www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(10): 337-340

www.thepharmajournal.com
Received: 29-08-2018
Accepted: 30-09-2018

Tanvi
Ph.D, Department of Microbiology, CCS Haryana Agricultural University, Hisar, Haryana, India

Sneh Goyal
Principal Scientist, Department of Microbiology, CCS Haryana Agricultural University, Hisar, Haryana, India

Correspondence
Tanvi
Ph.D, Department of Microbiology, CCS Haryana Agricultural University, Hisar, Haryana, India

Lignocellulose degradation by lignocellulolytic fungi: Influence of carbon and nitrogen sources

Tanvi and Sneh Goyal

Abstract
This study investigates the potential of lignocellulolytic fungi to produce lignocellulolytic enzymes under varying conditions of carbon and nitrogen sources. The four fungal isolates tested, showed significant production of lignocellulolytic enzymes under submerged conditions. Among two carbon sources used for cellulase production, carboxymethyl cellulose powder at 200 mg L^{-1} concentration was giving better results with isolate HST16 giving maximum FP ase and CM Case activity of 0.026 IU ml^{-1} and 0.006 IU ml^{-1} respectively. For ligninolytic activity, alkali lignin was used as carbon source and maximum enzyme activity was observed at 200 mg L^{-1} concentration with 9 IU ml^{-1} lac, 48.5 IU ml^{-1} LiP and 1.5 IU ml^{-1} Mn P activity by the fungal isolate HST16. Maximum lignocellulolytic enzyme activity occurred when ammonium sulfate was used as a nitrogen source. HST16 was giving maximum FPase (0.013 IU ml^{-1}), CM Case (0.013 IU ml^{-1}), lac (12 IU ml^{-1}), Lip (45.60 IU ml^{-1}) and Mn P activity (4 IU ml^{-1}). These results suggested that the carbon and nitrogen sources could be optimized for the growth and activity of lignocellulolytic fungi and these fungi could be exploited for compost preparation.

Keywords: Lignocellulolytic, carbon source, nitrogen source, alkali lignin, carboxymethyl cellulose powder

1. Introduction
Composting is a biological process in which organic matter undergoes many biochemical reactions particularly oxidation reaction to finally produce carbon dioxide, water and partially humified organic matter (He *et al*., 1992) [8]. The effectiveness of the process greatly depends on the nature of the substrate to be composted. In general, the C:N ratio decides the compostability of the substrate with optimal values between 25 to 30 parts carbon to one part nitrogen (25-30:1). Most of the plant residues with appropriate C:N ratio are composed of cellulose, hemicelluloses and lignin. The latter compound is difficult to biodegrade and hence restricts the availability of other polymers. However, lignin is the main compound involved in the formation of humus like substances therefore lignin biotransformation during composting is very crucial for lignocellulosic residues.

Many indigenous microorganisms of soil work in this direction and degrade cellulose, hemicelluloses and lignin polymers into simpler units. Among these, white rot fungi which are eukaryotic microorganisms belonging to the basidiomycetes group of fungi, can degrade cellulose and lignin by a fascinating developmental process of fruiting bodies formation (Sun and Cheng, 2002; Mtui, 2009) [16, 12]. They have exclusive complex enzymatic machinery for lignocelluloses transformation (Madhavi *et al*., 2009) [10]. Generally, they have two types of extracellular enzymatic systems, the first system which produces hydrolases that are responsible for polysaccharide degradation is known as hydrolytic enzyme system and second is the ligninolytic system which degrades lignin and opens phenyl rings using a unique oxidative and extracellular pathway (Sanchez, 2009) [18]. Lignocellulose degrading microbes produce various and multiple forms of cellulases, hemicellulases, pectinases and ligninases (lignases) (Blanchette *et al*., 1998; Behrendt *et al*., 2000) [2, 3].

Besides composting, the lignocellulolytic enzymes have a wide variety of other applications. These enzymes are being used for breakdown of lignocellulosic materials into fermentable sugars which can be converted into valuable products like ethanol, lactic acid and butanol. Cellulases are already being used at pilot and semi-industrial plants during the hydrolysis of cellulose derived from lignocellulosic biomass for fuel ethanol production (Sánchez and Montoya, 2012) [17]. Similarly, ligninases are being used for delignification of agricultural waste for animal feed, to decolorize synthetic dyes contained in the wastewater released from textile industries.

The appropriate physicochemical conditions also play an important role in the enzyme production by fungi. Media composition strongly affect the production of ligninolytic and cellulolytic enzymes and the extent of lignocellulose degradation (Tanvi *et al*., 2018). Therefore, there are immense possibilities for further increasing the production of lignocellulolytic enzyme by fungi under highly optimized conditions of carbon and nitrogen sources. In this study, lignocellulolytic fungi were subjected to optimization of carbon and nitrogen sources for enhanced production of lignocellulolytic enzymes.

2. Materials and Methods

Soil samples were collected from different sites like mushroom cultivation area, compost, vermicompost and garbage dumping sites of CCS Haryana Agricultural University campus, Hisar and surrounding areas. Fungal isolates were obtained from these soil samples by serial dilution plate method using Potato Dextrose Agar (PDA) media (300 ml potato extract, 20.0g dextrose and 20.0g agar) in 1L of deionised water. Morphologically different fungi were selected and screened for their lignocellulolytic activity. The fungal isolates were maintained by periodic transfer on PDA slants. These isolates were stored at 4°C for further use.

2.1 Quantitative estimation of lignocellulolytic activity

Fungal isolates were inoculated into 25 ml malt extract broth in 50 ml capacity flask. All the inoculated flasks were incubated at 28±2°C for 7 days. The growth suspension was centrifuged at 4,000 rpm for 10 minutes in the REMI Research Centrifuge to separate the supernatant from the hyphal growth and other debris. Five enzyme activities namely, laccase (lac), lignin peroxidase (LiP), manganese peroxidase (MnP), exoglucanases (filter paper degrading activity) and endoglucanases (carboxymethyl cellulase activity) were measured. Laccase (Shandilya and Munjal, 1983) [19], lignin peroxidase (Tien and Kirk, 1988) [21] and manganese peroxidase (Paszczynski *et. al.* 1988) production were measured with alkali lignin as substrate. CMCase and FPase production were measured by the method described by Ghose (1987) [7]. Reducing sugars produced due to enzymatic actions were determined by 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). One International Unit (IU) of enzyme was defined as the amount of enzyme required to produce 1 µmol of reducing sugars per minute under the assay conditions.

2.2 Optimization of carbon and nitrogen sources for efficient lignocellulolytic enzyme production

Lignocellulolytic enzyme production was determined in malt extract agar medium at pH 6 and 28±2°C temperature. The amendments of different carbon sources and nitrogen sources were made to find out optimum conditions for efficient enzyme production. For measuring the effect of carbon sources, fungal isolates were inoculated in 25 ml of malt extract broth amended with 50 mg L^{-1}, 100 mg L^{-1}, 200 mg L^{-1} and 300 mg L^{-1} cellulose powder, carboxymethyl cellulose powder and alkali lignin. To understand the effect of nitrogen sources on enzymes produced, malt extract broth was prepared using different forms of nitrogen in varying concentrations (Table 1). All the inoculated flasks were incubated at 28±2℃ for 7 days. The amount of enzymes released in the broths was estimated after incubation in comparison with uninoculated control.

Table 1: Concentration of nitrogen sources used for determination of lignocellulolytic enzymes

Metal ions	Concentration (g L^{-1})	
	For cellulase activity	For ligninases activity
$MnSO_4$	0.003, 0.006, 0.009	0.25, 0.50, 1.00
$FeSO_4$	0.0025, 0.005, 0.0075	0.25, 0.50, 1.00
$ZnSO_4$	0.0015, 0.003, 0.0045	0.25, 0.50, 1.00

3. Results and Discussion

In this study four lignocellulolytic fungi HST11, HST14, HST15 and HST16 were isolated from soil samples collected from different ecological sites of CCS Haryana Agricultural University, Hisar and surrounding area (Table 2). Fungal isolates were examined for their ability to produce lignocellulolytic enzymes. The efficient lignocellulolytic fungi were further subjected to varying carbon sources and nitrogen sources to understand the release of lignocellulolytic enzymes from them. Similarly, Banakar and Thippeswamy in 2014 isolated 8 effective fungal isolates from the forest soil samples for the production lignolytic enzymes, using guiaicol as carbon source.

Table 2: Fungal isolates and their site of isolation

Fungal isolate	Site of isolation
HST11	Mushroom waste
HST14	Vermicompost
HST15	Dumping site soil
HST16	Compost

3.1 Optimization of carbon and nitrogen sources for efficient lignocellulolytic enzyme production

The four fungal isolates selected on the basis of their zone of clearance and I_{CMC}, were further tested for optimization of conditions for efficient lignocellulolytic enzyme production under varying conditions of carbon and nitrogen sources. Fascinatingly, HST11 and HST15 were not at all giving any cellulolytic activity. Carbon source is considered as one of the most important factor effecting the cost and yield of enzyme production (Gao *et al*., 2008) [6]. In cellulose powder amended broth, fungal strain HST16 was showing maximum FPase and CM Case activity of 0.013 IU ml^{-1} and 0.006 IU ml^{-1} at a concentration of 200 mg L^{-1}. When cellulose powder was replaced with CMC powder, it was found that enzyme activity was comparatively more for all the fungal isolates. Among the different concentrations of alkali lignin tested, maximum enzyme activity was observed at a concentration of 200 mg L^{-1} with 9 IU ml^{-1} lac, 48.5 IU ml^{-1} LiP and 1.5 IU ml^{-1} MnP activity by the fungal isolate HST16 (Table 3). These results are supported by Chemelova *et al*. in 2011 that used cellulose, hemicellulose, lignin, glucose, maltose and starch as carbon sources. Among the carbon sources tested, lignin supported the maximum laccase production and protein content however, cellulase activities were similar in medium with different carbon sources. Likewise, effect of substrate on enzyme production was also noticed by Naseeb *et al.*, 2015. They noted that carboxymethyl cellulose, salicin and xylan induced the production of endoglucanases, β- glucosidase and xylanase respectively.

When amendment of different forms of nitrogen sources was made to the broth, it was found that enzyme production by all the fungal isolates was higher in ammonium sulfate amended medium broth than in ammonium chloride, ammonium nitrate and ammonium oxalate containing broth (Table 4). HST16 was giving maximum FPase (0.013 IU ml^{-1}), CMCase (0.013

IU ml^{-1}), lac (12 IU ml^{-1}), Lip (45.60 IU ml^{-1}) and MnP activity (4 IU ml^{-1}). This stimulating effect of additional nitrogen on enzyme production was due to higher biomass production. The role of these additional nitrogen compounds in the regulation of enzyme synthesis is interlinked with the physiology of the tested fungi and also on the medium composition, especially on presence of lignocellulosic substrate (Couto *et al*., 2004; Kapich *et al*., 2004) [5, 9]. Many other nitrogen sources like ammonium phosphate, potassium nitrate, ammonium oxalate, sodium nitrate (inorganic sources) and different amino acids (organic sources) were used by Prasher and Chauhan in 2015. They found that inorganic sources supported high enzyme activity whereas; the fungus did not express any enzyme activity with any organic nitrogen source.

Table 3: Enzyme activities of selected fungal isolates at different concentrations of alkali lignin, CMC powder and Cellulose powder

Fungal isolate	Cellulolytic activities (IU ml^{-1})				Ligninolytic activities (IU ml^{-1})		
	FPase	CMCase	FPase	CMCase	Lac	LiP	MnP
50 mg L^{-1}							
	Cellulose powder		CMC powder		Alkali lignin		
HST11	-	-	-	-	1.500	1.500	0.500
HST14	0.009	0.000	0.014	0.000	1.000	2.000	0.000
HST15	-	-	-	-	0.500	2.500	1.000
HST16	0.010	0.000	0.019	0.001	3.000	4.500	0.000
C.D. at 5%	0.002	0.000	0.005	0.001	0.526	0.440	0.372
100 mg L^{-1}							
	Cellulose powder		CMC powder		Alkali lignin		
HST11	-	-	-	-	6.000	8.500	0.500
HST14	0.009	0.002	0.016	0.003	2.000	3.000	0.500
HST15	-	-	-	-	5.500	15.000	0.500
HST16	0.011	0.003	0.020	0.003	8.500	40.500	1.000
C.D. at 5%	0.002	0.001	0.008	0.001	0.499	2.499	0.372
200 mg L^{-1}							
	Cellulose powder		CMC powder		Alkali lignin		
HST11	-	-	-	-	8.500	10.000	0.500
HST14	0.012	0.005	0.019	0.007	4.750	5.000	1.000
HST15	-	-	-	-	6.500	18.000	2.000
HST16	0.013	0.006	0.026	0.006	9.000	48.500	1.500
C.D. at 5%	0.001	0.003	0.002	0.001	1.558	3.478	0.499
300 mg L^{-1}							
	Cellulose powder		CMC powder		Alkali lignin		
HST11	-	-	-	-	7.000	9.000	0.500
HST14	0.010	0.004	0.019	0.006	4.000	4.000	1.000
HST15	-	-	-	-	6.000	17.500	1.500
HST16	0.011	0.006	0.021	0.006	7.000	48.000	1.000
C.D. at 5%	0.003	0.001	0.004	0.001	1.499	2.470	0.440

Table 4: Enzyme activities of selected fungal isolates at different concentrations of ammonium salt supplementations (0.5 g L^{-1})

Fungal isolate	Cellulolytic activities (IU ml^{-1})		Ligninolytic activities (IU ml^{-1})		
	FPase	CMCase	Lac	LiP	MnP
a) Ammonium chloride					
HST11	-	-	1.100	4.750	0.500
HST14	0.011	0.004	0.500	3.500	0.000
HST15	-	-	1.500	12.000	1.000
HST16	0.008	0.009	2.000	42.500	1.000
C.D. at 5%	0.002	0.001	1.034	5.519	0.407
b) Ammonium sulfate					
HST11	-	-	2.600	5.000	1.500
HST14	0.012	0.005	1.500	3.000	1.000
HST15	-	-	1.100	17.000	1.500
HST16	0.013	0.013	12.000	45.600	4.000
C.D. at 5%	0.004	0.001	0.478	5.065	0.470
c) Ammonium nitrate					
HST11	-	-	3.600	4.000	0.500
HST14	0.003	0.008	4.000	2.690	0.500
HST15	-	-	5.000	13.700	1.000
HST16	0.010	0.013	0.000	40.000	0.000
C.D. at 5%	0.003	0.002	0.402	5.362	0.288
d) Ammonium oxalate					
HST11	-	-	4.000	1.000	1.000
HST14	0.006	0.003	6.500	1.100	1.000
HST15	-	-	2.750	10.500	1.500
HST16	0.010	0.009	5.000	41.500	2.000
C.D. at 5%	0.002	0.002	0.381	4.373	0.576

4. Conclusion

The lignocellulosic plant biomass can be utilized for many Different applications in various industries like in paper and pulp industry, textile industry, biofuel generation, compost preparation and many more. Compost preparation requires digestion of lignocellulosic biomass to simple monomer units but the main hurdle in this process is the complex structure of lignin as it restricts the availability of other polymers. Recently, biodegradation of lignocellulosic material has emerged as a boon for the industries as well as environment. In this study, four lignocellulose degrading fungi were isolated from soil samples. They were further examined for optimization of carbon and nitrogen sources and it was found that maximum lignocelluloses degradation occurred at 200 mg L^{-1} concentration of carboxymethyl cellulose powder and alkali lignin in growth medium. Among all the nitrogen sources used, ammonium sulfate proved to be better nitrogen source. These results suggested that efficient lignocelluloses degrading strains could be further exploited for their maximum use under field conditions by regulating its nutritional conditions.

5. Acknowledgement

We greatly acknowledged the Department of Microbiology, College of Basic Sciences and Humanities, CCSHAU Hisar, Haryana, India for the help and support during execution of the experiments. We are highly thankful to the anonymous reviewers for their comments and suggestions which greatly enhanced the quality of old version.

6. References

1. Banakar SP, Thippeswamy B. Isolation and partial purification of fungal ligninolytic enzymes from the forest soil fungi isolated from Bhadra Wildlife Sanctuary. Frontiers in Biology. 2014; 9(4):291-299.
2. Behrendt CJ, Blanchette RA, Akhtar M, Enebak S, Iverson S and Williams D. Biomechanical pulping with *Phlebiopsis gigantea* reduced energy consumption and increased paper strength. TAPPI Journal. 2000; 83:65.
3. Blanchette RA, Behrendt CD, Williams D, Iverson S, Akhtar M and Enebak SA. A new approach to effective biopulping: Treating logs with *Phlebiopsis gigantean.* 7th International Conference on Biotechnology in the Pulp and Paper Industry, 2000: A51-A54.
4. Chmelová D, Ondrejovič V, Ondáš V and Šturdík E. Influence of cultivation conditions on production of lignocellulolytic enzymes by *Ceriporiopsis subvermispora.* Biologia. 2011; 66(5):748 754.
5. Couto SR, Rosales E, Gundin M and Sanroman MA. Exploitation of wastes from the brewing industry for laccase production by two *Trametes* species. Journal of Food Engineering. 2004; 64:423-428.
6. Gao J, Weng H, Daheng Z, Yuan M, Guan F and Xi Y. Production and characterization of cellulolytic enzymes from the thermo acidophilic fungal *Aspergillus terreus* M11 under solid-state cultivation of corn stover. Bioresource Technology. 2008; 99:7623-7629.
7. Ghose TK. Measurement of cellulase activities. Pure and Applied Chemistry. 1987; 59:257-268.
8. He X, Traina SJ, Logan TJ. Chemical Properties of Municipal Solid Waste Composts. Journal of Environmental Quality. 1992; 21:318-329.
9. Kapich AN, Prior BA, Botha A, Galkin S, Lundell T and Hatakka A. Effect of lignocellulose-containing substrate on production of ligninolytic peroxidases in submerged cultures of *Phanerochaete chrysosporium* ME-446. Enzyme and Microbial Technology. 2004; 34:187-195.
10. Madhavi SR, Lele SS, Madhavi V, Lele SS. Laccase: properties and application. Bioresources. 2009; 4:1694-1717.
11. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry. 1959; 31:426-428.
12. Mtui GYS. Recent advances in pretreatment of lignocellulosic wastes and production of value added products. African Journal of Biotechnology. 2009; 8:1398-1415.
13. Naseeb S, Sohail M, Ahmad A, Ahmed KSA. Production of xylanases and cellulases by *Aspergillus fumigatus* MS16 using crude lignocellulosic substrates. Pakistan Journal of Botany. 2015; 47(2):779-784.
14. Paszczynski A, Huynhv B and Crawford R. Enzymatic activities of an extracellular, manganese dependent peroxidase from *Phanerochaete chrysosporium*. FEMS Microbiology Letters. 1988; 29:37-41.
15. Prasher IB and Chauhan R. Effect of Carbon and Nitrogen Sources on the Growth, Reproduction and Ligninolytic Enzymes Activity of *Dictyoarthrinium Synnematicum* Somrith. Advances in Zoology and Botany. 2015; 3(2):24-30.
16. Sun Y and Cheng J. Hydrolysis of lignocellulosic materials for ethanol production: A review. Bioresource Technology. 2002; 83:1-11.
17. Sánchez, OJ and Montoya S. Production of Bioethanol from Biomass: An Overview. In: Gupta VK, Tuohy M (Eds) Biofuel Technologies- Recent Developments. Springer Verlag Berlin Heidelberg, Berlin. 2012; 397-441.
18. Sanchez C. Lignocellulosic residues: Biodegradation and bioconversion by fungi. Biotechnology Advances. 2009; 27:185-194.
19. Shandilya JR, Munjal RL. Some experiments with casing layer. Tai-wan-Yang-ku: Taiw Mush. 1983; 7(1):32-38.
20. Tanvi, Chaudhary S, Dhanker R, Devi S and Goyal S. Optimization of Physical and Nutritional Factors for Enhanced Production of Lignocellulolytic Enzymes by *Aspergillus terreus* FJAT-31011 under Submerged Conditions. International Journal of Current Microbiology and Applied Sciences. 2018; 7(7):150-162.
21. Tien M, Kirk TK. Lignin peroxidase of *Phanerochaete chrysosporium.* Methods in Enzymology. 1988; 161:238-249.