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Production of proteases and lignolytic enzymes during solid state fermentation (SSF) of finger millet straw (*Eleusine coracana*)

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ABSTRACT

The effect of fermentation on secretion of proteases and lignolytic enzymes was evaluated in non pretreated (UT), steamed for 10 min (ST) and 4% urea treated (UrT) finger millet straw for 5 days in bulk quantities under laboratory conditions with 4 species of white rot fungi, viz. *Pleurotus sajorcaju,Pleurotus ostreatus, Voriallae volvoraceae* and *Phanerochaete chrysosporium*. Fermentation with *V. volvoraceae* recorded the minimum increase in protein of 1.99,2.22 and 2.98% respectively in the untreated,steamed and urea treated straw. Dry matter showed a decrease for the 3 treatments upon fermentation with *P.chrysosporium* while the highest losses ranging between 10.99 for the untreated to 15.75% for the urea treated straw were observed upon fermentation with *V.volvoraceae*.Though the ash contents increased the difference was not significant. There were also consistent significant decreases in the values obtained for cell wall components (NDF, ADF ADL). *In vitro* dry matter digestibility increased with all the 3 treatments in the 4 fungi as compared to control value of 40.0±3.65. *P.chrysosporium* recorded highest values of 76.10±3.86for UT, 82.70±0.45 for ST and 87.20±2.02 for UrT followed by *P.ostreatus* and *P.sajor caju* while lowest increase in digestibility values of 58.09±0.54for UT, 59.23±0.64for ST and 62.57±2.6 for UrT was obtained in *V.volvoraceae*. A concomitant increase in the lignolytic enzymes laccase, manganese peroxidase and lignin peroxidase was obtained for all the 3 treatments up to the fifth day of fermentation. High protease activity was observed during the first 2 days of fermentation with steamed ragi straw fermented with *P. chrysosporium* recording the highest activity of 2641units on the second day. In conclusion, *P.chrysosporium* and *P. ostreatus* proved the most promising strains for improving the digestibility of finger millet straw for ruminants and secretion of these enzymes by these fungi for pretreating lignocellulosics for feeding ruminants can safely be manipulated.

Key words: Fermentation, Lignolytic enzymes, Proteases, Ruminant feed, White rot fungi.

Solid-state fermentation of lignocellulosic materials by white-rot fungi is receiving more attention, primarily because of the possibility of converting these materials to more digestible feedstuffs for ruminants. The white rot fungi are the most efficient to degrade lignin on account of their ligninolytic enzymes – the ligninases, and *Basidiomycete* mushrooms of the genus *Pleurotus* along with a few others like *Phanerochaete chrysosporium* are the most recognized (Arora *et al*. 1994). Finger millet *(Eleusine coracana*) accounts for 0.94 million hectares annual production in Karnataka with 3.4 million straw which enriched with microbial protein through a solid-state fermentation process could be profitably used for animal feeding. White-rot fungi produce various isoforms of extracellular oxidases including laccase, Mn peroxidase and lignin peroxidase (LiP), which are involved in the degradation of lignin in their natural lignocellulosic substrates. Even though the production of extracellular proteases is a common feature among fungi (Morihara 1974) in general and wood-degrading basidiomycetes (Kumagai *et al.* 1981) in particular, very little work has been done on this aspect. Eriksson and Pettersson (1982) reported the purification and partial characterization of 2 acidic proteases in shallow stationary cultures of *Sporotrichum pulverulentum (P.chrysosporium*, ME-446) grown on cellulose. Though these 2 proteases, isolated from 10-day-old cultures, played a role in the activation of the endo-1,4,B-glucanases, no data on their effect on the extracellular ligninase secreted by the fungus or their production and mode of action in shaken cultures was reported. The present study was aimed to study the changes in the composition of ragi straw after various pretreatments and during fermentation with few strains of white rot fungi and the effect on the production of proteases and the lignolytic enzymes.

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MATERIALS AND METHODS

Dried samples of finger millet straw were procured from the local market. The straw was hand chaffed and oven-treated at 65°C until a constant weight was obtained. The 4 strains of white rot fungi used in the present study *(Pleurotus Ostreatus* (NCIM1200), *Phanerochaete chrysosporium* (NCIM 1197), *Pleurotus sajor caju* (NCIM1133) *and Voriallae volvoraceae* (NCIM 1125) were obtained from the National collection of industrially important organisms (NCIM) of the National Chemical Laboratory, Pune. The cultures were initially sub cultured regularly and maintained as slants on PDA. The fully active mycelium was then transferred to bottles with sterilized wheat grains and incubated at 28ºC until colonization of the substrate was observed (approximately 5 days). These fermented grains were used as inocula (10% in weight) for the SSF of chaffed finger millet straw (1 to 1½ inch bits). Solid state fermentation of finger millet straw was carried out by following the method suggested by Ramesh and Lonsane (1987). Necessary changes were however, made in the medium composition and methodology after optimization of process parameters. Finger millet straw (1 kg) in 3 replicates in enamel trays was subjected to each of the following 3 pre treatments, viz. without any pretreatment (UT), after steaming for 10 min (ST) and after 4% urea treatment (UrT) for each of the 4 fungi respectively. After the pretreatments the chaffed straw (65% moisture) was inoculated aseptically with fungal inoculum (10%) so that 3 replicates of each fungus were available for each of the 3 treatments and the trays were then incubated at 39±2 °C for 5 days. Each day 5 g of straw was taken out asceptically, thoroughly homogenized with 0.1 M phosphate buffer, pH 7.5 and the extract used for enzyme assays after sonication and centrifugation at 10,000 rpm for 20 min at 4°C. After 5 days fermentation the straw was dried and analyzed for changes in the proximate composition.

Analytical techniques

Dry matter of the fermented samples was determined after drying at 100±5°C for 8 h. Nitrogen (N) content of the chaffed dried samples before and after fermentation was determined by the standard Kjeldhal method (AOAC 1995) and the crude protein (CP) was calculated (N × 6.25). Ash content was determined using muffle furnace. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined as per Van Soest *et al.* (1991). *In vitro* dry matter digestibility (IVDMD) was determined as per Tilley and Terry (1963) using triplicate samples. Protein of harvested media was estimated as per Lowry *et al*. (1951).

Enzymatic assays

Ligninase was measured according to Tien and Kirk (1988) and one unit (U) of activity represents 1 gmol veratryl alcohol oxidized to the aldehyde per minute. The Mn-peroxidase was measured using phenol red as substrate and activity was expressed as the absorbance change at 610 nm (A610) in 3 min for 20 µl sample (Kuwahara *et al.* 1984). Laccase was determinedfrom the oxidation of Veratryl alcohol to veratraldehyde by H_2O_2 and absorbance at 310 and one unit of activity (U) represents 1 g mol veratryl alcohol oxidized to the aldehyde per minute (Kirk *et al*. 1986). Protease activity was assayed using azocoll as substrate. The colored supernatant was assayed at 520 nm against a blank sample without enzyme and one unit of activity was defined as the amount of enzyme which catalyzes the release of azo dye causing an absorbance change of 0.001/min (Dosoretz *et al*. (1990).

Statistical analysis

The data on various parameters were tabulated, mean values were calculated and deviations from the means were calculated as standard deviations of the means according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Changes in proximate composition of ragi straw: The changes obtained in the proximate composition of ragi straw upon fermentation with white rot fungi (Table 1) showed a significant ($P<0.05$) increase in crude protein (CP) contents from 5.98% for the control unfermented ragi straw to values ranging between 7.82±0.06% in untreated straw fermented with *Pleurotus sajorcaju* to 11.82±0.10% in urea treated straw fermented with *Phanerochaete chrysosporium*. Fermentation with *Voriallae volvoraceae* recorded the minimum increase in protein of 1.99, 2.22 and 2.98% in the untreated,steamed and urea treated straw respectively. Pretreatment of straws proved beneficial in all the 4 fungi with urea treatment followed by steam treatment for 10 min, while lowest protein contents were recorded in straw fermented without any pre treatment (Table 1). Fermentation of finger millet straw caused solubilization and degradation of fungal protein which was observed as the increase in CP content (Belewu and Belewu 2005). CP increase could also be due to hydrolysis of starch to glucose and its subsequent use by same organism as a carbon source to synthesize fungal biomass rich in protein (Bender 1970, Hammond and Wood 1985). Our results are in lined with the findings of Zadrazil (1993) and Belewu and Okhawere (1998) who reported that the colonization of lignocellulosic waste by the fungi results in increase in their nutritional value probably caused by increase of the fungal biomass/reproduction in fungal treated straw.

Dry matter showed a decrease ranging between 3.08 to 4.35% for 3 treatments upon fermentation with *P. chrysosporium* while the highest losses ranging between 10.99 for the untreated to 15.75% for the urea treated straw were observed upon fermentation with *V.volvoraceae.* Losses in DM during fermentation with fungi during SSF are primarily due to consumption of carbohydrates, which are

mostly associated with the cell wall.

The ash contents also increased significantly ($P<0.05$). Although not significant the increase in the ash content of the fungi treated straw could be attributed to the growth of mushroom (hyphae).The NDF and ADF contents also recorded decreases with *Pleurotus ostreatus* again recording the maximum reduction in all the 3 treatments with values of 68.00 62.46 and 59.32% being obtained for NDF and values of 31.00, 30.54 and 29.42% respectively in ADF for the untreated, steamed and urea treated fermented ragi straw respectively (Table 1). A decrease in the ADL content was also recorded upon fermentation of ragi straw with the 4 white rot fungi but the best results were obtained in *P.chrysosporium* where ADL decreased from 11.21% in the unfermented ragi straw to 8.0% in untreated fermented ragi straw, 7.64% in steamed fermented ragi straw and to 7.05% in the urea treated fermented ragi straw. These results showed that vegetal cell-wall components were degraded during SSF incubation. Though promising the overall changes obtained in the proximate composition with bulk fermentation of manually chaffed ragi straw were not as superior to those obtained in our earlier studies of SSF carried out in conical flasks. The IVDMD (%) of the unfermented ragi straw was about 40.0 which showed a linear increase ($P<0.05$) with pretreatment from untreated, steamed to urea treated after SSF in all the 4 fungi studied (Table 1). The highest digestibility was obtained in *P.chrysosporium* where values of 76.1±3.86for UT, 82.7±0.45 for ST and 87.2±2.02 for UrT was obtained followed by *P.ostreatus* and *P.sajor caju* while lowest increase in digestibility values of 58.09±0.54for UT, 59.23±0.64for ST and 62.57±2.6for UrT was obtained.

Many authors have shown that some species of *Pleurotus* are able to colonize different types of vegetable wastes, increasing their digestibility (Platt *et al*. 1984, Commanday and Macy 1985,Rajarathnam and Bano 1989). Previous studies have shown the feasibility of using wastes to produce animal feed (Calzada *et al.* 1987, Adamovic *et al*. 1998). Rice straw soaked in water for 24 h was pasteurized at 100°C for 6 h and then inoculated with spawns of 4 *Pleurotus* fungi *(Pleurotus florida, Pleurotus djamor, Pleurotus sajor-caju and Pleurotus ostreatus*) packed in the plastic bags and incubated in a fermentation chamber at 23–27° C and 75–85% relative humidity. After 60th day, rice straw samples from all groups were taken and analyzed for chemical composition and *in vitro* digestibility. The data obtained were analyzed according to the complete randomized design model consisting of 4 treatments plus 1 control and 4 replicates. The results of this study showed that fungal treatment increased ($P<0.05$) the crude protein (CP), silica, Ca and P contents of the rice straw but the hemicellulose, organic matter (OM), acid detergent fiber (ADF), neutral detergent fiber (NDF) and acid detergent lignin (ADL) contents decreased. However, the ability of the fungi to degrade these components varied among the species. The ability of

Table 1.Changes in the proximate composition (g/100 g DM) of *Eleusine coracana* after bulk fermentation with white rot fungi in trays

Constituent	Control ragi straw (UF)	*Phanerochaete chrysosporium*			*Pleurotus ostreatus*			*Pleurotus sajorcaju*			*V.volvoracea*		
		UT	ST	UrT	UT	ST	UrT	UT	ST	UrT	UT	ST	UrT
DryMatter	90.08^{a}±0.03	87.00^{b}±0.17	86.80^{b}±0.07	85.73^{b}±0.06	85.09^{b}±0.03	84.31^{b}±0.09	79.42^{c}±0.10	85.73^{b}±0.05	85.09^{b}±0.07	84.31^{b}±0.04	79.42^{c}±0.08	79.01^{c}±0.08	74.33^{c}±0.12
CrudeProtein	5.98^{a}±0.07	9.98^{b}±0.06	10.62^{b}±0.05	11.82^{c}±0.10	8.93^{b}±0.09	9.68^{b}±0.11	10.97^{b}±0.04	7.82^{a}±0.06	8.93^{b}±0.05	10.68^{b}±0.03	7.97^{a}±0.09	8.20^{b}±0.09	8.96^{b}±0.08
Ash	7.70^{a}±0.02	11.73^{b}±0.07	12.24^{b}±0.15	12.11^{b}±0.07	13.63^{c}±0.03	13.91^{c}±0.02	13.21^{c}±0.05	12.11^{b}±0.03	13.63^{b}±0.12	13.91^{c}±0.11	13.21^{c}±0.07	13.46^{c}±0.07	13.98^{c}±0.04
NDF	74.20^{a}±10.14	68.0^{b}±9.05	65.00^{b}±8.03	64.47^{b}±2.09	64.88^{b}±6.08	62.19^{b}±7.05	61.30^{b}±4.26	69.47^{b}±0807	64.88^{b}±9.10	69.19^{b}±5.75	72.36^{a}±9.36	71.66^{a}±6.04	69.87^{b}±3.02
ADF	42.82^{a}±9.24	34.0^{b}±4.17	32.00^{b}±3.04	30.33^{c}±2.06	33.02^{b}±1.04	32.49^{b}±4.13	31.44^{c}±5.09	37.33^{b}±6.02	35.02^{b}±5.05	34.49^{b}±2.06	41.44^{a}±2.06	40.21^{a}±3.04	39.32^{b}±2.05
ADL	11.21^{a}±0.14	8.0^{a}±0.06	7.64^{b}±0.12	7.05^{b}±0.07	7.62^{b}±0.13	7.09^{b}±0.17	6.90^{b}±0.09	8.00^{a}±0.05	7.62^{b}±0.06	7.09^{b}±0.07	8.10^{a}±0.09	8.04^{a}±0.11	7.98^{a}±0.05
IVDMD (%)	40.00^{a}±3.65	76.10^{c}±3.86	82.70^{c}±0.45	87.20^{c}±2.02	66.05^{b}±0.56	67.40^{b}±0.62	69.25^{b}±1.2	57.13^{b}±0.78	62.77^{b}±2.21	65.03^{b}±2.31	58.09^{b}±0.54	59.23^{b}±0.64	62.57^{b}±2.6

Values are means±SE; a,b,c, means in the same row with different superscripts are significantly different ($P < 0.05$); UF, Unfermented control straw; UT, ragi straw fermented without any pretreatment;ST,ragi straw fermented after steaming for 10 min;UrT,ragi straw fermented after treating with urea.

Pleurotus sajor-caju and *Pleurotus ostreatus* were higher than the other species in decreasing the hemicellulose, NDF, ADF and ADL contents (Jafari *et al.* 2007).

Shrivastava *et al.* (2010) reported a significant decrease ($P<0.05$) in acid detergent fiber (ADF), neutral detergent fiber (NDF), hemicellulose, lignin and cellulose in *P. ostreatus* and *T. versicolor* fermented straw. The maximum efficiency of fermentation was observed on the 10th day for *P. ostreatus* and on the 30th day with *T. versicolor*. The myco-straw contained significantly high crude protein for *P. ostreatus and T. versicolor* treated straw, as compared to control straw a finding in agreement with our results.

Changes in the production of proteases and lignolytic enzymes

Lignolytic enzymes phenol oxidase (laccase) and peroxidases (lignin peroxidase (LiP) and manganese peroxidase (MnP) are widely considered to play a key role in the enzymatic degradation (Krause *et al.* 2003; Malherbe and Cloete 2003). Activities of the lignolytic enzymes in relation to time and protease activities were monitored upon fermentation by *Phanerochaete chrysosporium, Pleurotus ostreatus Pleurotus sajorcaju* and *V.volvoraceae* of chaffed ragi straw in trays in bulk (1 kg) quantities.

High protease activity was observed during the first 2 days of fermentation (Fig 1 A, B, C and D) with steamed ragi straw fermented with *P. chrysosporium* recording the highest activity of 2641units on the second day after which activity declined to as low as 13.67 units on the fifth day of fermentation. In urea treated straw protease activity showed an increase from 38 units in control straw to 568 units on day 1 and 2382 units on day 2 after which activity declined drastically to 34.25 units on day 3. A similar trend was also obtained with regard to protease activity in the other three fungi on all the treatments.

A concomitant increase in lignolytic enzymes Laccase, manganese peroxidase and lignin peroxidase was obtained for all the 3 treatments up to the fifth day of fermentation (Fig 2 A, B, C and D). Untreated ragi straw had 38 units of protease activity while no lignolytic enzyme activity was detected. High activities of MnP were recorded in all treatments in *Phanerochaete chrysosporium, Pleurotus ostreatus* and *Pleurotus sajorcaju.* No Li P activity was detected in ragi straw fermented without any pretreatment in all the four fungi and highest activity of 152±39.45 units was obtained in urea treated straw fermented using *Pleurotus ostreatus.* Though comparatively low as compared to MnP and LiP, the highest laccase activities of 238±43.33 and 422±109.56 respectively were also recorded in steamed and urea treated ragi straw upon fermentation with *P. ostreatus.* Our results clearly showed that lignolytic activity is predominant during the idiophase when nutrients start getting depleted while extracellular protease activity is predominant

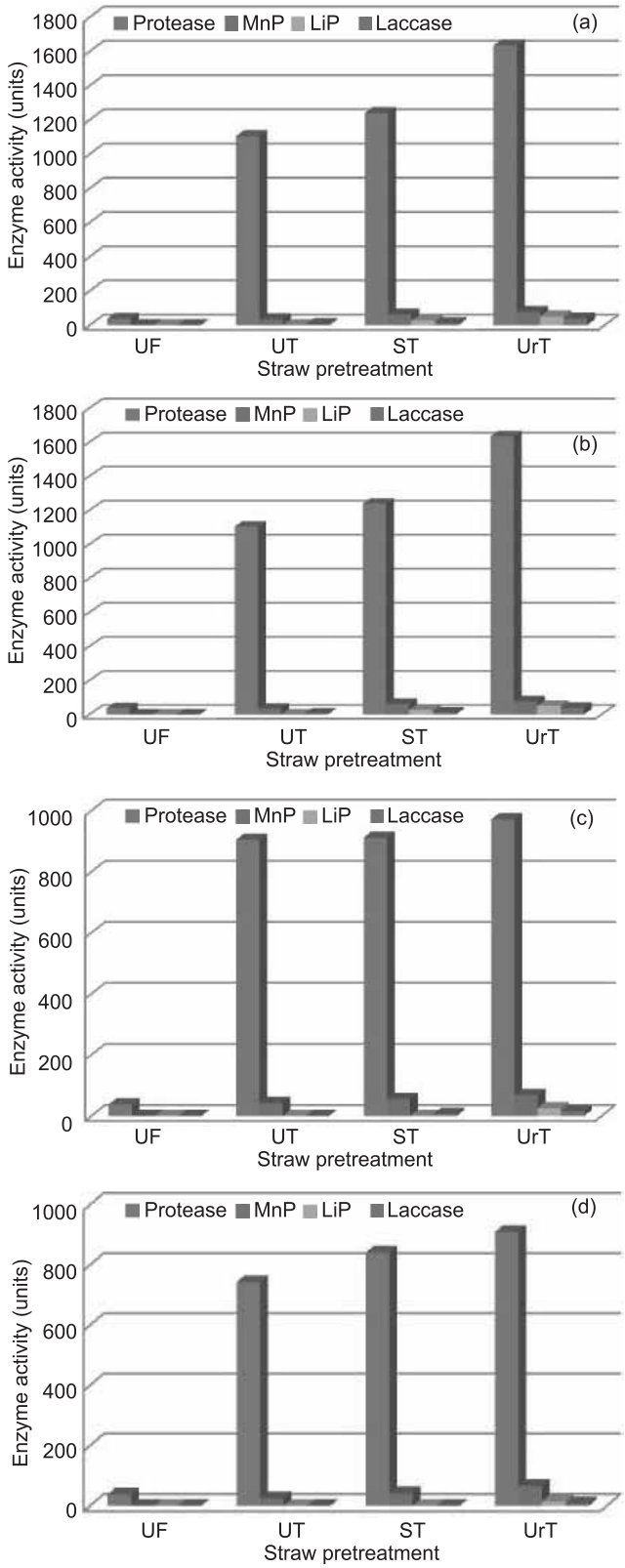

Fig. 1. Changes in the proteolytic and lignolytic enzyme profile of *Eleusine coracana* on the second day after fermentation with (a) *P.chrysosporium* (b) *P.ostreatus* (c) *P.sajorcaju* and (d) *V.volvoraceae.*

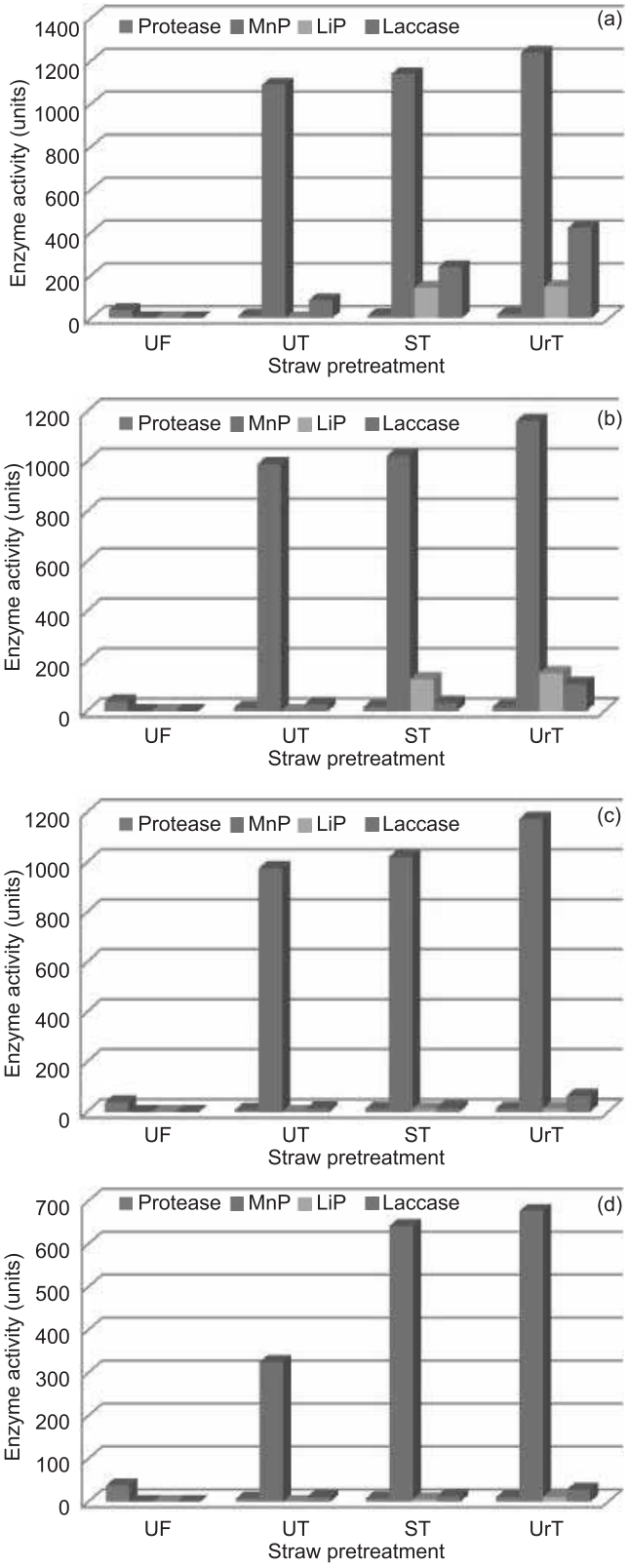

Fig. 2. Changes in the proteolytic and lignolytic enzyme profile of *Eleusine coracana* on the fifth day after fermentation with (a) *P.chrysosporium* (b) *P.ostreatus* (c) *P.sajorcaju* and (d) *V.volvoraceae*

during the trophophase when the media is rich in nutrients.

To promote the delignification of a lignocellulosic substrate, it is also essential to maximize the rate and specificity of lignin molecule degradation, avoiding polysaccharide consumption (Kerem and Hadar 1995). For some white-rot fungi, a high content of manganese in the culture medium increased activity of ligninolytic enzymes and a higher degradation level was observed when 600 mg/kg^{-1} of $MnSO_4$ was added to wheat straw, resulting in degradation of 50% of the lignin and only a slight increase in cellulose degradation as the Mn (III) ions, preferentially degraded the aromatic structures present in the lignocellulose (Kerem and Hadar 1993).Addition of manganese increased 61% straw digestibility during the fermentation whereas in the straw fermented without addition of manganese this increase was just 53%. We observed on an average 10% increase in the activity of the 3 lignolytic enzymes upon addition of 0.5% $MnSO_4$ to the culture medium at the start of fermentation and this condition was applied in the present study. Protease activity was however, not altered by this addition of manganese. A response surface methodology (RSM) based experiment designed by Sharma and Arora (2010) to optimize conditions for production of lignocellulolytic enzymes by *Phlebia floridensis* during solid state fermentation of wheat straw revealed that laccase production increased up to 34–fold with increase in moisture content, while manganese peroxidase was optimally produced in the presence of almost equal amount (50–55 mg/g of WS) of NH_4Cl and malt extract. In the present study we found that a moisture content of 65% accorded maximum lignolytic enzyme activity and was adopted for all the fermentations.

Eriksson and Pettersson (1982,1988) reported the purification and partial characterization of 2 acidic proteases in shallow stationary cultures of *Sporotrichum pulverulentum* (= *P. chrysosporium*, ME-446) grown on cellulose. These two proteases, isolated from 10–day-old cultures, played a role in the activation of the endo-1,4-,B-glucanases. However, no data on the effect of these proteases on the extracellular ligninase secreted by the fungus or their production and mode of action in shaken cultures with a soluble, noncellulolytic substrate were reported. Moreover, no data on the regulation of these proteases in either stationary or shaken lignolytic cultures of *P. chrysosporium* were reported.

Kole *et al*. (1988) reported that although the exact mechanism responsible for the cellular control of protease synthesis by microorganisms is unknown, production of the protease can be inhibited by amino acids, the carbon source, or both carbon and nitrogen sources. The major components of the lignolytic system of the white-rot fungus *P. chrysosporium* were idiophasic, triggered by nutrient limitation and particularly active in cultures grown under high oxygen (O_2) tension (Dosoretz *et al.* 1990). In all cases, the time courses of protease and ligninase activities were

negatively correlated, indicating that protease activity promotes the decline of ligninase activity. Our results also showed that these enzymes are produced during the secondary phase of growth i.e. after 2 days of fermentation, during the limitation of nutrients unlike the proteases which are produced during the primary growth phase and can thus be manipulated.

P.chrysosporium and *P. ostreatus* are appropriate strains to use for improvement in the digestibility of ragi straw. Higher protein levels, better conservation of the substrate and an increase in *in vitro* digestibility is observed. The relationship between the secretion of proteases and lignolytic enzymes by *Pleurotus sajorcaju*, *Pleurotus ostreatus*, *Voriallae volvoraceae* and *Phanerochaete chrysosporium* showed that the production of ligninase, laccase and Mn-peroxidase is predominant during the idiophase while extracellular protease activity is predominant during the trophophase. The secretion of these enzymes by these fungi for pretreating lignocellulosics for feeding ruminants or oral dosing them to enhance fiber digestibility can be safely manipulated.

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REFERENCES

Adamovic M, Grubic G, Milenkovic I, Jovanovic R, Protic R, Stretenovic L and Stoicevic L. 1998.The biodegradation of wheat straw By*pleurotus Ostreatus* mushrooms and Its use in cattle feeding. *Animal Feed Science And Technology* **71** (3–4) : 357–62.

AOAC 1995. *Official Methods of Analysis* 16th edn. Association of Official Analytical Chemist, Airlington VA.

Belewu M A and Belewu K Y. 2005. Cultivation of mushroom *(Volvariella volvacea*) on banana leaves. *African Journal of Biotechnology* **4** (1) : 1401–03. (Retrieved from: http://www.academicjournals.org/AJB/PDF/Pdf2005/Dec/Belewu%20and%20Belewu.pdf)

Belewu M A and Okhawere O C. 1998. Evaluation of Feeding Fungi Treated Rice Husk to Ram. *Proceeding of the 2*5th *Annual Conference and Silver Jubilee of the Nigerian Society Animal Production* 21–24th March 1998, pp 321–22.

Bender P F. 1970 *Under Utilized Resources as Animal Feedstuffs*. Pp100 National Academy Press Washington D C.

Calzada J F, Franco L F, De Arriola M C, Rolz C and Ortiz M A.1987. Acceptability body weight changes and digestibility of spent wheat straw after harvesting of *Pleurotus Sajor-Caju*. *Biological Wastes* **22** (4) : 303–09.

Commanday F and Macy J M.1985.effect of substrate nitrogen on lignin degradation by *Pleurotus ostreatus*. *Archives Of Microbiology* **142** (1) : 61–65.

Dosoretz CG, Chen H S Grethlein H E. 1990. The effect of environmental conditions on extracellular protease activity in lignolytic cultures of *Phanerochaete chrysosporium*. *Applied Environmental Microbiology* **56**: 395–400.

Eriksson K E and Pettersson B. 1982. Purification and partial characterization of two acidic proteases from the white-rot fungus Sporotrichum pulverulentum. *European Journal of Biochemistry* **124**: 635–42.

Eriksson K E and Pettersson B. 1988. Acid proteases from *Sporotrichum pulverulentum. Methods in Enzymo*logy **160**: 500–08.

Hammond J B W and Wood D A.1985 Metabolism biochemistry and physiology. *Biology and Technology of the Cultivated Mushrooms.* (Eds) Flagy P B,Spencer D M and Wood D A. pp 63–80.John wiley and Sons Chichester.

Jafari M A, Nikkhah A, Sadeghi A A, Chamani M. 2007.The effect of *Pleurotus* spp. fungi on chemical composition and *in vitro* digestibility of rice straw. *Pakistan Journal of Biological Sciences* **110** (15) : 2460–64.

Kerem Z, Hadar Y. 1993. Effect of manganese on lignin degradation by Pleurotus ostreatus during solid-state fermentation. *Applied Environment Microbiology* **59**: 4115–20.

Kerem Z and Hadar Y.1995. Effect of manganese on preferential degradation of lignin by *Pleurotus ostreatus* during solid-state fermentation. *Applied Environment Microbiology* **61**: 3057–62.

Kirk T K S, Croan M, Tien K E, Murtagh and Farrell R L. 1986. Production of multiple ligninases by *Phanerochaete chrysosporium*: effect of selected growth conditions and use of mutant strain. *Enzyme and Microbiol Technology* **8**: 27–32.

Kumagai H M, Matsue E, Majima K, Tomoda and Ichishima E.1981.Carboxyl proteinase from the wood deteriorating basidiomycete *Pycnoporus coccineus*: substrate specificity with oxidized insulin peptide B1–B16 and B15-B24 angiotensin and proangiotensin. *Agricultural Biological Chemistry* **45**: 981–85.

Kuwahara M, Glenn J K, Morgan M A, Gold M H. 1984. Separation and characterization of two extracellular H202-dependent oxidases from ligninolytic cultures of *Phanerochaete chrysosporium. FEBS Letter* **169**: 247–50.

Krause D O, Denman S E, Mackie R I. 2003.Opportunities to improve fiber degradation in the rumen: microbiologyecology and genomics. *FEMS Microbiological Review* **797**:1–31.

Lowry O H, Rosebrough N J, Farr L and Randall R J. 1951. Protein measurement with the Folin Phenol Reagent. *Journal of Biological Chemistry* **193:** 267–75.

Malherbe S, Cloete T E. 2003.Lignocellulose biodegradation: fundamentals and applications: A review. *Environmental Science Biotechnology* **1**: 105–14.

Morihara K. 1974. Comparative specificity of microbial proteinases. *Advances In Enzymology related areas. Molecular Biology* **41**: 179–243.

Platt W M, Hader Y and Chet I. 1984. Fungal activities involved in lignocellulose degradation by *pleurotus*. *Applied Microbiology and Biotechnology* **20** (2) : 150–54.

Rajarathnam S and Bano Z. 1989. *Pleurotus* mushroomsiii. Biotransformation of natural lignocellulosic wastes:commercial applications and implications. *Critical Reviews in Food Sciences and Nutrition* **28** (1) : 31–113.

Ramesh M V and Lonsane BK.1987. Solid state fermentation for production of amylase by *Bacillus megaterium* 16M. *Biotechnological Letters* **9:** 323–28.

Sharma R K Arora D S.2010.Production of lignocellulolytic enzymes and enhancement of in vitro digestibility during solid state fermentation of wheat straw by Phlebia floridensis.

Bioresources Technology **101** (23) : 9248–53.

Shrivastava B, Thakur S, Khasa Y P, Gupte A, Puniya A K, Kuhad RC.2010.White-rot fungal conversion of wheat straw to energy rich cattle feed. *Biodegradation.*

Steel R G D, Torrie J H. 1980. *Principles and Procedures of Statistics a Biometrical Approach*. McGraw-Hill International Book Company New York.

Tien M, Kirk T K. 1988.Lignin peroxidase of *Phanerochaete ehrysosporium. Methods Enzymology* **161**: 238– 49.

Tilley J M A and Terry R A. 1963. A two-stage technique for *in vitro* digestion of forage crops. *Journal of British Grassland Society* **18**: 104–11.

Van Soest P J, Robertson J B and Lewis B A. 1991. Methods for dietary fiber neutral detergent fiber and non–starch polysaccharides in relation to annual nutrition. *Journal of Dairy Science* **74**: 3583 –97. (Retrieved from:. http://jds.fass.org/cgi/reprint/74/10/3583)

Zadrazil F.1993.Conversion of Lignocellulose waste into animal feed with White rots Fungi. *Proceedings of the international conference of mushroom Biology*. Pp. 55–116.

Zadrazil F N D, Kamra O S, Isikhuemhen F, Schuchardt and Flachowsky G. 1966. Bioconversion of lignocelluloses into ruminal feed with white-rot fungi. *Journal of Applied Animal Research* **10:** 105–24.