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OPTIMIZATION OF CELLULASE ENZYME PRODUCTION FROM *PLEUROTUS OSTREATUS* AND *CALOCYBE INDICA*

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ABSTRACT

Mushrooms contain a number of bioactive compounds associated with beneficial effects on human health. In the present investigation the production of cellulase enzyme by Pleurotus ostreatus and Calocybe indica. The maximum cellulase activity (0.607 U/ml) was observed in Pleurotus ostreatus and Calocybe indica (0.490 U/ml). Cellulase from these mushroom species were assayed and optimized at different temperatures. The maximum cellulase production was observed in Pleurotus ostreatus (0.863 U/ml) and Calocybe indica (0.652 U/ml) at 70°C. The dependence of enzyme activity on reaction temperature for the cellulase enzyme activity in different mushrooms such as Pleurotus ostreatus and Calocybe indica has been studied. cellulase activity was high for Pleurotus ostreatus when compared to Calocybe indica at different range of temperature. The optimum temperature for maximum cellulase was production of observed at 70°C.

KEY WORDS

Pleurotus ostreatus, Calocybe indica, Cellulase enzyme activity

INTRODUCTION

Mushroom a form of fleshy edible fungi are essentially a rich source of good quality protein having most of the essential amino acids, minerals and vitamins with low calorific value. Though 20 genera of edible mushroom are being cultivated throughout the world only three type of mushrooms namely mushrooms are white button mushroom (*A. bisporus*), *Oyster mushroom* (*Pleurotus* sp.) and paddy straw mushroom (*Volvariella volvacea*) are grown commercially in India with the white button mushroom still contributing about 90% of the country's production as against its global share of about 35% (Rai *et al.,* 1993).

In recent years *Pleurotus* sp, has gained prominence as a type of edible mushroom *Pleurotus* species thrive over a wide range of such tropical climates and are representative of white rot fungi, which can degrade directly the lingo cellulosic organic wastes of nature (Ulezlo *et al.,* 1975; Tayama and Oguwu, 1976; Rajarathnam *et al.,* 1979).

Calocybe indica was first reported from India by Parkayastha and Chandra, 1974. This mushroom normally grows on the humus rich soil under roadside trees and in agricultural fields. In some place they are called as "Kuduk" but popularly known as "Dudhichhata". *Calocybe indica* has become the third commercially grown mushroom

in India after button and oyster mushroom (Beelman *et al.,* 1989).

MATERIALS AND METHODS

Sample Collection

Edible Mushroom of *Pleurotus ostreatus* (Oyster mushroom) and *Calocybe indica* (Milky mushroom) were procured from Department of microbiology, Jaya college of Arts and science, Thiruninravur, chennai, Tamil Nadu, India.

Pleurotus ostreatus and *Calocybe indica* was taken and cut into small pieces and surface sterilized with 80% ethyl alcohol. Then it was washed with sterile distilled water. Again surface sterilized with 0.1% mercuric chloride for 30 seconds and then this mushroom pieces were washed thrice with sterile distilled water.

Modified Czapek's cellulase medium(Cellulose 10g, KNO_3 3g, K_2HPo_4 1.0g, $MgSo_4$ 0.5g, $FeSo_4$ 0.01g, Distilled water 1L) Cotton plugged 250 ml Erlenmeyer flasks containing 50ml medium were autoclaved at 121°C for 30 minutes. The collected mushroom (*Pleurotus ostreatus* and *Calocybe indica*) were inoculated to the cellulase enzyme media were used in the present study. The flasks were then incubated at 30°C for 15 day under stationary conditions and three flasks were drawn at each 5 days interval for enzymatic determinations in cultural media.

Cellulase assay

Cellulase activity was determined by mixing 1 ml of 1% (w/v) Carboxymethyl cellulose (prepared in 50 mM sodium acetate buffer pH 5.3) with 1 ml of crude extracellular enzyme source and incubating at 50°C, 55°C, 60°C, 65°C, 70°C,75°C, 80°C for 15 min (Casimir *et al*., 1996). The reaction was stopped by the addition of 3 ml of 3, 5-dinitrosalicylic acid (DNS) and the contents were boiled for 15 minutes. The colour developed was read at 540 nm. The amount of reducing sugar liberated was quantified using glucose as standard. One unit (U) of enzyme activity was defined as the amount of enzyme required to liberate 1µM of glucose from CMC per min under the assay conditions. Cellulase activity was expressed as unit per mg (Miller, 1959).

Estimation of Protein

Protein concentration were determined according to (Lowry *et al.,* 1951)

RESULTS AND DISCUSSION

Pleurotus ostreatus and *Calocybe indica* were procured from Department of microbiology, Jaya college of Arts and science, Thiruninravur, chennai, Tamil Nadu, India. The collected mushrooms were tested for their cellulolytic activity. The maximum cellulase activity (0.607 U/ml) was observed in *Pleurotus ostreatus* and the minimum cellulase activity (0.490 U/ml) was exhibited in *Calocybe indica* (Table 1, Fig 1& Plate1).

In the present study the Cellulase from these mushroom species were assayed and optimized at different temperatures. Cellulase from both fungal species was optimized between the temperature ranges from 55°C – 80°C. Cellulase from *Pleurotus ostreatus* species had good activity between the temperature ranges of 55°C – 75°C. It attained maximum activity at 70°C (0.863U/ml). However in case of *Calocybe indica* mushrooms the cellulase was active between the temperature ranges of 60°C – 70°C and attained maximum activity at 70°C (0.652 U/ml). This means that the cellulase from milky species having some thermo stable property. Further increase in the temperature for both species resulted in the low activity of cellulose (Table 2 & Fig 2). Assay of cellulase from both mushroom species showed good activity. The cellulase activity was high for *Pleurotus ostreatus* when compared to *Calocybe indica*.

The *Pleurotus ostreatus* comprise the group of edible fungi with important medicinal properties and biotechnological and environmental

applications. They are highly adaptable to grow and fruit on wide variety of agro industrial lignocelluloses wastes due to their capability to synthesize cellulase and extracellular enzymes (Cohen *et al.,* 2002).

Three *Pleurotus* sp., *Pleurotus florida, Pleurotus ostreatus* and *Pleurotus sajar-caju*, were screened for cellulolytic enzyme production under submerged fermentation conditions. The optimum temperature and pH for maximum production of enzymes were 35 to 40°C (Meenakshi and Giridhar, 2011). Ramanathan *et al*., 2013 investigate the cultivation of oyster mushroom, *Pleurotus ostreatus* (local & exotic strains) and *P. sajarcaju* were conducted to find out the growth and yield performance on different substrates.

Our result from celullase activity support the finding of Omoanghe and Mikiashvili (2009), who observed that celullase activities, increased during primordial formation and fruiting. Ohga *et al*.,(1999) reported that high CMCase (CMCase carboxymethylcellulase) activity was strongly associated with the fruiting period. *Pleurotus sajor-caju* an edible mushroom elaborate both cellulose and xylanase activities on cellulose medium. The pH and temperature optima for cellulose activity were 5.0 and 45°C respectively (Madan and Bisaria, 1983). To study the potential of agrowastes as substrates for the production of cellulase and laccase by *Pleurotus* sp (Radhika *et al*., 2013 and José Maria Rodrigues da, *et al*., 2012) Cellulase is used for commercial food processing in coffee. It performs hydrolysis of cellulases are widely used in textile industry and laundry detergents. They have also been used in the pulp and paper industry for various purpose and they are even used for pharmaceutical application. Cellulase is used in the fermentation of biomass in to biofuels although this process is relatively experimental at present cellulose is used as a treatments for phytobezoars a form of cellulase bezoar found in the human stomach.

Plate 1.Cellulase production in *Pleurotus ostreatus* and *Calocybe indica*

Pleurotus ostreatus ***Calocybe indica***

Broth culture

A- *Pleurotus ostreatus*, B-*Calocybe indica*

Table 1. Production of cellulase from different mushroom

S.No.	Name of Mushroom	Enzyme Production U/ml
1.	*Pleurotus ostreatus*	0.607
2.	*Calocybe indica*	0.490

Fig 1. Production of cellulase from different mushroom

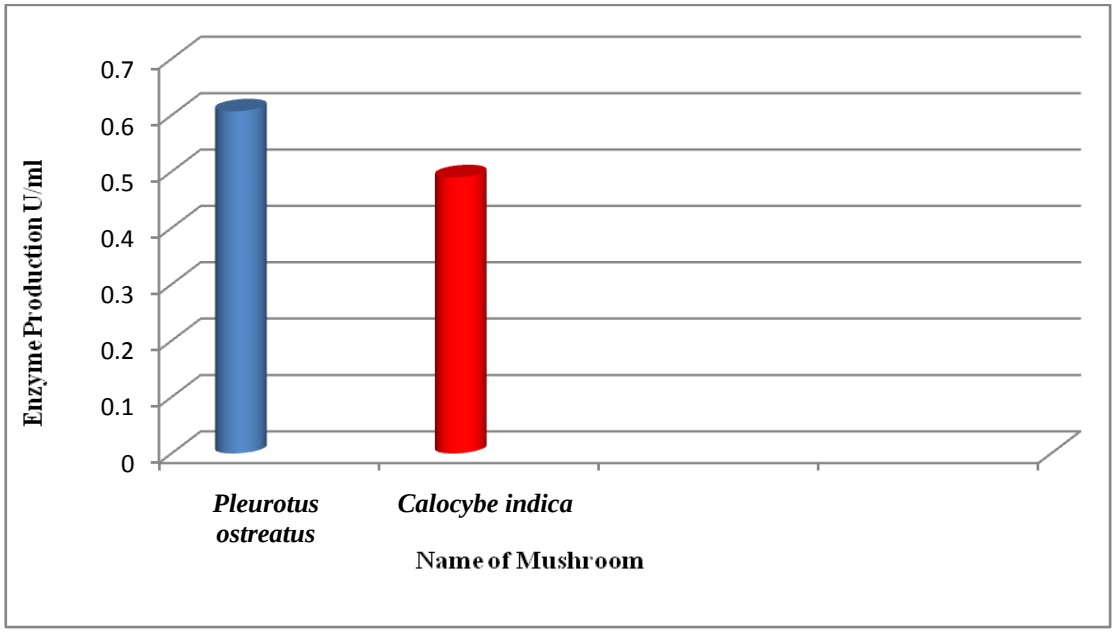
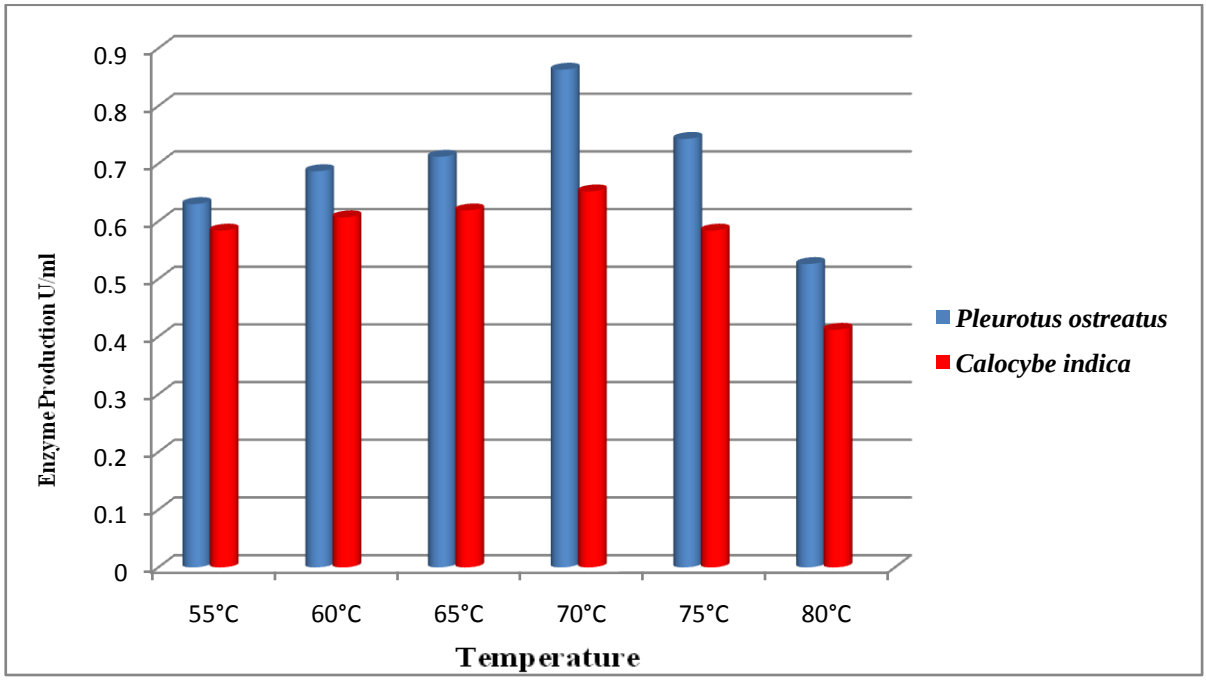


Table 2. Production of cellulase at different temperature

S.No.	Temperature	Enzyme Production U/ml	
		Pleurotus ostreatus	*Calocybe indica*
1.	55°C	0.630	0.584
2.	60°C	0.687	0.607
3.	65°C	0.712	0.619
4.	70°C	**0.863**	**0.652**
5.	75°C	0.743	0.584
6.	80°C	0.526	0.412

Fig 2. Production of cellulase at different temperature



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