.excavatus* recovered after 50 days of vermicomposting in various substrates. Values are mean ± SE. The same alphabets between substrates did not differ significantly at p < 0.05 by ANOVA.

Vermibed substrates	Worm biomass (g/trough)	Worms recovered (No./trough)	Cocoons recovered (No./trough)
P.ceylanensis			
MSW	64.1±0.7a	219.3±1.3a	70.0±0.8a
ST	55.3±0.6b	192.8±1.2b	65.7±0.7b
PS	59.5±0.7c	204.2±1.3b	58.8±0.7c
FYM	75.6±0.8d	272.9±1.5c	82.3±0.9d
P.excavatus			
MSW	56.2±0.7a	175.7±1.3a	79.3±0.9a
ST	44.7±0.8b	154.3±1.2b	72.0±0.8b
PS	49.3±0.9c	162.5±1.2b	75.1±0.8b
FYM	68.9±0.9d	198.2±1.4c	91.5±0.9c

The worm, *P.ceylanensis* was subjected to molecular characterization, in which the DNA isolated from the earthworm cultured in various substrates did not show any descriptive changes in the bands obtained (Fig.1). The molecular weight of the band was found to be 13400 bp for

P.ceylanensis in all the substrates. Adlouni *et al*. (1995) isolated the DNA from earthworm, *E.fetida* using a modified phenol:chloroform extraction method.

Fig. 1. Electrophoretic pattern of DNA of *P.ceylanensis* cultured in different organic wastes

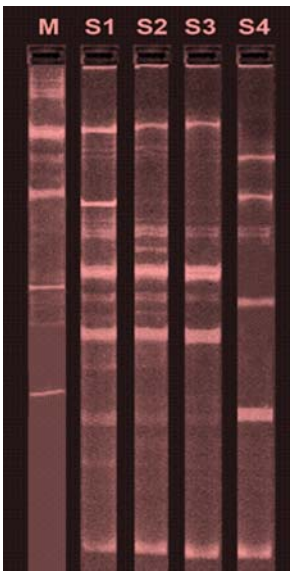


M - Marker, S1 - *P.ceylanensis* cultured in MSW; S2 - *P.ceylanensis* cultured in ST; S3 - *P.ceylanensis* cultured in PS; S4 - *P.ceylanensis* cultured in FYM.

The result of RAPD analysis is shown in Fig.2. Samples of *P.excavatus* collected from four different sites were analyzed and the polymorphic bands were observed. Among the four samples, the earthworms collected in Sirumalai Hills and Dindigul (S2& S3) has more taxonomical similarity than the samples collected from Vadipatti (S4). Dyer and Baker (1998) reported *E.fetida* collected from diverse locations of Himachal Pradesh, exhibited greater inter- and intra-population variation by molecular markers, and cluster analysis clearly discriminated each earthworm isolates according to their location. By using RAPD analysis, genetic variation

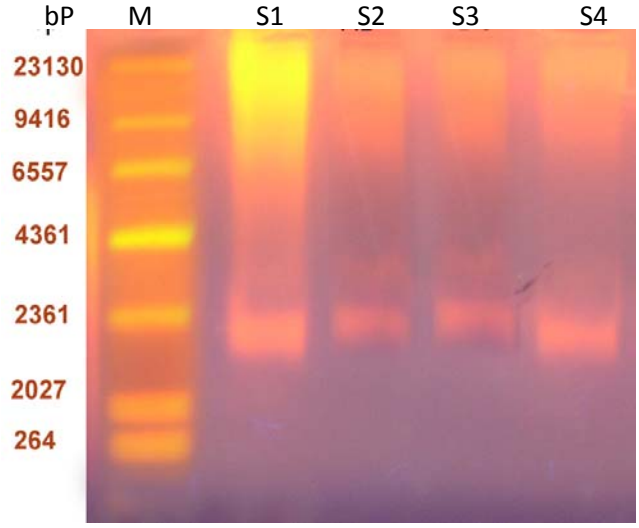
has identified in a similar species, this will help to identify the taxonomical nature of the earthworms.

Fig. 2. RAPD analysis of *P.excavatus* collected from various places



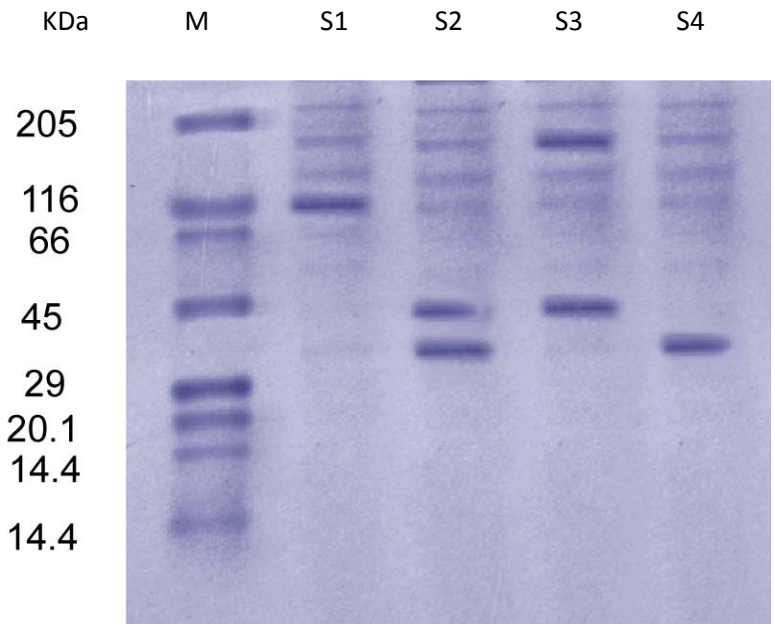
M - Marker; S1 - Sample collected from Gandhigram Rural Institute, Tamil Nadu; S2 - Sample collected from Sirumalai Hills, Tamil Nadu; S3 - Sample collected from Dindigul, Tamil Nadu; S4 - Sample collected from Vadipatti, Tamil Nadu.

Fig. 3. Electrophoretic pattern of RNA of P.ceylanensis cultured in different organic wastes



M – Marker; S1 - *P.ceylanensis* cultured in MSW; S2 - *P.ceylanensis* cultured in ST; S3 - *P.ceylanensis* cultured in PS; S4 - *P.ceylanensis* cultured in FYM.

Fig. 4. SDS-PAGE of vermiprotein of *P.ceylanensis* cultured in different organic wastes



M – Marker; S1 - *P.ceylanensis* cultured in MSW; S2 - *P.ceylanensis* cultured in ST; S3 - *P.ceylanensis* cultured in PS; S4 - *P.ceylanensis* cultured in FYM.

The RNA showed slight variation in the bands, because they are involved in protein synthesis (Fig. 3). The molecular weight was found to be 2190 bp, 2260 bp, 2275 bp and 2210 bp for *P.ceylanensis* cultured in MSW, ST, PS and FYM respectively. When different source of substrate used, the content of the vermiprotein was also varied. SDS-PAGE showed distinct changes in the protein pattern of *P.ceylanensis* cultured in MSW, ST, PS and FYM (Fig. 4). The vermiprotein of *P.ceylanensis*, which were grown in various substrates, showed several bands ranged from 37 to 250 kDa. The molecular weight of vermiprotein grown in MSW showed a single prominent band of 116 KDa when compared with marker protein. The vermiprotein separated from the earthworm grown in ST and PS showed two prominent bands of 45 KDa and 36 KDa and 190 KDa and 36KDa respectively. The molecular weight of vermiprotein cultured in FYM was found as 36 KDa. This showed the influence of the substrates. Similar band formation

was also reported by Changguo *et al.* (2006). They collected vermiprotein from *Metaphire california* was hydrolyzed and the aliquots was subjected to an amino acid auto-analyzer and suggested that the long-term pig manure application tended to increase earthworm protein content.

Conclusion

From this study it is revealed that the percentage decomposition, total biomass of the worms recovered, the total number of worms and the cocoons recovered were high in the treatment, which contained FYM and CD. Compare with these four substrates FYM was found suitable for vermiculture studies. First report on the molecular characterization of a newly reported native earthworm, *P. ceylanensis* opened up a way to vermitechnology.

References

Adlouni, M. J., Mukhopadhyay, P., Walsh, G., G. Poirier and D. Nadeau. 1995. Isolation of genomic DNA from the earthworm species *Eisenia fetida*, *Molecular and Cellular Biochemistry***,** 142(1):19-23.

Alberts, B., A. Johnson., J. Lewis., M. Raff., K. Roberts and P.Walters. 2002. Molecular Biology of the Cell; IV Ed. New York and London: Garland Science.

Avise, J.C. 1994. Molecular Markers, Natural History, and Evolution. Chapman & Hall, New York (511 pp.).

Changguo X. Pingjiu. Z., Genxing. P., Qiu, Duosheng, Q. and C. Qiuhua. 2005. Changes in diversity, protein content, and amino acid composititon of earthworms from a paddy soil under different long-term fertilizations in the Tai Lake Region, Chinna. *Acta Ecologica Sinica*, 26(6): 1667-1673.

Curry, J.P., and O. Schmidt. 2007. The feeding ecology of earthworms: a review. *Pedobiologia.* 50: 463–477.

Dyer, A.R., and G.H. Baker. 1998. Detecting genetic variability in exotic earthworms, *Aporrectodea* spp (Lumbricidae), in Australia soils using RAPD markers. *Soil Biol and Biochem*. 30: 159-165.

Fiers, W., R. Contreras., F. Duerinck., G. Haegeman., D. Iserentant., J. Merregaert, W. Min Jou., F.Molemans, A. Raeymaekers, A., Vandenberghe., G. Voickaert, and M. Ysebaert. 1976.

Complete nucleotide sequence of bacteriophage MS2-RNA: primary and secondary structure of replicase gene, *Nature*, 260, 500-507.

Goswami, B. and M.C. Kalita. 2000. Efficiency of some Indigenous earthworm species of Assam and its characterisation through vermitechnology. *Indian J. Environ & Ecoplan.* 3(2): 351-354.

Kale, R.D. and K. Bano. 1994. Laboratory studies on age specific survival and fecundity of *Eudrilus eugeniae*. *Mitt. Hamb. Zool. Mus. Inst.*, 89(2): 139-148.

Karmegam, N. and T. Daniel. 2009a. Investigating efficiency of *Lampito mauritii* (Kinberg) and *Perionyx ceylanensis* Michaelsen for vermicomposting of different types of organic substrates. *Environmentalist* 29, 287-300.

Karmegam, N. and T. Daniel. 2009b. Growth, reproductive biology and life cycle of the vermicomposting earthworm, *Perionyx ceylanensis* Mich. (Oligochaeta: Megascolecidae). *Bioresour. Technol.* 100, 4790-4796.

Karmegam, N., Paul, J.A.J and T. Daniel. 2003. Vermicomposting potential of some earthworm speices of south India. In:Environmental challenges of the 21st century(Ed:Aravind Kumar). APH publishing corporation. NewDelhi, India. 599-611.

Kaur, A., Singh, J., Vig, A.P., Dhaliwal, S.S., P.J. Rup. 2010. Cocomposting with and without *Eisenia fetida* for conversion of toxic paper mill sludge to a soil conditioner. *Bioresour. Technol.* 101, 8192-8198.

Paul, J.A., 2005. Municipal solid waste generation, characterization, microbial activity, vermicomposting and management in Dindigul Town. Ph.D., thesis submitted to The Gandhigram Rural Institute – Deemed University, Gandhigram, Tamil Nadu, India.

Paul, J.A., Karmegam, N. and T. Daniel. 2011. Municipal solid waste (MSW) vermicomposting with an epigeic earthworm, *Perionyx ceylanensis* Mich. *Bioresour. Technol.* 102, 6769–6773.

Pingod. A., Hogget. J. and C. Urbanke. 2002. Biochemical methods, Weiley publication London, 210–240.

Prakash, M. and N. Karmegam. 2010. Dynamics of nutrients and microflora during vermicomposting of mango leaf litter (*Mangifera indica*) using *Perionyx ceylanensis. Int. J. Global Environmental Issues* 10, 339-353.

Sambrook, J., Fritsch, E.F. and T. Maniatis. 1989. Molecular cloning in laboratory manual, Cold spring harbor laboratory press, 1, 314– 350.

Singh, J., Kaur, A., Vig, A.P. and P. J. Rup. 2010. Role of *Eisenia fetida* in rapid recycling of nutrients from biosludge of beverage industry. *Ecotoxicol. Environ. Safety* 73, 430-435.

Welsh, J., Petersen, C., and M. McClelland. 1990. Polymorphisms generated by arbitrarily primed PCR in the mouse: application to strain identication and genetic mapping. *Nucleic Acids Research*. 19: 303–306.