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Screening and characterization of extracellular cellulase enzyme produced by wild edible mushroom *Pleurotus giganteus*

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Cellulase is a class of enzyme that has great industrial importance. Cellulase catalyzes the hydrolysis of 1, 4 β-D glycosidic linkages in cellulose are mainly produced from a wide variety of microorganisms like fungi, bacteria and protozoan. The present study aimed at the screening and production of extracellular cellulase enzyme produced by a wild edible mushroom *Pleurotus giganteus.* Different physical and chemical parameters such as temperature, pH, substrate concentration and reaction time on cellulase activity were also studied. The clear yellowish zone around mycelial plug revealed that screening of extracellular cellulase activity of *P. giganteus* was positive. Cellulase production by using the mushroom was highest after 6 days of the incubation period. Extracellular cellulase activity was maximum at 50 ^{o}C temperature, pH 5, 1.4% of substrate concentration (Carboxy-methylcellulose) and 30 minutes of reaction time. The present investigation revealed that wild edible mushroom *P. giganteus* may be utilized as a source for the production of industrial cellulase.

Keywords: Cellulase, *Pleurotus giganteus,* pH, Reaction time, Screening, Substrate concentration, Temperature.

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Introduction

Cellulose is the most plentiful organic compound as a substrate for the production of biofuels, single-cell proteins and various other chemicals during enzymatic degradation by microbial cellulases[1]. Cellulase is an enzyme that catalyzes the conversion of insoluble cellulose to simple soluble products[2]. Cellulase enzyme gained reliable interest due to their extensive applications in the pulp and paper industry[3], detergent and textile industry[4], supplementation of animal feed[5], starch processing industry[6], as well as in the wine and brewing industry[7]. Cellulolytic enzymes are produced by a number of microorganisms; the main natural agents of cellulose degradation are fungi and bacteria[8]. According to Beguin *et al.*[9] large number of microorganisms are able of degrading cellulose, only some of these produce significant quantities of a cell-free enzyme capable of completely hydrolyzing crystalline cellulose. Cellulase enzymes synthesized by microorganisms like fungi, bacteria and protozoan that catalyze the hydrolysis of 1, 4 β-D glycosidic linkages in cellulose[10].

In recent years there have been increasing interests in mushrooms utilization worldwide; mushrooms are a treasure trove of several enzymes with biotechnological significance and industrial applications[11]. Mushrooms are an alternative source for the production of extracellular cellulolytic enzymes because during cultivation mycelia of mushrooms emit enzymes that degrade different components of plant material like cellulose and lignin which present in the substrate[12]. Mushrooms are an alternative and safe source of extracellular cellulolytic enzymes; of these, *Pleurotus* spp. is most efficient in utilizing lignocellulosics[13-15]. The present study focused on screening and production of extracellular cellulase by using wild edible mushroom *Pleurotus giganteus*. Effect of different temperatures, pH, substrate concentrations and reaction times on cellulase activity were also studied.

Material and Methods

Sample collection and identification

Wild edible mushroom *P. giganteus* having laboratory collection number MCCT 189 was collected from *Lake Chowmuhani* market, Agartala, West Tripura and brought into the laboratory for further process. Tissue culture of *P. giganteus* was done on Malt extract agar (MEA) and Potato dextrose agar

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(PDA) media. Slants were incubated for 5 to 7 days in a closed aseptic chamber. The mycelium collected from the growing edge was transferred into fresh media (MEA and PDA) and incubated further for 5 to 7 days. Such reinoculation repeated 2 to 3 times to get pure isolate. The mushroom was identified at the molecular level, on the basis of the sequence of the internal transcribed spacer (ITS) region and the obtained accession number from National Center for Biotechnology Information (NCBI) is MG198772.

Screening of extracellular cellulase enzyme

Screening of extracellular cellulase enzyme *P. giganteus* was done by the method of Hankin and Ananostakis[16]. Petri plates were prepared with Glucose Yeast Extract Peptone Agar (GYP) medium containing 0.5% Carboxy-methylcellulose (CMC). Five millimetres of mycelial plugs from 7 days old culture were inoculated on the prepared Petri plates. After 3-5 days of mycelial growth, the plates were flooded with 0.2% aqueous Congo red solution and destained with 1M sodium chloride (NaCl) for 15 minutes. The appearance of a clear zone around the mycelial culture indicates the activity of cellulase.

Cellulase enzyme production

Extracellular cellulase production was done by the method described by Deacon[17]. 250 mL of Erlenmeyer conical flask containing 50 mL of basal medium composed by (in g/L), yeast extract, 2.0; $NaNO_3$, 5.0; KH_2PO_4, 1.0; $MgSO_4.7H_2O$, 0.5; $FeCl_3$, 0.001 and 1% concentration of Carboxymethyl cellulose were inoculated with 9 mm mycelial plug of *P. giganteus.* The cultured conical flasks were incubated at 25 ℃ up to 10 days. Culture filtrate was extracted by filtration through Whatman filter paper No. 1 and the culture filtrate was used as the crude enzyme solution[18]. The culture broth was sampled at a different time during growth to determine the effect of incubation period on enzyme production.

Cellulase activity

Extracellular cellulase activity was determined by the method of Ghose[19]. Enzyme solution about 500 µL was added with 500 µL of 1% CMC in 0.05 M sodium citrate buffer (pH 4.8) and assay tubes were incubated in a water bath for 30 minutes at 50 °C. After incubation, DNS reagent (3 mL) was mixed to all the assay tubes and boiled in water for 10 minutes. Blank was prepared by replacing the enzyme solution with distilled water. The 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 carboxymethyl cellulase activity was expressed as µmol of reducing sugar released from per millilitre of culture filtrate as enzyme solution in per minute.

Cellulase activity on different temperatures

The optimum temperature of Cellulase activity produced by *P. giganteus* was determined by incubating 1 mL enzyme with 1 mL 1% CMC in citrate buffer pH 4.8 at different temperature (20–80 ℃) for 30 minutes.

Cellulase activity on different pH

The optimum pH of Cellulase activity was determined by incubating the 1 mL enzyme with 1 mL 1% CMC in the buffer of different pH (citrate buffer 3–6, phosphate buffer 7-8 and glycine NaOH buffer 9-10) for 30 minutes at 50 ℃ temperature.

Cellulase activity on different substrate concentrations

The optimum substrate concentration of Cellulase activity was determined by incubating the 1 mL enzyme mixed with 1 mL of different substrate concentrations (0.2-3% of CMC) in citrate buffer, pH 4.8 for 30 minutes at 50 ℃ temperature.

Cellulase activity in different reaction time

Cellulase activity in different reaction time was determined by incubating the 1 mL enzyme with 1 mL of substrate (1% of CMC) in citrate buffer, pH 4.8 for different reaction time (5-45 minutes) at 50 ℃ temperature.

Results and Discussion

Screening test of extracellular cellulase activity of *P. giganteus* was presented in Fig. 1. A clear yellowish zone around mycelial plug was formed in chromogenic media containing congo red dye. Congo red dye can only colourize the cellulose (CMC) and decolourized the area by the endoglucanases enzyme[20]. Ponnambalam *et al.*[21] reported that cellulose has been degraded into simple sugars by the enzymatic activity appearing the clear zone plug due to congo red dye stained that area washing with NaCl solution. In the present investigation, it was revealed that screening of extracellular cellulase activity of *P. giganteus* was positive.

Extracellular cellulase production by using *P. giganteus* in different incubation period was shown in Fig. 2. Cellulase production was increased up to 6 days of incubation period and then with increasing period of incubation cellulase production was decreased. Extracellular cellulase production was highest (6.35 µmol/mL/min) at 6 days of the

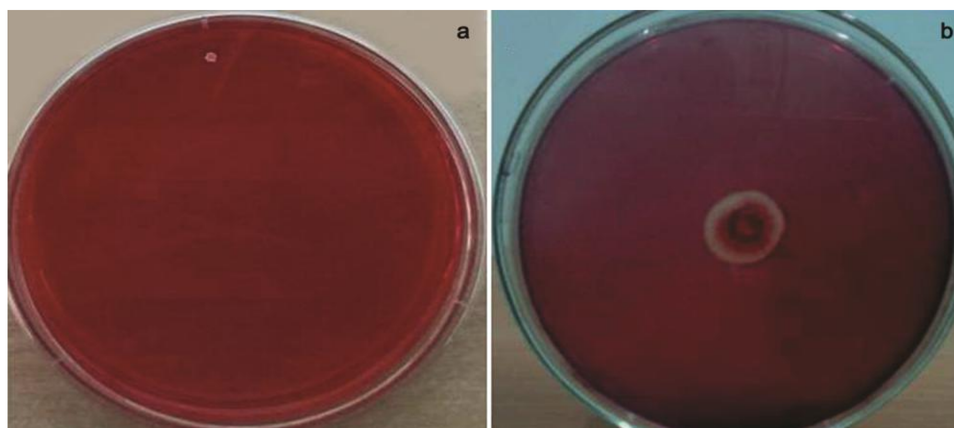

Fig. 1–Screening of extracellular cellulase activity of *P. giganteus*; a) Negative control (without mycelial plug and b) Positive activity of cellulase.

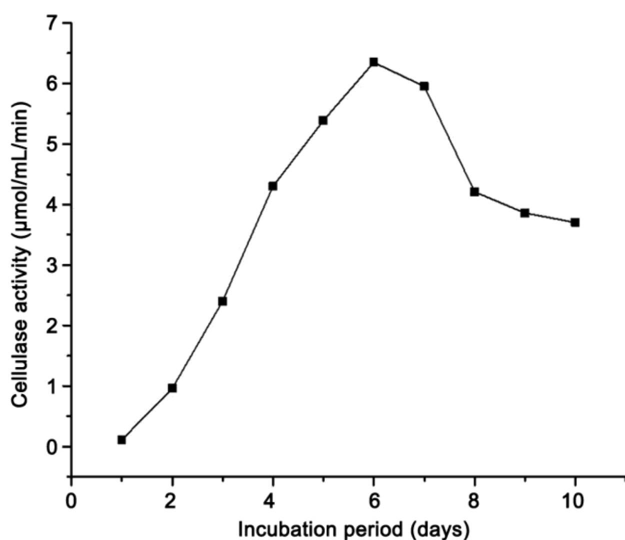

Fig. 2–Cellulase production using *P. giganteus* in different incubation periods.

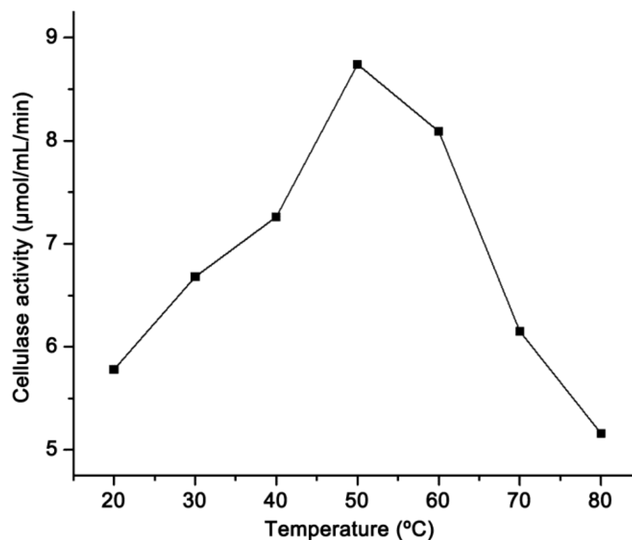

Fig. 3–Cellulase activity of *P. giganteus* in different temperatures.

incubation period. This study was closely similar to the findings of Debnath *et al.*[12] and Umbrin *et al.*[22].

The effect of temperature on cellulase activity produced by *P. giganteus* was determined (Fig. 3). In the present study, extracellular cellulase showed good activity between 40 and 60 °C temperature. Maximum cellulase activity (8.74 μmol/mL/min) was obtained at 50 °C temperature. Below 40 °C and above 60 °C temperatures may not be favourable during an enzymatic reaction of cellulase; closely similar results were reported by Petchluan *et al.*[23] and Debnath *et al.*[12] in their study.

Extracellular cellulase activity of *P. giganteus* in different pH was shown in Fig. 4. Cellulase activity was increased up to pH 5 and drastically decreased from pH 6 to 10. Optimum pH of cellulase activity

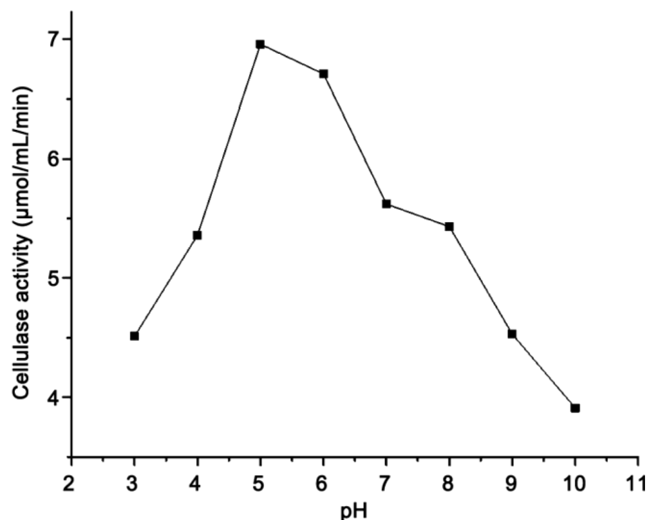

Fig. 4–Cellulase activity of *P. giganteus* in different pH.

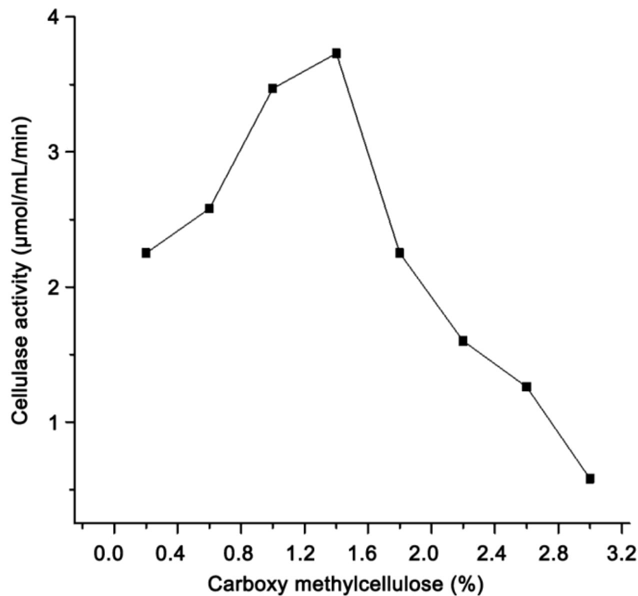

Fig. 5–Cellulase activity of *P. giganteus* in different substrate concentrations.

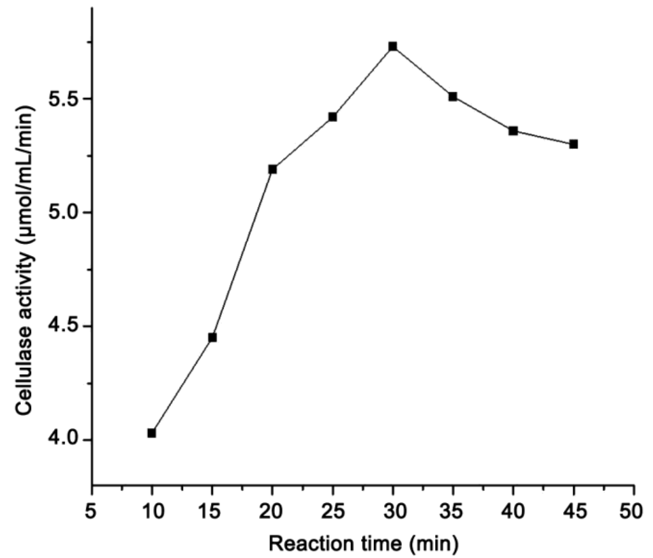

Fig. 6–Cellulase activity of *P. giganteus* in different reaction times.

(6.96 µmo/mL/min) was observed at pH 5. During the enzymatic reaction of cellulase activity pH below 4 and 6 may not be conducive. According to Haltrich and Steiner *et al.*[24] low or high pH values inactivate the enzyme.

Effect of substrate (CMC) concentration on cellulase activity was determined (Fig. 5). Maximum cellulase activity (3.73 µmol/mL/min) was obtained at 1.4% concentration of CMC; below and above this concentration cellulase activity was decreased. Higher concentrations of substrate (CMC) have resulted in a decline of cellulase activity; similar findings were reported by Debnath *et al.*[12].

Cellulase activity of *P. giganteus* was also studied in different reaction times. Cellulase activity was highest (5.73 µmol/mL/min) after 30 minutes of reaction time (Fig. 6). This study was revealed that 30 minutes of reaction time may be favourable for the enzymatic reaction of cellulase.

Conclusion

From the present investigation, it may be concluded that wild edible mushroom *P. giganteus* is a good source for the production of extracellular cellulase having maximum activity at 50 ^{o}C temperature, pH 5, 1.4% substrate (CMC) and 30 minutes of reaction time. The wild edible mushroom can be utilized as a source for the production of industrial cellulase in various applications like the manufacture of ethanol, detergent, weave, textile, coffee, pulp, paper and various pharmaceutical industries.

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