Lignocellulolytic Enzyme Production from Submerged Fermentation of Paddy Straw

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Abstract Five strains of cellulolytic bacteria and four strains of *Phanerochaete chrysosporium* were evaluated for the lignocellulolytic enzyme production during submerged fermentation (SmF) of paddy straw. Extra-cellular enzyme assay for CMCase, FPase, Cellobiase, Xylanase, Lignin peroxidase and Laccase enzymes was performed after 7 and 15 days of submerged fermentation. *Cellulomonas cellulans* MTCC 23, *Cytophaga hutchinsonii* NCIM 2338 and *Phanerochaete chrysosporium* MTCC 787 were found to produce higher lignocellulolytic enzyme activities than rest of the cultures after 15 days of fermentation.

Keywords Paddy straw · Submerged fermentation · Lignocellulolytic enzymes

Introduction

Paddy straw is a lignocellulosic material that contains cellulose, hemicellulose and lignin as major components. In nature, strains of fungi and bacteria, including species of genera *Aspergillus*, *Fusarium*, *Phoma* and those of *Cytophaga*, *Vibrio*, *Polyangium*, *Cellulomonas*, *Streptomyces*, and *Nocardia* exhibit significant cellulolytic activities¹. Free living cellulolytic bacteria such as *Cellulomonas*, *Cellovibrio* and *Cytophaga* have been studied from the point of understanding the enzyme systems involved in cellulose degradation². Many white rot fungi have been studied for their lignin-biodegradation activity, out of which *Phanerochaete chrysosporium* brings about a gradual depolymerization of lignin³ and produces many types of lignolytic enzymes viz. lignin peroxidase, Mn-peroxidase and laccase.

Synergistic action of cellulolytic and lignolytic enzymes results in the degradation of lignocellulosic matter^{4,5}. The promising cellulolytic and lignolytic microorganisms can be employed for production of lignocellulolytic enzymes viz., endo- and exo-cellulases, β -glucosidase, hemicellulases, lignin peroxidases, manganese peroxidases and laccases, by using different agro-wastes as carbon source during submerged fermentation. Large amount of agrowastes, rich in lignocelluloses, are generated in bulk throughout the world. In India substantial quantities of crop residues⁶ (376 million tones) are produced every year. Among crop residues paddy straw is not suitable for animal feed because of its high silica content⁷. Keeping in view the above facts, the present investigation was undertaken to assess the lignocellulolytic enzyme activity of the selected microbial strains during submerged fermentation of paddy straw.

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Table 1 Source of microbial cultures employed for submerged fermentation of paddy straw.

S.No.	Name of culture	Strain	Collection centre
1.	<i>Cellulomonas cellulans</i>	MTCC 23	Microbial type culture collection, IMTECH, Chandigarh, India
2.	<i>Cellulomonas fimi</i>	MTCC 24	-do-
3.	<i>Cellulomonas turbata</i>	MTCC 960	-do-
4.	<i>Cellulomonas uda</i>	NCIM 2353	National Collection of Industrially Important Microorganisms, NCL Pune, India
5.	<i>Cytophaga hutchinsonii</i>	NCIM 2338	-do-
6.	<i>Phanerochaete chrysosporium</i>	MTCC 787	Microbial Type Culture Collection, IMTECH, Chandigarh, India
7.	<i>P. chrysosporium</i>	NCIM 1073	National Collection of Industrially Important Microorganisms, NCL, Pune, India
8.	<i>P. chrysosporium</i>	NCIM 1106	-do-
9.	<i>P. chrysosporium</i>	NCIM 1197	-do-

Table 2 Reese's mineral medium.

Components	·	Quantity (g L ⁻¹)
KH ₂ PO ₄	-	2.0
(NH ₄) ₂ SO ₄	-	1.4
Urea*	-	0.3*
MgSO ₄ ·7H ₂ O	-	0.3
CaCl ₂	-	0.3
FeSO ₄ ·7H ₂ O	-	5.0 mg
MnSO ₄ ·H ₂ O	-	1.6 mg
CoCl ₂	-	2.0 mg
ZnSO ₄ ·7H ₂ O	-	1.4 mg
Agar – agar	-	18.0
pH	-	7.0

*Urea was filter sterilized and added to the medium before inoculation

Materials and Methods

Microorganisms: Four strains of *Cellulomonas* spp., one strain of *Cytophaga* spp. and four strains of *Phanerochaete chrysosporium* were procured from different culture collection centers in India (Table1). Bacterial cultures were maintained on Beef extract (0.1%) agar medium while fungal cultures on Malt extract (2%) agar medium.

Fermentation medium: Five gram dried chopped (1-2 cm) paddy straw suspended in 50 ml Reese's mineral solution⁸ (Table2) was inoculated @ 0.5% (v/v) bacterial culture after three days growth on beef extract agar medium. One mycelial disc (6.0mm dia.) each of three days old fungal cultures was used as inoculum in separate flasks.

Enzyme Assay: The activity of cellulases (FPU, CMCase and cellobiase) was estimated in the culture filtrates after 7

and 15 days submerged fermentation of paddy straw by the method described by Ghose *et al.*⁹ Xylanase was estimated by the method of Bailey *et al.*¹⁰ Lignin peroxidase and laccase was assayed in the culture filtrates by the method of Tien and Kirk¹¹ and Munoz *et al.*¹² respectively.

Results and Discussion

Among four strains of *P. chrysosporium*, only two strains (NCIM 1073 and MTCC 787) were found to possess higher CMCase (0.36 – 0.42 IU ml⁻¹), FPU (0.55 – 0.88 IU ml⁻¹) and cellobiase (1.07 – 1.18 IU ml⁻¹) activity after 15 days of submerged fermentation of paddy straw. Among bacterial cultures, *Cellulomonas cellulans* MTCC 23 demonstrated highest FPU (0.72 IU ml⁻¹) and cellobiase (1.05 IU ml⁻¹) activity while maximum CMCase (0.38 IU ml⁻¹) activity was recorded in *C. uda* NCIM 2353 (Table 2). In general an increase was recorded in all the three cellulases and xylanase activity from 7 days to 15 days fermentation in all the nine cultures as shown in Table 3.

Cellulase is an enzyme complex containing chiefly endo- β -glucanases (FPU/CMCase) and exo- β -glucanases (CMCase/FPU) and cellobiase. For complete hydrolysis of insoluble cellulose (paddy straw), synergistic action between components of the enzyme complex is required¹³. Results of cellulases' activity are in conformity with that of Lakshmikanth¹⁴. Kashem *et al.*¹⁵ have reported CMCase activity (13.78–82.68 μ g ml⁻¹) in *Cellulomonas cellulosa* with different carbon sources. They also reported maximum CMCase in presence of galactose and maximum FPase and cellobiase in presence of lactose as carbon source.

Cytophaga hutchinsonii: NCIM 2338 exhibited highest xylanase activity (0.659 IU ml⁻¹) after 7 days of SmF of

Table 3 Cellulolytic enzymes produced during submerged fermentation of paddy straw.

Cultures	CMCase*		FPU*		Cellobiase*	
	7days	15days	7days	15days	7days	15days
<i>Cellulomonas cellulans</i> MTCC 23	0.135	0.384	0.294	0.720	0.632	1.053
<i>C. fimi</i> MTCC 24	0.175	0.161	0.226	0.573	0.301	0.356
<i>C. turbata</i> MTCC 960	0.029	0.281	0.270	0.347	0.307	0.720
<i>C. uda</i> NCIM 2353	0.188	0.349	0.188	0.364	0.327	0.740
<i>Cytophaga hutchinsonii</i> NCIM 2338	0.076	0.280	0.284	0.523	0.198	0.904
<i>P. chrysosporium</i> MTCC 787	0.110	0.429	0.403	0.883	0.328	1.184
<i>P. chrysosporium</i> NCIM 1073	0.222	0.365	0.443	0.557	0.340	1.079
<i>P. chrysosporium</i> NCIM 1106	0.104	0.151	0.301	0.355	0.376	1.062
<i>P. chrysosporium</i> NCIM 1197	0.155	0.183	0.314	0.412	0.367	0.982
SEm±	0.005	0.010	0.009	0.064	0.004	0.005
CD at 5%	0.016	0.030	0.026	0.184	0.011	0.016

*IU ml⁻¹.**Table 4** Xylanolytic and Lignolytic enzymes produced during submerged fermentation of paddy straw.

Cultures	Xylanase*		Lignin peroxidase#		Laccase#	
	7days	15days	7days	15days	7days	15days
<i>Cellulomonas cellulans</i> MTCC 23	0.380	0.368	ND	ND	ND	ND
<i>C. fimi</i> MTCC 24	0.425	0.298	ND	ND	ND	ND
<i>C. turbata</i> MTCC 960	0.316	0.234	ND	ND	ND	ND
<i>C. uda</i> NCIM 2353	0.291	0.191	ND	ND	ND	ND
<i>Cytophaga hutchinsonii</i> NCIM 2338	0.659	0.443	ND	ND	ND	ND
<i>P. chrysosporium</i> MTCC 787	0.500	0.669	0.857	1.477	ND	0.116
<i>P. chrysosporium</i> NCIM 1073	0.645	0.637	0.943	1.517	ND	0.131
<i>P. chrysosporium</i> NCIM 1106	0.425	0.472	0.787	1.073	ND	0.104
<i>P. chrysosporium</i> NCIM 1197	0.462	0.540	0.867	1.143	ND	0.101
SEm±	0.011	0.012	0.012	0.031	-	0.003
CD at 5%	0.032	0.034	0.034	0.091	-	0.007

*IUml⁻¹ #Uml⁻¹ (one unit = 0.1unit change in O.D. per min.).

paddy straw whereas *P. chrysosporium* strains MTCC 787 and NCIM 1073 expressed maximum xylanase activity (0.669–0.637 IU ml⁻¹) after 15 days SmF of paddy straw (Table 4). Cellulase and xylanase systems generally contain multiple enzyme components that have a marked synergism against hemicellulosic residues^{16,17}. *Cellulomonas* sp. and *Cytophaga* sp. which are aerobic cellulolytic bacteria, have been studied for their enzyme systems involved in cellulose degradation² but they are also producing xylanase because the saccharification of lignocellulosic material can be accomplished when both cellulases and hemicellulases act on them. Ponce-Noyola and Torre¹⁸ reported that both the wild type and mutant strain of *Cellulomonas flavigena* were able to produce three to eight time higher levels of FPase with sugarcane bagasse as compared with xylose or cellobiose. Thus, cellulolytic activity is also dependent on the nature of the substrate used for fermentation.

In present study *P. chrysosporium* NCIM1073 produced highest lignin peroxidase activity both after 7 and 15 days of SmF however laccase was recorded only after 15 days of SmF of paddy straw in all the four strains of *P. chrysosporium* (Table4). These finding are in confirmation with Srinivasan *et al.*¹⁹ and Puniya & Singh²⁰, who had reported simultaneous production of laccase, LiP and MnP by *P. chrysosporium* with cellulose as the carbon source.

Vijaya and Singaracharya²¹ employed five fungal species of *Aspergillus*, *Humicola*, *Penicillium*, *Monilia* and *Trichoderma* for solid state fermentation of paddy straw and reported higher lignocellulolytic enzymes production by supplementing 1% urea as nitrogen source.

Fungal laccases are involved in lignin degradation together with lignin peroxidase (LiP) and manganese peroxidase (MnP)²². Although production of laccase is a common characteristic of white rot fungi, lignolytic peroxidases have

received much attention. This is due to the fact that most extensively studied white rot fungus, *P. chrysosporium* does not produce detectable levels of laccase under the culture conditions generally employed in the laboratories³. Ahlawat and coworkers²³ have reported highest level of induction of β -glucosidase and Xylanase in *Volvariella volvacea* when grown on wild grass rather than wheat straw or paddy straw. They have also reported that *Volvariella volvacea* produced more cellulase at 8 – 10 days while Xylanase, laccase and phenol oxidase were more at 10 – 13 day growth on paddy straw. Similarly in present investigation higher LiP and laccase recorded after 15 days submerged fermentation

Thus these results indicate that cellulases, xylanase, lignin peroxidase and laccase are produced by cellulolytic bacteria and white- rot fungi when grown on paddy straw as a sole carbon source. Further, investigations are required for large scale production of lignocellulolytic enzymes from submerged fermentation of paddy straw.

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