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Influence of pressmud on the enzymatic variations in the different reproductive stages of *Eudrilus eugeniae* (Kinberg)

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Posterior gut of reproductively active, clitellate stage of the compost worm *Eudrilus eugeniae* when reared in pressmud (a waste byproduct of sugar mill) exhibits enhanced amylase, protease, acid and alkaline phosphatase and cellulase activity compared to activity of these enzymes observed in immature, pre-clitellate stage worms reared in the same media as well as in worms raised in cow dung. These enzymes activities are correlated to the significant growth exhibited by the clitellate stage.

EARTHWORMS, the most important soil invertebrates considering the amount of soil they move through their body and in their effects in improving soil properties, rely on microorganisms, particularly fungi, as their primary source of food[1–3]. It has been recently shown that the clitellate stage of *Eudrilus eugeniae* reared in pressmud prey on and harbour more microorganisms[3]. Further, pressmud rich in organic matter (OM), nitrogen (N), phosphorus (P) and iron (Fe) has been shown to aid growth in terms of increased size with high fecundity in the clitellate stage[4].

Our present knowledge of enzyme physiology related to reproduction and biomass formation is not adequate in the present context of sustained organic farming using vermitechnology. The present paper analyses the enzymatic activities of the economically important compost worm *E. eugeniae* during its different phases of reproduction when the worms are reared in pressmud.

30-day-old immature pre-clitellate worms and 45-day-old mature clitellate worms were reared in separate cement tanks 50 × 35 × 30 cm, each containing 8 kg of feed with approx. 70% moisture, 29°C ± 1°C. Two-month-old, weathered, dry pressmud and dried powdered cow dung was used as feed. Required water was sprinkled to maintain moisture. The worms were preadapted to these feeds for three days and then were allowed to grow for 10 days. Homogenate of anterior gut (5–100 segments) cleared of contents and posterior gut (101–205 segments) was made in buffer solution. After centrifuging the homogenate at 2000 rpm for 15 min, the supernatent was used as an enzyme extract. The protein content of the gut homogenate was determined by the method of Lowry *et al.*[5] using bovine serum albumin as standard. Amylase activity was measured following the method of Raghuvamulu *et al.*[6], cellulase activity by the method of Malik and Singh[7], protease activity by the method of Gowenlock[8] and acid phospatase and alkaline phosphatase activities by the methods of Gutman and Gutman[9] and King and Armstrong[10] respectively. Statistical significance between anterior and posterior gut and preclitellate and clitellate stage of worms was tested at 1% level.

The activities of enzymes in the different regions of the gut of pre-clitellate and clitellate stages of *E. eugeniae* when fed on pressmud and cow dung are given in Table 1. Amylase, protease, acid and alkaline phosphatase activities were higher in the posterior gut of reproducing, mature, clitellate stages of worms reared in pressmud compared to preclitellate stage reared in pressmud as well as compared to worms (both stages) reared in cow dung.

Pressmud (OM 50%; N 1.2% and P, 2.9%) was earlier found[4] to support better growth measured as length and weight than when raised on cow dung (OM, 47%, N 1.1% and P 0.5%) in *E. eugeniae*. Significant growth and biomass was attained by this worm during sexual maturity (from 1500 mg to 3000 mg from day 30 to day 45) and probably this was supported by phenomenal increased activities of the amylase and protease in the clitellate, reproducing worm than pre-clitellate worms raised on pressmud as well as worms raised on cow dung (Table 1).

Enzyme activity in worms is regionally specialized as in other animals and influenced by physiological state, age and microorganisms[11]. The observed highest enzymatic activity in amylase followed by protease in the posterior gut of clitellate *E. eugeniae* raised on pressmud was likely due to (i) substrate availability, (ii) regional specialization, and (iii) enhanced presence of microorganisms[2–3]. Higher protease activity in the posterior region of other worms was reported by Mishra and

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Table 1. Variations of enzymatic activities in the gut of pre-clitellate and clitellate stages of *Eudrilus eugeniae* reared on cow dung and pressmud (values expressed as mg of starch hydrolysed/mg of protein/h (for amylase) mg of glucose released/mg of protein/h (for cellulase), millimoles of tyrosin released/mg of protein/h (for trypsin) and μmoles of phenol liberated/mg of protein/h (for acid and alkaline phosphatase)

Substrates		Cow dung		Pressmud	
Enzymes	Gut regions	Pre-clitellate	Clitellate	Pre-clitellate	Clitellate
Amylase	Anterior	12.15 ± 0.025	22.27 ± 0.56*	18.14 ± 0.029	26.56 ± 0.45*
	Posterior	19.21 ± 0.039*	31 ± 0.18*	31.17 ± 0.013*	64.7 ± 0.30*
Cellulase	Anterior	1.54 ± 0.03	8.52 ± 0.03*	1.32 ± 0.03	5.91 ± 0.01*
	Posterior	3.16 ± 0.07*	3.22 ± 0.07*	1.57 ± 0.01*	2.84 ± 0.03*
Trypsin	Anterior	1.07 ± 0.06	1.66 ± 0.01*	2.21 ± 0.03	2.88 ± 0.01*
	Posterior	1.98 ± 0.01*	3.27 ± 0.05*	3.75 ± 0.16*	7.69 ± 0.01*
Acid phosphatase	Anterior	1.15 ± 0.05	1.43 ± 0.04*	1.77 ± 0.08	2.01 ± 0.03*
	Posterior	1.54 ± 0.04*	1.87 ± 0.02*	2.22 ± 0.05*	3.43 ± 0.04*
Alkaline phosphatase	Anterior	0.526 ± 0.04	0.91 ± 0.01*	1.28 ± 0.07	1.84 ± 0.01*
	Posterior	0.57 ± 0.02*	1.24 ± 0.02*	1.50 ± 0.03*	2.89 ± 0.02*

Mean ± S.E. indicates the mean of five observations.
*Indicates significance at 1% level of *t* test.

Dash[11]. Acid phosphatase activity in earthworm is due to microorganisms and alkaline phosphatase is regarded as typical of earthworms[12]. Enhanced phophatases coinciding with increased protein synthesis in earthworm *Pontoscolex corethrurus*[13] have been positively correlated to growth and differentiation. The enhanced phosphatase activity in the present study is not only due to the rich P content of pressmud but also due to presence of microorganisms[12] and seems directly related to the significant biomass attained during 45–50 days which coincides with initial cocoon laying.

The growth of earthworm has been shown to necessarily require cellulose (carbohydrate), microorganisms and grit[14]. The cellulase activity, like the other enzymes studied, was higher in clitellated worms. With maturity (clitellated worms) there was however a shift in region of higher cellulase activity. The anterior gut recorded significantly higher cellulase activity in the case of both the feeds, pressmud and cow dung. Further investigations are required to explain this adaptation.

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