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ORIGINAL PAPER

Biodegradation of paddy straw obtained from different geographic locations by means of *Phlebia* spp. for animal feed

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Abstract Various cereal straws are used as feed by supplementing the green forage or other feed stuffs. An experiment was designed to see the effect of different geographic locations and climatological conditions on biochemical constituents, fungal degradation and in vitro digestibility of paddy straw. Paddy straw (PS) obtained from three different geographic locations of India was subjected to solid state fermentation using four white rot fungi i.e. *Phlebia brevispora*, *P. fascicularia*, *P. floridensis* and *P. radiata*. Changes in the biochemical constituents like water soluble content, hemicellulose, cellulose, lignin, total organic matter, and in vitro digestibility of paddy straw was analyzed over a period of 60 days along with lignocellulolytic enzymes i.e. laccase, xylanase and carboxymethyl cellulase. All the fungi degraded the straw samples and enhanced the in vitro digestibility. The paddy straw, obtained from north western zone (NWZ) suffered a maximum loss (228 g/kg) of lignin by *P. radiata,* while a maximum enhancement of in vitro digestibility from 185 to 256 g/kg was achieved by *P. brevispora*, which also caused minimum loss in total organic matter (98 g/kg). In PS obtained from central eastern zone (CEZ) and north eastern zone (NEZ), a maximum amount of lignin (210 and 195 g/kg, respectively) was degraded by *P. floridensis* and resulted into a respective enhancement of in vitro digestibility from 172 to 246 g/kg and 188 to 264 g/kg. The study demonstrates that geographic locations not only affect the biochemical constituents of paddy straw but the fungal degradation of fibers, their in vitro digestibility and lignocellulolytic enzyme activity of the fungus may also vary.

Keywords Geographic locations · In vitro digestibility · Paddy straw · *Phlebia* species

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Introduction

A variety of agricultural residues including wheat straw, corn stover, sugarcane bagasse and paddy straw are generally used to supplement the green forage as animal feed depending upon the availability of the residues in a particular geographic region. Paddy (*Oryza sativa*) is an important cereal crop and generates a huge amount of straw. Humid subtropical and tropical wet and dry land including northern, eastern and southern zones of India are main paddy producer regions. Much less agricultural land is used for the forage cultivation resulting in forage crisis. Paddy straw is higher in ash content primarily including silica as compared to other agricultural residues. Some of the workers have reported that

silica and lignin are the primary limiting factors in determining the quality of rice straw as feed (Balasta et al. 1989); while others could not find any correlation between microbial degradation and ash or silica content (Agbagla-Dohnani et al. 2001, 2003). Van Soest (2006), well reviewed the role of silica in rice straw and reported that the silicon is a nutrient element and involved in several roles in rice i.e. carbohydrate synthesis, grain yield, phenolic synthesis and plant cell wall protection.

The higher concentration of acid detergent fiber (ADF) is responsible for the lower digestibility and poor nutritive value of agricultural residues. Lignin is the main constituent of ADF which is resistant to most of the microbial enzymatic systems, as well as non digestible by ruminants. The presence of lignin and its hemicellulose binding matrix increases the unavailability of other energy rich constituents present in the agricultural residues for the ruminants. Biodelignification of such agro-residues has got the potential to convert this biomass into more nutritive and easily digestible feed of good quality (Agosin et al. 1985; Bisaria et al. 1997; Jalc et al. 1996). Among a variety of microorganisms, selective ligninolytic white rot fungi are potentially useful organisms (Jalc et al. 1998; Karunanandaa and Varga 1996). Most studied white rot fungus *Phanerochaete chrysosporium* is quite an efficient lignin degrader but it also degrades an enormous amount of organic matter thus limiting its practical use for improvement of animal feed quality (Jung et al. 1992), while the pretreated straw may be useful for other purposes including biofuel production (Bak et al. 2009). It necessitates the need to look for organisms which can degrade the lignin but with lower loss of other important constituents like hemicellulose and cellulose. *Phlebia* species are good producers of different ligninolytic enzymes including laccase, lignin peroxidase and manganese peroxidase (Niku-Paavola et al. 1988; Vares et al. 1994; Arora and Gill 2005). *Phlebia* species were also found to degrade lignin more selectively and leave behind more organic matter available for ruminants (Sharma and Arora 2010).

Degradation of lignin and other components is specific to the source of lignocellulosic substrate and fungal species. The effect of environmental conditions in governing the fungal degradation of wood components is also reported (Tuor et al. 1995). The biochemical composition of the lignocellulosic substrate may also vary with respect to climate, season, soil quality, temperature and environmental conditions, which are responsible for the difference in their quality and digestibility (Ford et al. 1979; Ouédraogo-Koné et al. 2008; Whalley et al. 2008). In the present study, the bioconversion of PS into more nutritive animal feed by four different *Phlebia* species have been evaluated for PS samples, collected from three different geographic locations of India.

Materials and methods

Substrate collection

Samples of paddy straw (PS) were collected from three different geographic locations of India i.e. north western zone (NWZ) Amritsar (31°38′ N 74°52′ E, altitude 234 meters) Punjab, central eastern zone (CEZ) Bilaspur (22°6′ N 82°14′ E, altitude 264 meters) Chhattisgarh and north eastern zone (NEZ) Gorakhpur (26°48′ N 83°23′ E, altitude 209 meters) Uttar Pradesh.

Organisms

Four white rot fungi *P. brevispora* (HHB-7030), *P. fascicularia* (FP- 70880), *P. floridensis* (HHB-5325) and *P. radiata* (MJL-1198) used in the present study were received from T. W. Jeffries (Forest Product Laboratories, Madison, USA). The cultures were maintained by regular subculturing on yeast extract glucose agar (YGA) slants and stored at 4°C as well as at −80°C in 10% (v/v) glycerol.

Experimental setup

Paddy straw obtained from different regions was ground (particle size 2 ± 0.5 mm), washed and dried at 90°C. Five gram of dried sample was taken in 250 ml conical flask and moistened with 25 ml of malt extract (0.5%, w/v). The flasks containing substrate were sterilized at 1.06 kg/cm^2 for 15 min and inoculated with three mycelial discs (7–8 mm) per flask, grown on YGA plates for 5–6 days. The inoculated flasks were incubated at 25°C. Triplicate set of flasks for each organism was processed at 10, 20, 30 and 60 days along with uninoculated control.

Enzyme extraction was done as described earlier (Arora et al. 2002). The contents of each flask were filtered on a tared filter paper and dried at 90°C till constant weight. Loss in total organic matter (TOM) was calculated from the difference between the blank and inoculated flasks. Dried residue was used for further analytical tests.

Enzyme assays

Laccase (1.10.3.2) activity was measured according to previously described method (Arora and Gill 2005). Laccase activity was measured by using guaiacol as a substrate and expressed in colorimetric units per ml (CU ml^{-1}). Xylanase (EC 3.2.1.8) and carboxymethyl cellulase (CMCase) (EC 3.2.1.4) activity was determined by measuring the release of reducing sugars using dinitrosalicylic acid (DNS) method (Miller 1959), using birch wood xylan and carboxymethyl cellulose as substrate, respectively. The activity was represented as μM of xylose (xylanase) or glucose (CMCase) equivalent released per minute per ml by using xylose or glucose standard curve.

Analytical methods

A sequential fractionation was carried out for the estimation of various components of PS according to Datta (1981) with slight modifications (Sharma and Arora 2010). One gram of PS was suspended in 100 ml distilled water, kept at 100°C for 2 h in a water bath and filtered through a tared crucible, residue was dried at 90°C till constant weight and the loss was considered as water soluble part. Dried residue was suspended in 100 ml of 0.5 M H_2SO_4 and after keeping it at 100°C for 2 h in a water bath, the contents were filtered, dried and weighed as described in the first step and the loss was represented as hemicellulose. For cellulose and lignin, the dried residue was mixed with 10 ml of 72% H_2SO_4 and kept at 30°C for 1 h on a rotary shaker at 200 rpm. The contents were then diluted up to 4% concentration of H_2SO_4 and autoclaved at 1.06 kg/cm^2 for 40 min. Thereafter the contents were filtered, dried and weighed according to first step and the loss was considered as cellulose while residue was considered as lignin.

In vitro digestibility

In vitro digestibility of uninoculated and fungal treated PS was estimated according to Akhter et al. (1999), as described earlier (Sharma and Arora 2010). Fecal inoculum was prepared by mixing fresh fecal matter of cow (100 g/l) in pre warmed (39°C) artificial saliva [$NaHCO_3$ 9.80 g, $Na_2HPO_4 \cdot 7H_2O$ 7.00 g, KCl 0.57 g, NaCl 0.47 g, $MgSO_4 \cdot 7H_2O$ 0.12 g and 1 ml $CaCl_2$ (4%, w/v) in 1,000 ml of distilled water] and filtered through four layered muslin cloth. Five hundred milligram PS was taken in 50 ml centrifuge tube and suspended in 35 ml of fecal inoculum. After flushing with CO_2 gas, these tubes were kept at 39°C for 48 h in a water bath. Supernatant was discarded and 35 ml of acidified pepsin was added to the residue. Tubes were again incubated at the same conditions for 48 h and then residue was filtered on a tared filter paper and dried. The weight loss in dry matter during the processing has been expressed as in vitro digestibility.

Statistical analysis

Data was represented as mean value and significance was established by ANOVA and Pearson's correlation.

Results

Paddy straw obtained from three different geographic regions suggested certain variations in their biochemical constituents (Table 1). PS obtained from NWZ and NEZ was lower in lignin content (203 and 205 g/kg, respectively) and showed higher in vitro digestibility as compared to the straw collected from central eastern zone (CEZ), having a relatively higher lignin content (225 g/kg) and poor in vitro digestibility. Solid state fermentation of straw obtained from different regions was carried out with four different *Phlebia* spp. *viz. P. brevispora, P. fascicularia, P. floridensis* and *P. radiata* for 60 days. All the tested fungi were able to grow on PS under the experimental conditions and degraded lignin selectively by degrading relatively low levels of other components i.e. hemicellulose, cellulose etc. thus resulting in minimum loss in total organic matter.

Table 1 Biochemical constituents of paddy straw (g/kg) collected from different geographic locations of India

	NWZ	CEZ	NEZ
Water solubles	104 ± 5	85 ± 3	94 ± 3
Hemicellulose	292 ± 7	315 ± 5	335 ± 8
Cellulose	400 ± 5	372 ± 6	365 ± 8
Lignin	203 ± 4	225 ± 7	205 ± 5
Digestibility	185 ± 4	172 ± 4	188 ± 5

Each value represents mean of four individual samples along with standard deviation

NWZ north western zone, *CEZ* central eastern zone, *NEZ* north eastern zone

Solid state fermentation of paddy straw collected from north western zone (NWZ)

Maximum loss in TOM (139 g/kg) was caused by *P. radiata* during 60 days of incubation followed by *P. floridensis* and *P. fascicularia* while *P. brevispora* caused minimum loss in TOM (98 g/kg) (Table 2). Maximum water solubles (125 g/kg) were liberated from PS inoculated with *P. radiata* on 30th day without any subsequent change during next 30 days, whereas *P. fascicularia* released the similar amount of water solubles during 20 days. Hemicellulose was degraded maximally by *P. radiata* (216 g/kg) followed by *P. fascicularia* (196 g/kg) and *P. floridensis* (188 g/kg) whereas *P. brevispora* degraded only 128 g/kg during 60 days of incubation. Maximum cellulose degradation was caused by *P. radiata* (134 g/kg) followed by *P. floridensis*, *P. fascicularia* with a respective loss of 114 and 103 g/kg whereas *P. brevispora* degraded least amount of cellulose (87 g/kg). During initial 10 days of incubation, cellulose degradation was not detected in PS inoculated with different fungi. Lignin degradation was maximum in *P. radiata* (228 g/kg), which was insignificantly ($P > 0.05$) followed by *P. floridensis* (218 g/kg). *P. fascicularia* and *P. brevispora* degraded 210 and 195 g/kg of lignin, respectively. *P. brevispora* enhanced the in vitro digestibility of PS from 185 to 256 g/kg followed by *P. radiata* (248 g/kg), *P. fascicularia* (237 g/kg) and *P. floridensis* (232 g/kg).

All the fungi showed laccase, xylanase and CMCase activity (Fig. 1). *P. floridensis*, *P. radiata* and *P. brevispora* showed maximum laccase activity on 30th day, while *P. fascicularia* showed lower laccase activity. Maximum xylanase activity (3.69 and 3.32 μmol/min/ml) was shown by *P. radiata* on 30th and 60th day of incubation, which was statistically insignificant ($P > 0.05$) and followed by *P. fascicularia* while *P. floridensis* and *P. brevispora* showed maximum xylanase activity on 60th day. Maximum CMCase activity was shown by *P. radiata*, *P. floridensis* and *P. brevispora* on 30th day, which ranged from 3.80 to 3.92 μmol/min/ml (statistically insignificant $P > 0.05$), while *P. fascicularia* showed maximum activity on day 60th.

Solid state fermentation of paddy straw collected from central eastern zone (CEZ)

A maximum amount of TOM (147 g/kg) was degraded by *P. floridensis* while *P. brevispora* and *P. radiata* caused a similar loss of 101 g/kg, which was followed by *P. fascicularia* (68 g/kg) during 60 days of incubation (Table 2). Maximum water solubles were liberated by *P. radiata* (145–148 g/kg) during 20 and 30 days of incubation followed by *P. floridensis* (142 g/kg, 20 days). Maximum hemicellulose (184 g/kg) was degraded by *P. floridensis* whereas *P. fascicularia* degraded only 111 g/kg of hemicellulose. Except *P. radiata,* none of the fungi was able to degrade cellulose up to a detectable level during initial 10 days, which however was maximum in PS inoculated with *P. floridensis* (167 g/kg) during 60 days of incubation and closely followed by *P. radiata* (163 g/kg). A maximum amount of 210 and 208 g/kg of lignin was degraded by *P. floridensis* and *P. radiata*, respectively, which was followed by *P. brevispora* (198 g/kg) and *P. fascicularia* (104 g/kg). In vitro digestibility was maximum in PS inoculated with *P. floridensis* (246 g/kg) followed by *P. brevispora* (238 g/kg) and *P. radiata* (235 g/kg), while *P. fascicularia* enhanced the in vitro digestibility up to 227 g/kg.

A significantly ($P < 0.05$) higher laccase activity (2.70 CU/ml) was shown by *P. floridensis* on 20th day of incubation. *Phlebia fascicularia* and *P. radiata* also showed maximum laccase activity during the same period of incubation, while *P. brevispora* showed a maximum laccase activity on 30th day. All the organisms gave a similar xylanase activity ranging between 0.90–0.98 μmol/min/ml (statistically insignificant $P > 0.05$) on 30th day of incubation, except *P. fascicularia,* which showed maximum activity on 60th day. *Phlebia floridensis* and *P. brevispora* followed a similar pattern of CMCase activity, which

Table 2 Changes in biochemical constituents of paddy straw (g/kg) obtained from north western zone (NWZ); central eastern zone (CEZ) and north eastern zone (NEZ) during 60 days of its solid state fermentation with white rot fungi

Organisms	*P. brevispora*				*P. fascicularia*				*P. floridensis*				*P. radiata*				SE	A	B
Days of Incubation	10	20	30	60	10	20	30	60	10	20	30	60	10	20	30	60			
NWZ																			
TOM loss	15^{a}	37^{b}	57^{d}	98^{f}	19^{a}	35^{b}	86^{e}	107^{fg}	14^{a}	47^{c}	79^{e}	108^{g}	20^{a}	51^{cd}	96^{f}	139^{h}	9.8	*	***
Water solubles	100^{abcd}	95^{ab}	107^{de}	116^{fg}	98^{ab}	125^{h}	92^{a}	114^{ef}	97^{abc}	105^{cd}	119^{fgh}	116^{fg}	115^{ef}	102^{bcd}	125^{h}	124^{gh}	4	NS	NS
Hemicellulose loss	15^{ab}	28^{bc}	70^{e}	128^{f}	17^{abc}	28^{bc}	133^{f}	196^{g}	10^{a}	32^{c}	132^{f}	188^{g}	10^{a}	48^{d}	128^{f}	216^{h}	18.2	NS	***
Cellulose loss	ND	8^{a}	15^{ab}	87^{g}	ND	15^{ab}	40^{d}	103^{h}	ND	20^{b}	50^{e}	114^{i}	ND	30^{c}	67^{f}	134^{j}	11.2	*	***
Lignin loss	10^{a}	29^{bc}	106^{d}	195^{g}	11^{a}	33^{bc}	142^{e}	210^{h}	5^{a}	27^{b}	180^{f}	218^{hi}	7^{a}	40^{c}	140^{e}	228^{i}	21.7	NS	***
Digestibility	189^{cd}	195^{e}	228^{i}	256^{l}	180^{a}	182^{ab}	212^{g}	237^{j}	182^{ab}	193^{de}	202^{f}	232^{i}	186^{bc}	189^{cd}	217^{h}	248^{k}	6.3	*	***
CEZ																			
TOM loss	21^{ab}	58^{d}	88^{e}	101^{ef}	11^{a}	20^{a}	45^{c}	68^{d}	31^{b}	62^{d}	111^{f}	147^{h}	44^{c}	97^{e}	101^{ef}	131^{g}	10.3	***	***
Water solubles	82^{b}	112^{e}	112^{e}	130^{g}	63^{a}	88^{bc}	84^{bc}	92^{cd}	92^{cd}	142^{h}	125^{fg}	133^{g}	98^{d}	145^{h}	148^{h}	121^{f}	6.4	***	**
Hemicellulose loss	20^{a}	109^{c}	125^{c}	150^{d}	5^{a}	7^{a}	64^{b}	111^{c}	22^{a}	145^{d}	172^{e}	184^{e}	52^{b}	116^{c}	145^{d}	152^{d}	15.3	**	***
Cellulose loss	ND	60^{c}	83^{d}	106^{e}	ND	28^{b}	43^{b}	90^{d}	ND	117^{e}	128^{f}	167^{g}	12^{a}	106^{e}	130^{f}	163^{g}	14.5	**	***
Lignin loss	43^{b}	94^{cd}	143^{f}	198^{h}	14^{a}	40^{b}	93^{c}	104^{cd}	43^{b}	108^{d}	160^{g}	210^{h}	31^{b}	127^{e}	173^{g}	208^{h}	16.5	**	***
Digestibility	168^{ab}	184^{c}	225^{f}	238^{h}	164^{a}	183^{c}	214^{e}	227^{fg}	170^{b}	204^{d}	230^{g}	246^{i}	165^{a}	200^{d}	224^{f}	235^{h}	7.2	*	***
NEZ																			
TOM loss	24^{a}	65^{d}	92^{f}	120^{i}	20^{a}	38^{b}	52^{c}	78^{e}	34^{b}	74^{e}	100^{g}	122^{i}	32^{b}	59^{d}	87^{f}	108^{h}	8.5	***	***
Water solubles	90^{bc}	105^{e}	114^{fg}	126^{h}	86^{ab}	92^{c}	104^{e}	117^{g}	85^{a}	107^{e}	128^{h}	138^{i}	92^{c}	98^{d}	112^{f}	118^{g}	4	*	***
Hemicellulose loss	20^{ab}	44^{c}	64^{d}	138^{g}	15^{a}	27^{b}	42^{c}	115^{f}	20^{ab}	45^{c}	82^{e}	153^{h}	18^{ab}	47^{c}	92^{e}	106^{f}	11.2	NS	***
Cellulose loss	12^{a}	32^{b}	83^{e}	104^{g}	10^{a}	18^{a}	44^{c}	65^{d}	12^{a}	32^{b}	83^{e}	102^{g}	12^{a}	30^{b}	92^{f}	125^{h}	10	*	***
Lignin loss	22^{a}	87^{c}	163^{f}	185^{g}	14^{a}	37^{b}	66^{c}	98^{cd}	28^{ab}	104^{d}	162^{f}	195^{g}	25^{a}	85^{c}	126^{e}	164^{f}	15.6	**	***
Digestibility	188^{a}	197^{b}	245^{f}	260^{g}	184^{a}	190^{a}	217^{d}	235^{e}	190^{a}	210^{c}	243^{f}	264^{g}	190^{a}	205^{c}	220^{d}	246^{f}	6.9	**	***

Each value represents mean of four individual samples; Different superscripts within row shows significant difference ($P < 0.05$)

TOM total organic matter, *SE* standard error, *ND* not detected, *NS* non significant ($P > 0.05$), *A* level of significance for fungus type, *B* level of significance for incubation time

* $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$

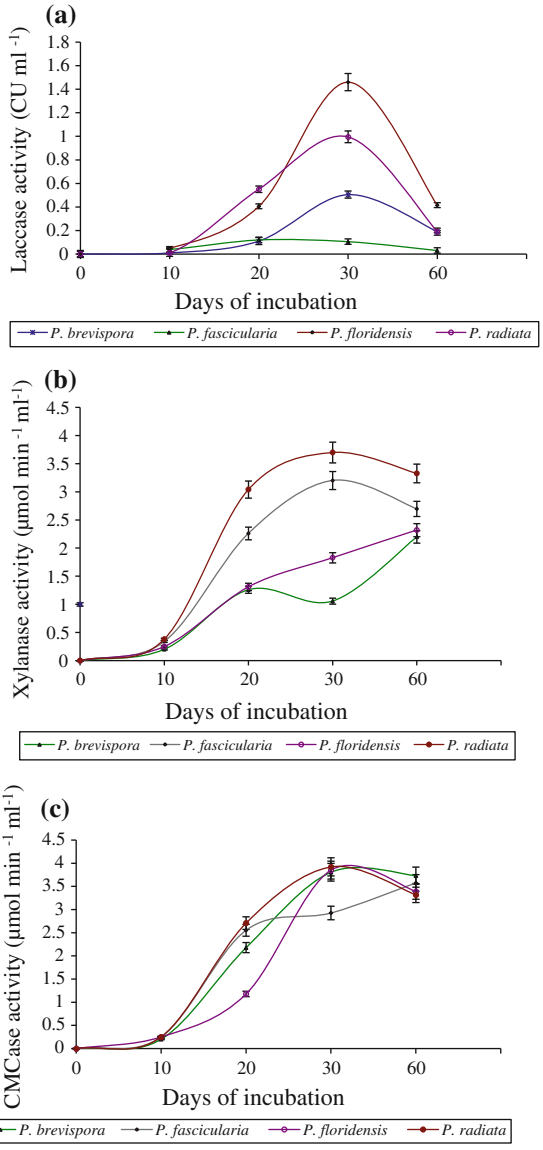

Fig. 1 Enzyme activities by different white rot fungi during 60 days of solid state fermentation of paddy straw obtained from north western zone: **a** laccase, **b** xylanase, **c** CMCase

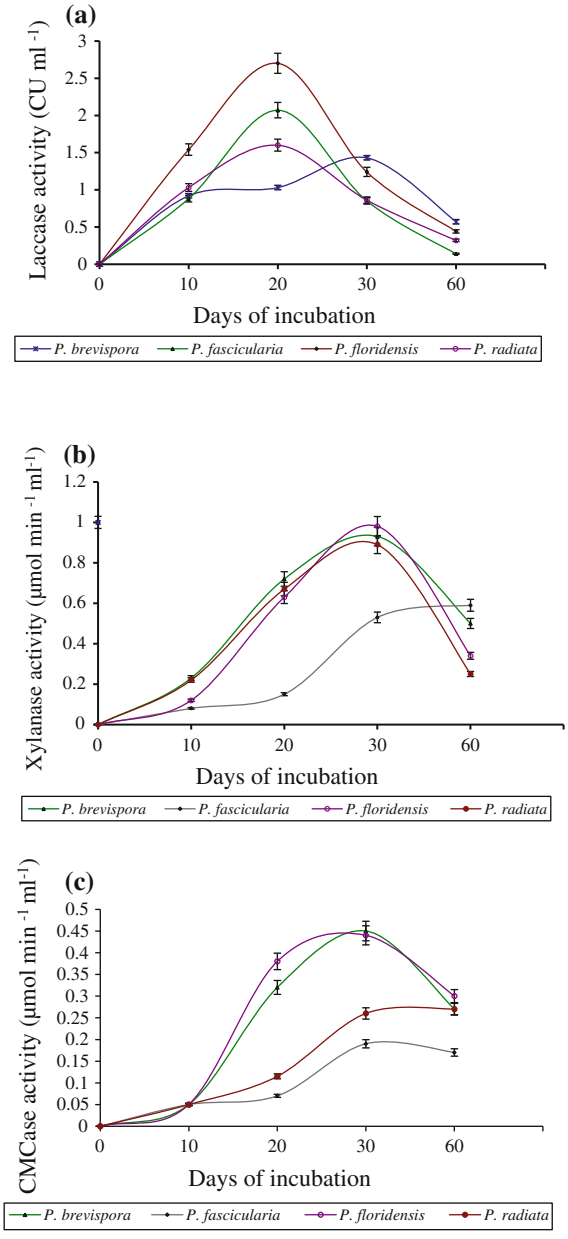

Fig. 2 Enzyme activities by different white rot fungi during 60 days of solid state fermentation of paddy straw obtained from central eastern zone: **a** laccase, **b** xylanase, **c** CMCase

was maximum on 30th day (0.45 µmol/min/ml) while other two organisms *P. radiata* and *P. fascicularia* showed significantly ($P < 0.05$) lower activity and also followed the similar pattern (Fig. 2).

Solid state fermentation of paddy straw collected from north eastern zone (NEZ)

During 60 days of incubation, maximum loss in TOM was caused by *P. floridensis* (122 g/kg) and *P. brevispora* (120 g/kg) followed by *P. radiata* (108 g/kg) and *P. fascicularia* (78 g/kg) (Table 2). Maximum water solubles (128 and 138 g/kg) were liberated by *P. floridensis* during 30 and 60 days, respectively. Maximum hemicellulose was degraded by *P. floridensis* (153 g/kg) followed by *P. brevispora* (138 g/kg), *P. fascicularia* (115 g/kg) and

P. radiata (106 g/kg) during 60 days. A maximum amount of cellulose (125 g/kg) was degraded by *P. radiata. P. brevispora* and *P. floridensis* degraded almost similar amount (104 and 102 g/kg), while *P. fascicularia* degraded only 65 g/kg of cellulose. Maximum lignin was degraded by *P. floridensis* (195 g/kg) followed by *P. brevispora* (185 g/kg), *P. radiata* (164 g/kg) and *P. fascicularia* (98 g/kg). *P. floridensis* and *P. brevispora* enhanced maximum in vitro digestibility of PS from 188 to 264 and 260 g/kg respectively, followed by *P. radiata* (246 g/kg) and *P. fascicularia* (235 g/kg).

Phlebia floridensis and *P. radiata* showed maximum laccase activity on 20th day of incubation (2.52 and 1.60 CU/ml, respectively). *Phlebia fascicularia* showed almost similar laccase activity (2.00 CU/ml) on 10th and 20th day, while in *P. brevispora* the activity peaked on 10th and 30th day of incubation. Maximum xylanase activity (from 2.71 to 3.19 µmol/min/ml) was shown by *P. radiata* during 20th to 60th day of incubation. A similar pattern was followed by *P. brevispora*, but the activity declined after 30 days of incubation. Other two fungi *P. floridensis* and *P. fascicularia* showed a maximum xylanase activity on 60th day (statistically insignificant $P > 0.05$). A significantly ($P < 0.05$) higher CMCase activity (4.60 µmol/min/ml) was shown by *P. radiata* and followed by *P. floridensis* (3.51 µmol/min/ml) on 30th day of incubation. *Phlebia brevispora* showed almost similar CMCase activity (3.5–3.6 µmol/min/ml) on 30th and 60th day, while *P. fascicularia* showed a maximum activity on 60th day (Fig. 3).

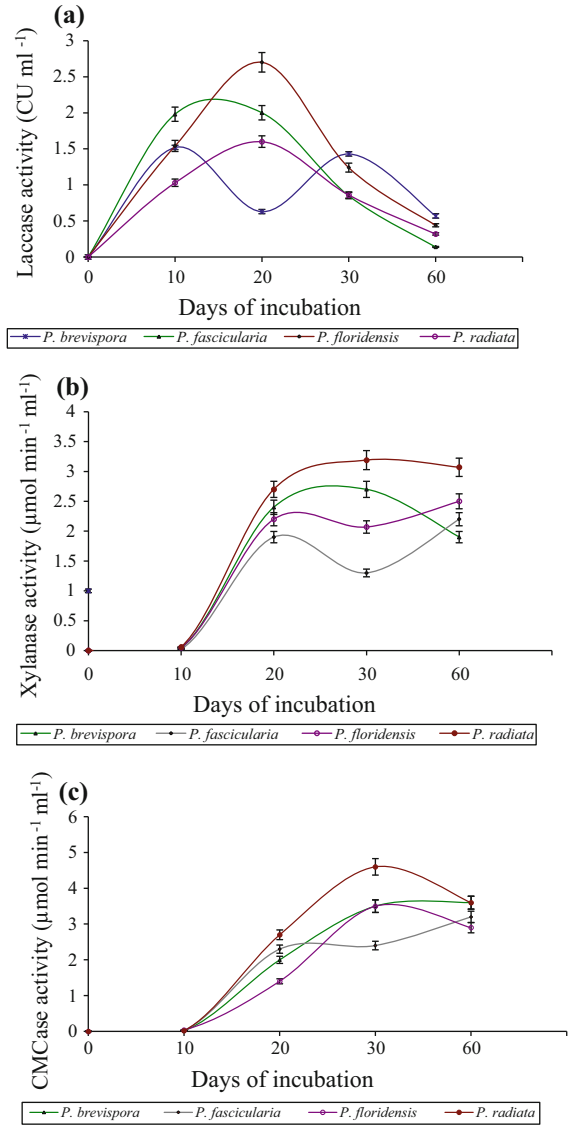

Fig. 3 Enzyme activities by different white rot fungi during 60 days of solid state fermentation of paddy straw obtained from north eastern zone: **a** laccase, **b** xylanase, **c** CMCase

Discussion

The plant cell wall consists of complex polymers like cellulose, hemicellulose and lignin. The ratio of these plant cell wall constituents vary from plant to plant as well as in the same plant depending upon the various environmental factors, which is governed by their geographic location of cultivation (Cherney et al. 2003; Whalley et al. 2008). Lignin is the most abundant heterogeneous natural biopolymer after cellulose on the earth, which comprises of three main phenolic propanoid units. The ratio of these monomeric alcohols vary in lignin of different origin and affects the degradability of plant cell wall (Grabber 2005). Studies have demonstrated that lignification in the cambial layer and early developing xylem is affected by seasonal variations (Christiernin 2006). It is also reported that the in vitro digestibility may differ from one to another part of the same plant (Vadiveloo 2000).

Cell wall constituents of straw play an important role in determining its quality as animal feed. In undecayed PS samples, lignin content and in vitro digestibility showed a very strong negative correlation ($r = -0.966$), while a strong positive correlation

($r = 0.869$) existed between lignin loss and in vitro digestibility in degraded PS. It strengthens the viewpoint that lignin plays an important role in determining the digestibility and quality of straw. In several studies lignin loss enhanced the in vitro digestibility and selective lignin degradation minimized the TOM loss (Cohen et al. 2002). Correlation between TOM loss and lignin loss was higher ($r = 0.955$) as compared to both the polysaccharides ($r = 0.921$ and 0.908 for cellulose and hemicellulose, respectively), which showed that the lignin loss contributed more in TOM loss in comparison to hemicellulose and cellulose; thus, giving a selective ligninolysis of the PS. More selective ligninolysis in PS minimized the TOM; thus, leaving behind more digestible organic matter for the ruminants.

All the fungi were selective in lignin degradation of the PS obtained from NWZ and degraded about 20–23% of lignin, while none of the fungi could degrade more than 20% of the lignin in PS collected from NEZ. This may be due to variation in the constituents and concentration of the fibers and enzyme production profile of the fungus. The enzyme activity could not be well correlated with its corresponding fiber degradation at the same time period, as the laccase activity was lower on 60th day in all the cases while the maximum lignin degradation was recorded on 60th day of incubation. The estimated amount of degraded lignin was the cumulative effect of 60 days of fermentation while the enzyme production started earlier and showed better activity during 10–30 days.

The fungal lignocellulolytic enzyme system plays an important role in governing the degradation of plant cell wall fibers. This system mainly consists of cellulases, hemicellulases and ligninolytic enzymes. *Phlebia* spp. produce most of the lignocellulolytic enzymes and also show selective ligninolysis. Three important enzymes (laccase, xylanase and CMCase) were selected for the present study.

Phlebia floridensis produced significantly ($P < 0.05$) higher laccase and resulted in good ligninolysis of PS irrespective of its zone of procurement. A maximum amount of lignin (228 g/kg) was degraded by *P. radiata* in the sample collected from NWZ while it degraded a lower amount of lignin (164 g/kg) in the sample collected from NEZ (Fig. 4), reflecting a specific profile of fiber degradation depending upon the origin of substrate. *Phlebia fascicularia* degraded

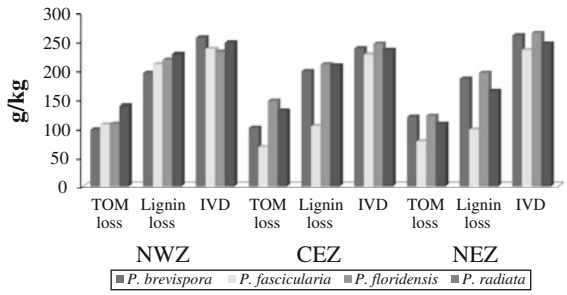

Fig. 4 Changes in total organic matter loss (TOM), lignin and in vitro digestibility (IVD) of paddy straw obtained from north western zone (NWZ), central eastern zone (CEZ) and north eastern zone (NEZ) during 60 days of degradation by white rot fungi

21% of lignin in PS obtained from NWZ while it degraded only 10% lignin in the other two samples of PS. On the other hand, *P. brevispora* degraded almost same amount of lignin and TOM in all the PS samples (Fig. 4). It is also suggested that beside the constituents and concentration of lignin in the substrate; the holocellulose binding capacity of lignin polymer may also be responsible for the fungal behaviour towards cell wall degradation.

In undecayed PS, in vitro digestibility showed a weak positive correlation with both the polysaccharide contents and a fairly positive correlation with water soluble content. The water soluble part provides easily digestible components which can be used by rumen microflora and contributes in the digestibility of straw. Cellulase and hemicellulase like enzymes break down the complex polysaccharides into simple sugars, which directly contribute the water soluble part of the feed. However, a very strong correlation could not be established between the water soluble contents and in vitro digestibility, as the later depends upon the availability of other polysaccharides as well as the structure of polymers (Rolz et al. 1986). It may also be possible that the fungus break the polymers by excreting the lignocellulolytic enzymes and use the easily available sugar units for its own growth also, which thus lead to underestimations of soluble components and thus may be interfering with the interpretation of the results.

Studies have reported that the use of xylanases and cellulases as feed additives can be attributed mainly to improved ruminal fiber digestion resulting in

increased digestible energy intake. These enzymes also break down the holocellulosic part of the cell wall of lignocellulosic biomass and liberate simpler molecules freely available to the animals (Beauchemin et al. 2003).

During the degradation of wood and other lignocellulosic biomass, hemicellulose lignin matrix is primarily attacked by white rot fungi (Martínez et al. 2005). Similar pattern was observed during the study, where all the fungi degraded relatively higher amount of lignin and hemicellulose as compared to cellulose. In the PS collected from NWZ and CEZ, no significant amount of cellulose loss could be detected during initial 10 days of incubation. *Phlebia brevispora* degraded lignin and hemicellulose efficiently in all the PS samples while *P. fascicularia* degraded more hemicellulose than lignin in the PS obtained from CEZ and NEZ.

Lignocellulosic substrate significantly affects the fungal enzyme production. When the same fungal spp. (*Phlebia*) were allowed to degrade wheat straw of different origin, the laccase activity was higher than paddy straw and the fungi could degrade more than 30% lignin, thus resulted in improved digestibility (Arora and Sharma 2009). Similarly, during the present study, the *Phlebia* spp. degraded the lignin selectively and improved the digestibility of paddy straw too. From both the studies it can also be concluded that the fungal degradation pattern depends upon the biochemical character of the substrate which in turn is influenced or governed by their geographic and climatological conditions.

Studies suggest that fungal profile of lignin degradation and improvement in digestibility depends on the nature of lignocellulosic material and the fungal strain employed (Okano et al. 2006; Chalamcherla et al. 2009). It is like treating the different PS with the same chemical resulting in variable effect on the biochemical properties of the substrate (Vadiveloo and Fade 2009). Studies on paddy straw reported that production and composition of the straw may also vary due to the difference in the altitude of the land as well as the season of cultivation (Williams et al. 1996), resulting in variable susceptibility of such straw to fungal degradation as observed in present study. Thus, it can be concluded that biochemical constitution of PS varied from region to region depending upon their site of origin which governed its fungal degradation profile.

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