

Profiles of enzyme activity in the gut of *Lampito mauritii* and *Eudrilus eugeniae* reared on various substrates

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The digestive enzymes of the litter feeding animals, particularly oligochaetes, are responsible for decomposition and humification processes. Earthworms derive their nutrition from organic matter in the form of plant materials, living bacteria, fungi, diatoms, algae, protozoans, nematodes, actinomycetes and also from decomposing remains of large and small animals (Flack & Hartenstein 1984; Ranganathan & Parthasarathi 1999). Activities of amylase, cellulase, chitinase, lichenase, protease, lipase, urease, acid and alkaline phosphatase have been recorded in the gut of *Dichogaster bolau*, *Drawida calebi*, *Drawida willsi*, *Eutyphocus* spp, *Perionyx millardi* and *Pontoscolex corethrurus* (Baskaran *et al.* 1986; Mishra & Dash 1980; Mishra 1993; Parle 1963). Quantitative differences in the activities of enzymes in the different regions of the gut of worms indicate regional specialization. Such differential activities of enzyme was related to (1) types of food and rate of feeding of each species (Mishra & Dash 1980); (2) physiological state of worms (Baskaran *et al.* 1986); (3) reproductive stage (Ranganathan & Vinotha 1998); (4) differential pH and microflora (Parthasarathi 1997) and (5) rate of casting (Mishra 1993).

However, our knowledge on (1) the activities of enzymes related to different stages of reproduction in tropical earthworms and (2) influence of different substrate media on the enzymatic activities of different earthworm species in different stages of

reproduction is limited only to the work of Baskaran *et al.* (1986) and Ranganathan & Vinotha (1998). Therefore, an attempt has been made to quantify the activities of amylase, cellulase, trypsin, acid and alkaline phosphatase in the preclitellate, early clitellate and late clitellate stages of *Lampito mauritii* and *Eudrilus eugeniae* raised on clay loam soil, sawdust, pressmud and sawdust-pressmud mixture.

Clay loam soil (CLS), Sawdust (SD), Pressmud (PM) and Sawdust – Pressmud mixture (SD – PM) (1:1) (v/v) were used as feeding substrates for *Lampito mauritii* and *Eudrilus eugeniae*. *L. mauritii* was collected in and around Annamalainagar and *E. eugeniae* was obtained from Dr. Radha D. Kale of University of Agricultural Science, Bangalore. Sixty worms of 12 days old were reared in cement tanks of dimension 50 x 35 x 30 cm, each with 8 kg of substrate, sprinkled with water. The substrate was maintained with 60-70% moisture, 29 ± 1°C temperature and 70 – 75% relative humidity. The substrate was changed once in twenty days and the casts removed once in a week.

Enzymes were analysed in the gut (3-4 cm, ranging from about 20-100 segments for *L. mauritii* and 4-5.5 cm, ranging from about 18-185 segments for *E. eugeniae*) in the three stages of worms like preclitellate (30 & 20 days old) (Pre-CS), early clitellate (75 & 45 days old) (Early – CS) and late clitellate (130 & 70 days old) (Late – CS) fed on different substrates. The gut was cleared by feed-

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ing the worms with wet blotting paper for 10-12 hours. The enzyme extract was made after homogenizing the gut tissues (20 mg) free from gut contents with respective buffers (5 ml). The homogenate was centrifuged at 2000 rpm for 15 minutes. The supernatant was used as enzyme extract.

The protein content of the gut homogenate was determined as per Lowry *et al.* (1951) using bovine serum albumin as standard and the enzyme activities were determined according to the methods of Raghuvamulu *et al.* (1983). The activities are expressed in μg of starch hydrolysed/mg of protein/h (amylase), μg of glucose released/ mg of protein/h (cellulase), millimoles of tyrosine released/mg of protein/h (trypsin) and μ moles of phenol liberated/mg of protein/h (acid and alkaline phosphatase). The results are tested statistically by using analysis of variance at 0.05% level.

The enzymatic activities of amylase (a), cellulase (b), trypsin (c), acid (d) and alkaline phosphatase (e) in the gut of Pre-CS, Early-CS and Late-CS of *L. mauritii* and *E. eugeniae* reared in different substrates are summarized in Table 1. In general, the maximum enzymatic activity is found in the gut of Late – CS of both worms reared in SD-PM, followed by PM than other stages and substrates. The only exception is cellulase which is more in the gut of worms reared in SD followed by SD-PM.

Enzyme activity, of the various factors, is influenced also by type of food. Earthworm which feed and depend on microbes, litter, grit present in soil should contain battery of enzymes. Flack & Hartenstein (1984); Ranganathan & Parthasarathi (1999) have demonstrated that earthworms predate microbes as a source of their food. During their passage through the gut the microflora get enhanced in population, very particularly during Late – CS of *L. mauritii* and *E. eugeniae* (Parthasarathi & Ranganathan 1998), which may be responsible for the increased enzyme activities (Parthasarathi & Ranganathan 2000). Positive correlation between increased enzyme activity and increased microorganisms have been established by Parle (1963); Parthasarathi & Ranganathan (1999). Due to the presence of rich cellulose in SD (Ramalingam 1997), sugar in PM (Ranganathan 1999) and abundance of amylase producing fungi (*Aspergillus* spp., *Fusarium* spp. *Mucor* spp. and *Rhizopus* spp.) and bacteria (*Bacillus* spp) in PM (Parthasarathi & Ranganathan 1998) worms raised on SD – PM followed by PM exhibit higher

amylase activity. SD contains more cellulose, lignin and cellulolytic enzyme producing fungi – *Aspergilli* and *Fusarium* spp and bacteria – *Bacillus* spp and *Pseudomonas* spp (Parthasarathi & Ranganathan 1998) resulting in more cellulase activity. Presence of more proteins/amino acids (Ranganathan 1999) and proteolytic enzyme producing microbes (*Aspergillus niger*, *Aspergillus flavus* and *Bacillus subtilis* in PM (Parthasarathi & Ranganathan 1998) supports the enhanced trypsin activity. The higher acid and alkaline phosphatase activities are supported by rich phosphate (3.6%) and phospho solubilizing microbes (*Pseudomonas* spp., *Bacillus* spp., *Micrococcus*, *Fusarium* spp and *Aspergillus* spp) (Parthasarathi & Ranganathan 1998) found in PM and also enhanced mineralization on N taking place during the transit through the gut of worm helps to release P (Parthasarathi & Ranganathan 1999).

During development and growth of animals, there is a great fluctuation in enzymes activity (Baskaran *et al.* 1986). Earthworms for their growth and reproduction (Flack & Hartenstein 1984; Ranganathan & Parthasarathi 1999) require combination of carbohydrates (cellulase), microorganisms (proteins) and grit etc. Increased amylase, cellulase and tryptic activities in Late-CS seem to suggest that more carbohydrates and proteins are metabolized by the reproductively active worms. Worms being hermaphrodites with simultaneous functioning gonads, may require more energy and increased enzyme activities during this active phase of reproduction (Late – CS).

Phosphatases, the architectural enzymes, play important role during important phases like embryogenesis, regeneration, maintenance and differentiation of cells, growth and differentiation, histogenesis of organ and organ system and development in animals. (Baskaran *et al.* 1986; Parthasarathi 1997). Since late clitellate stage in earthworm has to support functions related to reproduction like gametogenesis, accessory reproductive structure development and functioning, probably enhanced phosphatases help in these activities.

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