



EXTRACELLULAR CELLULASE PRODUCED BY *LENTINUS SQUARROSULUS* A WILD EDIBLE MUSHROOM OF TRIPURA

Gopal Debnath*, Panna Das and Ajay Krishna Saha

Department of Botany, Tripura University, Suryamaninagar- 799022, Tripura, India

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ABSTRACT

Cellulase is an enzyme has great industrial importance, it catalyzes the conversion of insoluble cellulose to simple, water-soluble products. Cellulases are produced from a wide variety of microorganisms including fungi and bacteria. The present study investigated to the production of extracellular cellulase enzyme by wild edible mushroom *Lentinus squarrosulus*. The mycellial culture was screened for enzymatic activity using Carboxyl Methyl Cellulose (CMC) as substrate. The result revealed a clear zone of inhibition in the agar plates. Extracellular cellulase activities were measured at different physical and chemical condition such as pH, temperature, days and substrate concentration. The highest cellulase production was obtained on the 5th day by measuring 1.9 mg reducing sugar of per ml enzyme activity per min. The optimum cellulase activity was recorded at pH 5 (2.070 mg/ml/min), 50^o C temperature (2.83 mg/ml/min) and 1% CMC (1.91 mg/ml/min). This study revealed that *L. squarrosulus* has the ability to produce cellulase and it may be utilized as a source for the production of industrial cellulase.

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INTRODUCTION

Cellulase is an enzyme that has the capability to degrade cellulose, it is the major enzyme used for production of biofuels by sachharification of many natural substrates and also widely used for useful adulterations of pulp and paper characteristics (Kibbelwhite *et al.*, 1996). Cellulase enzyme produced by fungi, bacteria and protozoans that catalyze the hydrolysis of 1, 4 β -D glycosidic linkages in cellulose (Singh *et al.*, 2012). Huge number of microorganisms are able of degrading cellose, only some of these produce significant quantities of cell free enzyme capable of completely hydrolyzing crystalline cellulose (Beguin *et al.*, 1994). Enzymatic hydrolysis of cellulosic materials for the production of fermentation sugar are squat yield and the cost of cellulases (Lee *et al.*, 2010). Cellulosic material is the most abundant renewable carbon source for the production of different useful products all over the world (Ali *et al.*, 2008). Production of cellulase from agrowastes is economical than production from pure cellulose (Chahal *et al.*, 1985). Cellulose hydrolyzation by using enzymes to produce glucose which can be used for the production of ethanol, organic acids and other chemicals (Koomnok *et al.*, 2005). Various enzymes produced by bacteria, fungi and yeast but the most extensively studied cellulases are those produced by efficient lignocellulose degrading fungi (Narsimha *et al.*, 2006).

During vegetative growth mycelia of mushroom emit enzymes that degrade different components of plant material like cellose and lignin which present in the substrate, so mushrooms are an alternative source for production of extracellular cellulolytic enzymes. Optimal conditions are required for fungal growth which leads to the release of extracellular enzymes and enzyme activity, culture conditions for extracellular enzyme production and activities are different among isolates. The present study focus on extracellular cellulase production by using wild edible mushroom *Lentinus squarrosulus*.

MATERIAL AND MATHODS

Sample Collection and Identification

Wild edible mushroom *Lentinus squarrosulus* (fig1) was collected from market Lake chomohini, Agartala, West Tripura and brought into the laboratory for further process. The Mushroom was identified by comparing the morphological data of Pegler (1997) published in the book "A Preliminary Agaric Flora of East Africa" ISBN 011 241101 0*

*Corresponding author: Gopal Debnath

Department of Botany, Tripura University,
Suryamaninagar- 799022, Tripura, India



Fig 1 Fruit bodies of *Lentinus squarrosulus*

Isolation and Maintenance of Mycelial Culture

Tissue culture of *L. squarrosulus* was done on malt extract agar (MEA) and Potato dextrose agar (PDA) by tissue taken from the basidiocarp. Slants were incubated for 5 to 7 days in a closed aseptic chamber. The mycelium collected from the growing edge was transferred into new media (MEA and PDA) and incubated further for 5 to 7 days. Such reinoculation repeated 2 to 3 times to get pure isolate.

Screening of Extracellular Cellulase Enzyme

Screening of extracellular cellulase enzyme of *L. squarrosulus* was done by placing of 5mm mycelial plugs on the Glucose Yeast Extract Peptone Agar (GYP) medium containing 0.5% Carboxy-methylcellulose (CMC). After 3-5 days of mycelial growth, the plates were flooded with 0.2% aqueous Congo red solution and destained with 1M NaCl for 15 minutes. Appearance of clear zone around the mycelia indicates cellulase activity (Hankin and Ananostakis, 1975).

Cellulase Enzyme Production

Nine mm mycelial plug of *L. squarrosulus* was placed in 50 ml basal medium containing (in g/l), yeast extract, 2.0; NaNO₃, 5.0; KH₂PO₄, 1.0; MgSO₄·7H₂O, 0.5; FeCl₃, 0.001 and 1% concentration of Carboxymethyl cellulose (Deacon, 1985). The culture was incubated at 25°C and culture filtrate was extracted by filtration through Whatman No. 1 filter paper and the culture filtrate served as the crude enzyme solution (Singh *et al.*, 1988). Culture broth was sampled at different time during growth to determine effect of incubation period on enzyme production.

Cellulase Activity

Activity of cellulase enzyme produced by *L. squarrosulus* was determined by estimating the reducing sugars formed by the method described by Ghose (1987), with slight modifications. Enzyme solution about 500µl was added with 500µl of 1% CMC in 0.05 M sodium citrate buffer (pH 4.8) and assay tubes were incubated in a water bath for 30 min at 50 °C. After incubation, DNS reagent (3 ml) was mixed to all the assay tubes and boiled in water for 10 min. Blank were prepared by replacing the enzyme solution with distilled water. Absorbance value of each assay tube was recorded against 540 nm wavelength. Standard curve of reducing sugar was prepared by

using glucose. One unit of CMCase activity was expressed as milligram of glucose released per milliliter enzyme per minute.

Effect of Temperature on Cellulase Activity

The optimum temperature of Cellulase activity from *L. squarrosulus* was determined by incubating 1ml enzyme with 1 ml 1% CMC in citrate buffer pH 5.5 at different temperature (20 – 80°C) for 30 min.

Effect of pH on Cellulase Activity

The optimum pH of Cellulase activity from *L. squarrosulus* was determined by incubating the 1 ml enzyme mixed with 1 ml 1% CMC in buffer of different pH (citrate buffer 3 – 6, phosphate buffer 7-8 and glycine NaOH buffer 9-10) for 30 min. at 50°C temperature.

Effect of Substrate Concentration on Cellulase Activity

The optimum substrate concentration of Cellulase activity from *L. squarrosulus* was determined by incubating the 1 ml enzyme mixed with 1ml of different concentration (0.2 - 3% CMC in citrate buffer, pH 5.5 for 30 min. at 50°C temperature.

RESULT AND DISCUSSION

Clear zone formed in chromogenic media containing congo red by extracellular cellulase activity was shown in fig 2. In the present study screening of extracellular cellulase produced by *L. squarrosulus* was positive. In the present investigation, chemically modified CMC was used to resemble the cellulose. Thormann *et al.* (2002) reported that congo red can only colorize the cellulose and decolorized the area by the endoglucanases enzyme. According to Ponnambalam *et al.* (2011), cellulose has been degraded into simple sugars by the enzymatic activity appearing the clear zone around mycelial plug due to congo red dye stained that area washing with NaCl solution. The present work was closely similar to the report of Hwan *et al.* (2007).

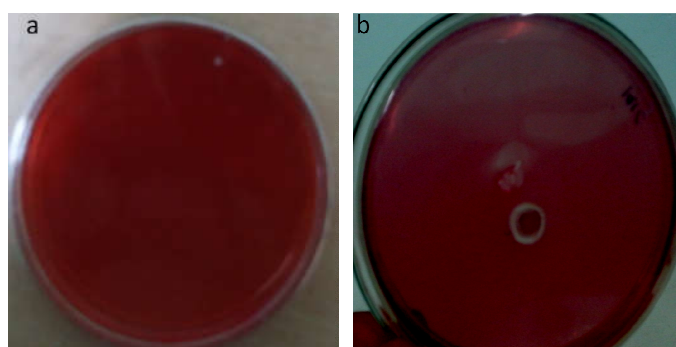


Fig 2 Screening of extracellular cellulase activity of GYP medium, a) Negative control and b) Mycelial plug of *L. squarrosulus* was showed positive in cellulase activity.

Incubation period on cellulase production of *L. squarrosulus* was investigated (fig 3). It was seen that the extracellular cellulase production is high (1.91 mg/ml/min) after 5 days of incubation. This study was closely similar to the findings of Umbrin *et al.* (2011).

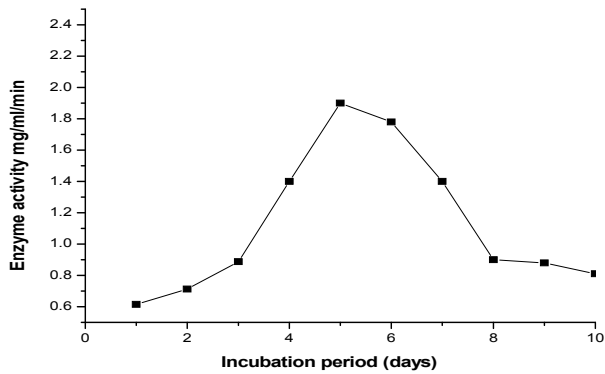


Fig 3 Incubation period on cellulase enzyme production.

Cellulase activity on different pH was shown in fig 4. The increasing cellulase activities were obtained from pH 3.0 to pH 5.0 and decreased drastically with an increase in pH 5 to pH 10.0. Maximum cellulase activity was found at pH 5.0 (2.070 mg/ml/min). The present work closely similar to the work of Petchlua *et al.*(2013). Cellulase activity at pH 3.0 and 9.0 confirming with the observation of Haltrich and Steiner *et al.*, (1994) that low or high pH values inactivate the enzyme.

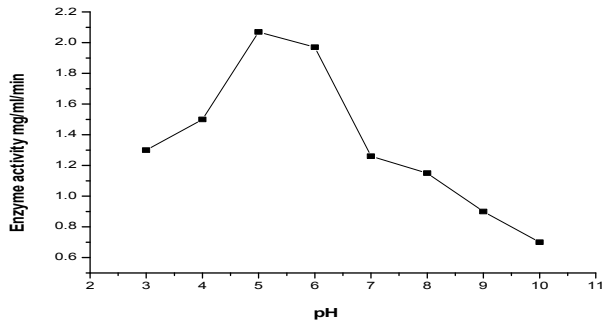


Fig 4 cellulase activity in different pH

Extracellular cellulase activity on different temperature was shown fig 5. The cellulase showed good activity between 30°C to 60°C. The highest cellulase activities was obtained at 50°C (2.83 mg/ml/min). Cellulase enzyme activities decreased in values above 60°C. Temperatures below 30°C and above 50°C may not be conducive for cellulase activity. The present study showed closely similar to the work of Petchlua *et al.* (2013).

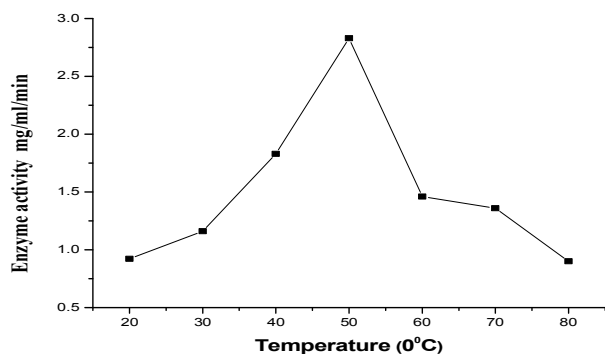


Fig 5 Cellulase activity in different temperature

Cellulase activity in different substrate concentration was shown in fig:6 was influenced by the concentration of substrate (CMC). The highest activity occurred at 1.0% CMC concentration (1.91 mg/ml/min) and below and above this activity decreased. There was an increase in cellulase activity as the CMC concentration in the reaction mixture was raised

from 0.5 to 1.0%. However, higher concentrations of CMC resulted in a decline of cellulase activity. This finding was closely similar to the report of Jaafar *et al.* (2010).

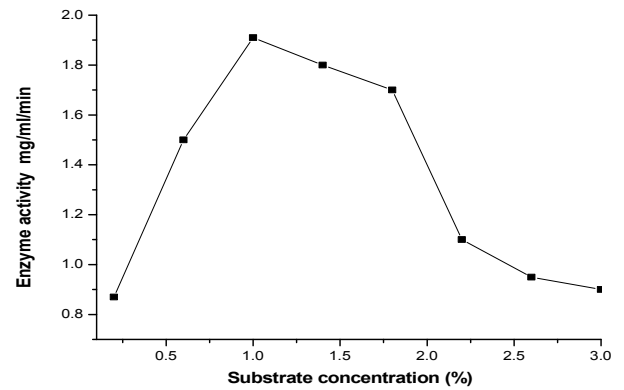


Fig 6 Effect of substrate concentration on cellulase activity

CONCLUSION

From the above study it may be concluded that wild edible mushroom *L. squarrosulus* can be exploited for the production of cellulase enzyme, which can be used in production of ethanol, detergent, weave, textile, coffee, pulp and paper and pharmaceutical industries.

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