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Production of Xylanase Enzyme by *Pleurotus eryngii* and *Flamulina velutipes* Grown on Different Carbon Sources under Submerged Fermentation

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Abstract: The ability to produce xylanlytic enzyme in submerged (SF) fermentation by *Pleurotus eryngii* and *Flamulina velutipes* was checked first time. The cultivation in identical culture conditions revealed wide differences among xylanase production by both species. Among the carbon sources used, the maximum xylanase was produced by *Flamulina velutipes* 5.3 IU/ml (Xylose) and *Pleurotus eryngii* 6.83 IU/ml (starch) in comparison to other carbon sources. This study pointed out that the both organisms are capable to produce sufficient amount of xylanlytic enzymes.

Key words: *Pleurotus eryngii* · *Flamulina velutipes* · Xylanase · Production · Culture conditions

INTRODUCTION

Recently, extensive research on basidiomycetous fungi has been conducted with aim to isolate new organisms with tremendous secretion of ligninolytic enzymes as well as enzymes with potential industrial applications [1, 2]. The major enzymes associated with lignin-degrading ability of white rot fungi are lignin peroxidase, manganese peroxidase, laccase and xylanases. Some white rot fungi produce all these enzymes while others produce only one or two of them. *Flamulina velutipes* and *Pleurotus eryngii* (oyster mushrooms) comprises the group of edible fungi with important medicinal properties, biotechnological and environmental applications. They are able to produce the relevant hydrolytic (cellulases and hemicellulases) and oxidative (ligninolytic) extracellular enzymes required to degrade the major components of lignocellulosic biomass into the low molecular weight compounds that can be assimilated for fungi nutrition [3-5]. However, very little or no work is reported on xylanolytic enzymes production by Pleurotus eryngii and Flamulina velutipes, but especially little attention has been given to the evaluation of the hydrolytic system of these fungi.

Since the biotechnological applications require large amounts of low cost enzymes, one of the appropriate approaches for this purpose is to utilize the potential of lignocellulosic wastes. Some lignocellulosic waste may contain significant concentrations of soluble carbohydrates and induces of enzyme synthesis ensuring efficient production of ligninolytic enzymes [6, 5, 7].

This paper deals with the evaluation of xylanolytic enzyme activity of Pleurotus eryngii and Flamulina velutipes in submerged fermentation using various carbon sources of different composition.

MATERIALS AND METHODS

Fungal Strain: In this study, mushroom strains *Pleurotus eryngii* and *Flamulina velutipes* were purchased from Edible Fungi Institute, Shanghai Academy of Agricultural Sciences, Shanghai, China. Stock cultures of these fungi were maintained on Potato dextrose agar slants at 4°C.

Inoculum Preparation: The inoculum was prepared by growing mushrooms on a rotary shaker 120rpm and at 27 ± 2°C in 250ml Erlenmeyer flasks containing 50ml of following synthetic medium (per liter) 6.0g glucose, 0.2g yeast extract, 0.5g peptone, 1.0g KH_2PO_4 and 0.5g $MgSO_4$•$7H_2O$. The medium was adjusted to pH 5.5 with 0.2M NaOH. After 4 days of cultivation mycelial pellets of *Pleurotus eryngii* and *Flamulina velutipes* were harvested and homogenized with a laboratory blender.

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Culture Conditions: Submerged fermentation has been carried out on a rotary shaker 120 rpm and at 27±2°C in 250ml Erlenmeyer flasks containing 50ml of above-mentioned medium with various carbon sources (0.2, 0.4 and 0.6% concentrations g/L) instead of glucose. The initial pH of the medium was adjusted to 5.5 prior to sterilization by adding 0.2M NaOH. 1.0 ml of mycelial homogenate was used to inoculate the Erlenmeyer flasks containing media. After 96 hours of mushrooms cultivation (when cultures reached end of logarithmic stationary phase of growth), the extracellular enzymes were checked from culture broth. The micelial biomass was separated by filtration through whatman filter paper No.1 and followed by centrifugation (5000g; 15min) at 4°C. Reducing sugar in the culture broth was determined by Miller [8] method and the final pH was measured by WPA pH meter.

Enzyme Assay: Xylanase activity was determined by mixing 0.5ml sample (broth) with 0.5ml of oat to xylan (Fluka, Germany) (1% w/v) in 50mM citrate buffer (pH 5.3) at 60°C for 15min [9]. Xylose standard curve was used to calculate the xylanase activity. In assay the release of reducing sugars was measured using the dinitrosalicylic acid reagent method [8].

One International unit of enzyme activity was defined as the amount of enzyme, releasing 1 mol of reducing group per minute per ml.

RESULT AND DISCUSSION

The production of metabolites by different organisms is highly effected by their growth, which is determined by the presence of the nutrients in the medium. Addition of different carbon sources will improve the nutritional value for the growth of *Pleurotus eryngii* and *Flammulina velutipes* grown on different carbon sources and results shown in Table-1. The highest xylanase production was obtained with 0.2% xylan and xylose as sole carbon sources for both species. Glucose, starch and sucrose could not simulate enzyme production but they support the growth of the both species while rice husk and wood straw are not good inducers. These results obtained are in agreement with those reported by Techapun *et al.,* [10, 11].

Table 2: shows the supplementation of 0.4% carbon sources. Sucrose xylan and starch were moderate carbon sources for the xylanases production in both species while higher production of xylanase was achieved with xylose and glucose. As shown in Table-2, rice husk and wood straw are poor producers of xylanase.

Table 1: The effect of 0.2% Carbon Sources on the Production of Xylanase

	Flamulina velutipes			*Pleurotus eryngii*		
Carbon sources	*pH*	*R.Sugar*	*Enzyme Units/ ml*	*pH*	*R.Sugar*	*Enzyme Units/ ml*
Xylose	4.2	2.39	3.69	5.5	0.51	1.64
Glucose	4.2	1.66	3.34	7.5	1.87	0.46
Sucrose	5.5	0.99	1.16	6.6	0.39	1.21
Starch	6.4	2.35	2.68	6.5	0.69	1.44
Xylan	7.2	1.43	2.62	6.8	0.64	2.38
Rice husk	7.1	0.39	1.38	7.3	0.43	0.91
Wood straw	7.3	0.82	1.85	4.8	0.12	0.96

Table 2: The effect of 0.4% Carbon Sources on the Production of Xylanase

	Flamulina velutipes			*Pleurotus eryngii*		
Carbon sources	*pH*	*R.Sugar*	*Enzyme Units/ ml*	*pH*	*R.Sugar*	*Enzyme Units/ ml*
Xylose	6.8	1.97	4.31	4.4	1.84	5.37
Glucose	4.3	2.00	4.48	7.3	2.68	0.88
Sucrose	7.8	1.57	2.13	4.1	0.58	2.205
Starch	6.2	1.98	4.09	5.6	0.36	2.46
Xylan	7.6	2.12	3.06	6.9	0.77	3.77
Rice husk	7.6	0.37	1.66	7.0	0.406	1.82
Wood straw	7.6	0.41	0.94	4.8	0.201	0.83

Table 3: The effect of 0.6% Carbon Sources on the Production of Xylanase

	Flamulina velutipes			*Pleurotus eryngii*		
Carbon sources	*pH*	*R.Sugar*	*Enzyme Units/ ml*	*pH*	*R.Sugar*	*Enzyme Units/ ml*
Xylose	6.1	1.95	5.3	5.2	0.39	6.68
Glucose	4.3	1.97	5.11	6.7	2.73	0.92
Sucrose	4.4	1.98	4.32	5.4	0.18	6.53
Starch	6.9	1.96	4.12	5.1	0.59	6.83
Xylan	7.4	1.93	4.36	6.2	0.61	1.26
Rice husk	7.1	0.08	0.85	6.7	0.57	3.07
Wood straw	6.3	0.15	1.12	6.6	0.31	0.85

The effect of 0.6% carbon sources on the production of xylanbase was checked. The higher production of xylanase was achieved with xylose and starch respectively in *Flammulina velutipes and Pleurotus eryngii* as shown in Table-3, rice husk and wood straw are poor producers of xylanase. It is reported that that the type and size of carbon sources were an important factor in xylanase production [12].

CONCLUSION

The results obtained from this comparative study that significant improvement of xylanase production by Pleurotus eryngii and Flamulina velutipes could be obtained by selective use of carbon source. Since oat spelt xylan is an expensive substrate in comparison to starch, so it could be used as a less expensive carbon source for xylanase production.

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