Production of cellulolytic enzymes by a mushroom—Stereum ostrea

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Abstract

Stereum ostrea (S. ostrea) is a colorful mushroom belongs to Stereaceae, Basidiomycota. It is inedible due to its tough, leathery texture and is often called as ‘False turkey tail’. Since S. ostrea has not been studied yet. The aim of this study was to analyze its ability to produce cellulolytic enzymes – filter paper assay, carboxy methyl cellulase and β-D-glucosidase under submerged conditions by an unexplored organism. Though growth and secretion of extracellular protein by S. ostrea were comparable to those of Phanerochaete chrysosporium (P. chrysosporium), but activities of cellulolytic enzymes by S. ostrea were greater than P. chrysosporium. Stereum ostrea exhibited activities of cellulolytic enzymes higher than the reference culture P. chrysosporium. Yields of β-D-glucosidase by S. ostrea were higher than β-D-glucosidase titres of P. chrysosporium by more than 3 folds on the peak production time interval (6th day of incubation). S. ostrea yielded titres of 5.51 units of β-D-glucosidase/ml as against 1.40 units of β-D-glucosidase/ml on the 6th day of incubation. S. ostrea appears to be a promising culture with complete cellulolytic system.

Key words: S. ostrea, P. chrysosporium, FPase, CMCase, β-D-glucosidase

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1. Introduction

Cellulose is a linear polymer of glucose subunits linked together by β-1,4-glucosidic bonds with the degree of polymerization up to 15000. In wood cell wall cellulose forms microfibrils and fibers stabilized by hydrogen bonds between hydroxyl groups of the adjacent cellulose chains. Hemicelluloses are heteropolysaccharides, which comprise of β-1,4-linked polysaccharide backbone with different degree of substitution. The carboxyl groups of hemicellulose are covalently bonded with lignin via ether and benzyl ester linkages [1]. The main hemicelluloses in softwood and hardwood are galactoglucomannan and arabinogluconoxylan, respectively. Depending on the species, wood contains extractives from 2 to 5% of dry weight [2]. Extractives comprise of triglycerides, fatty acids, resin acids, steryl esters, and phenolic substances, which provide the wood resistance towards microbial attack [3]. Phanerochaete chrysosporium and Trametes versicolor have been the focus of intensive research and a greater understanding of physiology, biochemical and molecular biology of lignocellulolytic enzymes in the above organisms have been gained [4]. Much of the knowledge gained about lignocellulolytic enzymes has emanated from work on Phanerochaete chrysosporium (P. chrysosporium). No reports are available on cellulolytic enzymes of Stereum ostrea (S. ostrea). This is the first report on production of cellulolytic enzymes by S. ostrea. Moreover, the enzyme levels are very high as compared to P. coccineus and C. versicolor [5]. Hence, the present work focuses on the distribution of cellulolytic enzymes in the least understood white-rot fungus S. ostrea in comparison to the reference culture P. chrysosporium.

2.0. MATERIALS AND METHODS

2.1. Microorganism and growth medium

S. ostrea was kindly supplied by Prof. M.A. Singaracharya,
Department of Microbiology, Kakatiya University, Andhra Pradesh, India and was isolated from wood logs. The reference culture, *P. chrysosporium* was obtained from IMTECH, India. Both the cultures were maintained on Koroljova-Skorobogat’Ko medium [6]. Sterile Koroljova-Skorobogat’Ko medium was dispensed into sterile 250 ml Erlenmeyer flasks at a rate of 50 ml of medium per flask. The flasks were inoculated with homogenized mycelial suspension and incubated in an orbital shaker (Orbitek, Chennai, India) at 30°C and speed of 200 rpm. The flasks with growing cultures of *S. ostrea* and *P. chrysosporium* were withdrawn at different time intervals during the course of the experiment for processing. The entire culture medium in flasks was used for processing in the same manner as mentioned earlier [7]. The fungal cultures were aseptically filtered through preweighed Whatman No.1 filter paper to separate mycelial mat and the culture filtrate. The filter paper along with mycelial mat was dried at 70°C in an oven until constant weight. Difference between the weight of the filter paper having mycelial mat and weight of only filter paper represented biomass of fungal mat. Fungal growth was expressed in terms of mg/flask. pH of the culture filtrate was measured. Content of extracellular protein in culture filtrates of both fungi was estimated according to Lowry et al. [8].

### 3.0 Enzyme Assay

Activities of cellulytic enzymes in the cultural filtrate of both fungal cultures were estimated following the standard protocols.

#### 3.1. Filter paper assay

Filter paper degrading activity of cellulase (FPAase) is a measure of total cellulytic activity resulting from combined action of different enzyme components present in the culture filtrate [9-10]. Whatman filter paper strip with 50 mg weight was suspended in one ml of 0.05M sodium citrate buffer (pH 4.8) at 50°C in a water bath. Suitable aliquots of enzyme source with/without dilution was added to the above mixture and incubated for 60 minutes at 50°C. Enzyme blank (without enzyme) were run simultaneously in the same manner as specified above. After incubation, addition of 3,5-dinitrosalicyclic acid (DNS) was made and the contents were mixed. All samples, enzyme blanks and glucose standards were vigorously boiled for exactly 5 minutes in a boiling water bath. After cooling colour developed in tubes were read at 540nm in a Spectronic 20-D-Spectrophotometer (Milton Roy). Activity of cellulase was expressed in filter paper units. One unit of filter paper unit (FPU) was defined as the amount of enzyme releasing one μmole of reducing sugar from filter paper per minute.

#### 3.1.1. Assay of carboxy methyl cellulase

The reaction mixture contained 1.0ml of 1% carboxy methyl cellulose in 0.2 M acetate buffer (pH 5.0). This reaction mixture was pre-incubated at 50°C in a water bath for 20 minutes. An aliquot of enzyme source with appropriate dilution was added to the reaction mixture and incubated at 50°C in a water bath for 60 minutes. Appropriate controls devoid of substrate or enzyme were simultaneously run. The reducing sugar produced in the reaction mixture was determined by dinitrosalicyclic acid method [11]. 3, 5-Dinitrosalicyclic acid reagent was added to aliquots of the reaction mixture and the colour developed was read at wavelength 540 nm in a Spectronic 20-D-Spectrophotometer (Milton Roy). One unit of endoglucanase activity was defined as the amount of enzyme releasing one μmole of reducing sugar per minute.

#### 3.1.2. Assay of β-D-glucosidase

Activity of β-D-glucosidase activity in the culture filtrate was determined according to the method of [12]. β-D-glucosidase (EC 3.2.1.21) activity was measured in assay mixture containing 0.2 ml of 5 mM p-nitro phenyl β-D-glucopyranoside (PNPG) (Merck)dissolved in 0.05 M citrate buffer (pH 4.8) and 0.2ml of diluted enzyme solution with appropriate controls. After incubation for 30 min at 50°C, the reaction was stopped by adding 4 ml of 0.05M NaOH-glycine buffer (pH 10.6). The yellow colored p-nitrophenol liberated was determined in Spectronic 20-D-Spectrophotometer (Milton Roy) at 405nm. β-D-glucosidase activity was defined as the amount of enzyme liberating one μmole of p-nitrophenol per minute under standard assay conditions.

### 4.0. Results

*S. ostrea* and *P. chrysosporium* were grown in liquid Koroljova-Skorobogat’Ko medium under shaking conditions was analyzed and presented in (Figure 1). Growth of the both cultures was increased with increase in incubation time. *S. ostrea* produced maximum dry mycelium weight of 1.25 g/flask on the 10th day of incubation as against 1.05 g/flask in respect of *P. chrysosporium*. The secretion of protein content into liquid medium under shaking conditions for 10 days was measured (Figure 2). The secretion of protein content by both fungal cultures increased with increase in incubation time and thence onwards dropped. *S. ostrea* secreted maximum protein content of 990µg/ml into medium as against 756µg/ml by *P. chrysosporium* on 6th day of incubation.

The selected fungal cultures were tested in liquid Koroljova medium to find out whether cellulytic enzymes – FPass, CMCase and β-D-glucosidase are present in *S. ostrea*. All the three enzymes were detected in the culture filtrate of *S. ostrea* and *P. chrysosporium* throughout the incubation period (Table 1). There was increase in enzyme activity of FPass, CMCase and β-D-glucosidase up to 6th day of incubation followed by declining trend. Maximum enzyme activity of FPass was recorded in respect of *S. ostrea* and *P. chrysosporium* on 6th day of incubation were 4.67 and 0.84 U/ml respectively. *S. ostrea* displayed activity of even FPass
TABLE 1: Activities of cellulolytic enzymes by fungal cultures

<table>
<thead>
<tr>
<th>Incubation Period</th>
<th>S. ostrea (U/ml)</th>
<th>P. chrysosporium (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FPase</td>
<td>CMCase</td>
</tr>
<tr>
<td>II</td>
<td>0.46</td>
<td>0.72</td>
</tr>
<tr>
<td>IV</td>
<td>1.10</td>
<td>0.89</td>
</tr>
<tr>
<td>VI</td>
<td>4.67</td>
<td>4.15</td>
</tr>
<tr>
<td>VIII</td>
<td>2.64</td>
<td>2.81</td>
</tr>
<tr>
<td>X</td>
<td>1.15</td>
<td>0.89</td>
</tr>
</tbody>
</table>

5.0. Discussion

Cellulase enzyme is responsible in releasing cellobiose from long cello-oligomers and cellulose, to be further converted to glucose by the action of beta-glucosidases [9]. As for white-rot fungi, cellulase synthesis is induced by cellulose and repressed by glucose [12]. Since in the presence of glucose, ligninolytic enzymes production seemed to be induced [13] and in turn cellulase production was repressed, the present study demonstrated parallel relationship between lignolytic and cellulase activities. It is also found that cellulase activities (Table 2) produced from S. ostrea culture showed higher activities rate than P. chrysosporium culture. This indicates that S. ostrea had the capacity to degrade lignin preferentially with limited attack on cellulose. This preferential degradation is useful in an environmentally friendly biotechnological delignification process in paper pulp manufacturing [14]. Cellulose degradation by fungi is generally a result of the induction of a family of cellulolytic enzymes which could be classified into three major classes: endoglucanases, exoglucanases (cellobiohydrolases) and glucosidases [15]. It was well established that the degradation of cellulose by white rot fungi, similar to that of other fungal cellulases, was carried out by a multicomponent enzyme complex in which the individual components interacted synergistically to degrade cellulose to glucose. The precise mechanism/s of cellulase induction is not known. The most accepted view of the induction process was that the organisms produce a basic level or a constitutive amount of cellulase that subsequently produce soluble hydrolysis products of cellulose that function as inducers. Cellobiose, a product of cellulase action, both induced and inhibited cellulase of P. chrysosporium [9].

Thus these enzyme activities clearly indicated that the S. ostrea was relatively more cellulolytic than the reference culture P. chrysosporium. But cellulolytic activities of these two cultures were high when compared to yields of cellulase enzyme by cellulolytic cultures such as Trichoderma viridae [16], Chaetomium globosum [17] Aspergillus niger [18] in submerged fermentation. The productions of cellulolytic enzymes by these cultures exceed 3 FPU/ml. The white-rot basidiomycetes have a capability to produce simultaneously the hydrolytic and ligninolytic enzymes in fermentation of lignocellulose [19-21]. It is clear from the present study that S. ostrea and the reference culture P. chrysosporium possesses cellulolytic activity.
6.0. Conclusion

The white-rot fungus *S. ostrea* produces a complete cellulolytic system – filter paper assay, carboxy methyl cellulase and β-D-glucosidase under submerged conditions. This study revealed that β-D-glucosidase production from *S. ostrea* was the highest compared to other cellulolytic enzymes produced indicating it was induced in a nitrogen rich condition. For production of cellulolytic enzymes, *S. ostrea* culture is more promising and potential culture than the reference culture *P. chrysosporium.*

References


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