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Comparative studies on the digestive enzymes in the gut of earthworms, *Eudrilus eugeniae* and *Eisenia fetida*

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Solid waste and resource recycling are the only sensible ways to tackle the problem of municipal solid waste (MSW). The digestive enzymes and the intestinal microflora of earthworms seem to play an important role in the digestion of soil organic matter. The present investigation was aimed to study the levels of various enzymes viz. amylase, cellulase, xylanase, cellbiase, endonuclease, acid phosphatase, alkaline phosphatase and nitrate reductase and their activities in the gut of the two selected earthworms, *Eudrilus eugeniae* and *Eisenia fetida*. Overall, amylase, cellobiase, endoglucanase, acid phosphatase and nitrate reductase showed higher enzymatic activity.

Keywords: *Eudrilus eugenia*, *Eisenia fetida*, amylase, cellulase, nitrate reductase, acid phosphatase, alkaline phosphatase

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India produces about 3,000 million metric tonnes of organic wastes annually which are disposed of by ocean dumping, incineration and land application. Wastes from domestic, agriculture, urban and industrial sources are the main cause of organic soil pollution[1]. Vermiculture farming involves the use of earthworms as versatile natural bioreactors for cleaning up the environment with cost-effective waste management technology for sustainable agriculture[2].

Earthworms are physically aerators, crushers and mixers; chemically degraders; and biologically stimulators in the decomposer system. They effectively harness the beneficial soil microflora, destroy soil pathogens and convert organic wastes into vitamins, enzymes, antibiotics, growth hormones and protein rich casts. Earthworm bioreactors have an in-house supply of enzymes such as amylase, cellulose, nitrate reductase, and acid and alkaline phosphatases. These enzymes biodegrade the complex biomolecules into simple compounds. The digestive enzymes of earthworms are responsible for the decomposition and humification of organic matter. These enzymes are active at a very narrow *p*H range and efficiently maintain the highly non-linear *p*H parameters[3].

Organic wastes, broken down and fragmented rapidly by earthworms, result in a stable non-toxic material with good structure, which has a potentially high economic value as soil conditioner for plant growth[4]. Hence, an attempt was made to assess the activities of amylase, cellulase, xylanase, cellobiase, endoglucanase, acid phosphatase, alkaline phosphatase and nitrate reductase in the gut of *Eudrilus eugeniae* and *Eisenia fetida*.

The exotic earthworms, *Eudrilus eugenia* and *Eisenia fetida*, were collected from Aarthi Farm, Kondegoundampalayam Village, Pollachi Taluk, Coimbatore, Tamil Nadu, India. The species were cultured at Kongunadu Arts and Science College premises for six months. The guts of fully-grown earthworms were dissected and the material inside (5 g) was sampled for the extraction and activity of amylase, cellulase, xylanase, cellobiase, endonuclease, acid phosphatase, alkaline phosphatase and nitrate reductase following the standard procedures[5-11]. Duncan's multiple range test was applied for comparing the levels of enzymes in the gut of the earthworms.

Eathworm's gut is an effective tubular bioreactor, which maintains a stable temperature through novel temperature regulatory mechanisms, thus accelerating the rates of the bioprocesses and preventing enzyme inactivation caused by high temperatures. Earthworm gizzard is a novel colloidal mill in which the feed is ground into particles smaller than 2 mm giving, thereby, an enhanced surface area for microbial processing[12]. They also have an in-house supply of various enzymes, which act upon the complex biomolecules and thereby metabolise the phosphate and nitrogen present in the organic waste. The activity of amylase, cellulase, xylanase, cellobiase, endoglucanase, acid phosphatase, alkaline phosphatase and nitrate reductase assayed in the gut of *E. eugeniae* and *E. fetida* is given in Table 1 and

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Table 1—The activity of enzymes in the gut of *E. eugeniae* and *E. fetida*

Enzymes	*E. eugeniae*	*E. fetida*	SED
Amylase	0.290**	0.248**	0.004
Cellulase	0.154**	0.198**	0.004
Xylanase	0.154**	0.192**	0.002
Cellobiase	0.453**	0.400**	0.006
Endoglucanase	0.361**	0.326**	0.006
Acid phosphatase	0.361**	0.400**	0.006
Alkaline phosphatase	0.098**	0.125**	0.004
Nitrate reductase	0.608**	0.549**	0.010

Units: Amylase, mg of maltose/min/mg of starch; Cellulase, mg of glucose/min/mg protein; Xylanase, mmoles/mg protein; Cellobiase, µmoles/mg protein; and Endoglucanase, µmoles/mg protein; Acid Phosphatase, mg of phosphorus/min/mg protein; Alkaline phosphatase, mg of phosphorus/min/mg protein; and Nitrate reductase, µM of nitrate/min/mg protein. Values are means of six replicates ● $P < 0.01$

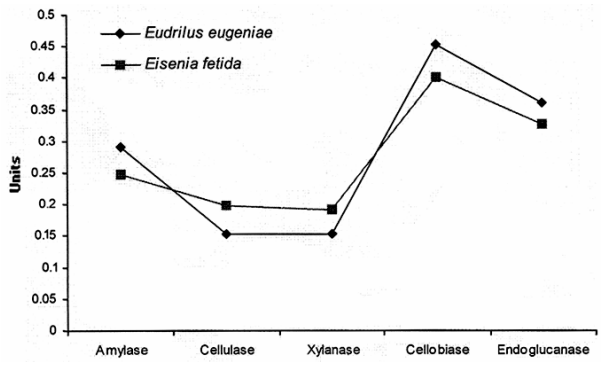

Fig. 1—Activity of enzymes in the gut of *E. eugeniae* and *E. fetida*. Units: Amylase, mg of maltose/min/mg of starch; Cellulase, mg of glucose/min/mg protein; Xylanase, mmoles/mg protein; Cellobiase, µmoles/mg protein; and Endoglucanase, µmoles/mg protein.

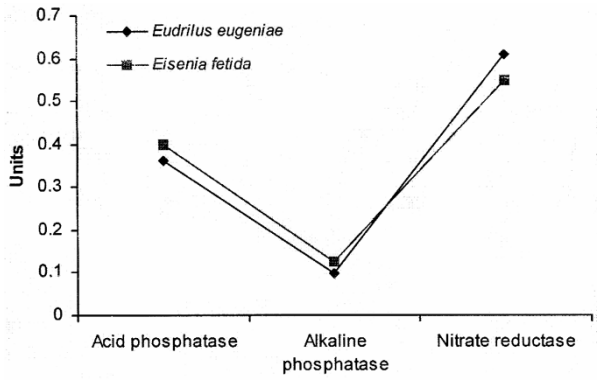

Fig. 2—Activity of enzymes in the gut of *E. eugeniae* and *E. fetida*. Units: Acid phosphatase, mg of phosphorus/min/mg protein; Alkaline phosphatase, mg of phosphorus/min/mg protein; and Nitrate reductase, µmoles of nitrate/min/mg protein.

Figs 1 and 2. Overall, the enzymes nitrate reductase, cellobiase, endoglucanase acid phosphatase, and amylase showed higher activity in the guts of both the earthworms.

Enzyme activity in earthworms is regionally specialized and influenced by physiological state, age and microorganisms. Digestive enzymes like cellulase, xylanase, acid phosphatase and alkaline phosphates were found to be more in the gut of *E. fetida* as compared to *E. eugeniae.* While the activity of amylase cellobiase, endoglucanase and nitrate reductase was higher in the gut of *E. eugeniae* as compared to *E. fetida*. The mean difference in the levels of enzymes studied in the two selected earthworms was found to be significant at 1% level. Amylase, cellulase, acid phosphatase, alkaline phosphatase and nitrate reductase were secreted in the gut of the earthworms due to increased presence of microorganisms in it. The present results are in accordance with those of Ranganathan and Vinotha[13]. The enzymes secreted by *E. eugeniae* and *E. fetida* and/or in association with the gut flora might be breaking down the food containing plant matters and decomposing remains of small animals. Naturally occurring plant-remains ingested by earthworms are very complex, consisting of starch, cellulose, xylan, galactine and pectin substances. These complex organic molecules are digested through a mutualist earthworm microflora-digestion system. Amylase, cellulase, xylanase, endoglucanase, cellobiase, acid phosphatase, alkaline phosphatase and nitrate reductase produced jointly by earthworms and gut microflora are supposed to play a central role in the process of digestion and humification of soil organic matter. Amylase, cellulase, xylanase, endoglucanase and cellobiase act upon the complex biomolecules such as starch, cellulose, xylan and cellodextrins. Acid phsophatase, alkaline phosphatases and nitrate and nitrate reductase are involved in the metabolism of phosphate and nitrogen.

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