Indian Journal of Agricultural Sciences **84** (8): 1022–3, August 2014/Short communication
https://doi.org/10.56093/ijas.v84i8.43143

Enzymatic properties of oyster mushroom (*Pleurotus florida*) exudate

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Received: 21 August 2013; Revised accepted: 6 May 2014

Key words: β-glucosidase, Cellulose, Endoglucanase, Oyster mushroom exudate, Pectinase, *Pleurotus florida*

Mushrooms are rich source of proteins, vitamins, minerals, natural antibiotics and popularly called as the vegetarian's meat. The oyster mushroom, *Pleurotus florida* Mont. is an efficient lignin- degrader, which can grow well on different types of lignocellulolosic materials in variable temperature conditions. The cell wall glucans are well known for their immunomodulatory properties, and the secondary metabolites act against bacteria and viruses (Jose and Janardhanan 2000). Extracts from mushroom mycelia are active against protozoa such as the malarial parasite *Plasmodium falciparum* (Lovy *et al*. 1999).

During spawn formation stage, sometimes exudate production is quite significant and accumulates as droplets on the growth medium. Volz (2000) has reported antitumor and antimicrobial property of the exudate from *Pleurotus ostreatus*. The present study reports biochemical and enzymatic properties of the exudate of *Pleurotus florida* grown on mango peel waste.

Pleurotus florida obtained from Pathology lab of CISH, Lucknow was multiplied and spawn was prepared as per method described by Yadav *et al.* (2012). Mango peel residue and wheat straw in 10:1 ratio were soaked in water to get a final moisture of 30%, packed in polypropylene bags (35 cm × 45 cm size) and steam sterilized at 121° C for 20 min (Bano and Srivastava 1962). Multi layered technique was adopted for spawning @ 2% inoculums on wet weight basis of substrate and kept in chamber maintained at 25 ºC and 80% RH, with sufficient light and ventilation for 21 days. The polypropylene bags were torn off and the exudate appearing as reddish-brown droplets of fluid on the surface of spawn run were collected and analysed.

The total soluble solid (TSS) of exudates was measured using Brix refractometer. The antioxidant property juice in terms of FRAP values was measured as per method developed by Benzie and Szeto (1999). The protein concentration was estimated as per Lowry *et al*. (1951), while the xylase, pectinase, amylase, β glucosidase, endoglucanase and cellulase activities were assayed as per method of Wood and Bhat (1998). To 0.4 mL of 1% substrate in 0.05M acetate buffer (pH 5.0), 0.1 mL of culture filtrate was added and incubated at 35°C for 60 min followed by addition of 0.5mL double distilled water and reaction termination with 1.0 mL of DNS reagent and incubating at 100°C for 10 min. The absorbance was recorded at 550 nm. The enzyme activity (units/g of substrate) was calculated as the amount of enzyme required to release one micromole equivalent of glucose per minute under assay condition.

The pectinase specific activity was optimized at temperature (25, 35, 45, 55, and 65°C) in 0.05M sodium acetate buffer with pH 5.5; pH (3.0, 4.0, 5.0 and 6.0) in 0.05M sodium acetate buffer at 35 °C. The experiment was laid in two factor CRD design using values in triplicate and data was subjected to statistical analysis applying statistical package for agricultural workers developed by O P Sheoran of CCSHAU, Hisar (www.hau.ernet.in/opstat.html.)

Pleurotus florida produced reddish-brown fluid exudate under spawn run conditions, which accumulated as droplets in bags after 21 days representing the biodegradation potential of the mushroom. The TSS of the exudate was found to be 1.2 °Brix. Jose and Janardhanan (2000) reported that fruiting bodies of *P. florida* showed IC_{50} (inhibition concentration 50%) value 496 μg/ml indicating antioxidant potential in terms of lipid peroxidation inhibition activity. In the present study, the P. *florida* exudate possessed total antioxidant value as FRAP value (ferric reducing-antioxidant power) of 920 mM/mL and 1.67 mg/mL protein . The high value of protein might be due to presence of large number of enzymes in the exudate. The enzymatic analysis of the crude exudate revealed dominance of pectinase over other enzymes since former exhibited maximum activity (0.35 U/mL) followed by amylase (0.15 U/mL) and cellulase (0.12 U/mL). In addition, little β- glucosidase (0.08 U/mL) and endoglucanase (0.05 U/mL) activities were also recorded (Table 1). High pectinase activity in exudate might be because of the fact that mango peel is a rich source of pectin (Garg and Ashfaque 2010). Optimization studies revealed that maximum pectinase activity (1.36 U/mL) was achieved

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Table 1 Enzyme profiling of *Pleurotus florida* exudate

Enzyme	Enzyme activity (U/mL) ± Standard deviation
Xylase	0.002 ± 0.001
Pectinase	0.355 ± 0.023
Amylase	0.148 ± 0.015
β-Glucosidase	0.082 ± 0.034
Endoglucanase	0.050 ± 0.018
Cellulase	0.119 ± 0.018

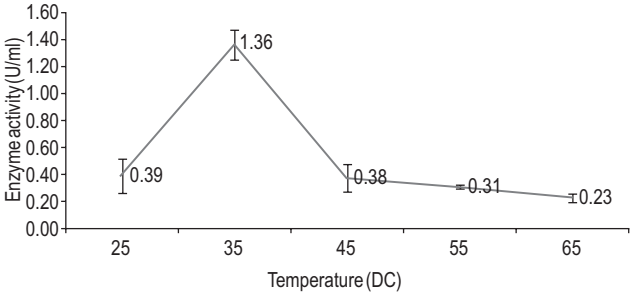

Fig 1 Optimization of temperature for pectinase of *Pleurotus florida* exudates

at a temperature of 35°C. The activity was fairly low at 25°C (0.39 U/mL) and 45°C (0.38 U/mL) (Fig 1). Similarly, the peak of pectinase activity was observed at 5.0 pH (2.22U/mL) and declined sharply to reach zero activity at pH 6.0. Kinetic values of pectinase in terms of Michaelis-Menten Constant (Km), velocity max. (V_{max}) were found to be 0.54 mg/mL and 0.25 mole/mL/min confirming higher activity of pectinase over the other enzymes.

The results of the present investigations indicate that exudate of *Pleurotus florida* exhibited maximum pectinase activity at 35°C temperature and 5.0 pH. It may, therefore, be stated that *Pleurotus florida* exudate possess antioxidant as well as enzymatic properties and may be used as a source of enzymes.

ACKNOWLEDGMENT

The research was funded by AMAAS networking project of ICAR.

SUMMARY

Pleurotus florida Mont. exudate, appearing as reddish-brown droplets of fluid was characterized for enzymatic properties. Oyster mushroom exudate grown on mango peel residue and wheat straw in 10:1 ratio at 25±1°C possessed high antioxidant value (920 mM/mL) and exhibited pectinase, cellulase, β-glucosidase and endoglucanase activities. Predominant enzyme activity was identified as pectinase with maximum activity of 0.35 U/ml. The temperature of 35 ºC and pH 5.0 were found optimum for maximum pectinase specific activity. Kinetic analyses of pectinase in terms of Michaelis-Menten constant (Km), velocity maximum (V_{max}) were 0.54 mg/ml, 0.25 mole/ml/min, respectively. This is first report of mushroom exudate enzyme characterization.

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