

Cellulase and Xylanase Production by *Pleurotus* sp. on Mixed Substrate System

Premkumar G^{1*}, Muthuramkumar S¹, Kannan M², Varatharaju G³ and Rajarathinam K¹

¹Department of Botany, Virudhunagar Hindu Nadar's Senthikumara Nadar College (Autonomous), Virudhunagar, India

²Department of Zoology, Virudhunagar Hindu Nadar's Senthikumara Nadar College (Autonomous), Virudhunagar, India

³Department of Microbial Technology, School of Biological Science, Madurai Kamaraj University, Tamilnadu, India

Abstract

In this study, *Pleurotus florida* and *Pleurotus djamor* is used for solid state fermentation studies and sugarcane bagasse, wheat bran and sugarcane bagasse with wheat bran (in different ratio) are used as the substrate. The fermentation studies are carried out in 7 days intervals up to 28 days. Sugarcane bagasse and wheat brane has the highest degradation of cellulose and hemicelluloses. The mixed substrate (sugarcane bagasse with wheat bran) has comparatively low degradation of cellulose and hemicellulose. *P.djamor* plays better performance in cellulase and xylanase production. Cellulase production is enhanced in sugarcane bagasse by *P.djamor* mean while xylanase production is enhanced in wheat bran by *P.djamor*. Both *P.djamor* and *P.florida* has obstructed on cellulase and xylanase production on mixed substrate (sugarcane bagasse mixed with wheat bran)

Keywords: *P.florida*; *P.djamor*; Cellulose; Xylanase; Fermentation

Introduction

Pleurotus sp. are wood destroying saprophytic fungi which occur widely in the tropical and temperate zones [1]. Species of *Pleurotus* are commonly known as "Oyster Mushroom". *Psajor-caju*, *P.fabellatus*, *Perungii*, *P.citrinipileatus* are commonly cultivated [2]. Bioconversion of lignocellulosic residues through mushroom cultivation offers the potential of converting them into protein rich, palatable food [3]. As early as straw is found to be a good substrate for growing *Pleurotus* [4] since that time, it's use has been studied in most of the rice producing countries of the world. The ability of *Pleurotus* species to excrete hydrolyzing and oxidizing enzymes has enabled them to flourish over a wide range of natural lignocellulosic waste materials [5,6]. India has great future to cultivate mushrooms. It can be artificially cultivated on different agricultural waste like paddy straw, wheat straw, sugar cane bagasse and etc.. The artificial cultivation of oyster mushroom has not only economically efficient for farmers and has the ability to produce extracellular lignocellulolytic enzyme like cellulase, xylanase, lignin peroxidase, manganese peroxidase and laccase [7,8].

In recent years, the lignolytic degradation and extra-cellular enzyme production by *Pleurotus* sp. has been extensively studied. The enzyme excretion have varied during colonization and fructification stages of *Pleurotus* growth [9]. To overcome these crises, various methods have been used for the production of extra-cellular enzyme [10]. Enumerated the advantages of extra-cellular enzymes production by *Pleurotus* through Solid State Fermentation (SSF) [11]. Showed about ligninolytic and cellulolytic enzyme pattern and activities were influenced by substrate. Thus, the capacity of particular substrate to induce or increase production of lignocellulases is another factor that indirectly confers ability of extra-cellular enzymes production. The present study is focused on the production of cellulase and xylanase enzymes using *Pleurotus florida* and *P.djamor* on sugarcane bagasse, wheat bran and sugarcane bagasse mixed with wheat bran (co substrate) through Solid State Fermentation.

Materials and Methods

Pure cultures and maintenances

Pure culture of *Pleurotus florida* and *P.djamor* were obtained from Tamilnadu Agriculture University, Coimbatore, Tamilnadu, India. They are

maintained in Maltose Extract (ME) Medium, at pH 5.8 and incubated for at 25°C and are sub-cultured regular interval of three weeks.

Substrate preparation and inoculation

Agriculture residues, Sugarcane bagasse and wheat bran were procured locally and allowed to dry. The agricultural residues were milled and powders made which were passes through 1 mm sieve.

Determination of cellulose and hemicellulose

Cellulose is estimated colorimetrically by the method described by [12]. Pure cellulose is used as the standard and it is obtained from Sigma-Aldrich. Hemicellulose is estimated colorimetrically by the method described by [13]. Pure xylose is used as the standard and it is obtained from Sigma-Aldrich.

Solid state fermentation studies

Ten gram of the substrate by dry weight is taken in 250ml flask and 60% of moisture is set for the substrate with distilled water and sterilized at 121°C for 30min. After cooling, each flask is inoculated with five agar discs from the edges of actively growing colonies of *P.florida* and *P.djamor*. After inoculation, the flasks were incubated at 27 ± 1°C. SSF is carried out in once every seven days.

Enzyme extraction

Sodium citrate buffer is used for enzyme extraction. The substrate is squeezed with sodium citrate using cheese cloth to get the culture filtrate. This is used as an enzyme source and is stored in refrigerator at 0°C until use. Enzymatic assays are done in triplicate.

***Corresponding author:** Premkumar G, Virudhunagar Hindu Nadar's Senthikumara Nadar College (Autonomous), Virudhunagar, India, E-mail: premkumar@vhsnc.edu.in

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Cellulase and xylanase assay

Carboxymethylcellulose (low viscosity) and Oat spelt xylan were used as a substrate to determine the cellulase and xylanase activity respectively. The amount of reducing sugars released during the reaction time is determined by the DNS methods as mentioned by [14].

Result

In the present investigation, *Pleurotus florida* and *P.djamor* are used to evaluate the production of lignocellulolytic enzyme viz., cellulase and xylanase and efficiency of cellulose and hemicellulose degradation through solid state fermentation using Sugarcane bagasse, Wheat bran and mixture of Sugarcane bagasse with Wheat bran as a substrate. Five different composition of substrate is prepared by using Sugarcane bagasse with Wheat bran viz., 100% SB, 75% SB+ 25% WB, 50% SB+ 50% WB, 25% SB+ 75% WB, and 100% WB. In this study, cellulase and xylanase production is studied and also estimate the cellulose and hemicellulose during spawn run stage of *Pleurotus* growth.

Chemical composition of sugarcane bagasse and wheat barn

The future scope and importance of the fermentation biotechnology industry will depend upon the lignocellulose raw materials [15]. Therefore much attention is given to analysis of the chemical composition of lignocellulosic biomass (Table 1). Shows the Cellulose, Hemicellulose and Lignin composition of sugarcane bagasse and wheat bran. Sugarcane bagasse has higher cellulose and lignin contents. Wheat bran has attained highest hemicellulose content.

Degradation of cellulose and production cellulase by *P.flordia* and *P.djamor*

White rot fungi are the most efficient ligninolytic microorganisms, and they are excellent producers of ligninolytic enzymes in the

Substrate	Cellulose	Hemicellulose	Lignin
Sugarcane Bagasse	28.8	26.7	22.4
Wheat Bran	21.5	35.8	18.4

Table 1: Chemical composition of the substrates: (% dry weight).

fermentation of agricultural materials due to their compositions [16]. After one week growth period nearly 20% of cellulose content is degraded by *Pleurotus* sp. Sugarcane bagasse shows highest amount of cellulose degradation pattern by both species of *Pleurotus* on 28 days growth. At the same time, Wheat bran shows least cellulose degradation on 28 days growth. Mixture composition of sugarcane bagasse with wheat bran stood in mid-level in cellulose degradation by *Pleurotus* sp. at end. *P.djamor* shows superior in cellulose degradation in 100% sugarcane bagasse as a substrate (Table 2).

Among the two mushrooms, *P.djamor* produced more cellulase enzyme (Figure 1). During initial time, both mushrooms produces approximately same amount of cellulase enzyme. From 21st day onwards *P.djamor* shows superior on cellulase production on 100% sugarcane bagasse as substrate. Comparing to other substrate, sugarcane bagasse is most suitable substrate for cellulase enzyme production. Mixture compositions of sugarcane with wheat bran and wheat bran alone have no significant on cellulase production at the end of 28th days cultivation. But at the same till 14 days cultivation there is no significant difference in cellulase production.

Degradation of Hemi-cellulose and production cellulase by *P.flordia* and *P.djamor*

Hemicellulose is the second most abundant polysaccharide after cellulose. Hemicelluloses are biodegraded to monomeric sugars and acetic acid. Endo-1,4- β -xylanase and xylan 1,4- β -xylosidase are the important enzyme to degrade the hemicellulose. Accessory enzymes viz., xylanesterases, ferulic and p -coumaricesterases, α -1-arabinofuranosidases and α -4-O-methyl glucuronosidases also acting synergistically to efficiently hydrolyze the agricultural xylan and wood xylan [17].

Analysis of chemical composition of wheat bran indicated that wheat bran has the highest hemicellulose content than the sugarcane bagasse. Wheat bran nearly has 35% dry weight of hemicellulose content. Hemicellulose degradation process is also same to cellulose degradation. Here also after one week growth period nearly 20% of hemicellulose content is degraded by *Pleurotus* sp. At last nearly 65%

Substrate	<i>P.flordia</i>				<i>P. djamor</i>			
	7 th day	14 th day	21 th day	28 th day	7 th day	14 th day	21 th day	28 th day
100% Sugarcane Bagasse (SB)	20.62	32.45	52.45	65.5	21.25	35.45	57.54	68.45
75% SB+ 25% WB	18.75	25.45	32.45	58.25	18.25	29.45	39.45	65.25
50% SB + 50% WB	17.95	31.24	39.45	55.25	19.45	35.45	58.45	62.45
25% SB + 75% WB	19.54	28.45	38.45	57.70	18.75	25.45	49.45	61.75
100% Wheat Bran (WB)	21.45	32.45	41.45	49.50	22.45	35.45	49.45	55.75

Table 2: Degradation percentage of cellulose content in the substrate after *Pleurotus* sp. growth.

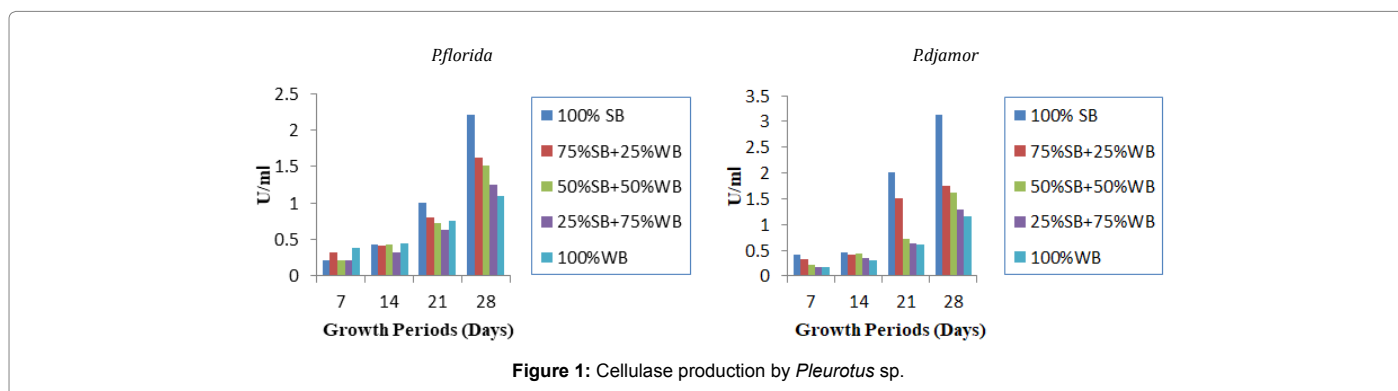


Figure 1: Cellulase production by *Pleurotus* sp.

Substrate	<i>P.florida</i>				<i>P.djamor</i>			
	7 th day	14 th day	21 th day	28 th day	7 th day	14 th day	21 th day	28 th day
100% Sugarcane Bagasse (SB)	22.45	35.45	55.55	65.50	25.45	35.50	54.50	65.75
75% SB+ 25% WB	24.25	34.45	51.25	62.25	25.45	35.45	50.25	63.45
50% SB + 50% WB	25.25	35.50	48.45	58.45	26.45	38.45	49.25	61.25
25% SB + 75% WB	23.15	38.45	49.2	52.45	24.25	31.45	51.45	60.75
100% Wheat Bran (WB)	20.50	31.45	45.45	49.25	21.45	32.45	52.75	67.45

Table 3: Degradation percentage of hemi-cellulose content in the substrate after *Pleurotus* sp. growth.

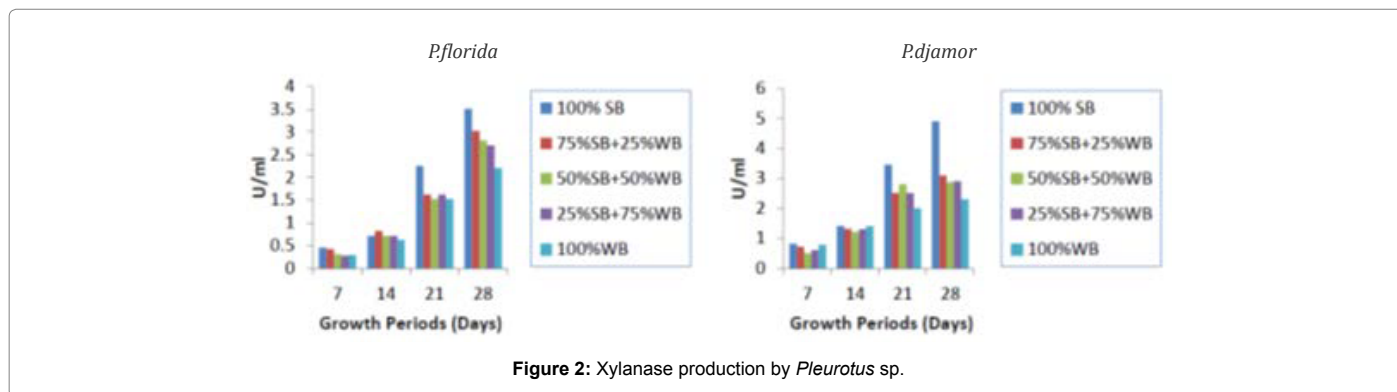


Figure 2: Xylanase production by *Pleurotus* sp.

of hemicellulose content is degraded by two *Pleurotus* sp. In difference, *P.florida* attained 65.5% of hemicellulose degradation when substrate as 100% sugarcane bagasse at the end of 28th day culture. In contrast, *P.djamor* is attained 67.45% of hemicellulose degradation when wheat bran alone used as substrate Table 3. Degradation percentage of Hemicellulose content in the substrate after *Pleurotus* sp. growth. Xylanase production in *P.djamor* is found to be highest after 7 days of growth (Figure 2). 100% Sugarcane bagasse supported maximum xylanase production when compared with mixture sugarcane bagasse and wheat brone alone. Xylanase production by *P.florida* also steadily increased during the growth.

Discussion

Two different species of *Pleurotus* species are compared for their ability to produce lignocellulolytic enzyme viz, cellulase and xylanase on sugarcane bagasse, wheat brone and mixture of sugarcane bagasse with wheat bran. The main aim of the most research works is the bioconversion of plant residues into valuable products. In plant residues, lignin content is the important parameter for the digestibility of lignocelluloses materials [18]. In this study, sugarcane bagasse shows highest lignin content compare to wheat borne [19,20] are also reported that sugarcane bagasse contains more lignin content when compare to wheat bran.

The production of cellulase and xylanase enzyme and digestion of cellulose and hemi-cellulose by *P.florida* and *P.djamor* may be influence the lignin content of the substrate. Cellulase production is more on when sugarcane bagasse alone used as substrate by *P.florida* and *P.djamor*. Mean while cellulase production is impeded on wheat bran alone and mixture of sugarcane bagasse with wheat bran by *P.florida* and *P.djamor*. Similarly xylanase production was also more on sugarcane bagasse and lesser on wheat bran and sugarcane bagasse with wheat bran by *Pleurotus* sp. Many reports suggested and point out the role of *Pleurotus* sp. in cellulase and xylanase production on agricultural residues [21] also reported that mixes of agro waste are neutralized the optimization of cellulase and xylanase production [22]. Suggested that enzyme activity is strongly depends upon the composition of the

substrate [23]. Pointed out the role of lignin in the substrate on the production of cellulase and xylanase.

Conclusion

In this study, *P.djamor* has played dominant role in cellulase and xylanase production and also sugarcane bagasse is the most suitable substrate for cellulase production. In contrast, wheat bran is the most suitable substrate for xylanase production. Meanwhile, the sugarcane bagasse with wheat bran has no significant effect for enzyme production.

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