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Production of cellulases from *Humicola fuscoatra* MTCC 1409: Role of enzymes in paddy straw digestion

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Cellulases were produced from fungus *Humicola fuscoatra* MTCC 1409 by solid state fermentation under different cultural conditions viz. pH, incubation temperature, inoculum size and days of incubation in order to optimize the conditions for maximum enzyme production. The potential of cellulase pretreatment to increase the digestibility of paddy straw was also ascertained. Maximum enzyme production was achieved at pH 6.0 of Mandel media and at temperature 45°C. Inoculum size of 1×10^7 spores/ml was found to be optimum for maximum enzyme production. Enzyme production increased with the increase in days of incubation from 2 to 6 days and then declined thereafter. Cellulase units at the concentration of 1, 1.5 and 2 µmole/g were exogenously added to paddy straw and change in chemical composition of paddy straw was determined after 18, 24, 30 and 36 h of treatment. With increase in enzyme concentration and incubation period, the content of neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose and hemicellulose reduced gradually with simultaneous increase in lignin and silica content. The concentrations of NDF, ADF, cellulose and hemicellulose decreased by 9.2, 5.9, 10.8 and 23.4% respectively, however lignin and silica content increased by 9.7 and 6.4% respectively as compared to control (with no cellulase added) at 2 µmole enzyme concentration after 36 h of pretreatment. These results show that the enzyme produced from cellulolytic fungus *H. fuscoatra* is capable of increasing paddy straw digestibility and thus enhancing the utilization of paddy straw for different purposes.

Key words: Cellulase production, *Humicola fuscoatra*, paddy straw, paddy straw digestibility.

INTRODUCTION

Rice being the major cereal crop is produced in large quantities in India. About 136.5-150 million tons of paddy straw is estimated to be produced annually in India (Anonymous, 2010). Punjab had contributed 10.86% of the total rice production in India during 2012, with a production of 104.32 million tons. About 1-1.5 kg of straw is produced from every kilogram of grain harvested (Maiorella, 1985). Paddy straw is a big challenge for agriculture scientists, engineers and environmentalists, as a huge quantity of straw is difficult to handle. In India,

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approximately 75-80% (85-95 million tons) of paddy straw is disposed off by burning. One ton of paddy straw burning releases 3 kg particulate matter, 60 kg CO, 1460 kg CO_2, 199 kg ash and 2 kg SO_2 (Jenkins and Bhatnagar, 2003). Burning of paddy straw in the open fields led to various medical and environmental problems. It is a major source of environmental pollution and climate change. It may also lead to many medical problems such as skin irritation, bronchitis, asthma, eye ailments, respire-tory troubles etc. The straw burning further destroys the soil texture.

Paddy straw is an attractive lignocellulosic material which is one of the most abundant renewable resources. It predominantly contains cellulose (35-40%), hemicellulose (20-24%), lignin (8-12%), ash (14-16%) and extractives (10-12%) (Maiorella, 1985; Saha, 2003). Bioconversion of lignocellulosic biomass contributes significantly to the production of organic chemicals. High cellulose and hemi-cellulose content of paddy straw can also be readily hydrolysed into fermentable sugars. Cellulase is the enzyme that degrades the cellulose into glucose, which in turn can be converted into ethanol, single cell protein and other valuable chemicals. Paddy straw can be mainly used as a source of feed for ruminant livestock, but the high level of lignification and silicification, slow and limited ruminal degradation of the carbohydrates and the low content of nitrogen affect its value as a feed for ruminants (Van Soest, 2006).

There occur several challenges and limitations in the process of bioconversion of rice straw. The barrier to the production and recovery of valuable materials from paddy straw is the structure of lignocellulose. An efficient multi-enzyme system is required for the hydrolysis of agricultural biomass to fermentable sugars. But the lignin and silica complex surrounding the cellulose fibers shields the microbial/enzyme action. Therefore, the paddy straw needs to be pretreated in order to enable cellulose to be more accessible to the microbial or enzymatic attack and to increase its digestibility by removing lignocellulolytic complex. Several pretreatments such as physical (grinding, steaming, γ-irradiation), chemical (alkaline hydrolysis, acid hydrolysis, oxidative delignification and solvent extraction), physico-chemical (ammonia fiber explosion, CO_2, steam explosion) and biological (microorganisms and enzymes) pretreatments have been used to improve rice straw utilization (Sarnklong et al., 2010). However, the physical and chemical pretreatments require high energy and corrosion resistant high pressure reactors, which increase the cost of pretreatment. The physical, chemical and physico-chemical treatments are still restricted in terms of safety concerns, costs and potential negative environmental consequences (Phutela et al., 2011). Biological treatments such as the use of ligninolytic fungus, with their ligninolytic enzymes or specific enzymes degrading cellulose or hemicelluloses is an alternative approach to improve the nutritive value of rice straw. Enzymatic hydrolysis of cellulosic wastes may give a relatively pure product with the consumption of less energy during the process (Fennington et al., 1982).

Complete enzymatic hydrolysis of lignocellulosic waste requires the synergistic action of three types of cellulases; namely exoglucanase or cellobiohydrolase, endoglucanase or carboxymethyl cellulase and β-glucosidases. Endoglu-canase (endo-1,4-glucano-hydrolase) attacks regions of low crystallinity in the cellulose fiber and creates free side chains. Exoglucanase or cellobiohydrolase (1,4-glucan cellobiohydrolase) degrades the molecule further by removing cellobiose units from the free chain ends. β-glucosidase hydrolyses cellobiose to produce glucose. Cellulolytic enzymes are synthesized by a number of microorganisms commonly by bacteria and fungi (Lederberg, 1992). Fungal cellulases have proved to be a better candidate than other microbial cellulases (Lynd et al., 2002), with their secreted free cellulase complexes comprising all the three components of cellulase (endoglucanases, exoglucanases and cellobiases). Filamentous fungi like *Aspergillus*, *Penicillium* and *Trichoderma* had demonstrated a great capability for secreting a wide range of cellulolytic enzymes. Since most industrial processes are carried out at high temperatures, therefore, there is a great demand for thermophilic enzymes (Haki and Rakshit, 2003). Thermophiles are a good source of novel catalysts that are of great industrial interest. The thermophiles have more stable enzymes as compared to mesophiles (Li et al., 2005). Thermophilic enzymes are also active at low temperatures. Thermophiles developed more rapidly to higher peaks as compared to mesophiles and stability of obligate thermophiles increased with process temperature. No doubt, reports are available for biological pretreatment of paddy straw by using mesophilic fungi; however less work has been done on pretreatment using thermophilic fungi. Recently, Phutela and Dar (2014) showed enhancement in paddy straw digestibility by pretreatment with thermophilic fungus *Thermoascus aurantiacus* MTCC 375. However, till now the data on the production of cellulases by thermophilic fungi is very scarce. Therefore, the need of the hour is to produce efficient cellulases which can enhance paddy straw digestibility. So, the present investigation was undertaken with the objective of the optimization of production of cellulases from microbial source i.e. *Humicola fuscoatra* MTCC 1409 and application of cellulases to increase paddy straw digestibility.

MATERIALS AND METHODS

Procurement of materials and maintenance of culture

Paddy straw was procured from the research field of Punjab Agricultural University, Ludhiana after harvesting of the crop. The paddy straw was chopped to 3-4 cm with a chopping machine and ground with a blender and was stored in polythene bags at room

temperature. The culture of *Humicola fuscoatra* MTCC 1409 was procured from the Institute of Microbial Technology, Chandigarh, India. The culture was maintained by sub-culturing on potato dextrose agar (PDA) slants at 45±2°C by monthly transfers.

Preparation of media and production of enzyme

Enzyme cellulase was produced on Mandel media (Mandels et al., 1976). Composition of Mandel medium (per L) was: 0.3 g urea, 0.75 g peptone, 0.25 g yeast extract, 1.4 g $(NH_4)_2$ SO_4, 2.0 g KH_2PO_4, 0.3 g $CaCl_2$, 0.3 g $MgSO_4.7H_2O$, 0.005 g $FeSO_4.7H_2O$, 0.0016 g $MnSO_4.4H_2O$, 0.0014 g $ZnSO_4.7H_2O$ and 0.020 g $CaCl_2.6H_2O$. Enzyme production was carried out by adding 3 g of paddy straw (substrate) to flasks containing 12 ml of Mandel media. The pH of media was adjusted to 6.0 and media was autoclaved. After cooling to room temperature, the flasks were inoculated with 1 ml of 10^7 spores /ml of spore suspension and were incubated at 45±2°C for 6 days in an incubator. After 6 days of incubation, the enzyme was extracted with acetate buffer (10 times of substrate) and the fermented medium was centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was used as crude enzyme preparation.

Effect of different cultural conditions on cellulase production

The effect of initial pH on enzyme production was investigated by adjusting the pH of Mandel's medium containing substrate in the range of pH 4.0-8.0 with the interval of pH 1.0 and incubating the flasks for 6 days at 45°C in an incubator. For investigating the effect of incubation temperature on enzyme production, flasks containing Mandel media and substrate having pH 6.0 were incubated at different temperatures ranging from 35 to 60°C with 5°C interval for 6 days in an incubator. The effect of inoculum size was determined by adding the spore suspension of concentrations 10^6, 10^7 and 10^8 spores/ml using 1 ml of spore suspension to each flask containing Mandel media and substrate having pH 6.0 and the flasks were incubated at 45°C for 6 days. To study the effect of days of incubation on enzyme production, spore suspension of 1 ml of 10^7 spores/ml was grown on substrate with Mandel medium at pH 6.0 and incubated at 45°C and the enzyme was extracted after 2, 4, 6 and 8 days interval.

Assay of cellulolytic enzymes

Filter paper assay

To 0.5 ml of enzyme, 1 ml of sodium citrate buffer (pH 4.8) and one whatman # 1 filter paper strip (6×1 cm) was added to each tube. Tubes were incubated in a water bath at 50°C for 1 h. After incubation, 3 ml of DNS reagent was added and tubes were then placed in a boiling water bath for 15 min and 1 ml of sodium potassium tartarate was added. The contents were cooled at room temperature followed by addition of 2 ml distilled water in each test tube. The absorbance was recorded at 575 nm in a UV-VIS spectrophotometer (Miller, 1959). The corresponding enzyme activity was calculated from the standard curve prepared simultaneously using glucose as a standard (10 to 100 µg/ml). One unit of cellulase is defined as the amount of enzyme which will release 1 µmole of reducing sugar in one min per gram paddy straw.

Carboxymethyl cellulase assay

To 0.5 ml of enzyme extract 0.5 ml of substrate (carboxymethyl cellulose) was added and the tubes were incubated at 50°C for 30 min. Reducing sugar produced during this reaction was estimated using DNS as described above.

Cellobiase assay

To 0.5 ml of enzyme extract, 0.5 ml of cellobiose solution was added and the mixture was incubated at 50°C for 10 min. Reducing sugar produced during this reaction was estimated using DNS.

Effect of cellulase on paddy straw digestibility

Chopped paddy straw (10 g) was soaked in water overnight. The excess water was drained off, so as to have approximately 65-70% moisture content. Then, 1, 1.5 and 2 units of enzyme cellulase were added per gram of paddy straw. After proper mixing, paddy straw was incubated at 45±2°C for different time intervals i.e. 18, 24, 30 and 36 h, respectively. After the completion of each incubation period, the paddy straw was oven dried and then each set of paddy straw was analysed for its proximate composition that is total solids, volatile solids, ash, cellulose, hemi-cellulose, lignin and silica content by Standard methods of AOAC(AOAC, 2000). Total sugars were estimated by phenol-sulphuric acid method using glucose as a standard (100 µg/ml) (Dubois et al., 1956).

Statistical analysis

All treatments were completed in triplicate. Critical difference at 5% level was performed for proximate and chemical analysis using Completely Randomized Designs (CRD) in the CPCS software developed by Department of Statistics, PAU, Ludhiana. Standard error was calculated manually for all the experiments.

RESULTS AND DISCUSSION

Optimization of production of cellulose

Effect of different cultural conditions on cellulase production

Effect of initial pH on cellulase production: Production of cellulases increased with increase in pH value, reaching the maximum at pH 6.0, followed by gradual decrease thereafter (Figure 1). Cellobiase activity was higher than CMCase and Fpase activity. In medium at pH 6.0, the activity of enzyme cellobiase was 10.8 U/g, CMCase was 2.99 U/g, and Fpase was 1.42 U/g. Ong et al. (2012) also reported maximum exoglucanase activity of 46.45 FPU/gat pH 6.0 by *Aspergillus niger.* However, Ahmed et al. (2009) observed maximum production of exoglucanase, endoglucanase and cellobiase at pH 5.5 by *Trichoderma harzianum*. While Devi and Kumar (2012) showed maximum cellulase production of 3.9 U/ml by *A. niger* at pH 5.0. Pushalkar et al. (1995) observed that cellobiase activity was very high as compared to CMCase and Fpase activity with the pH in range of 4.0-5.5 when produced from *Aspergillus terreus*.

Effect of temperature on cellulase production:

Maximum activity of cellobiase (9.30 U/g), CMCase (3.83

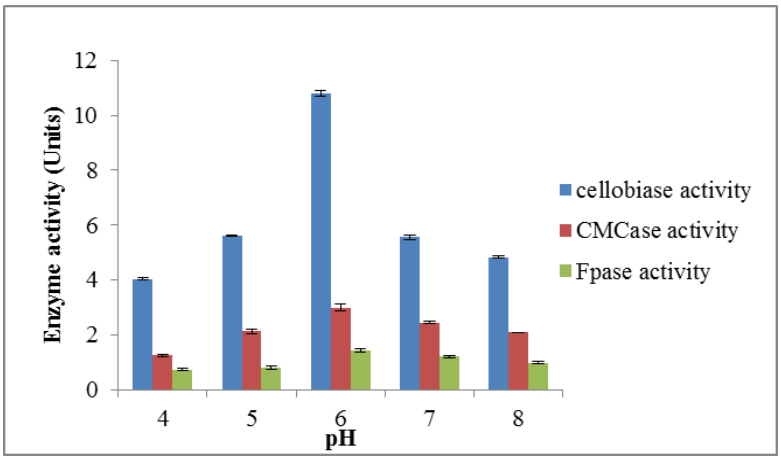

Figure 1. Effect of pH on cellulase production.

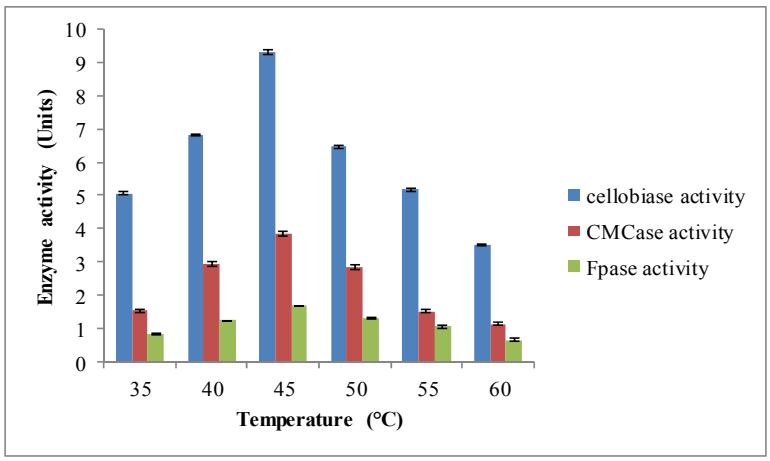

Figure 2. Effect of temperature on cellulase production.

U/g) and Fpase (1.67 U/g) was observed at 45°C (Figure 2). Further increase or decrease in temperature resulted in decrease in enzyme activity indicating that 45°C was the optimum temperature for the maximum production of enzyme. Less production of enzyme at low temperature (35-40°C) and at high temperature (50-60°C) as compared to 45°C might be due to slow growth of fungus at low temperature and inactivation of the enzyme at high temperature. Kaur et al. (2007) also reported maximum cellobiase activity of 132.4 U/g at 45°C from a new strain of thermophilic fungus *Melanocarpus* sp. MTCC 3922. Devi and Kumar (2012) showed that the highest cellulase activity (3.9 U/ml) was obtained at 45°C by *A. niger*. However, Ahmed et al. (2009) reported maximum cellulase production at 28°C by *Trichoderma harzianum*. Ali and El-Dein (2008) showed that optimum temperature for three cellulases was 35°C for *A. niger* and 30°C for *Aspergillus nidulans*.

Effect of inoculum density on enzyme production

Increase in inoculum size from 10^6 spores/ml to 10^7 spores/ml resulted in increase in enzyme production from 5.13 U/g for cellobiase, 1.24 U/g for CMCase and 0.817 U/g for Fpase to 9.72 U/g for Cellobiase, 2.89 U/g for CMCase and 1.60 U/g for Fpase. Further increase in inoculum size that is at 10^8 spores/ml resulted in decrease in all the three enzyme activities which might be due to fast degradation of substrate (Figure 3). At inoculum size of 10^8 spores/ml, 32.71% decline in the production of enzyme was observed with respect to 10^7 spores/ml indicating inoculum size of 1ml of 10^7 spores was found to be optimum for maximum production of cellulases. Grover et al. (2013) showed that with increase in inoculum size from $1\times10^5$to 1×10^7 spores/ml, cellulase production increased from 0.237 to 0.541 IU. However, Pankaj and Satyanarayana (2004) observed maximum

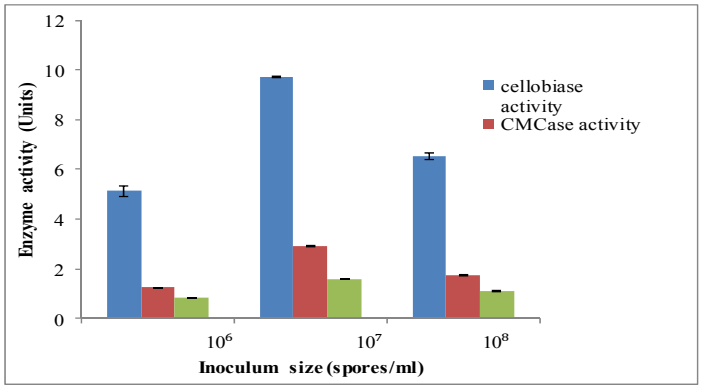

Figure 3. Effect of inoculum size on cellulase production.

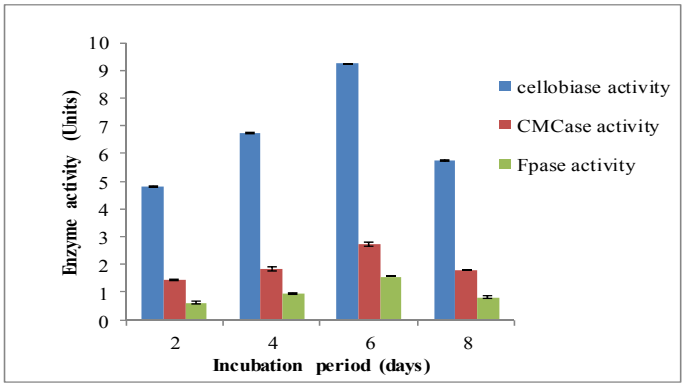

Figure 4. Effect of incubation days on cellulase production.

cellulase production of 7832 U/g of dry moldy bran with an inoculum level of 3×10^6 spores of *Humicola lanuginose*.

Effect of incubation days on cellulase production

After 2 days of incubation the activity was 4.82 U/g for cellobiase, 1.44 U/g for CMCase and 0.62 U/g for Fpase which increased to 6.73 U/g for cellobiase, 1.84 U/g for CMCase and 0.94 U/g for Fpase after 4 days of incubation. After 6 days of incubation the activity further enhanced to 9.24 U/g for cellobiase, 2.74 U/g for CMCase and 1.55U/g for Fpase. Further increase in time did not show any increment in the level of enzyme production as activity of enzyme declined to 5.74 U/g for cellobiase, 1.79 U/g for CMCase and 0.82 U/g for Fpase after 8 days of incubation period. Hence maximum yield of cellulase was observed after 6 days of incubation (Figure 4). The period required for incubation depends on the growth rate of microorganism, on substrate concentration and its enzyme production pattern. Kang et al. (2004) reported the highest cellulase activity after 5-6 days of fermentation by *A. niger* on rice straw. Devi and Kumar (2012) reported maximum cellulase production at 7^{th} day of incubation by *A. niger*. However, *Trichoderma harzianum* showed maximum cellulase activity at 5^{th} day of incubation (Ahmed et al., 2009). Ali and El-Dein (2008) reported that 7 days of incubation was best for maximum cellulase production by *A. niger* and *A. nidulans.*

Evaluation of effect of enzymatic pretreatment on paddy straw digestibility

Effect of enzymatic pretreatment and incubation period on paddy straw digestibility

Pretreatment of paddy straw was done using varied enzyme concentrations (1, 1.5 and 2 units/g paddy straw) and was kept for 18, 24, 30 and 36 h in an incubator at 45°C. Results show that with the increase in enzyme concentration and incubation period; neutral detergent fiber, acid detergent fiber, cellulose and hemicellulose content of paddy straw decreased whereas lignin and silica content increased. After 36 h of incubation, neutral detergent fiber decreased by 5.3% at 1 unit/g, 8.8% at

Table 1. Effect of enzyme concentration and incubation time on lignocellulose composition of paddy straw.

Composition	Enzyme units (µmoles)	Time (h)				CD (5%)
		18	24	30	36	
NDF (%)	1	74.5±0.26 (3.1↓)	73.8±0.37 (4.03↓)	73.4±0.26 (4.5↓)	72.8±0.35 (5.3↓)	T= 0.494 E= 0.427 T×E= 0.855
	1.5	73.5±0.24 (4.4↓)	72.9±0.26 (5.2↓)	72.2±0.26 (6.1↓)	70.1±0.35 (8.8↓)	
	2	72.1±0.21 (6.2↓)	71.0±0.26 (7.7↓)	69.8±0.23 (9.2↓)	69.6±0.38 (9.2↓)	
ADF (%)	1	51.9±0.26 (0.4↓)	51.3±0.29 (1.5↓)	50.9±0.52 (2.3↓)	50.4±0.40 (3.3↓)	T= 0.545 E= 0.472 T×E= NS
	1.5	51.2±0.26 (1.7↓)	50.5±0.32 (3.1↓)	50.3±0.35 (3.4↓)	49.9±0.36 (4.2↓)	
	2	50.8±0.14 (2.5↓)	50.1±0.29 (3.8↓)	49.7±0.18 (4.6↓)	49.0±0.32 (5.9↓)	
Cellulose (%)	1	37.9±0.17 (2.0↓)	37.3±0.26 (3.6↓)	36.5±0.35 (5.7↓)	35.6±0.29 (8.0↓)	T= 0.492 E= 0.426 T×E=NS
	1.5	37.4±0.32 (3.3↓)	36.7±0.32 (4.4↓)	35.7±0.28 (7.7↓)	34.8±0.43 (10.1↓)	
	2	37.0±0.26 (4.4↓)	36.1±0.18 (6.7↓)	35.1±0.21 (9.3↓)	34.5±0.32 (10.8↓)	
Hemicellulose (%)	1	23.1±0.32 (6.8↓)	22.2±0.33 (10.5↓)	21.1±0.32 (14.9↓)	20.0±0.56 (19.3↓)	T= 0.56 E= 0.485 T×E= NS
	1.5	22.4±0.26 (9.7↓)	21.3±0.23 (14.1↓)	20.1±0.30 (18.9↓)	19.2±0.35 (22.6↓)	
	2	21.9±0.31 (11.7↓)	20.9±0.26 (15.7↓)	19.6±0.26 (21.0↓)	19.0±0.34 (23.4↓)	
Lignin (%)	1	7.20±0.06 (2.9↑)	7.35±0.03 (5.0↑)	7.44±0.04 (6.3↑)	7.47±0.01 (6.7↑)	T= 0.058 E=0.051 T×E= NS
	1.5	7.38±0.03 (5.4↑)	7.54±0.03 (7.7↑)	7.57±0.02 (8.1↑)	7.61±0.02 (8.7↑)	
	2	7.55±0.02 (7.8↑)	7.60±0.02 (8.6↑)	7.64±0.03 (9.1↑)	7.68±0.03 (9.7↑)	
Silica (%)	1	6.55±0.03 (2.34↑)	6.63±0.03 (3.6↑)	6.71±0.03 (4.8↑)	6.76±0.02 (5.6↑)	T= 0.039 E= 0.034 T×E= NS
	1.5	6.62±0.02 (3.4↑)	6.66±0.03 (4.1↑)	6.73±0.02 (5.15↑)	6.79±0.02 (6.1↑)	
	2	6.67±0.01 (4.2↑)	6.70±0.02 (4.7↑)	6.77±0.02 (5.9↑)	6.81±0.01 (6.4↑)	

#Data in parenthesis represent percentage increase or decrease as compared to control i.e. untreated paddy straw; T: time, E: enzyme concentration; Untreated paddy straw composition (%): NDF=76.9; ADF=52.1; cellulose=38.7; hemicellulose=24.8; lignin= 7.0; silica=6.4; ± values indicate % standard error for triplicate data; (↓), decrease; (↑), increase.

1.5 units/g and 9.2% at 2 units/g of enzyme concentration whereas acid detergent fiber decreased by 3.3% at 1 unit/g, 4.2% at 1.5 units/g and 5.9% at 2 units/g of enzyme concentration. Cellulose and hemicellulose content also decreased gradually during 36 h of incubation and percentage decrease was 8 to 10.8% for cellulose and 19.3 to 23.4% for hemicellulose (from 1 to 2 units/g of enzyme concentration). Decrease in cellulose and hemicellulose content might be the result of breakdown of cellulose and hemicellulose into fermentable sugars. Lignin content before the start of treatment was 7% which increased to 7.47, 7.61 and 7.68% after treatment with 1, 1.5 and 2 units/g of enzyme concentration respectively. Silica content also increased by 5.6, 6.1 and 6.4 % at 1, 1.5 and 2 units/g of enzyme treatment respectively (Table 1). Similar results were reported by Zafar et al. (1980)

Table 2. Effect of enzymatic pretreatment on total solids, ash, volatile solids and total sugars.

Composition	Enzyme units (µmoles)	Time (h)				CD (5%)
		18	24	30	36	
Total solids	1	24.1±0.32(6.9↓)	23.7±0.17(8.5↓)	23.1±0.23(10.8↓)	22.7±0.26(12.3↓)	T= 0.426
	1.5	23.5±0.23(9.3↓)	23.3±0.23(10.0↓)	22.6±0.29(12.7↓)	22.3±0.32(13.9↓)	E= 0.369
	2	23.0±0.23(11.2↓)	22.5±0.26(13.1↓)	22.2±0.23(14.3↓)	21.9±0.23(15.4↓)	T×E= NS
Ash	1	17.6±0.29(4.8↑)	18.2±0.14(8.3↑)	19.0±0.17(13.1↑)	20.4±0.26(21.4↑)	T= 0.392
	1.5	17.9±0.26(6.5↑)	18.4±0.21(9.5↑)	19.8±0.23(17.8↑)	20.7±0.24(23.2↑)	E= 0.34
	2	18.1±0.20(7.7↑)	18.9±0.20(12.5↑)	20.9±0.20(24.4↑)	21.3±0.31(26.8↑)	T×E= NS
Volatile solids	1	82.4±0.29(0.96↓)	81.8±0.23(0.607↓)	81.1±0.21(1.45↓)	80.0±0.20(2.8↓)	T= 0.413
	1.5	82.0±0.28(0.36↓)	81.3±0.26(1.25↓)	80.2±0.23(2.55↓)	79.6±0.23(3.3↓)	E= 0.357
	2	81.7±0.23(0.73↓)	80.5±0.28(2.2↓)	79.4±0.23(3.5↓)	79.1±0.20(3.9↓)	T×E= NS
Total sugars	1	36.9±0.52(3.4↑)	37.4±0.23(4.8↑)	38.4±0.20(7.6↑)	38.7±0.23(8.4↑)	T= 0.417
	1.5	37.3±0.14(4.5↑)	37.7±0.26(5.6↑)	38.8±0.20(8.7↑)	39.3±0.18(10.1↑)	E= 0.362
	2	37.9±0.18(6.2↑)	38.2±0.18(7.0↑)	39.0±0.26(9.2↑)	39.7±0.14(11.2↑)	T×E= NS

#Data in parenthesis represent percentage increase or decrease as compared to control i.e. untreated paddy straw composition; T, time; E, enzyme concentration; Untreated paddy straw composition (%): Total solids=25.9, volatile solids=83.2, ash= 16.8, total sugars=35.7; ± values indicate % standard error for triplicate data; (↓): decrease; (↑): increase.

who showed decrease in cellulose content after treatment of paddy straw by *Pleurotus sajor caju*. Jafari et al. (2007) also reported decrease in hemicellulose, acid detergent fiber andneutral detergent fiber after pretreatment of rice straw with *Pleurotus spp*. Sahni (2013) also reported reduction in neutral detergent fiber, acid detergent fiber, cellulose and hemicellulose content and increase in lignin and silica content after pretreatment of paddy straw with *Humicola fuscoatra*. Phutela and Dar (2014) investigated the potential of microbial pretreatment under aerobic conditions on paddy straw digestibility by pretreating it with *Thermoascus aurantiacus* MTCC 375 at regular intervals of 1, 2, 3, 4 and 5 days and reported that pretreatment of 5 days significantly reduced the concentrations of cellulose, hemicelluloses, lignin and silica content of paddy straw. A maximum of 30% increase in biogas production was observed from one day pretreated paddy straw as compared to untreated paddy straw.

Effect of enzymatic pretreatment on total solids, ash, volatile solids and total sugars

Treatment of paddy straw with different enzyme concentrations and incubation periods resulted in decrease in total solids and volatile solids and increase in ash content. This increase or decrease in parameters increased with increase in enzyme concentration and incubation period. During 36 h of incubation total solids gradually decreased from 25.9 to 22.7, 22.3 and 21.9% at 1, 1.5 and 2 units/g of enzyme concentration respectively. However, ash content increased by 21.4, 23.2 and 26.8 % respectively at 1, 1.5 and 2 units/g of enzyme concentration. From 18 to 36 h volatile solids decreased from 82.4 to 80% at 1 unit/g, 82 to 79.6% at 1.5 units/g and 81.7 to 79.1% at 2 units/g of enzyme concentration. The percentage increase in total sugars was 8.4 to 11.2% from 1 to 2 units/g of enzyme concentration at 36 h. Total sugars increased gradually with increase in enzyme concentration and incubation period because of hydrolysis of cellulose into fermentable sugars by cellulase action (Table 2) as also indicated by decrease in cellulose and hemicellulose content (Table 1). Phutela et al. (2011) showed that treatment of rice straw with *Trichoderma reesei* MTCC 164 and *Coriolus versicolor* MTCC 138 resulted in decrease in total solids, volatile solids and increase in ash &total sugars. Phutela and Dar (2014) also reported reduction in total solids, volatile solids and enhancement in ash content of paddy straw when pretreated with thermophilic fungus *Thermoascus aurantiacus* MTCC 375.

Conclusion

Cellulases were produced from thermophilic fungus *H. fuscoatra* by solid state fermentation and maximum production was achieved at 6th day of incubation at pH 6.0 and temperature 45°C when spore suspension containing 10^7 spores/ml was used. The pretreatment of paddy straw with this enzyme resulted in decrease in NDF, ADF, cellulose and hemicellulose content of paddy straw. Thus, the cellulases produced from thermophilic fungus *Humicola fuscoatra* are capable of increasing paddy straw digestibility and thus enhancing the utilization of

paddy straw for different purposes such as for biogas production, ethanol production or as a feed for ruminants etc.

Conflict of interests

The authors did not declare any conflict of interest.

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