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Research article

PURIFICATION OF CATALASE ENZYME FROM *PLEUROTUS OSTREATUS*

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ABSTRACT: The oyster mushroom *Pleurotus ostreatus* is the most commonly cultivated mushroom, and are effective for antitumor, antibacterial, anti viral and hematological agents and in immune modulating treatments. Several compounds from oyster mushrooms, potentially beneficial for human health have been isolated and studied. The aim of this research is to purify an enzyme catalase from *Pleurotus ostreatus* through Sephadox G-75 column, its molecular weight was determined by polyacrylamide gel electrophoresis and the catalase enzyme stability were observed at various temperature and different pH condition. Under denaturing conditions, polyacrylamide gel electrophoresis revealed dissociation of a major component of molecular weight 62,000 kDa, which constituted 90% of the total protein of the stained gel, suggesting that the native enzyme is tetrameric. The optimum temperature and pH for the purified enzyme catalase from *Pleurotus ostreatus* enzymatic reaction were 30°C and pH 7.5.
Keywords: *Pleurotus ostreatus*, Catalase, polyacrylamide gel electrophoresis.

INTRODUCTION

Mushrooms have been widely known and used as a source of food from ancient time. They are spore bearing fruiting bodies of fungi, typically produced above ground on soil or on food. Many species of mushrooms are used as medicine. They are very appreciated, not only for their texture, flavor, but also for their nutritional properties. Mushrooms have been demonstrated antitumor, antifungal, antibacterial activities [Chirinang *et al*., 2009]. Many mushrooms possess significant medicinal attributes in preventing hypocholesterolemic, hypoglycemic, hypertension, atherosclerosis and cancer [Ribeiro *et al*., 2006]. These functional characteristics are mainly due to their chemical composition [Bernas *et al*., 2006]. FAO recognizes mushrooms as the right source of protein. Mushrooms are a low-calorie, high protein diet, with no starch and sugars. They are called as the diabetics delight. These are also rich in B-Complex and iron. Vitamin B-12 and Folic acid, which are normally not found in vegetarian items, are present in mushrooms. Commercially button mushroom ranks first followed malignancies such as lung cancer; cardiac failure etc. *Pleurotus* species belongs to phylum Basidiomycota that produce oyster shaped mushrooms (basidiocarps) and accordingly they have been called as oyster mushroom (*OM*). Oyster mushroom grows saprophytically at a temperature range of 12-32°C. Their cap is normally shell-like (about 5-20 cm in diameter; 1.9-7.8 inches), fleshy, with eccentric or lateral stipe; and their color can be white, cream, yellow, pink, brownish, or dark gray. The protein content of oyster mushrooms can be considered as their main nutritional attribute. Average values ranging from 10.5-30.4%, on a dry weight basis, have been reported. The concentration of essential amino acids varies from 33.4-46.0 grams/ 100 grams of corrected crude protein. The fat content reported as 1.1-2.2% on a dry weight basis, having a high proportion of unsaturated fatty acids (79.3%). The carbohydrate content varies from 46.6-81.8% on a dry weight basis. Several compounds from oyster mushrooms, potentially beneficial for human health have been isolated and studied.

Natural antioxidants present in the diet increase the resistance toward oxidative damages and they may have a substantial impact on human health [Boskou, 2006]. Antioxidant compounds play an important role in our body due to favorable effects on human health. Consumption of food containing phytochemical with potential antioxidant properties can reduce the risk of human disease [Temple, 2000]. Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases.

Catalase (E.C. 1.11.1.6; H_2O_2: H_2O_2-oxidoreductase) belonging to the oxidoreductase family are a group of metallo enzymes with the ability to catalyze the decomposition of hydrogen peroxide into water and dioxygen. Four classes of catalases have been defined, including mono functional hem-containing catalases, catalase peroxidases, manganese catalases, and minor catalases. CAT reacts very efficiently with H2O2 to form water and molecular oxygen; and with H donors (methanol, ethanol, formic acid, or phenols) with peroxidase activity:

$$2\ H_2O_2O \xrightarrow{\quad} CAT \qquad 2\ H_2O + O_2$$

$$ROOH + AH_2 \xrightarrow{\quad} CAT \qquad H_2O + ROH + A$$

Catalase protects cells from hydrogen peroxide generated within them. Even though CAT is not essential for some cell types under normal conditions, it plays an important role in the acquisition of tolerance to oxidative stress in the adaptive response of cells. There is an increasing demand for different enzymes in modern industries [Dordick, 1991]. In spite of the outstanding progress in chemistry, there is not yet efficient reagent for synthesizing some chemical substances. The present study investigates the enzymatic activity and purification of industrial and therapeutically important catalase enzyme from locally available edible mushroom.

MATERIALS AND METHODS

Collection of sample

The mushroom *Pleurotus ostreatus* was collected from Tamilnadu Agriculture University, Coimbatore and washed well in tap water followed by distilled water. The cleaned fruiting bodies were sliced and stored at 4 °C for further study.

Catalase enzyme extraction

The enzyme extract was prepared by homogenizing 200g of fruiting bodies in pre-chilled mortar and pestle with 1000ml of cold phosphate buffer at pH 7. The homogenate was centrifuged at 10000 rpm for 30min and supernatant was collected. The sediment was mixed with cold phosphate buffer, allowed to stand in cold condition with occasional shaking. Then the sediment containing buffer was subject to centrifugation once again to collect supernatant. Finally, the collected supernatant was used as a source of enzyme.

Phytochemical screening

All phytochemical screening tests were carried out using the supernatant. Followings are the phytochemical screening tests were performed for this study.

Test for alkaloids

To test alkaloids, 1ml of each supernatant was mixed with 5ml of 27% HCl and heated gently in a steam bath for 1 minute. Then 0.5ml of Wagner's Reagent was added to each mixture and observed for the development of Brick red color.

Test for saponins (frothing test)

3.0 ml of supernatant was taken into test tubes and shaken vigorously. It was allowed to stand on the bench for 1 minute and observed for the development of forth formation.

Test for flavonoids

1ml of 5% lead acetate was added to 1ml of mushroom supernatant and the mixture was allowed to stand on the bench. The precipitate formation in the samples was observed for the presence of flavonoids.

Test for tannins

2ml of supernatant was added with 3 drops of 10% of $FeCl_3$. The appearance of blackish-blue or blackish-green coloration was observed for the presence of tannins.

Protein Determination [Lowry *et al*., 1951]

Protein content in the supernatant was estimated by the method described by Lowry *et al*. (1951). 0.2ml and 0.4ml of mushroom supernatants was used to determine the protein content.

Catalase assay [Sadasivam and Manickam, 2008]

Catalase activity was measured spectrophotometrically by monitoring the decrease in A_{240} resulting from the elimination of H_2O_2, using a Hitachi U-3210 spectrophotometer. 3ml of H_2O_2 –PO_4 buffer was taken in a cuvette and mixed with 0.01-0.04ml of supernatant. Enzyme activity was read against a control contained enzyme solution containing PO_4 buffer without H_2O_2 at 240nm. The time required to decrease in A_{240} from 0.45 to 0.40 was noted and the rate of H_2O_2 decomposition was calculated.

Purification of catalase enzyme [Tony ching *et al*., 1973]

The supernatant was brought to 45-90% saturation with solid ammonium sulfate and it was kept overnight at 4°C. The resultant precipitate was collected by centrifugation at 7000 rpm for 30 min, dissolved in 20ml of 50mM potassium phosphate buffer (pH 7), and dialyzed against the 3 liters of the same buffer. A column (1 by 3cm) of DEAE-cellulose equilibrated with potassium phosphate buffer. 30µl of the dialyzed enzyme solution was applied to the column and washed with 4 ml of potassium phosphate buffer. The column was eluted with distilled water. Active fraction detected for catalase activity by monitoring the decrease in $A_{240.}$ The fractions were collected and pooled for subsequent sephadex G-75 column.

A column of (0.5 X 5 cm) of sephadex G-75 that had been equilibrated with potassium phosphate buffer. The active fractions (8 ml) were diluted to 32 ml with the phosphate buffer in order to reduce the salt concentration. The fraction was then applied to the column and washed with the 3 ml of potassium phosphate buffer. Elution was performed with distilled water (total volume 15 ml). The volume of 1 fraction was 1 ml. The active fractions (2 ml) were further purified by rechromatography on the same column and under elution conditions. Then the enzyme were stored at 4°C and used for the characterization of the enzyme.

Molecular weight determination of catalase [Sambrook and Russel, 2001]

Purified catalases was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in 12% gel along with standard molecular weight protein markers. The sample and marker proteins were treated with 2% SDS and 5% β- mercaptoethanol at 100°C for 5 min just before loading. The gel was stained with Coomassie-brilliant blue R-250. The standards used to make a plot of log molecular weight *versus* mobility of the protein band were: lysozyme (14kDa) trypsin inhibitor (20 KDa), pepsin (36k Da), ovalbumin (43 KDa) egg albumin (45 kDa), bovine serum albumin (67 kDa) and phosphorylase B (94 KDa).

Analysis of pH profile

The pH profiles for the activity of catalase was obtained using 0.067M potassium phosphate buffer (pH 3 to pH 8). In each pH buffer (1 ml), 30µl of enzyme solution was mixed and kept for overnight at 4 °C. After this period, enzyme activity of samples was analyzed under the standard assay condition.

Analysis of thermostability

10µl of catalase was placed in water bath at a temperature of 10, 20, 30, 40, 50 and 60°C for periods of 60 min. Enzyme activity after treatment was analyzed under the standard assay condition.

Storage stability

30µl of catalase was mixed with 1ml of potassium phosphate (pH7) buffer and stored at the 4°C. Enzyme activity was analyzed every day up to 1 week in order to find out the storage stability of enzymes.

RESULTS

Phytochemical analysis

Phytochemical analysis in *Pleurotus ostreatus* extract revealed the presence of saponins, flavanoid and tannins but absence of alkaloids.

Protein estimation

Standard graph was plotted for bovine serum albumin using spectrophotometric reading at 660 nm. The total protein contents were 3.2 mg/ml and 5.25mg/ml respectively for 20 µl and 40 µl [Table 1].

Table 1. Total protein content of *Agaricus bisporous* and *Pleurotus ostreatus*

Sample volume (µl)	*Pleurotus ostreatus* (µg/ml)
20	3.2
40	5.25

Catalase assay

Catalase assay was performed for the mushroom extracts supernatant, enzyme activity were measured at 240nm wavelength. Time taken for the reduction of A_{240} values from 0.45-0.4 was noticed. The enzyme activity for *Pleurotus ostreatus* is 14.66 U/ml of the enzyme.

Purification and molecular weight of catalase from *Pleurotus ostreatus*

The homogeneity of purified catalase enzyme from *Pleurotus ostreatus* was produced single band in SDS-PAGE and its molecular mass was about 62 kDa [Fig.1]. Table 2 summarizes the results of each step of the catalase purification. The enzyme was purified about 51.11 fold, with a final specific activity of 13.8U/mg. The overall recovery of the purification was 10.9%.

Table 2. Purification of catalse from *Pleurotus ostreatus*

S.No	Purification step	Total amount of protein (mg)	Total activity (U)	Specific activity (U/mg)	Recovery (%)	Purification (fold)
1	Supernatant	525	142	0.27	100	1
2	Dialysate	112	97	0.86	68	3.18
3	DEAE- cellulose	108	87	1.24	61	4.59
4	Sephadox G-75 fraction	1.12	15.5	13.8	10.9	51.11

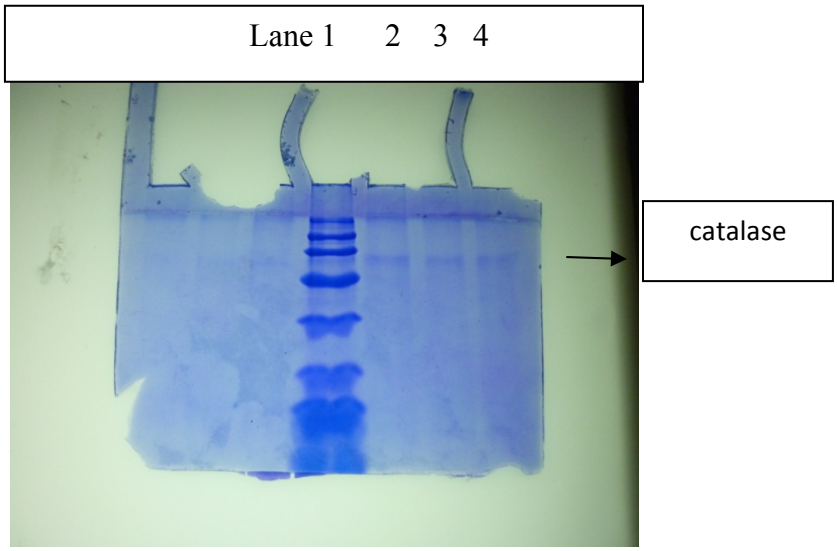

Fig. 1 Molecular weight of purified catalase from *Pleurotus ostreatus*

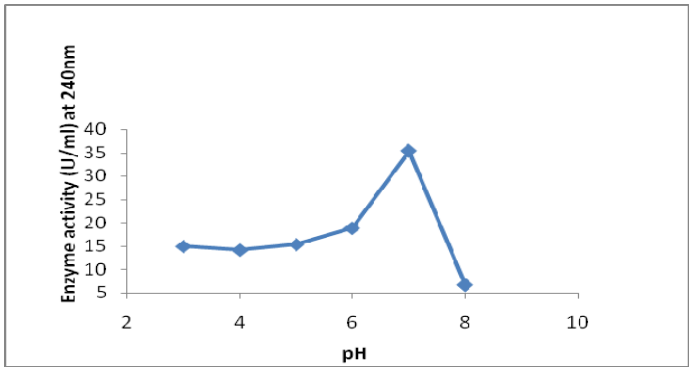

Fig. 2 pH stability of catalase enzyme from *Pleurotus ostreatus*

Effect of pH on catalase activity

The pH profile on the activity of catalase enzymes from mushroom was shown in fig.2. The optimum pH was 7.5 for *Pleurotus ostreatus* catalase and optimum pH was 7. All the catalase enzymes had a minimum activity at pH 3.5.

Effect of temperature on catalase activity

Purified catalase from *Pleurotus ostreatus* was stable in potassium phosphate buffer (pH 7) for more than 4 days at 4°C, as a decrease in activity observed after 5 days [Fig.3]. *Pleurotus ostreatus* catalase was completely inactive under heat treatment between 40-60°C for 30 minutes. This catalase enzyme has 28.08 units/ml of activity after being heated at 10-30°C for 60 minutes. The thermostability of *Pleurotus ostreatus* catalase was shown in [Fig.4].

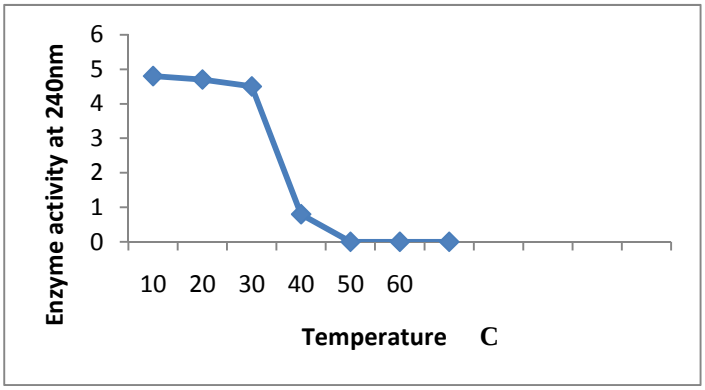

Fig. 3 Temperature stability of catalase enzyme from *Pleurotus ostreatus*

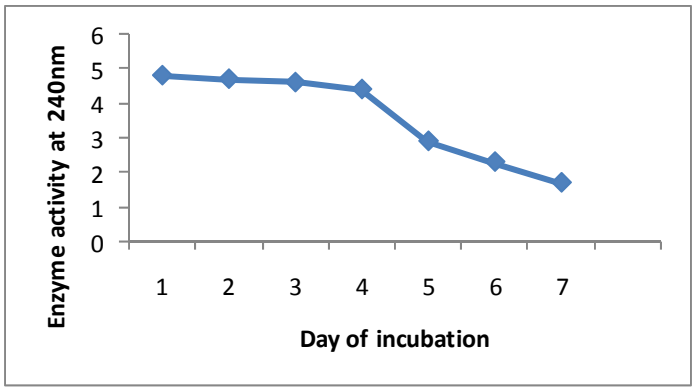

Fig. 4 Storage stability of catalase enzyme from *Pleurotus ostreatus*

DISCUSSION

The main aim of analyzing the phytochemical properties of mushroom is to determine its biochemical composition. In fact, the final status of a mushroom is conditioned by these compounds and the status of the catalase enzyme is really the result of interaction of these factors. The phytochemical screening of *Pleurotus ostreatus* extract have rich in flavonoids (aurones, chalcones, flavones, flavonols and leucoanthocyanins), saponins and tannins but absence of alkaloids. The alkaloids and flavonoids confirmed in the present studies were compared with those components observed by Basu *et al.*, 2007. Basu *et al.* studied that the presence of phytochemical components from *Pleurotus ostreatus* and suggested that all these have potential health promoting effects, at least under some circumstances.

Edible mushrooms contain high amount of proteins, and are excellent source of fibers, vitamins and minerals [Cheung, 1996]. Similarly, protein content of edible mushrooms taken for analysis revealed that the presence of total protein about 3.2µg/ml in *Pleurotus ostreatus.* Mattila *et al.* ,2002 also studied several species of mushrooms grown in Finland and reported that protein content of *P. ostreatus* and *L. edodes* were 24.6 and 21.4% of dry matter, respectively. From the observed results, it was concluded that higher amount of protein present in *Pleurotus ostreatus*.

Catalase contains four porphyrin heme groups that allow the enzyme to react with the hydrogen peroxide [Regelsberger *et al*., 2002]. Our study revealed the decomposition of hydrogen peroxide by catalase, the absorption decreased with time. The enzyme activity could be arrived at from this decrease. The peak specific activity of catalase was 13.8 U/mg observed for *Pleurotus ostreatus*. There was rise in specific activity in each purification step. Loewen and Switala, 1987 were observed the rise of specific enzyme activity in every purification step.

The molecular weight of purified catalase of *Pleurotus ostreatus* was 62 kDa. Molecular weight of *Saccharomyces cerevisiae* and *Escherichia coli* determined by gel filtration (66 kDa and 240 kDa) was in consonance with that obtained by SDS-PAGE reported by Leowen *et al*., 1987 and Ventsislava *et* al., 2002. There is no previous report about catalase molecular weight from mushroom. This may be the first kind of report about catalase from mushroom.

Since the separated enzyme appeared as a single band, it was concluded catalase enzyme to be tetrameric. From the reported data, it can be concluded that catalase varies in its MW depending upon the source from which it is isolated.

The optimum temperature and optimum pH of purified catalase from *Pleurotus ostreatus* enzymatic reaction was 30°C and pH 7.5, respectively. Rahul Singh *et al*., 2007 were studied on *Archaeoglobus fulgidus,* and reported similar charectrization.

CONCLUSION

Mushroom research revealed that it is used not only for its nutritive and medicinal values but many other bio-potentialities. Enzymes are widely used in medicinal applications. Antioxidant compounds play an important role in our body due to favorable effects on human health. Many medicinal properties have been attributed to mushroom catalase including inhibition of platelet aggregation reduction of blood cholesterol concentrations prevention or alleviation of heart disease and reduction of blood glucose levels, also prevention or alleviation of infections caused by bacterial, viral, fungal, and parasitic pathogens. Consumption of mushroom containing phytochemical with potential antioxidant properties can reduce the risk of human disease.

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