Enzymatic changes during the mycelial growth and yield of protein by the dikaryon and the protoplast fusant mycelia of *Pleurotus sajor-caju*

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J. Mycopathol, Res, 54(2) : 307 -309, 2016;
ISSN 0971-3719

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J. Mycopathol. Res. ***54(2)*** *: 307-309, 2016;* (ISSN 0971-3719)

SHORT COMMUNICATION

Enzymatic changes during the mycelial growth and yield of protein by the dikaryon and the protoplast fusant mycelia of *Pleurotus sajor-caju*

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Received : 13.10.2015 RMS Accepted : 16.03.2016 Published : 25.07.2016

In this study the enzymatic changes during the mycelial growth and yield of protein by the dikaryon and protoplast-fusant mycelia of *Pleurotus sajor-caju* under submerged culture was determined. The different enzymes were assayed during the mycelial growth, pin head formations and mature basidiocarp of the dikaryon and fusant protoplasts mycelia. The enzymes studied were β-glucosidase, cellulase, xylanase,laccase and polyphenol oxidase (PPO). The experimental data revealed that in the case of dikaryon mycelia β-glucosidase activity was maximum in the mycelia which was followed by pin head and then there was again increase in the basidiocarp. Similarly, the cellulase activity was maximumin the mycelia and minimum in the pin head and there was a rise in the basidiocarp. Xylanase, laccase and PRO activities were maximum in the mycelial stage followed by basidiocarp pin head stages. The data revealed in case of fusant protoplast mycelial stage followed by basidiocarp pin head stages. The data revealed in case of fusant protoplast mycelia a similar trend was observed though the activities of the enzymnes were more in comparison to dikaryon ones.

Key words: *Pleurotus sajor-caju,* dikaryon and protoplasts fusant mycelia, mycelia growth, yield of protein, enzymes.

Increasing population, high rate of industrialisation and urbanisation, lead to the increasing food crisis world over and undernourishment or malnutrition, especially in the developing and under developing countries. Among the malnutrition, the low protein content of the food is one of the most important factors. The animal protein is beyond the reach of majority of Indian population due to low economic condition.

In order to provide adequate amount of protein in the diet, new sources of protein rich foods have to be developed which can be produced economically on a large scale under such socio-economic condition. Cultivation of edible mushroom will be an ideal proposal to counteract the acute protein malnutrition. India is fast gaining its position as a major mushroom producing country in the world (about 35-40% annual growth rate). The cultivation of *Pleurotus* has increased significantly in the last few years. Out of the 15 species cultivated in India, *Pleurotus sajor-caju* (Fr.) Singer is cultivated extensively and commercially.

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In this study the effect of enzyme sources on the growth and yield of protein by the dikaryon and fusant mycelia of *P. sajor-caju* is studied.

Fresh and healthy basidiocarps of *P. sajor-caju* were collected from different localities of West Bengal during rainy season. The surface of the basidiocarps were cleaned with sterilized and distilled water and with 0.1% mercuric chloride solution and rinsed with sterile distilled water. From the pileus tissue of the freshly collected basidiocarps, tissue cultures were prepared on PDA medium. These PDA cultures were made pure by repeated subculturing and maintained in the laboratory under aseptic condition.

In order to determine the activities of enzymes the mycelia of the mushroom were grown in liquid medium for 15 days at 30^{o} C. After the incubation period the mycelia were harvested by filtration and the clear culture filtrate was used as source of the extracellular enzymes. The harvested mycelia were ground with cleaned sand in mortar and pestle (previously cooled in sodium acetate : acetic acid buffer (pH 5.2) for cellulase and 0.1M phosphate buffer (pH 6.0) for laccase enzyme. After grinding the extracts was refrigerated centrifuged for 17000 g for 20 minutes and the clear supernatant was taken for enzyme assay.

For cellulase assay 4.0 ml of 0.5% CMC was taken in each of a test tube and 1.0 ml of sodium acetate acetic acid buffer, (pH 5.2) and 2.0 ml of enzyme extract was added to it. The tubes were incubated at 30^{o} C in the water bath. At regular intervals 1.0 ml of this reaction mixture was withdrawn and the amount of reducing sugar released was determined.The enzyme activity was expressed as the amount of glucose released/ml of the enzyme extract/unit time.

For xylanase assay 4.0ml of 0.5% xylan was taken in each of a test tube and 1.0ml of sodium acetate acid buffer (pH 5.2) and 2.0ml of enzyme extract were added to it. The tubes were incubated at 30^{o} C in the water bath. At regular intervals 1.0ml of this reaction mixture was withdrawn and the amount of reducing sugar released was determined following the method of Dubois *et al,* (1956). The enzyme activity was expressed as the amount of glucose released/ml of the enzyme extract/unit time.

For laccase assay, 5.0 ml of 0.1M sodium phosphate buffer (pH 6.0) containing 10mM guaicol was taken in a test tube and 2.0 ml of enzyme extract was added to it. The reaction mixture was incubated at 25^{o} C for 5 minutes. After the incubation period the absorbance data was taken in a colorimeter at 470nm .The data are expressed as enzyme unit (1 unit= change in absorbance of 0.01 per minute).

For PPO assay, 5.0 ml of 0.1M sodium phosphate buffer (pH 6.0) containing 10 mM tyrosine was taken in a test tube and 2.0 ml of enzyme extract was added to it. The reaction mixture was incubated at 25^{o} C for 5 mins. After the incubation period the absorbance data was taken in a colorimeter at 470 nm.

The data are expressed as enzyme unit (1 unit= change in absorbance of 0.01 per minute). For â-glucosidase assay 0.4 ml of 0.1M acetate buffer (pH 5.0) containing 1.6 u mol cellobiose was taken in a test tube and 0.6 ug enzyme extract was added to it. The reaction mixture was incubated at 40^{o} C for 15 mins. After the incubation period the reaction was stopped by placing the assay mixture in a boiling water bath for 5 mins. The enzyme activity was expressed by glucose liberated was estimated by the method of Somogyi (1952).

In this experiment different enzymes were assayed during the mycelial growth, pin head formations and mature basidiocarp of the dikaryon and fusant protoplasts mycelia of *P. sajor-caju*. The enzymes studied were β-glucosidase, cellulase, xylanase, laccase and polyphenol oxidase.

The experimental data are given in Tables 1 and 2

The data in Table 1 revealed that in the case of dikaryon mycelia β-glucosidase activity was maximum in the mycelia which was followed by pin head and then there was again increase in the basidiocarp. Similarly, the cellulase activity was also maximum in the mycelia and minimum in the pin head and there was a rise in the basidiocarp. Xylanase, laccase and PPO activities were maximum in the mycelia stage followed by basidiocarp and pin head stages.

The data in Table 2 revealed that in the case of fusant protoplasts mycelia a similar trend was observed through the activities of the enzymes, they were more in comparison to an dikaryon ones.

Table 1 : Activities of different enzymes during mycelial growth, pin head production and mature basidiopcarp production by the dikaryon mycelium of *Pleurotus sajor-caju*

Enzyme Activity	Unit	Dikaryon mycelia of *Pleurotus sajor-caju*		
		Mycelial growth	Pinhead production	Mature basidiocarp
β-glucosidase	u $h^{-1}ml^{-1}$	28.00	13.00	18.00
Cellulase	u g^{-1}	0.62	0.48	1.44
Xylanase	u h^{-1} ml^{-1}	0.66	0.48	0.62
Laccase	u $min^{-1}ml^{-1}$	0.82	0.52	0.74
PPO	u $min^{-1}ml^{-1}$	0.76	0.58	0.67

Table 2 : Activities of different enzymes during mycelial growth, pin head production and mature basidiopcarp production by the fusant protoplast mycelium of *Pleurotus sajor-caju*

Enzyme Activity	Unit	Fusant protoplast mycelia of *Pleurotus sajor-caju*		
		Mycelial growth	Pinhead production	Mature basidiocarp
β-glucosidase	u $h^{-1}ml^{-1}$	30.00	14.00	21.00
Cellulase	u g^{-1}	0.64	0.58	1.56
Xylanase	u h^{-1} ml^{-1}	0.78	0.51	0.67
Laccase	u $min^{-1}ml^{-1}$	0.86	0.58	0.78
PPO	u $min^{-1}ml^{-1}$	0.78	0.59	0.68

The experimental data revealed that the lytic enzyme mixture of Novozyme 234, cellulase and chitinase is most efficient for release of protoplasts from 4 days old mycelia of *Pleurotus sajor-caju* under the osmotic stabilizer of magnesium sulphate and maleate buffer and MYG medium.

The degradation of cellulase is a synergistic action of several enzymes to produce sugars which are utilized by the mycelia of mushrooms. In the present investigation the β-glucosidase and cellulase activitiesare found to be directly involved with the basidiocarp suggest its role in the development of the basidiocarp and as well as a function in the nutrition of *P. sajor-caju.* The increased activity of xylanese, laccase and PRO in the mycelia stage indicates that these enzymes are responsible for the degradation of lignocellulosic substrate in nature

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