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Studies on the Assay of Digestive Enzymes in the Gut Extracts and Gut Bacterial Population in the Vermicomposting earthworm *Lampito mauriti*

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Abstract

Digestive enzymes of *Lampito mauriti* have been assayed and its gut bacterial population were isolated, the physico chemical characteristics of vermicast has been discussed. Protease, amylase, cellulase invertase were detected in *Lampito mauriti.* The eartworm showed maximum activity of protease and Amylase and increasing activity of amylase and protease in according to time in seconds. Enzyme activity was found to be maximum at 3.6 x 10 -3 seconds. There were seven bacterial species were isolated in this present study.

Introduction

Domestic and industrial wastes are increasingly disposed to the environment causing long term effect to ecosystem. Fertilizers play a major role in the production of agricultural outputs. Available plant nutrients in soil are not uniform in their distribution that restricts the output of agricultural sectors. To overcome this problem, soil has to be fortified with fertilizers such as inorganic and organic fertilizers. Inorganic fertilizers though support plant growth and increase yield, but in long run, they affect the soil biota and make the soil infertile due to leaching, change in pH etc.

Vermicomposting is the phenomenon of compost formation by earthworm. Obviously earthworms play an important role in the cycling of plant nutrients, turnover of organic matter and maintenance of soil structure. They can consume 10-20% of their own biomass per day. The most important effect of earthworms in agro systems is the increase in nutrient cycling, particularly nitrogen. They ingest organic matter with a relatively wide C:N ratio and convert it to tissue with a lower C:N ratio. Thus they affect the physico-chemical properties of soil.

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 cellulose activity compared to activity of these enzymes observed in immature, pre-clitellate stage worms raised in cow dung. These enzymes activities are correlated to the significant growth exhibited by the clitellate stage (Ranganathan and Vinotha 1998). The main objectives of this study are (1) To analyze the organic and inorganic composition of soil (Physico chemical characterization) (2) To isolate the bacterial species from the gut extract of the earthworm (3) To study the enzyme activity in the digestive system of the earthworm *Lampito maruti* species.

Methods

Sixty adult earthworms were reared in separate cement tanks(50x35x30 cm) with vermibed., water was sprinkled as required to maintain the moisture content.The physico chemical characteristics of vermicast was analysed by the standard methods. Invertase, Amylase and cellulose were estimated by the procedures of Galstyan (1965). For invertase activity, 1 g substrate sample (PM, FC, 15dc, 30dc) placed in a 50-ml Erlenmeyer flask was incubated with 1 ml acetate buffer (pH 5.2), 0.3 ml toluene, 1ml sucrose (4%) and 3ml distilled H2o at 28+2c for 24h. Subsequently, 1ml supernatant was withdrawn and reducing sugars were estimated by Nelson`s reagent (Nelson 1944). To a 1-ml aliquot at boiling point in a test tube, 1 ml reagent was added, and the volume was made up to 25ml with distilled H2O. The absorbance was determined by a Spectronic 20 colorimeter at 495nm. The standard curve was prepared from a serial dilution of glucose. Enzyme activities were expressed in mg glucose g^{-1} oven dry substrate sample for a 24-hr. incubation. Amylase activity was assayed at pH 5.2 employing sodium acetate-acetic acid buffer with 8% soluble starch as the substrate. Cellulase activity was assayed at the substrate. The reducing sugars released were estimated as outlined earlier and enzyme activity was expressed as detailed earlier

Protease activities were estimated photometrically according to the method of Hoffmann and Teicher (1957). A 1-g substrate sample in a 50-ml Erlenmeyer flask was incubated at 28± 2°C for 24hr with 1 ml phosphate buffer (pH 7.0), 2ml of 2% gelatin, 0.5ml toluene and 2ml distilled H2O. Subsequently, 5ml of 5% trichloro acetic acid was added to the flask, was shaken thoroughly to affect deproteinisation for 30 min, and the contents later centrifuged at 2, 100 g for 15min. An aliquot of the supernatant was used for estimation of amino-N. To a 1-ml aliquot in a test tube, 1 drop of methyl red indicator was added and solution was neutralized with 0.1 N NaOH. To this, 1ml ninhydrin reagent was added. This reagent was made up by dissolving 20 g ninhydrin in 500ml methyl cellusolve, which was added to a mixture 0.8 g stannous chloride. Citrate buffer (500ml: pH 5.0) was added, and the mixture thoroughly shaken. Glass bulbs were placed on the mouth of the test tubes ad their contents boiled for 20min. Then 5ml diluent solution [distilled H2O and n-prophyl alcohol (v/v)] was added, the tubes were cooled, and the absorbance of the purplish pink colour which developed was read in a Spectonic- 20 at 475nm. Standards

prepared from glutamic acid were treated similarly and used to determine a standard curve. Protease activity was expressed as mg glutamic acid g^{-1} oven dry substrate sample incubated for 24hr.

Results

Table I: Physico Chemical Characteristics of Vermicasts.

S.No	Texture	Calcium Carbonate	EC	pH	N	P	K
1	Sandy	Medium	1.3 dS/m	6.9	62%	4.5%	10%
2	Sandy clay	No	2.0 dS/m	6.6	67%	5.4%	12%
3	Sandy	Trace	2.1 dS/m	6.5	68%	5.3%	11%
4	Sandy	No	2.0 dS/m	6.6	67%	5.4%	12%

Table 2: Bacterial isolates from earthworm samples.

S.No.	Microorganism	% of isolate
1	*Escherichia coli*	60.22
2	*Staphylococcus aureus*	10.14
3	*Bacillus cereus*	7.50
4	*Pseudomonas aeruginosa*	6.50
5	*Proteus mirabilis*	10.20
6	*Serratia marcescens*	2.22
7	*Micrococcus luteus*	3.12

Table 3: Activity of Amylase and Invertase in Earthworm(concentration).

Enzyme	Enzyme activity in different sample of Earthworm				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
INVERTASE	0.030	0.015	0.064	0.007	0.076
CELLULASE	0.003	0.006	0.015	0.005	0.004

Table 4: Activity of Amylase Enzyme in Earthworm.

S.No	Time in minutes	Starch + Buffer + Enzyme	DNSA	D/W	Standard			Earthworm enzyme		
					O.D	O.D/T	Con of Maltose	O.D	O.D/T	Conc. of maltose
01	0	30 + 30+30	1	1	-----	------	------	-----	-----	----------
02	5	1+1+1	1	1	0.13	0.004	0.35	0.31	0.010	0.85

03	10	1+1+1	1	1	0.16	0.0053	0.425	0.34	0.011	0.925
04	15	1+1+1	1	1	0.17	0.0056	0.45	0.34	0.011	0.925
05	20	1+1+1	1	1	0.18	0.006	0.475	0.35	0.011	0.95
06	25	1+1+1	1	1	0.19	0.0063	0.525	0.37	0.012	1
07	30	1+1+1	1	1	0.18	0.006	0.475	0.39	0.013	1.05
08	35	1+1+1	1	1	0.18	0.006	0.475	0.50	0.016	1.375
09	40	1+1+1	1	1	0.14	0.0046	0.375	0.46	0.015	1.25
10	45	1+1+1	1	1	0.14	0.0046	0.375	0.46	0.015	1.25
11	50	1+1+1	1	1	0.14	0.005	0.375	0.36	0.012	0.975
12	55	1+1+1	1	1	0.14	0.0046	0.375	0.35	0.011	0.93
13	60	1+1+1	1	1	0.14	0.0046	0.375	0.35	0.011	0.93

Table 5: Protease Activity.

STD	BLANK	TEST	DIFFERENCE
Neat	0.169	2.034	1.865
1:10	0.170	0.471	0.301
1:20	0.165	0.175	0.010
1:30	0.132	0.137	0.005
Sample			
Neat	0.276	0.300	0.024
1:10	0.201	0.208	0.007
1:20	0.142	0.164	0.022
1:30	0.112	0.167	0.055

Calculation
Standard - 1mg/ml = 200 IU/mg
1ml = 1mg = 1.865
X = 0.024
X = 200*0.024/1.865
= 2.573 IU/ml

Total Volume = 4.5 ml
= 2.573*4500µl
= 11578.5 IU/4500µl

Specific Activity
Activity/Protein Content
2.573 units = 8.332 mg/ml

Specific Activity = 2.573/8.332
= 0.309 unit/mg of protein.

Amylase
A = 0.1525
Tv = 2.9ml
t = 60mimutes
v = 2ml

$$\text{Amylase activity} = \frac{A \times Tv}{t \times V}$$

$$= \frac{0.1525 \times 2.9}{60 \times 2}$$

$$= (0.0036854 \mu g/min$$

Invertase
A = -0.3649
Tv = 4.5ml
t = 1 hour
v = 2.5m

$$\textbf{Invertase activity} = \frac{A \times Tv}{t \times V}$$

$$= \frac{0.3649 \times 4.5}{1 \times 2.5}$$

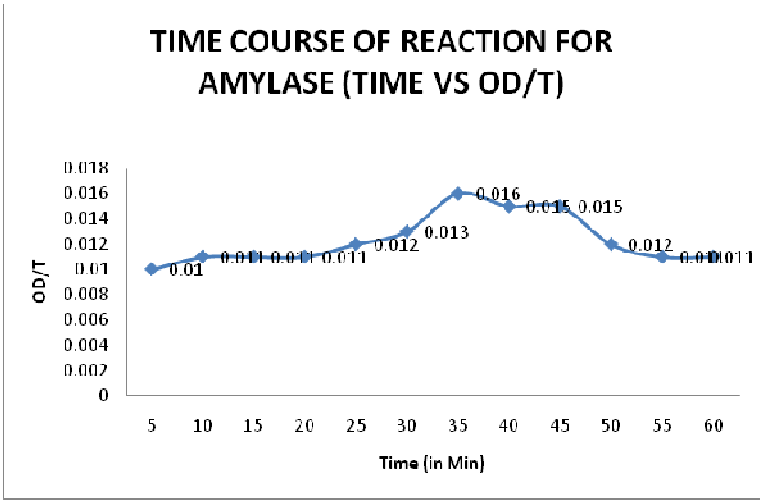
$$= 0.010947 \ \mu g/min$$

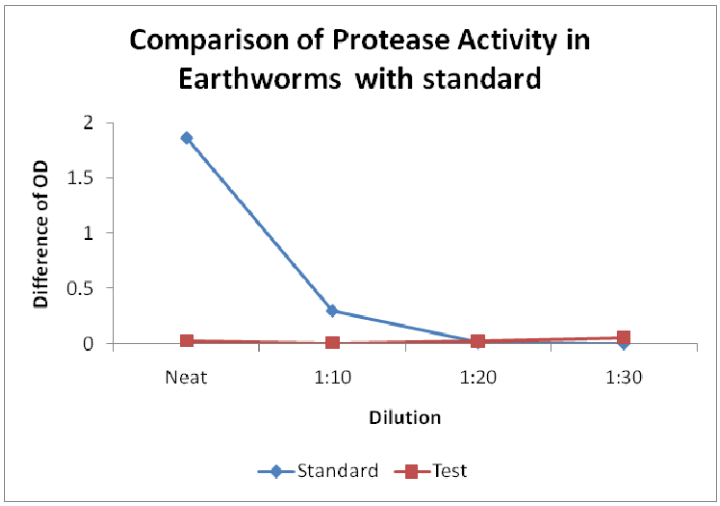
Studies on the physico chemical analysis of vermicasts has been shown in Table 1.There were four vermicasts samples were analysed in this present study and increased amount of N, P, K found in vermicast is more than that of ordinary soil and vermicast observed by Parthasarathy and Ranganathan (2000).

Activities of protease, amylase, cellulose and invertase could be detected in *Lampito mauriti* (Table 2-5).*Enzyme activity in Lampito mauriti* has been converted to micrograms referring the calculation made by Mishra and Dash (1980).Table 4 and 5 shows that protease and amylase activities are more and invertase cellulose activities are less in *Lampito mauriti* in comparison to the activities recorded in other tropical earthworms (Swarnalata mishra 1993).

The higher protease and amylase activities might be related to the presence of microorganisms in the gut capable of breaking down protein and starch.The activity of Amylase and protease enzymes is also linked to the type of substrate and rate of feeding and the enzyme activities are shown in graph I and II.

Table 2 presents the list of bacterial species isolated from the gut extracts of earthworm. There were seven bacterial species were isolated in this present study, Bacterial groups isolated from the digestive tracts of earthworms were significantly different from those isolated from soil and excrements (Hendriksen, N.B. 1990) Some bacterial taxa occurred in different sections of intestines of different earthworm species; however, the overall composition of bacterial communities in these objects is different. Existence of bacterial groupings symbiotically associated with intestines is proposed.





Graph I

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