Efficacy of Laccases Obtained from the White Rot Basidiomycete *Schizophyllum commune*-NI 07 in Enhancing the *in Vitro* Digestibility of Crop Residues for Ruminants

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Efficacy of laccases obtained from the white rot basidiomycete *Schizophyllum commune*-NI 07 in enhancing the *in vitro* digestibility of crop residues for ruminants.

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Introduction

Crop residues like maize stover, finger millet straw, wheat straw, rice straw, etc. generally constitute the main dietary component for animals. The rumen microbial utilization of these crop residues is hindered by the presence of lignin, which limits its overall digestion process and can significantly influence animal performance, because it is resistant to most of the enzymatic hydrolysis by microorganisms. In nature lignin is degraded by lignolytic enzymes of white rot fungi (WRF). These residues can thus be converted into high quality feed by means of fungal delignification improving their nutritive value. Fungal ligninolysis breaks down the cellulose-hemicellulose matrix liberating degradable components utilizable by rumen microflora. Earlier we reported on the enhancement in digestibility of ragi straw with lignolytic enzyme extracts. Laccase is one amongst these lignolytic enzymes holding immense potential in biodelignification of crop residues (Sridhar *et al*., 2014). However, its low level in the native state limits its practical use in the degradation of lingo cellulosics for ruminants necessitating the need for enhancing production. In the current work we report the efficacy of laccases isolated from *Schizophyllum commune*, in enhancing *in vitro* digestibility of some commonly used crop residues for ruminants.

Materials and Methods

S. commune (MTCC 11893, Bank It 1679236 *Schizophyllum* KF911323) was isolated in the current work. The culture was maintained on Malt Extract Agar (MEA) and stored at 4°C. Laccases obtained in solid state fermentation (SSF), submerged culture, after immobilization to enhance production and after purification were used in the study. The purified enzyme was also cloned and successfully expressed into *Pichi pastoris*. Paddy(PS) , finger millet (FMS) wheat straw (WS) maize (MS) and sorghum stover (SS) were procured from the local market, manually chaffed into 2 cm length and treated by spraying with laccase enzyme extracts stated above at an enzyme to straw ratio of 1:2.5 (Sridhar *et al*., 2014). Untreated straws served as control.

Laccase activity was determined by the oxidation of ABTS [Bourbonnais *et al*., 1998] at 4^{o}C. One unit of enzyme activity was expressed as the amount of enzyme that oxidized 1.0 µmole substrate per min under assay conditions. Protein in media and biomass was determined using BSA as standard. Dry matter (DM) of dried untreated and treated samples was determined at 100 ±5 ^{o} C for 8 hours. Nitrogen (N) content was determined by the standard Kjeldhal method and the crude protein (CP) was calculated (N x 6.25). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined using the standard method. *In Vitro* dry matter digestibility (IVDMD) or apparent digestibility was estimated as per the standard procedure.The data on various nutritional parameters was tabulated, mean values and deviations from the means were calculated and correlation coefficient between two parameters was established using proc CORR of SAS (9.3).

Results and Discussion

White rot fungi (WRF) attack cellulose and lignin simultaneously during normal degradation of cellulosic material, resulting in DM loss, thereby, rendering the straw fiber more accessible to rumen enzymes for subsequent digestion, thus providing the scope for utilization of ample quantity of DM as animal feed which would otherwise remain under utilized. The major enzymes involved in this degradation are lignin peroxidase, manganese peroxidase and laccase. Thus delignification results in changes in the structure of the cell wall beyond simple removal of lignin and the cell content is thus accessible. Improvement in nutritional quality and digestibility of feed material is not only dependent upon the fungal strain used but also on the type of substrate employed for the purpose (Chalamcherla *et al.*, 2009).

Laccase was the sole enzyme secreted by *S. commune*-NI07 in all the production methods. Treatment of various straws by spraying laccase extracts showed minimal DM losses (1 to 2 %) coupled with a marginal increase in protein content

(Fig.1), attributed to addition of the enzyme extract, a protein by nature. NDF content recorded a gradual reduction in untreated straw < treated with laccase from submerged culture < laccase obtained from immobilized *S. commune*. Treatment with partially purified laccase was observed to be most effective recording reduction in NDF content of 7% in sorghum and 14% in finger millet (Fig. 1) to the untreated straws. ADF showed a similar trend with maize and wheat showing 3% and finger millet 7% reduction. Though all the laccases studied were effective in delignification as evident from ADL values (Fig. 1), purified laccase proved most efficient in reducing lignin content up to 3% in all the straws. The decrease NDF, ADF and ADL contents in treated samples suggested that vegetal cell wall components of the straws were degraded, on account of laccase, the only enzyme present and capable of oxidizing lignin, facilitating enhanced digestibility of the straws. *In vitro* dry matter digestibility of all straws increased upon treatment with purified laccases with sorghum stover recording lowest increase of 8 % whereas paddy straw showed 14% (Fig. 1). Recombinant laccase degraded relatively higher amount of lignin and treatment of FMS enhanced digestibility. Thus by using it steps involved in the purification of laccase could be reduced and costs involved in purification also minimized. NDF, ADF values for ragi straw treated with recombinant laccase were 64.03% and 33.56% respectively and the DM losses were minimized as protein was directly used to treat the straw.

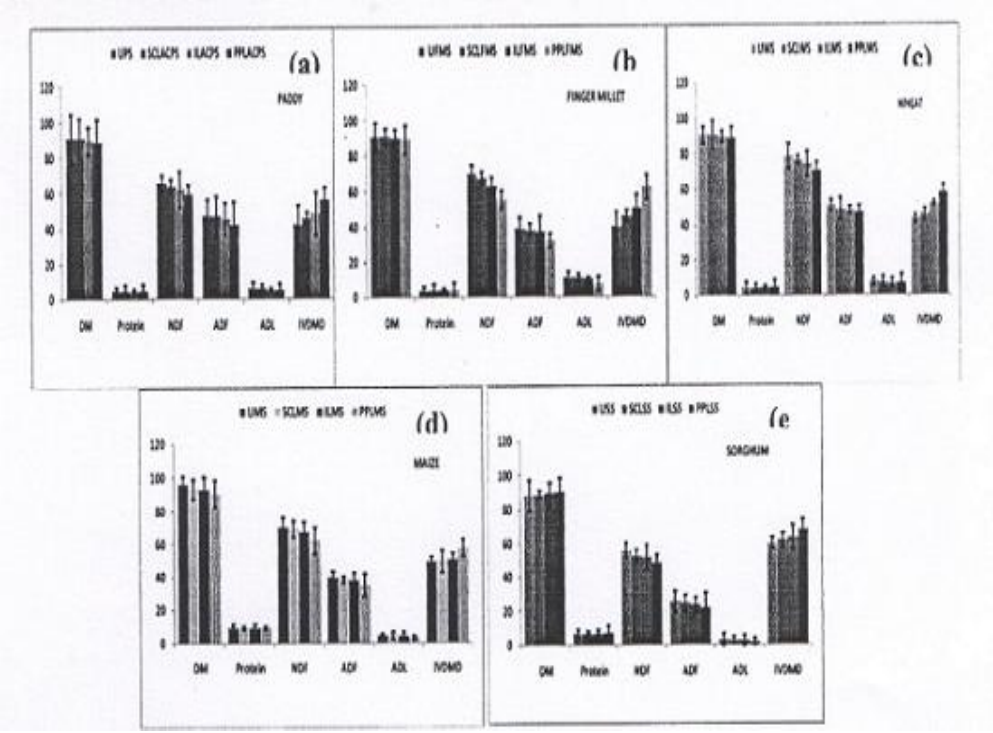

Fig. 1: Composition of some of the commonly used crop residues tested for deconstruction of lignin after treatment with laccase produced by *S. commune* NI-07. (a) – paddy straw; (b) – finger millet straw; (c) – maize straw; (d) wheat straw; (e) – sorghum straw. In (a-e): 1 – untreated straw; 2 – straw treated with laccase produced by S. commune NI-07 grown in submerged culture; 3 – straw treated with laccase produced by immobilized *S. commune* NI-07; 4 – straw treated with purified laccase produced by *S. commune* NI-07

There are few reports on changes in proximate composition of crop residues upon treatment with lignolytic enzymes in general and laccases in particular. A reduction in the NDF and ADL contents of straw after treatment with laccases obtained from three white rot isolates compared to untreated straw was attributed to lignin breakdown (Crowe and Olsson, 2001).

The ADL and IVDMD values obtained upon treatment of finger millet straw with laccases obtained in the various production methods employed by us were taken into consideration for establishing the relationship between digestibility and lignin degradation by plotting a scatter graph using proc CORR of SAS (9.3). Correlation was established in terms of lignin degradation and *in vitro* digestibility. There was a strong negative correlation (correlation coefficient r=-0.94333) (F value 97.82) between enhancement *in vitro* digestibility and lignin degradation between the various methods. Purified and recombinant laccase accorded highest degradation and enhancement in digestibility. Recombinant laccase expressed in *P. pastoris* yielded 55 % lignin breakdown as compared to untreated straw. IVDMD increased to 66 % in straw treated with recombinant laccase as compared to 39.67 % recorded in untreated ragi straw (Table 1). Thus an overall enhancement in digestibility of 26.33 % was obtained. Also the recombinant laccase obtained in the present study was biologically active and effective in enhancing the digestibility of straw.

Table 1: Comprehensive scenario of lignin degradation and enhancement in digestibility of crop residues using laccases

Laccase source	ADL	IVDMD	Sum of square	Mean square	F value	P value
SSF	9.26±0.15	43.0±01.09	1742.680000	348.536000	97.82	<.0001
Submerged	9.01±0.21	42.16±02.29				
Submerged (after optimization)	8.16±0.26	50.82±1.89				
After immobilization on PUF	7.63±0.35	54.33±3.09				
After purification	6.62±0.42	64.33±2.18				
Recombinant laccase	6.26±0.71	66.00±1.42				
Control straw without enzyme treatment	11.26±0.15	39.67±1.09				

Conclusion

From results it is evident that lignin was successfully degraded by laccases. To our knowledge there is no report on the changes obtained in proximate composition of crop residues on account of treatment with laccases. Laccases obtained from white rot basidiomycete *Schizophyllum commune*-NI 07 were highly effective in bio delignification and could be produced in bulk for enhancing digestibility of crop residues in ruminants.

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