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Biochemical Screening Of Cellulase In The Gut Of Glossoscolex Paulistus Used For Composting Of Textile Dye Treated Eichhornia Crassipes

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Abstract: Bioremediation is considered as the best technique to treat and reduce biodegaradable wastes that gets accumulating day by day. A study was conducted to reduce the accumulation of biodegradable wastes by using earthworm sps. by evaluating the level of digestive enzymes in the gut of earthworm (Glossoscolex paulistus). Glossoscolex paulistus used to compost Eichhornia crassipes grown in vat dye solution was used for this study. Glossoscolex paulistus used to compost Eichhornia crassipes grown in water without dye was used as control. Digestive enzyme activities in the earthworm gut was done on the casein agar plate. The screening results revealed the quantitiy of enzymes produced as 153.80 ± 0.02 U/L for cellulase in the gut of earthworms from the vat dye solution than from control. Furthermore, cellulase activity was estimated in the gut of Glossoscolex paulistus from the vat dye solution. The maximum activity of 137.20±0.023 U/L was observed at the pH of 7.0 and 7.5 for cellulase enzyme. The enzyme activity was maximum at the temperature of 40ºC to70°C for the enzyme. Cellulase showed peak activitiy at 40ºC (137.22 ± 0.022 U/L). The enzyme production varies with respect to time for different enzymes. The maximum cellulase activity was seen at 72 hours (137.22 ± 0.022 U/L) in the test conducted from 12 hrs to 120 hrs time period. *Copper* ***sulphate salt showed more profound effect in the production of cellulase (137.22 ± 0.02 U/L).***

Keywords: Glossoscolex paulistus, Eichhornia crassipes, Cellulase, Bioremediation and temperature.

1. INTRODUCTION

Microbes have to breakdown macromolecules in their surrounding into smaller absorbable units in order to obtain the nutrients. The breaking down of macromolecular nutrients into smaller molecules is accomplished by the enzymes secreted by the microbes themselves. More than 3000 different enzymes have been described to date; the majority have been isolated from mesophilic organisms. The microorganisms from diverse and exotic environments called as extremophiles, are an important source of enzymes, whose specific features are exploited into novel applications (Kumar and Takagi, 1999). With better knowledge and purification techniques of enzymes the number of applications of it has increased many folds, and with the availability of engineered enzymes a number of new possibilities for commercial processes have emerged (Beg *et al*., 2003). It is the most abundant biomass and a major structural component of plants and a renewable source of

energy in the biosphere (Saha *et al*, 2006, Klemm *et al* 2005, Bhat , 2000). Cellulose is mainly degraded by cellulase enzyme which is commonly produced by bacteria and fungi (Immanuel *et al*, 2006). The growth rate of bacteria is faster than fungi and has been widely used in cellulase production under different culture conditions [Nakamura and Kappamura (1982)]. In order to ascertain these with the aim of setting up a bioremediation model using earthworms, there is the need to compare the activities of digestive enzymes and microflora in the gut of earthworms. This study therefore aims at evaluating the effect of gut digestive enzymes of the earthworms used to compost *Eicchornia crassipes* grown in vat dye.

Proper understanding about the food requirement and digestive ability of the earthworms is necessary to understand the decomposition process during vermicomposting. The enzymes and the enzyme activity in the gut of earthworms and in casts are extremely essential because they play a key role in the breaking down of organic materials and to make the nutrients in available form for plants. Devi and Prakash (2015) studied the activities of enzymes, amlylase, cellulose, invertase, phosphatase and protease in vermicomposts of three different vermibed substrates. The study was conducted to screening, optimization, purification and characterization of cellulase from cellulase producing bacteria from gut of *Glossoscolex paulistus* used to compost *Eichhornia crassipes* grown in Purple vat dye solution.

2. MATERIALS AND METHODS

The plants (*Eichhornia crassipes*) grown in Purple vat dye solution and control for 15 days were subjected for the process of composting. The phytoremediated water hyacinth plants were cut into small pieces of 4 to 5 cm length and kept ready for composting. The precomposted compost of the treatment and control were further subjected to vermicomposting using the earthworm species- *Glossoscolex paulistus* for about 60 days. After 60 days the macerated gut samples were spread on the cellulose agar media. An isolate of *Glossocolex paulistus* grown in vat dye showed good result. As it showed good growth it was selected for further microscopic studies for morphological characterization. The plates were examined for Gram staining and microscopic viewing for identification of bacterial strains (Apun *et al*, 2000).

The bacterial isolate were identified by performing several biochemical tests like Fermentation test, Catalase test, Citrate utilization test, Methyl-red test, H_2S production and Voges–Proskauer test by standard methods (Buchanan and Gibbons, 1974). The estimation of cellulase production was done on the cellulose agar media by following the method of Gamez *et al*., 2006. In these tests reducing sugars were estimated spectrophotometrically with 3, 5-dinitrosalicylic acid using glucose as standards (Ghose, 1987). Then enzymatic activities of total endoglucanase were defined in Enzyme unit. One unit of enzymatic activity is defined as the amount of enzyme that release 1µ mole reducing sugars (measured as glucose per min) (Ghosh, 1987). Conformation of cellulose degrading ability of bacterial isolates was performed by streaking on the cellulose Congo Red agar media. Colonies showing discoloration of Congo-Red were taken as positive cellulose- degrading bacterial colonies and only these were taken for further study. The bacterial isolates were streaked on separate fresh nutrient agar media. The plates were incubated at 37ºC for 24 hours. The culture was collected and assayed (Gamez *et al*., 2006) to study their effect, temperature, time period and metal ions on cellulose activity of the selected isolate *Bacillus* species.

PURIFICATION OF CELLULASE

Protein estimation was used for the determination of protein concentration using crystalline bovine serum albumin (BSA) as a standard (Lowry *et al*., 1951). The purification of the crude enzyme extract was carried out by using ammonium sulphate precipitation to obtain saturation. After centrifugation, the precipitate was dissolved in PBS and then purified by dialysis against double distilled water over night. This after purification with ion exchange chromatography.

PROTEIN ESTIMATION AND MOLECULAR WEIGHT DETERMINATION

Protein concentrations in the crude sample were estimated by using Lowry *et al*., 1951, method with bovine serum albumin (BSA) as a standard (Lowry *et al*, 1951) and SDS-PAGE was used for molecular weight determination Laemmli (1970). The standard proteins markers were loaded next to the purified protein, followed by the crude and dialyzed sample. Statistical analysis was done using ANOVA $p<0.05$ to test the data.

3. RESULTS

Based on the experiments conducted the newly isolated bacteria from the sample of *Glossocolex paulistus* grown in purple vat dye produced the enzyme cellulose. The microscopic studies revealed that the isolate was rod shaped gram positive, motile and spore forming bacteria (*Bacillus sp.*). The quantity of enzymes produced is 153.80 ± 0.02 U/L for cellulose. Different culture parameters such pH, temperature, time period and metal ions on enzyme activity were optimized for enzyme production. The optimization of culture conditions on cellulose production are presented in Table 1.The maximum activity of 137.20 ± 0.023 U/L was observed at the pH of 7.0 and 7.5 for cellulase enzyme. The enzyme activity was maximum at the temperature of 40 º C to70°C for the enzyme. Cellulase showed peak activitiy at 40 º C (137.22 ± 0.022 U/L) for cellulase (Table: 2). The enzyme production varies with respect to time for different enzymes. The maximum cellulase activity was seen at 72 hours (137.22 ± 0.022 U/L) in the test conducted from 12 hrs to 120 hrs time period (Table: 3) Copper sulphate salt showed more profound effect in the production of cellulase (137.22 ± 0.02 U/L) (Table: 4).

PROTEIN ESTIMATION LOWRY METHOD

Protein estimation by Lowry method was used for the determination of protein concentration using crystalline bovine serum albumin (BSA) as a standard (Lowry et al., 1951). The crude enzyme was 23.2 U/mg and ammonium sulphate precipitation sample showed 38.3 U/mg.

SDS PAGE FOR THE CONFORMATION OF CELLULASE ENZYMES

SDS Page for the conformation of cellulose enzyme (Laemmli, 1970) was performed. The molecular weight of the enzyme was found to be 63 kDa, a homogenous monomer protein with a single band on SDS page comparing with the marker proteins.

4. DISCUSSION

In present study, *Bacillus sp*. showed maximum cellulase activity at pH 7.0 -7.5, 40 °C temperature on 24 hrs at 72 hrs and in copper sulphate salt. The presence of active enzymes such as amylase, cellulase, protease, chitinase, lipase and lichenase in the

earthworm's gut emphasize the digestive capability of the earthworms. These enzymes are reported to operate in a stable pH range of 6.3 to 7.3 throughout the length of the intestine which encourages the growth of bacterial colonies as reported by Senapati (1993). Taking into account highest cellulase activity, *Bacillus sp*. strain was selected for enzyme purification, molecular weight determination. In the last stage of enzyme purification and yield were 23.2 U/mg and ammonium sulphate precipitation sample showed 38.3 U/mg. The molecular weight of the purified protein was 63 kDa.

5. CONCLUSION

Results of this study indicate that cellulase producing bacterial strain *Bacillus sps.* can be grown at different optimized conditions. The isolated strain *Bacillus sps.* showed maximum cellulase activity at pH 7.0-7.5, 40°C temperature, time profile of 72 hrs and copper sulphate salt. The worms also harboured some bacteria which in turn helped the degradation process by producing enzymes which accelerates the process of degradation of different organic compounds like cellulose, amylase which is the major component of plants and proteins.

Table: 1 Effect of different pH on the selected isolate *Bacillus sp.* and maximum enzyme activities.

S.NO.	pH	Enzyme Activity (U/L)
		Cellulase
1.	4.0	128.90±0.012
2.	4.5	131.42±0.015
3.	5	135.86±0.021
4.	5.5	136.74±0.025
5.	6	137.03±0.022
6.	6.5	137.20±0.027
7.	7	137.20±0.023
8.	7.5	137.18±0.020
9.	8	136.20±0.019
10.	8.8	136.20±0.019
p value		0.053ns

Values are mean ± SD of three samples in each group

Table :2 Effect of different temperatures on the isolate *Bacillus sp.* and maximum enzyme activities

S.No.	Temperature (ºC)	Enzyme Activity (U/L)
1	30	137.20 ±0.023
2	40	137.22 ±0.022
3	50	137.16±0.021
4	60	90.87±0.018
5	70	87.68±0.016

p value		0.0564ns

Values are mean ± SD of three samples in each group

Table : 3 Time profile for the new isolate *Bacillus sp.* on its enzyme activity

S.No.	Time period (Hours)	Enzyme Activity (U/L)
1	12	0.14±0.020
2	24	10.10 ±0.021
3	36	48.95±*0.022*
4	48	87.64±0.028
5	60	102.48±0.021
6	72	137.22 ±0.022
7	84	137.01 ±0.021
8	96	130.66 ±0.02
9	108	126.78 ±0.024
10	120	101.34 ±0.032
P value		0.0473ns

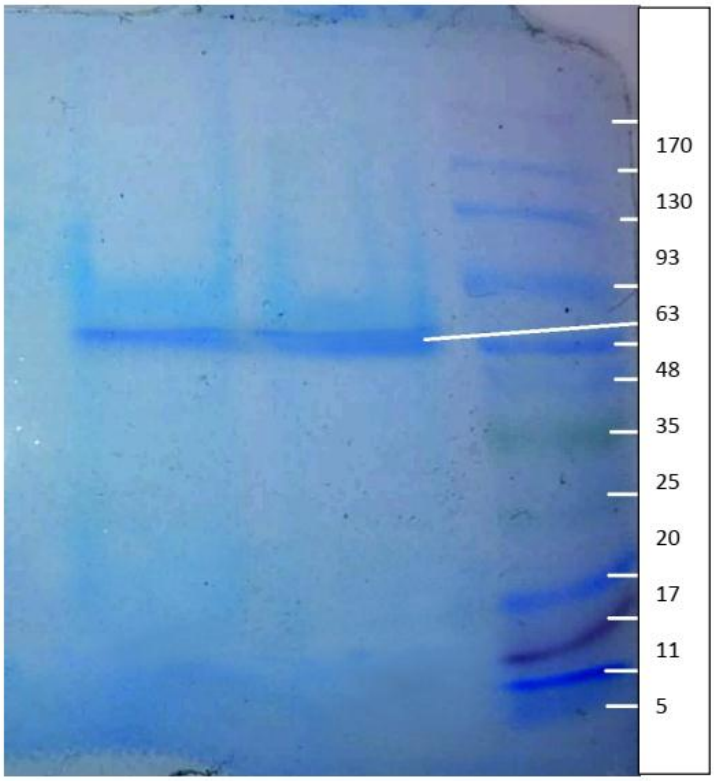
Values are mean ± SD of three samples in each group

Table 4: Effect of various salts on the enzyme activity of the isolate *Bacillus sp.* from *G. paulistus* grown on vat dye (Gamez *et al*., 2005)

S.No.	Salts	Enzyme Activity (U/L)
1	MgSO4	137.12 ± 0.02
2	$ZnSO_4$	137.10 ± 0.02
3	$MnSO_4$	137.00 ±0.02
4	$FeCl_2$	136.90±0.02
5	$CuSO_4$	137.22±0.02

Values are mean ± SD of three samples in each group

Fig: 1 SDS PAGE for cellulose showing single band corresponding to 63 kDa



Molecular weight determination by SDS-PAGE method. Lane 1, marker protein: Phosphorylase B (97 kDa), Bovine serum albumin (67 kDa), Ovalbumin (45 kDa), Carbonic anhydrase (29 kDa), Trypsin inhibitor (20 kDa) and Lysozyme (14.6 kDa). Lane 2, crude enzyme. Lane 3, DEAE-cellulose unbound fraction and Lane 4, CM-cellulose bound (purified protein). The migration position of cellulase is indicated as 67 kDa

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