Selection of Fungal Isolates for Biopulping of Rice Straw

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Sixty-two fungal isolates were screened for lignin peroxidase production. The most potent isolates for lignin peroxidase production were identified using the DNA sequence of the internal transcribed spacer (ITS) region as *Phanerochaete chrysosporium* and *Pleurotus ostreatus*. The pretreatment of rice straw with *P. chrysosporium*, *Pl. ostreatus*, or lignin peroxidase for use in the biopulping process was studied. Great variations in the loss of pulp yield and kappa number were recorded with different fungal and enzyme treatments. Pretreatment of rice straw with *P. chrysosporium* for 25 days resulted in a substantial decrease in pulp yield (by 9.1%) and kappa number (by 25.6%). Losses of pulp yield and kappa number were considerably lower with lignin peroxidase treatment (3.7 and 14.1%, respectively). However, the pretreatment of rice straw with the *Pl. ostreatus* isolate caused moderate pulp yield losses (5.8%) and preferential lignin degradation (kappa number losses of 34.6%). This indicated that the *Pl. ostreatus* isolate might be superior to both the isolate of *P. chrysosporium* and lignin peroxidase for use in the biopulping process or other processes in which preferential lignin degradation is desired.

Keywords: Lignin peroxidase; Biopulping; Rice straw; Phanerochaete chrysosporium; Pleurotus ostreatus

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INTRODUCTION

Lignocellulosic material can be obtained from wood, grass, agricultural residues, forestry wastes, and municipal solid wastes. Several biological methods for lignocellulose recycling, based on the enzymology of cellulose, hemi-cellulose, and lignin degradation, have been suggested. Among them, composting and use as a raw material for the production of ethanol as an alternative combustible form seem to be the most economically feasible. Moreover, the general use of alternative, environmentally friendly technologies that introduce lignocellulose enzymes at different stages of pulp and paper manufacture as a pretreatment to pulping (biopulping) and bleaching (biobleaching) have allowed considerable electrical power savings, improvements in paper strength, and a reduction of pollutants in the waste water from these industries (Akhtar *et al*. 1998; Shukla *et al*. 2004; Singh *et al*. 2010; Isroi *et al*. 2011; Garmaroody *et al*. 2011). In addition, pretreatment of agricultural wastes with ligninolytic fungi enables the use of the wastes as raw material for paper manufacturing.

Increase of demand for paper production and limited wood resources have directed researchers to look for appropriate additional resources of non-wood materials for pulp and paper manufacturing. Several kinds of non-wood lignocellulosic by-products of agricultural cultivation have been investigated, of which wheat, rice, and barley straw

are the most prominent (Yaghoubi *et al*. 2008; Xu *et al*. 2013; Wulandari *et al*. 2013), particularly in the countries with deficient wood resource, such as China, India, and Egypt. In Egypt, huge amounts of rice straw are produced annually as fibrous by-product. The disposal of these by-products is becoming a major problem. The microbial pretreatment is a natural process; therefore, no adverse environmental consequences are foreseen.

Biological pretreatment employs microorganisms and their enzymatic machineries to break down lignin and alter lignocellulosic structures. Some of the most promising microorganisms for biological pretreatment are white-rot fungi that can mineralize lignin to CO_2 and water in pure culture (Eriksson *et al.* 1990; Akhtar *et al*. 1998). Several white-rot fungi such as *Phanerochaete chrysosporium*, *Ceriporiopsis subvermispora*, *Phlebia subserialis*, and *Pleurotus ostreatus* are capable of efficiently metabolizing lignin in a variety of lignocellulosic materials (Eriksson *et al.* 1990; Martinez *et al*. 1994; Dorado *et al*. 1999; Yaghoubi *et al*. 2008). The fungi have been studied in connection with several ligninolytic enzymes, such as lignin peroxidases (LiP), manganese peroxidases (MnP), laccase (Lac), and versatile peroxidases (VP) (Rodrigues *et al*. 2008; Singh *et al*. 2010; Isroi *et al*. 2011).

White rot fungi and their enzymes (especially ligninases and xylanases) have been considered for wood chip treatment prior to pulping. Although ligninases attack the lignin content of wood, xylanases degrade hemicelluloses and make the pulp more permeable for the removal of residual lignin. Thus, the biopulping process removes not only lignin but also some of the wood extractives, thus reducing the pitch content and effluent toxicity (Ali and Sreekrishnan 2001).

Lignin degradation by white rot fungi is a complex secondary metabolic process mediated by the action of several extracellular enzymes, of which lignin peroxidases are the most important (Buswell and Odier 1987; Akhtar *et al.* 1998; Rodrigues *et al*. 2008; Isroi *et al*. 2011). Consequently, they and their extracellular ligninolytic enzymes have been considered for various applications in environmental biotechnology. The present work aims to isolate and select fungal strains with high lignin peroxidase contents to use as a pretreatment for pulping (biopulping) of rice straw.

MATERIALS AND METHODS

Rice Straw

Rice straw was obtained from Kom Hamada City, El-Behira Province, Egypt. It was 2.0 to 2.5 mm in length. The main composition of the rice straw was as follows: cellulose, 40%; hemicellulose, 19%; lignin, 25%; and ash, 16%. The components (cellulose, hemicellulose, and lignin) of rice straw were analyzed according to the procedures of Goering and Van Soest (1971). Ash content was determined by ignited a straw sample of 2 gram in muffle furnace at 550 ^{o}C to constant weight.

Microorganisms

In the present study, 62 local fungal strains were isolated from different rice straw compost samples in Egypt. The serial dilution plate method was used. The potato dextrose agar (PDA) medium was poured into sterile petri plates, then inoculated with 1.0 mL from each dilution. The plates were incubated at 30 ^{o}C for 4 days, and individual colonies were grown out on slants of PDA medium. The isolated fungi were purified

three times by restreaking on the same medium. Pure cultures of fungi were maintained on agar slants of PDA at 4 ^{o}C. The isolated pure cultures were tested for enzyme activity.

Selection of the Potent Isolates for Lignin Peroxidase Production

Erlenmeyer flasks (250 mL capacity) containing 50 mL of sterile potato dextrose broth medium were autoclaved. Each flask was inoculated with 2 fungal discs (0.8 cm) of a 4-day old culture and incubated at 30 ^{o}C in rotary shakers at 100 rpm for 2, 3, 4, 5, and 6 days. The cultures were filtered using Whatman no.1 filter paper for the removal of fungal growth. The filtrate was used as the source of crude enzyme.

Lignin peroxidase Assays

Lignin peroxidase activity was measured by the veratryl alcohol method (Tien and Kirk 1988). The assay mixture contained in a final volume of 2.5 mL was as follows: 1.8 mL of the diluted supernatant, 0.1 mL of 50 mM veratryl alcohol, 0.5 mL of 0.5 M sodium tartrate buffer (pH 3.0), and 0.1 mL of 10 mM H_2O_2. Oxidation of the substrate at room temperature was monitored by an absorbance increase at 310 nm due to the formation of veratraldehyde (ε_{310} = 9300 M^{-1} cm^{-1}). One unit of enzyme activity was defined as the amount of enzyme needed to produce 1 µmol of veratraldehyde per minute.

Dry Biomass Measurement

Fungal biomass determination was conducted by filtration through preweighed Whatman no.1 filter paper, washing with distilled water, and then drying in an oven until two successive equal weights for the same sample were obtained. The biomass was calculated in terms of gram per liter of growth medium.

Extraction of Genomic DNA

Fungal mycelia were grown on potato dextrose agar (PDA), harvested using a spatula, transferred into 1.5-mL Eppendorf tubes, suspended in 1 mL of $T_{10}E_1$ buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]), and centrifuged at 13,000 rpm for 5 min. The pellet was resuspended in 200 µL of 50 mM NaOH, vortexed, and incubated at 95 °C for 10 min. The mixture was then neutralized with 200 µL of 0.1 mM Tris-HCl (pH 7.0) and centrifuged for 5 min at 13,000 rpm. The pellet was resuspended in 500 µL of sterile H_2O and centrifuged at 13,000 rpm for 5 min. The supernatant was removed, followed by the addition of 200 µL of cracking buffer (2% Triton X-100, 1% sodium dodecyl sulfate, 100 mM NaCl, 20 mM Tris [pH 8.0], 10 mM EDTA [pH 8.0]). Extraction of genomic DNA was achieved by addition of glass beads to 3/4 of the liquid volume, followed by the addition of 200 µL of cold phenol-chloroform-isoamyl alcohol in a 25:24:1 ratio. The mixture was subjected to constant vortexing for 30 min. The sample was then centrifuged at 13,000 rpm for 5 min. The aqueous (top) layer was transferred to a new tube, and 1 mL of 100% ethanol was added for DNA precipitation. The sample was centrifuged at 13,000 rpm for 2 min. The supernatant was removed, and the pellet was resuspended in 400 µL of $T_{10}E_1$ buffer plus 30 mg of RNase A (Sigma, St. Louis, MO) for a 1-h water bath incubation at 35 °C. The reaction was stopped, and the DNA was precipitated with 10 µL of 3 M sodium acetate and 1,000 µL of cold 100% ethanol. The sample was centrifuged at 13,000 rpm for 2 min, the supernatant was removed, and the pellet was air-dried. The DNA pellet was resuspended in 50 µL of TE buffer and was used as the DNA template for amplification after 30 min or stored at -20 ^{o}C for future use (Turenne *et al*. 1999).

PCR Amplification

The primers used for universal fungal amplification were ITS4 (reverse primer [5'-tcc tcc gct tat tga tat gc-3']) (White *et al.* 1990) and fluorescently labeled (5' HEX) ITS86 (forward primer [5' -gtg aat cat cga atc ttt gaa c-3']) (Life Technologies, Burlington, Ontario, Canada). The 50-µL PCR reaction mixture contained 5 µL of DNA template; 5 µL of 25 mM $MgCl_2$–10 x PCR buffer; 1.25 mM deoxynucleoside triphosphate; dATP, dGTP, dCTP, and an 8:1 ratio of dUTP to dTTP; 0.5 µL of 100 mM of each primer; 0.5 units of uracil DNA glycosylase (UDG); 2.5 units of *Taq* DNA polymerase; and 30 µL of sterile distilled H_2O. The PCR was performed in a GeneAmp PCR system 9600 (Perkin-Elmer Applied Biosystems, Foster City, Calif.) with cycles of 37 °C for 10 min (UDG activation) and 94 °C for 10 min (UDG inactivation) and 30 cycles of 94, 55, and 72 °C for 1 min each; the mixture was then incubated at 72 °C for 10 min for a final extension (Turenne *et al.* 1999).

DNA Sequencing

The PCR reaction products were sequenced directly using a big Dye terminator reagent kit including *Taq* polymerase and the protocol recommended by the manufacture (Perkin-Elmer). The reactions were run on an ABI 310 automated DNA sequencer (Applied Biosystems Incorporation, California, USA).

Phylogenetic Analysis

The blast program (www.ncbi.nlm.gov/blast) was used to assess DNA similarities; multiple sequence alignment and molecular phylogeny were performed using BioEdit software (Hall 1999). The phylogenetic tree was displayed using the TreeView program (Page 1996).

Inoculum Preparation

In preparing the liquid inoculum of the selected fungal isolates, 1-L flasks containing 200 mL of the fermentation medium were inoculated with 10 plugs cut with a number-8-size cork pore from a potato dextrose agar plate containing 10-day-old fungal cultures. The flasks were then incubated at 30 ^{o}C for 10 days. Flasks containing the fungal biomass were decanted and washed with sterilized distilled water to remove excess medium from the fungal biomass. The fungal biomass was then placed in sterilized distilled water and blended in a Waring blender twice for 15 s, each time at high speed. Distilled water was then added to the suspension to a total volume 100 mL (stock). About 1.0 mL of fungal suspension stock produced 0.005% (dry weight basis) inoculum of each strain.

Partial Purification of Lignin Peroxidase

The culture fluid was centrifuged at 10,000 rpm for 5 min at 4 ^{o}C to remove fungal mycelium. $(NH_4)_2SO_4$ was added to the supernatant to 85% saturation at 4 ^{o}C. The precipitated crude lignin peroxidase fraction was recovered by centrifugation (10,000 rpm, 10 min). The pellet was resuspended in deionized water and dialyzed several times against 20 mM succinate (pH 3.5) for 2 days at room temperature. The protein content and lignin peroxidase activity were determined in the enzyme fraction. Protein content was determined as described by Lowry *et al.* (1951) using bovine serum albumin as a standard.

Fungal Pretreatment of Rice Straw

Wide-neck jars (2 L) containing 100 g of rice straw (dry weight), with 2.0-2.5 in length, were autoclaved at 121 ^{o}C for 20 min. After cooling the bioreactors, 440 mL of sterile distilled water containing 25 mg of mycelium (dry weight) of *Phanerochaete chrysosporium* or *Pleurotus ostreatus* or 10 mL of partially-purified enzyme solution were introduced into each of the jars to bring the final moisture of rice straw to about 60% (wet weight basis) and weighed. Rice straw and fungal biomass were mixed and incubated at 30 ^{o}C for 5, 10, 15, 20, and 25 days. The moisture content was kept for each jar at 60% of water holding capacity (WHC) during the experimental period. Controls were prepared under the same conditions but without inoculation.

Soda Pulping

Alkaline pulping was carried out by soaking straw (25 g oven dry weight) in a 1% solution of sodium hydroxide solution at a 10:1 liquor to straw ratio at 45 ^{o}C for 10 min. The saturated mass was squeezed and cooked with an addition of 12% sodium hydroxide at a straw to liquor ratio of 1:6 and 0.1 g anthraquinone % oven-dry straw with maximum temperatures at 127 ^{o}C and cooking time up to 60 min (El-Ashmawy *et al.* 1984). The resulting pulp was washed over filter paper and dried, and the yield and kappa number were determined. Kappa number was determined using the method described by Tasman and Berzins (1957).

RESULTS

Screening for Lignin Peroxidase Production from Isolated Fungi

The results in Table 1 show that the different fungal isolates exhibited a great variation in the lignin peroxidase activity. Of 62 fungal isolates, 11 isolates had lignin peroxidase activity. The highest enzyme activities were recorded after 4 days of incubation. The enzyme activities of such isolates ranged from 0.465 to 7.440 U/mL after 4 days of incubation. The most potent isolates for lignin peroxidase production were isolates 21 and 43. The enzyme activities of these isolates ranged from 5.673 to 7.440 U/mL after 4 days of incubation. Thus, those isolates were selected for further study.

Table 1. Lignin Peroxidase Activities for Fungal Isolates (U/mL)

Isolates No.	Time of incubation in days				
	2	3	4	5	6
1	0.372	0.465	0.465	0.279	0.093
2	0.558	0.744	1.116	0.837	0.651
5	0.372	0.651	0.837	0.558	0.279
14	0.093	0.372	0.465	0.279	0.093
16	0.279	0.558	0.651	0.465	0.186
21	1.767	2.976	5.673	3.999	3.255
34	0.093	0.186	0.651	0.744	0.372
43	3.720	6.975	7.440	5.859	2.883
51	0.651	0.744	0.930	0.558	0.279
59	0.186	0.372	0.465	0.186	0.093
62	0.615	0.701	0.880	0.511	0.324

PCR and DNA Sequencing

PCR products of the ITS (internal transcribed spacer) region (ITS1 + 5.8S + ITS2) amplified with primers ITS1 and ITS4 were visualized as a single band in agarose gels. The DNA sequences of the ITS region for isolates 21 and 43 are represented in Figs. 1 and 2, respectively.

AAAATACGATACTGAACAGGTTGTAAGCTGGCCTCTCGGGGCATGTGCACGCCTGGCTCAT
CCACTCTTCAACCTCTGTGCACTTGTTGTAGGTCGGTAGAAGAGCGAGCATCCTCTGATGCT
TTGCTTGGAAGCCTTCCTATGTTTTACTACAAACGCCTTCAGTTTAAGAATGTCTACCTGCGT
ACAACGCATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACG
CAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGC
ACCTTGCGCTCCCTGGTATTCCGGGGGAGCATGCCTGTTTGAGTGTCATGGTATCCTCAAC
CTTCATAACTTTTTTGTTACCGAAGGCTTGGACTTGGAAGGTTGTGCTGGCTTCAAGTCAAGT
CGGCTCCCCTTAAATGTATTAGCGGGGGGGGTAACGGAATCCCTTCGGGGGGAAAATTATC
TGCCCCCGGGGTCCTGAAGAAACAAAAGCCTGGGGCTTTCTAACCGTCCTTCAGTTGGACA
ACTTACTTTGACATCTGGCCCCAAATCAGGGAGAACACCCCCTGAACTTAACCTT

Fig. 1. DNA sequences of ITS region of isolate 21

CTTTCAACCACGAGAGAACTTTTGATAGATCTGGTGAAGTCGTCTCTCCAGTCGTCAGACTT
GGTTGCTGGGATTTAAACGTCTCGGTGTGACTACGCAGTCTATTTACTTACACACCCCAAAT
GTATGTCTACGAATGTCATTTAATGGGCCTTGTGCCTTTAAACCATAATACAACTTTCAACAA
CGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTAATTGC
AGAATTCAGTGGAATCATCGAATCTTTGAACGCACCTTGCGCCCCCTTGGTATTCCCGAGGG
GCATGCCTGGTTTGAGTGTCATTAAAATTTCTCAAACGCCACTTT

Fig. 2. DNA sequences of ITS region of isolate 43

Phylogenetic Analysis

The sequence data of isolates 21 and 43 were then analyzed using the Blast program (www.ncbi.nlm.gov/blast) to assess the DNA similarities. Multiple sequence alignment and molecular phylogeny were performed using BioEdit software; the phylogenetic trees are displayed, using the TreeView program, in Figs. 3 and 4.

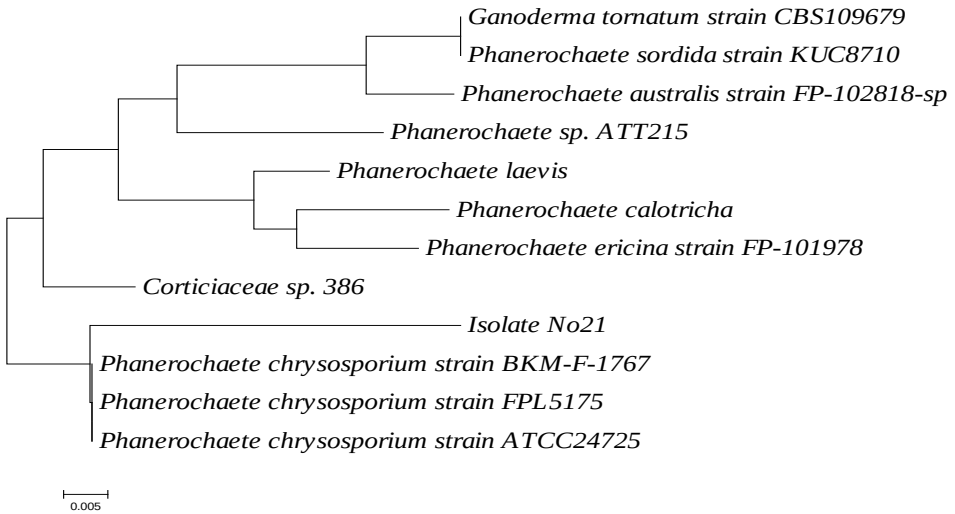

Fig. 3. The phylogenetic tree showing the relationship between isolate 21 and other known sequences using the neighbor-joining method

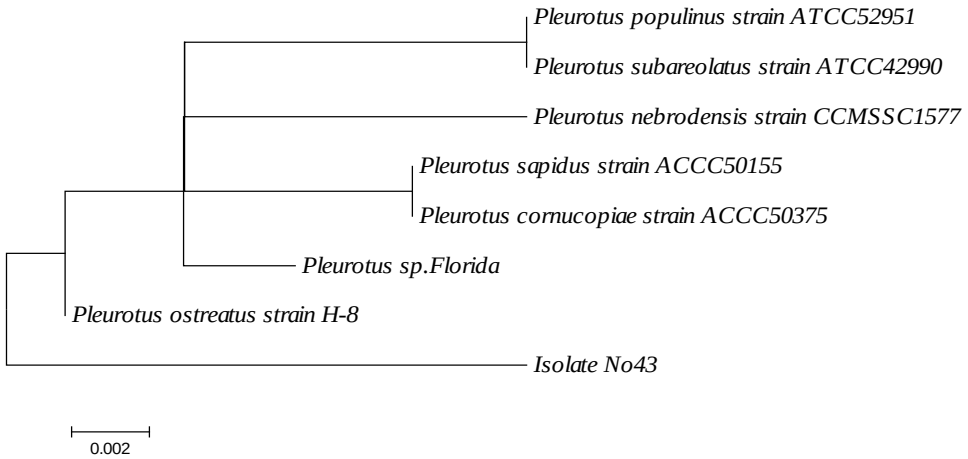

Fig. 4. The phylogenetic tree showing the relationship between isolate 43 and other known sequences using the neighbor-joining method

The obtained results revealed that the sequence of isolate 21 showed the highest similarity (95%) with *Phanerochaete chrysosporium* (*P. chrysosporium*), and the sequence of isolate 43 showed the highest similarity (96%) with *Pleurotus ostreatus* (*Pl. ostreatus*).

Fungal Pretreatment of Rice Straw

The results in Table 2 showed considerable variation in the pulp yield losses of rice straw with different fungal and enzyme treatments. In all treatments, the pulp yield of rice straw was decreased gradually as time increased, as compared to the control treatment. The pulp yield losses ranged from 3.7 to 9.1% as compared to the control after 25 days incubation time. The greatest loss in pulp yield of rice straw was recorded after 25 days with *P. chrysosporium* treatment. The lowest loss in pulp yield of rice straw was detected after 25 days with lignin peroxidase treatment. Treatment of rice straw with *Pl. ostreatus* caused moderate losses of pulp yield.

The increase in the percentages of ash content (Table 2) in the pulp yield paralleled the losses in total dry weight in all the treatments throughout the experimental period. However, the actual amount of ash in all treatments did not show any remarkable changes. The highest increase in ash content in pulp yield occurred with the *P. chrysosporium* treatment. The lowest increase of ash content was observed with lignin peroxidase treatment.

The results also showed great variations in the kappa number of pulp with different fungal and enzyme treatments. During the experimental period, the kappa number reduced gradually from that of the control with all treatments. The fungal strains *P. chrysosporium* and *Pl. ostreatus* reduced the kappa number of pulp from that of the control by 25.6 and 34.6%, respectively. Lignin peroxidase treatment reduced the kappa number from that of the control by 14.1%.

Therefore, the pretreatment of rice straw with the *Pl. ostreatus* isolate caused moderate pulp yield losses and preferential lignin degradation. This indicated that the isolate of *Pl. ostreatus* might be superior to both the isolate of *P. chrysosporium* and lignin peroxidase enzyme for use in biopulping processes or other processes in which preferential lignin degradation is desired.

Table 2. Effect of Fungal and Lignin Peroxidase Pretreatment of Rice Straw on Pulp Yield, Ash Content, and Kappa Number

Treatments	Time in days				
	5	10	15	20	25
Pulp yield %					
Control	48.7	48.1	47.4	46.7	46.2
P. chrysosporium	48.2	46.5	45.0	43.4	42.0
Pl. ostreatus	48.3	47.0	45.8	44.6	43.5
Lignin peroxidase	48.4	47.3	46.2	45.3	44.5
Ash %					
Control	15.2	15.6	15.8	16.1	16.3
P. chrysosporium	16.2	16.9	17.5	18.2	18.7
Pl. ostreatus	15.8	16.5	17.0	17.6	18.1
Lignin peroxidase	15.5	15.8	16.2	16.5	16.8
Kappa number					
Control	8.6	8.4	8.3	8.1	7.8
P. chrysosporium	8.1	7.5	7.0	6.4	5.8
Pl. ostreatus	7.8	7.0	6.4	5.7	5.1
Lignin peroxidase	8.3	7.8	7.4	7.0	6.7

DISCUSSION

The decomposition of lignin in nature has been believed for a long time to occur by the action of wood-rot fungi, mostly the *Basidiomycete* class. These microorganisms simultaneously decompose lignin and wood polysaccharides. Lignin degradation by white rot fungi is a complex secondary metabolic process mediated by the action of several extracellular enzymes, of which lignin peroxidases are the most important. In the course of screening for the lignin peroxidase production from fungi, 11 isolates out of 62 fungal isolates had lignin peroxidase activity. The most potent isolates for lignin peroxidase production were isolates 21 and 43.

The DNA sequences of the internal transcribed spacer (ITS) region for isolates 21 and 43 were analyzed by the Blast program for identification of fungal isolates. The obtained results revealed that the sequence of isolate 21 showed the highest similarity (95%) with *Phanerochaete chrysosporium*, and the sequence of isolate 43 showed the highest similarity (96%) with *Pleurotus ostreatus*. Several screening studies to find suitable fungi for biopulping of wood or agricultural wastes have revealed that, under certain conditions, fungi preferentially degrade lignin over cellulose. Such lignin-selective fungi include *P. chrysosporium*, *Ceriporiopsis subvermispora* (Kirk and Farrell 1987; Eriksson *et al.* 1990; Taniguchi *et al*. 2005), *Pycnoporus cinnabarinus* (Ander and Eriksson 1977), *Pl. ostreatus* (Martínez *et al*. 1994; Yaghoubi *et al*. 2008), *Pl. eryngii* (Martínez *et al*. 1994; Dorado *et al*. 1999), *Phlebia radiata* (Ander and Eriksson 1977), *Phlebia tremellosa* (Eriksson *et al*. 1990), *Phlebia subserialis* (Akhtar *et al*. 1998), *Phellinus pini* (Eriksson *et al*. 1990), and *Dichomitus squalens* (Eriksson *et al*. 1990).

In recent years, it has been demonstrated that the pretreatment of wood and agricultural wastes with lignin-degrading fungi could be beneficial not only to the process of mechanical pulping (Akhtar *et al*. 1998; Scott *et al.* 2002; Singh and Chen 2008) but also in chemical pulping (Messner and Srebotnik 1994; Akhtar *et al*. 1998; Isroi *et al*.

2011). Reports on biochemical pulping indicate a reduction in kappa number at a given pulp yield (Oriaran *et al*. 1990; Blanchette *et al*. 1992; Mosai *et al*. 1999; Yaghoubi *et al*. 2008) as well as improvements in certain physicochemical properties of paper handsheets, such as brightness and strength properties (Akhtar *et al*. 1998; Shukla *et al*. 2004; Singh *et al*. 2010; Isroi *et al*. 2011; Garmaroody *et al*. 2011) and a reduction of pollutants in the waste water from these industries (Yadav *et al*. 2010).

In the present study, great variations in the loss of pulp yield and kappa number were recorded with different fungal and enzyme treatments. Pretreatment of rice straw with *P. chrysosporium* for 25 days resulted in a substantial decrease in pulp yield (by 9.1%) and kappa number (by 25.6%) from that of the control. Losses of pulp yield and kappa number (3.7 and 14.1% from that of the control, respectively) were considerably lower with lignin peroxidase treatment of rice straw as compared with other fungal pretreatments. Pulp yield and kappa number were reduced by 5.8 and 34.6% from that of the control, respectively, with pretreatment by *Pl. ostreatus* of rice straw for 25 days. Similarly, Scott *et al*. (1996) reported that biosulfite pulping with *C. subvermispora* SS-3 for 2 weeks reduced the kappa number of pine by 21%, whereas pretreatment of spruce with *C. subvermispora* CZ-3 for 2 weeks resulted in a 22% kappa number decrease from that of the control (Fischer *et al*. 1994). Mosai *et al*. (1999) found that pretreatment of eucalyptus wood chips with *C. subvermispora* SS-3 for 10 days resulted in a decrease in kappa number by 29% and increase in brightness by 12%. Yaghoubi *et al*. (2008) used *C. subvermispora* for biochemical pulping of agricultural residues, and the results were compared with chemical pulping. Biological treatment of rice, wheat, and barley straw samples resulted in a decrease of the kappa number by 34, 21, and 19%, respectively, as compared with control samples.

In the present study, pretreatment of rice straw with the *Pl. ostreatus* isolate caused moderate pulp yield losses and preferential lignin degradation. Similarly, Taniguchi *et al*. (2005) reported that *Pl. ostreatus* preferentially degraded lignin over polysaccharides in rice straw, while *P. chrysosporium and C. subvermispora* degraded both lignin and polysaccharides in the straw. This indicated that the isolate of *Pl. ostreatus* might be superior to both the isolate of *P. chrysosporium* and lignin peroxidase enzyme for use in biopulping processes or other processes in which preferential lignin degradation is desired.

CONCLUSIONS

Of 62 fungal isolates, the most potent isolates for lignin peroxidase production were identified as *Phanerochaete chrysosporium* and *Pleurotus ostreatus*. Great variations in the loss of pulp yield and kappa number were recorded with the pretreatment of rice straw with *P. chrysosporium, Pl. ostreatus*, or lignin peroxidase. The *Pl. ostreatus* isolate may be superior to both the isolate of *P. chrysosporium* and lignin peroxidase for use in the biopulping process or other processes in which preferential lignin degradation is desired.

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