International Biodeterioration & Biodegradation 64 (2010) 594–600

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Development of compatible lignocellulolytic fungal consortium for rapid composting of rice straw

H. Kausar [a], M. Sariah [a,*], H. Mohd Saud [a], M. Zahangir Alam [b], M. Razi Ismail [a]

[a] Laboratory of Food Crops and Floriculture, Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[b] Department of Biotechnology Engineering, Faculty of Engineering, International Islamic University Malaysia, Jalan Gombak, 53100 Kuala Lumpur, Malaysia

ARTICLE INFO

Article history:
Received 22 February 2010
Received in revised form
13 June 2010
Accepted 17 June 2010
Available online 15 August 2010

Keywords:
Rice straw
Biodegradation
Lignocellulolytic
Fungal species

ABSTRACT

An experiment was conducted to evaluate the potential of lignocellulolytic fungi for rapid composting of rice straw. Forty-nine isolates of fungi were isolated from several natural and induced rice straw composting sources. Ten isolates were tested for their potential to decompose lignocellulosic rice straw by assessing their growth rate and biomass production, as well as their ability to decompose lignin and cellulose on rice-straw-powder-amended media. Four isolates (F26, F28, F29, and F44) were selected as potential lignocellulolytic agents for in-vitro compatibility study based on their optimum growth rate, biomass production, and lignocellulolytic activities. Six different interactions were found among four interacting isolates in the form of mutual intermingling, partial mutual intermingling, and inhibition at the contact point. Finally, a consortium of *Aspergillus niger* (F44) and *Trichoderma viride* (F26) was tested for in-vitro biodegradation of rice straw. The fungal consortium was able to decompose cellulose, hemicelluloses, lignin, and total carbon significantly ($p \leq 0.05$) over the control. The C/N ratio was reduced to 19.5 from an initial value of 29.3 in three weeks of the biodegradation process, thus showing the potential of this method for use in large-scale composting of rice straw.

1. Introduction

Rice is the main staple crop in the world, where 661.811 million tons of rice was produced from 155.711 million hectares of land in 2008 (USDA 2009). Annually a large amount of straw is accumulated as a byproduct from rice cultivation, as straw makes up about 50% of the dry weight of the rice plant. Farmers do not incorporate rice straw in the crop field because of its slow degradation rate, disease infestation, unstable nutrients, and reduced yield caused by the short-term negative effect of nitrogen immobilization (Pandey et al. 2009). They usually dispose of it through open field burning. As a consequence, carbon dioxide, carbon monoxide, methane, nitrous oxide, and sulphur dioxide are emitted into the atmosphere. This process also emits harmful air pollutants such as polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), which have toxic properties and are, notably, potential carcinogens that can cause severe impacts on human health (Gadde et al. 2009).

Thus, proper management and disposal of bulky rice straw is a serious concern all over the world. Attention has been focused on nonhazardous, environment friendly, and sustainable techniques for safe disposal of rice straw in a short period of time. Microbial composting is an effective environmentally sound alternative for the recycling of rice straw into compost. It promotes sustainable agriculture and environmental protection, improving the soil's physical, chemical, and biological properties (Perez-Piqueres et al. 2006; Rasool et al. 2008; Mylavarapu and Zinati 2009), which ultimately results in better plant growth and yield.

Composting of lignocellulosic rice straw requires a process that ensures rapid biodegradation despite the fact that the lignin matrix shields cellulose and hemicelluloses from biodegradation. Naturally a few microbes have the potential to depolymerize lignin. Fungi have an advantage in the composting of lignocellulosic waste because they are filamentous and have the ability to produce prolific spores, which can invade substrates quickly. Moreover, mixed cultures can better influence colonization of the substrate through increased production of enzymes as well as resistance to contamination by other microbes. The most important determinant in mixed cultures is strain compatibility, which influences the organization, distribution, and density of the microhabitat population and the ecological balance of the communities (Gutierrez-Correa and Tengerdy 1997; Molla et al. 2001). Hence, a compatible lignocellulolytic fungal consortium might play a vital role in the rapid disposal of rice straw. In order to address the foregoing issues, this study was undertaken to isolate, screen, and evaluate the compatible lignocellulolytic

* Corresponding author. Tel.: +603 89468967.
E-mail address: sariahm@putra.upm.edu.my (M. Sariah).

0964-8305/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ibiod.2010.06.012

fungal consortium from ecologically related habitats for rapid and environmentally friendly composting of rice straw.

2. Materials and methods

2.1. Isolation of lignocellulolytic fungi

In-situ samples were collected from several sources: decomposed rice straw and soils from dairy and goat farms and rice fields of the Universiti Putra Malaysia (UPM) and Kuala Selangor, Malaysia. In-vitro samples were obtained from different phases of a composting process conducted at Composting Unit UPM, Malaysia. Isolation was performed by the dilution plate method on potato dextrose agar (Difco, USA) and *Trichoderma* Medium E (TME) incubated at room temperature (28 ± 2 °C) for 7 days. The single colony was transferred aseptically onto PDA media to obtain the pure culture.

2.2. In-vitro screening for lignocellulolytic activity

2.2.1. Enzymatic degradation of lignin

The presence of polyphenol oxidase (EC 1.10.3.1) was tested using tannic acid medium (Thormann et al. 2002). A 5-mm mycelia disc from a 5-day-old PDA culture was placed in the centre of the plate and incubated in the dark at room temperature (28 ± 2 °C). Formation of a dark brown pigment surrounding the point of inoculation was used as an indicator of polyphenol oxidase (PPO) activity on tannic acid media within 4 days after inoculation.

2.2.2. Enzymatic degradation of cellulose

The presence of endoglucanase (EC 3.2.1.4) was tested using the media proposed by Hart et al. (2002) with some modifications. A 5-mm fungal inoculum disc was cut from the hyphal edge of a 5-day-old PDA culture. Each inoculum disc was placed at the centre of the plate and incubated at room temperature (28 ± 2 °C) for 7 days. At day 7, the medium was flooded with an aqueous solution of Congo red (1 mg ml^{-1} media) for 15 min. The Congo red solution was then poured off and plates were further treated by flooding with 1 M NaCl for 15 min. Degradation of cellulose was visualized as a clearing zone around the fungal colony. The diameter of the clearing zone around the colony was used to assay the degree of endoglucanase activity.

2.3. Lignocellulolytic activity of selected fungi to rice-straw-powder-amended media

Ten selected lignocellulolytic fungi (F23, F25, F26, F27, F28, F29, F37, F39, F41, and F44) based on previous in-vitro biochemical screening were further screened for their adaptability to different percentages (0, 10, 20, and 25%) of rice-straw-powder-amended media. A 5-mm mycelia disc from a 5-day-old PDA culture of the test fungi was placed in the centre of the plate and incubated at room temperature (28 ± 2 °C) for 2 weeks. Radial growth was recorded daily. Dry fungal biomass (Molla et al. 2002), and lignin and cellulose contents (Van Soest et al. 1991) were assayed at the end of the study.

2.4. In-vitro compatibility evaluation of fungal isolates

Based on their lignocellulolytic characteristics and adaptability to rice-straw-powder-amended media, four isolates (F26, F28, F29, and F44) were selected for their growth and compatibility/interaction in mixed culture.

To study the interactions in mixed culture, mycelial discs from two different isolates were inoculated 40 mm apart from each other in the same petri dish. A single culture served as the control. The co-inoculated plates were incubated at room temperature (28 ± 2 °C) for 15 days. Interactions were assayed using the key based on the observations of Molla et al. (2001).

2.5. Identification of the selected lignocellulolytic fungal consortium (F26 and F44)

2.5.1. Morphological and cultural identification

Macro-and micro-morphological studies were done for tentative identification of the fungal isolates. For macro-morphological studies, the isolates were grown on PDA for 10 days. The mycelia growth, color, and changes of media color of each isolate were observed daily. For micro-morphological studies, slide cultures were prepared and viewed with a microscope and with the Image Processing and Analytical System (Leica Q500 IW, Germany). The size, shape, and arrangement of conidiophores, phialides, and conidiospores or phialospores were observed for identification.

2.5.2. Molecular identification

Species identification was confirmed by sequence analysis of rRNA gene. The DNA extraction was performed using a modified CTAB method adopted from Edwards et al. (1991). The purification and the concentration of extracted DNAs were measured using a Nanodrop spectrophotometer. The DNA concentration was about 100 to 150 ng μl^{-1}, stored in a refrigerator at 4 °C, and used as mother stock to prepare DNA template before running polymerase chain reaction (PCR).

A nuclear rDNA region containing the internal transcribed spacer 1, the internal transcribed spacer 2, the 18S small subunit (SSU), and the 5.8S and the 28S large subunit (LSU) genes was amplified by PCR using primers ITS1 and ITS4 (White et al. 1990) synthesized by NHK Bioscience Solutions Sdn. Bhd. Polymerase chain reaction amplifications were performed in a total volume of 50 µl by mixing 25 µl $2 \times$ Taq Master Mix, 3 µl $MgCl_2$ (50 mM), 0.2 µM concentrations of each primer, and 19 µl nuclease free water. For isolate F26, the reaction was subjected to an initial denaturation of 3 min at 95 °C, followed by 30 cycles of 30 s at 94 °C, annealing for 30 s at 56 °C, extension 1 min at 72 °C, with a final extension of 5 min at 72 °C. For isolate F44, the reaction was subjected to an initial denaturation of 5 min at 95 °C, followed by 34 cycles of 1 min at 95 °C, annealing for 1 min at 55 °C, extension 2 min at 72 °C, with a final extension of 5 min at 72 °C. After PCR amplification, 10-µl aliquots were analyzed by electrophoresis in 1.2% (w/v) agarose gel in 1X TAE buffer [40 mM Tris, 20 mM acetic acid, 1 mM EDTA (pH 8)] stained with ethidium bromide, and photographed over a transilluminator. The molecular size marker was estimated using 100 bp GeneRuler from Fermentas.

2.6. Evaluation of the fungal consortium (F26 and F44) to in-vitro biodegradation of rice straw

A fungal consortium (F44 and F26) was chosen for in-vitro rice straw biodegradation. Ligninolytic isolate F44 was inoculated at the onset of the experiment and cellulolytic isolate F26 on day 10. Non-inoculated substrate served as the control.

Rice straw (Table 1) was ground and sieved through a 2-mm filter. Fifty grams of ground rice straw and an equal amount of chicken manure (Table 1) were mixed together and amended with distilled water to obtain a moisture content of about 60% (w/w). The moisture content was adjusted with a moisture meter (HH2 Meter, ΔT Delta Devices, Cambridge, England). The substrate was inoculated with 10% (v/w) of microbial inoculum at a concentration 10^6 cell ml^{-1}. The prepared substrate was transferred into individual plastic bags containing 100 g of substrate and incubated for 6

Table 1
Chemical characteristics of initial substrates used for biodegradation of rice straw.

Parameters	Rice straw	Chicken manure	Composting substrate
Carbon (%)	45.96 ± 0.82	30.69 ± 0.67	38.32 ± 0.68
Nitrogen (%)	0.89 ± 0.08	4.43 ± 0.31	1.31 ± 0.31
C/N ratio	51.45 ± 4.01	6.95 ± 0.33	29.25 ± 0.32
Lignin (%)	12.45 ± 0.59	–	11.19 ± 0.65
Cellulose (%)	39.95 ± 1.36	–	30.31 ± 1.27
Hemicelluloses (%)	30.20 ± 1.47	–	22.24 ± 0.63

Each value represents the average of five replicated samples with standard error.

weeks. The substrate was turned twice in a week. Samples were collected at 3 and 6 weeks during the biodegradation process. The cellulose, hemicellulose, and lignin contents were analyzed using the neutral detergent fibre (NDF) and acid detergent fibre (ADF) method (Van Soest et al. 1991) and the C/N ratio was determined using the digestion method (Chefetz et al. 1996).

2.7. Experimental design and data analysis

All the experiments were conducted using completely randomized design (CRD) with five replications. The data were subjected to analysis of variance (ANOVA) and tested for significance using least significant difference (LSD) by PC-SAS software (SAS Institute, Cary, NC, USA, 2001). To group the microbial isolates based on their biochemical activities, data were subjected to cluster analysis and a clustering tree was constructed using S-PLUS (Struyf et al. 1997).

3. Results and discussion

3.1. In-vitro lignocellulolytic activity

All of the 49 fungal isolates exhibited a different ability to degrade lignin and cellulose when tested on tannic acid and CM-cellulose-amended media, respectively (Table 2).

Isolate F44 produced a significantly ($p \leq 0.05$) higher dark brown zone (84.71%) on tannic acid media. Development of a dark brown zone on tannic acid medium confirmed the polyphenol oxidase (PPO) activity of the fungal isolate. Polyphenol oxidase—a mixture of monophenol oxidase and catechol oxidase—catalyzed the reaction between polyphenol and molecular oxygen to form dark brown complexes, which play a vital role in the degradation of the phenolic compound in lignin. Fungi have been shown to produce ligninolytic enzymes during the biodegradation of lignocellulosic materials (Rodrigues et al. 2008; Zhang et al. 2008). The activities of lignin peroxidases, manganese peroxidases, and laccase during solid-state fermentation of lignocellulosic materials were also reported by Dinis et al. (2009). Lignin depolymerization is crucial in the bioconversion of lignocellulosic materials in rice straw as lignin encrusts the cellulose and hemicellulose and protects them from biodegradation. Therefore, screening of fungal isolates having a ligninolytic potential was quite logical for the rapid composting of rice straw.

Isolate F26 produced a significantly ($p \leq 0.05$) higher clearing zone (94.12%) on CM-cellulose-amended media. The clearing zone on CMC-amended media proved the presence of endoglucanase enzymes. Effective biodegradation of cellulose to glucose depends on synergistic action of cellulase enzymes, i.e., endoglucanases, exoglucanases, cellobiohydrolases, and glucosidases (Lynd et al. 2002). Cellulose is the key component and comprises approximately 45% of the dry weight of the rice plant. Hence, screening of fungal isolates having the cellulolytic capability is another critical step for the rapid composting of rice straw.

The results of 49 fungal isolates to biochemical degradation of lignin and cellulose were combined in a single cluster analysis to produce a dendrogram derived from S-PLUS software to facilitate their comparison (Fig. 1). The divisive coefficient was high (0.91), which indicates a good clustering structure. A clustering tree provides four clusters based on the ability of the fungal isolates to catalyze the oxidation and hydrolysis of lignin and cellulose. Cluster 1 consisted of 11 isolates with a high degree of ability to degrade the tannic acid and CM-cellulose as carbon sources. Based on mean separation by $LSD_{0.05}$ (Table 2) and the cluster analysis (Fig. 1), ten isolates from cluster 1 were selected for second-stage screening on rice-straw-powder-amended media to explore their potential as lignocellulolytic agents. These isolates produced more than 60% dark brown zone on tannic acid media and above 70% clearing zone on CMC-amended media.

Table 2
The ability of fungal isolates to degrade lignin and cellulose on media containing tannic acid and carboxymethyl cellulose.

Isolate No	Lignin degradation		Cellulose degradation	
	Colony diameter (%)	Dark brown zone (%)	Colony diameter (%)	Clearing zone (%)
In-situ sources				
F1	0.00 o	0.00m	21.56 nq	23.13 l
F2	18.04 lm	26.27 gh	36.86 j	37.27 h
F3	0.00 o	0.00 m	25.88 ln	24.31 l
F4	0.00 o	0.00 m	30.98 k	28.24 k
F5	21.95 ik	22.35 hi	36.07 j	32.54 ij
F6	23.13 ij	21.56 ij	30.19 kl	29.41 jk
F7	0.00 o	0.00 m	12.98t	12.15 pq
F8	0.00 o	0.00 m	20.39 r	16.47 no
F9	0.00 o	0.00 m	14.12 st	13.33 op
F10	0.00 o	0.00 m	0.00 u	0.00 r
F11	0.00 o	0.00 m	0.00 u	0.00 r
F12	29.8o fg	27.84 g	48.24 h	48.62 f
F13	0.00 o	0.00 m	19.60 pr	18.04 mn
F14	0.00 o	0.00 m	0.00 u	0.00 r
F15	0.00 o	0.00 m	18.42 qs	14.89 np
F16	0.00 o	0.00 m	15.68 rt	14.51 np
F17	0.00 o	0.00 m	27.45 km	24.31 l
F18	30.98 f	27.84 g	63.92 g	65.88 d
F19	0.00 o	0.00 m	0.00 u	0.00 r
F20	14.89 mn	20.39 j	42.74 i	36.07 hi
F21	0.00 o	0.00 m	20.39 or	15.68 np
F22	0.00 o	0.00 m	0.00 u	0.00 r
F23	0.00 o	0.00 m	85.88 ac	82.35 b
F24	19.60 kl	21.56 ij	73.21 f	49.80 f
F25	71.76 bc	74.12 bc	84.31 cd	79.21 b
F26	41.95 e	46.66 e	89.41 a	94.12 a
F27	23.53 i	32.94 f	83.53 be	74.51 b
F28	74.89 b	77.65 b	80.78 e	72.54 c
F29	58.42 d	60.39 d	87.84 ab	92.94 a
F30	20.00 jl	25.09 gi	45.09 hi	30.59 jk
In-vitro source				
F31	0.00 o	0.00 m	0.00 u	0.00 r
F32	25.09 hi	23.13 hi	0.00 u	0.00 r
F33	0.00 o	0.00 m	25.48 ln	23.13 l
F34	58.82 d	56.07 cd	24.71 mo	28.62 k
F35	23.53 i	22.35 hi	0.00 u	0.00 r
F36	21.95 ik	18.04 jk	23.53 mp	22.74 l
F37	70.59 c	73.72 bc	82.74 ce	78.82 b
F38	0.00 o	0.00 m	0.00 u	0.00 r
F39	59.21 d	60.00 d	19.60 pr	24.31 l
F40	0.00 o	0.00 m	26.27 kn	21.18 lm
F41	72.94 bc	70.98 c	60.78 g	60.00 e
F42	0.00 o	0.00 m	0.00 u	0.00 r
F43	27.06 gh	34.89 f	47.45 hi	42.74 g
F44	81.18 a	84.71 a	78.82 e	72.94 c
F45	14.51 n	14.12 kl	13.33 t	9.80 q
F46	14.89 nm	12.94 l	16.86 qt	12.54 pq
F47	0.00 o	0.00 m	0.00 u	0.00 r
F48	0.00 o	0.00 m	14.51 st	13.72 op
F49	13.33 mn	13.29 l	0.00 u	0.00 r

Values having the same letter(s) in a column do not differ significantly at the 5% level of probability.

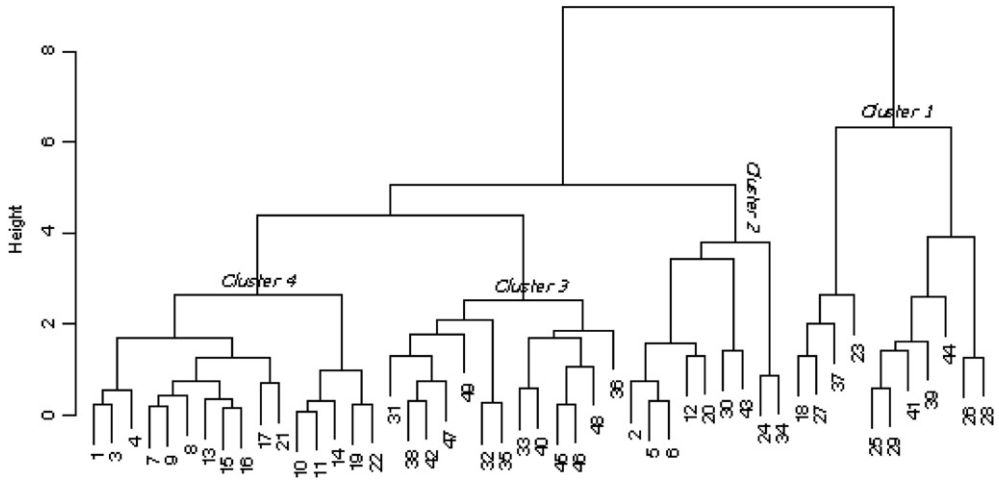

Fig. 1. Dendrogram of 49 fungal isolates based on their lignocellulolytic ability prepared using S-Plus software, version 2008.

3.2. Lignocellulolytic activity of selected fungal isolates on rice-straw-powder-amended PDA media

The growth rate, biomass production, and ability to degrade lignin and cellulose of the ten lignocellulolytic fungal isolates tested are shown in Tables 3–5, respectively. Isolate F26 showed a significantly ($p \leq 0.05$) higher growth rate at the 20 and 25% levels of rice-straw-powder-amended media over other isolates (Table 3). Isolate F44 produced the highest amount of biomass, followed by F28, F26, F29, and F25 at the 25% level of rice-straw-powder-amended media (Table 4). The significantly ($p \leq 0.05$) high growth rate and cell biomass of the studied isolates confirmed their strong affinity to the rice straw environment. The lignocellulolytic activities increased with increase in the concentration of rice straw powder in the media, which showed the ability to utilize complex lignocellulosic materials in rice straw by producing oxidative and hydrolytic enzymes. This observation was supported by previous reports that microfungi produced an array of endoglucanases, cellobiohydrolases, glucosidases, polyphenol oxidases, lignin peroxidases, and laccases for total degradation of lignocellulosic materials in rice straw during the composting process (Rodrigues et al. 2008; Zhang et al. 2008; Dinis et al. 2009). A similar observation was also reported by Molla et al. (2002), who used sludge-powder-amended media to screen effective fungi for the composting of domestic wastewater sludge.

Isolates F44 and F28 were found to decompose lignin, and isolates F26 and F29 to decompose cellulose significantly ($p \leq 0.05$) over the other isolates (Table 5). Considering their adaptability to rice-straw-powder-amended media and the ability to decompose lignin and cellulose, two ligninolytic (F44 and F28) and two cellulolytic (F26 and F29) fungal isolates were selected for the development of lignocellulolytic consortium towards effective biodegradation of rice straw. The lignin shield is oxidized by ligninolytic enzyme systems (Elisashvili et al. 2008; Rodrigues et al. 2008) to expose cellulose for microbial degradation. Subsequently, the exposed substrates are converted to glucose by cellulase, hemicellulase, and esterase enzyme systems (Panagiotou et al. 2007). Therefore, selection of ligninolytic and cellulolytic fungal isolates is essential for the rapid composting of rice straw.

3.3. In-vitro compatibility evaluation of fungal isolates

A total of three different interaction patterns were observed during co-incubation of the four fungal isolates as tested in vitro: F26 & F29 showed mutual intermingling, F26 & F28 and F26 & F44 showed partial mutual intermingling, and three, F28 & F29, F28 & F44, and F29 & F44, 50% were inhibited/deadlocked at the contact point (Fig. 2).

In mutual intermingling association with 16% of the total interactions, both isolates advanced forward and grew in each other's territory with hyphal anastomosis. The advancing mode of uninhibited mycelia growth of both fungal isolates proved their mutual intermingling association on PDA media (Fig. 2A). This finding (16% mutual intermingling) was consistent with the findings of Webber and Hedger (1986) where they reported 14% mutual

Table 3
Effect of different concentrations of rice-straw-powder-amended media on percentage radial growth rate (h^{-1}) of ten selected lignocellulolytic fungal isolates.

Isolate No	PDA	PDA + 10% RSP	PDA + 20% RSP	PDA + 25% RSP
F23	1.33 de	1.37 d	1.38 e	1.43 e
F25	1.66 a	1.70 a	1.93 b	2.11 b
F26	1.37 cd	1.59 b	2.08 a	2.42 a
F27	1.53 b	1.53 c	1.54 d	1.56 d
F28	1.40 c	1.42 d	1.60 c	1.85 c
F29	1.29 e	1.39 d	1.59 c	1.87 c
F37	0.36 j	0.47 h	0.54 j	0.66 j
F39	1.02 f	1.22 e	1.28 f	1.07
F41	0.64 g	0.93 f	1.03 g	0.92 h
F44	0.63 g	0.67 g	0.84 h	1.14 f
$LSD_{0.05}$	0.05	0.08	0.06	0.08

PDA – potato dextrose agar; RSP – rice straw powder.
Values having the same letter(s) in a column do not differ significantly at the 5% level of probability.

Table 4
Effect of different concentrations of rice-straw-powder-amended media on dry cell biomass (in milligrams) of ten selected lignocellulolytic fungal isolates.

Isolate No	PDA	PDA + 10% RSP	PDA + 20% RSP	PDA + 25% RSP
F23	63 cd	76def	94de	81 f
F25	57ef	69eg	84g	101d
F26	68bc	80dc	98cd	113c
F27	69b	78de	92ef	56h
F28	71b	95a	110a	122b
F29	72b	91abc	93df	105d
F37	49g	60gh	82g	43 i
F39	72 b	83bcd	102bc	85f
F41	61de	73def	82g	71g
F44	83a	92ab	107ab	155a
$LSD_{0.05}$	6.94	10.61	8.98	9.08

PDA – potato dextrose agar; RSP – rice straw powder.
Values having the same letter(s) in a column do not differ significantly at the 5% level of probability.

Table 5
Lignin and cellulose content (as a percentage) in decomposed rice-straw-powder-amended media after 15 days of fungal growth.

Isolate No	Lignin (%)	Cellulose (%)
F23	10.60 b	31.63 bc
F25	10.63 b	31.90 bc
F26	10.23 b	28.17 a
F27	10.53 b	33.77 de
F28	8.97 a	30.67 b
F29	10.17 b	28.33 a
F37	10.27 b	34.50 e
F39	10.30 b	32.90 ce
F41	10.27b	33.40 ce
F44	8.73 a	32.57 cd
$LSD_{0.05}$	0.91	1.85

Values having the same letter(s) in a column do not differ significantly at the 5% level of probability.

intermingling relationship out of 22 pairings among *Ceratocystis ulmi* and other associated species in an in-vitro study. However, 47% mutual intermingling interactions among soil micro-fungal communities was observed using high (Malt agar) and low resources (diluted cornmeal) media (Stahl and Christensen 1992), where higher frequency was found in low resource based media than in high resource media. Molla et al. (2001), on the other hand, reported only 7% mutual intermingling association among six lignocellulolytic interacting fungi on high resource media.

Thirty-two percent of the interaction patterns (F26 & F28 and F26 & F44) observed were categorized as partial mutual intermingling where only one isolate grew into the territory of the other, causing inhibition in the growth and development of the weaker isolates (Fig. 2B). The findings support the observations reported by Molla et al. (2001) where they found 27% partial intermingling outcomes in a compatibility study of lignocellulolytic fungi.

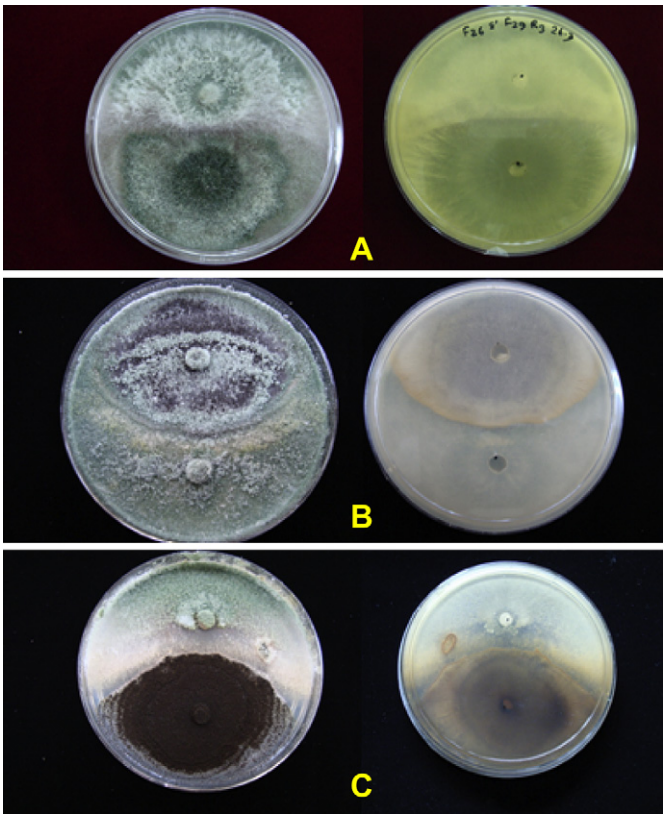

Fig. 2. Interaction outcomes of different fungal isolates grown on PDA media. A. Mutual intermingling; B. Partial mutual intermingling; C. Deadlock at contact point.

F28 & F44 were deadlocked at the contact point where the two fungal isolates met each other and did not allow further growth after the contact point (Fig. 2C). The frequency of deadlock interaction was usually higher than that of the other types of interactions (Shearer and Zare-Maivan 1988; Molla et al. 2001). The capacity for deadlock could be viewed as a defense strategy of fungi where they protect their territory from invading mycelia of potential competitors. Considering the biochemical characteristics, the lignocellulolytic potential on rice-straw-amended media and in-vitro compatibility interactions, a partial mutual lignocellulolytic consortium (F44 & F26) was selected to be evaluated for the biodegradation of rice straw.

3.4. Identification of the selected lignocellulolytic fungal consortium

Based on their morphological and cultural characteristics (Raper et al. 1965; Rifai 1969) F26 and F44 were tentatively identified as *Trichoderma viride* and *Aspergillus niger*, respectively. To confirm their identification the internal transcribed spacer regions of the isolates F26 and F44 were amplified (Fig. 3A, B) and sequenced. BLAST (Altschul et al. 1990) searches of their DNA sequences showed 99% and 100% identity with the deposited *T. viride* and *A. niger* sequences in database GQ221864 and GU183168, respectively. *A. niger* and *T. viride* are known to be effective in the composting of lignocellulosic materials by producing oxidative and hydrolytic enzymes (Zayed and Abdel-Motaal 2005). *A. niger* is a thermo-tolerant (Sanchez et al. 2000) ligninolytic microbe (Conesa et al. 2000). *T. viride* is a cellulolytic microbe used as a biocontrol amendment to prepare disease-suppressive compost (Clarkson et al. 2004). They inhibit phytopathogens by competition for nutrients, antibiosis, production of lytic enzymes, and increasing plant resistance against pathogens. Therefore, their complementary enzymatic potential in the rice straw composting process might lead to the production of an improved end product in the shortest period of time.

3.5. Evaluation of the fungal consortium for in-vitro biodegradation of rice straw

3.5.1. Changes in cellulose, hemicelluloses, and lignin during biodegradation of rice straw

Lignin content of the inoculated substrates decreased significantly ($p \leq 0.05$) during the first three weeks of the biodegradation process (Table 6), which proved that the fungal consortium (*A. niger*–*T. viride*) depolymerized the lignin in the rice straw. Lignin in rice straw contains three monolignol units (hydroxyphenyl–guaiacyl–syringyl) that make the substrate more resistant to biodegradation. The fungal consortium can overcome these constraints by virtue of the extracellular ligninolytic enzymes that act through a mechanism involving free radical formation. Lignin in lignocellulosic substrates is depolymerized by ligninolytic enzymes (Elisashvili et al. 2008; Rodrigues et al. 2008; Zhang et al. 2008). *A. niger* has been documented to depolymerize the lignin-hemicelluloses matrix in lignocellulosic materials (Panagiotou et al. 2007). However, after six weeks of biodegradation, lignin content was found to increase both in the inoculated and non-inoculated substrates. This could be due to the un-decomposed lignin, which was partly transformed into relatively inert humic substances during mineralization and transformed to complex organic substrates with low solubility in water. These tend to flocculate out the solution (Said-Pullicino et al. 2007) through the repolymerization and condensation pathways that increase the lignin content at the end of the biodegradation process. However, the content of cellulose and hemicelluloses decreased significantly ($p \leq 0.05$) in the inoculated substrates compared to the control. These

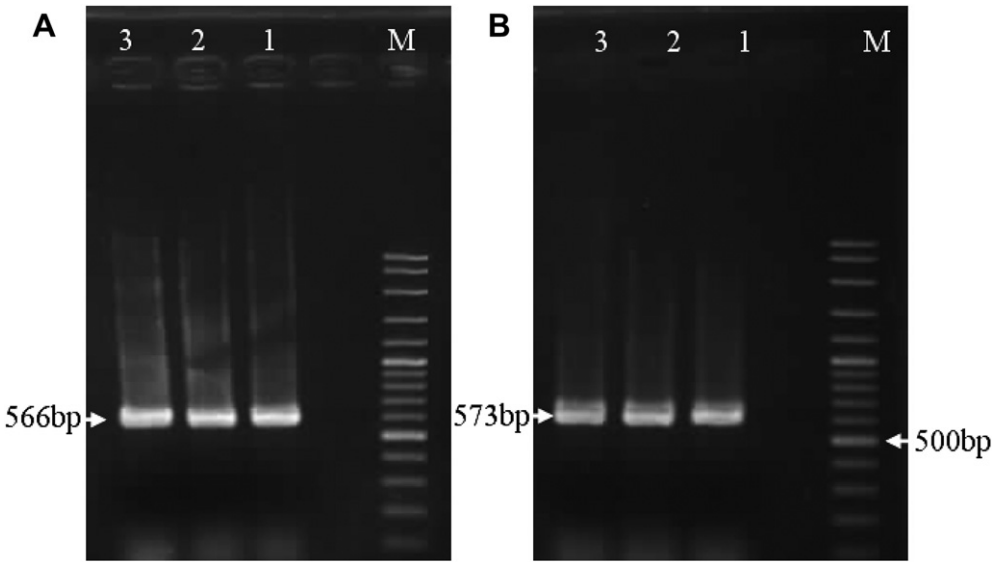

Fig. 3. Polymerase chain reaction (PCR) amplification of ITS region of (a) *T. viride* (F26); (b) *A. Niger* (F44). PCR product was electrophorsed on 1.2% (w/v) agarose gel; Lane M: 100 bp; DNA marker (Vivantis), Lane 1–3: F26 (A), Lane 1–3: F44 (B).

trends of biodegradation by lignocellulolytic consortia of fungi were expected because after cleavage of the lignin barrier, cellulose and hemicelluloses were converted to simple sugar by cellulase, hemicellulase, and esterase enzyme systems (Panagiotou et al. 2007). In this way, these enzymes acting together performed a complementary action in order to facilitate the biodegradation of rice straw.

3.5.2. Changes in carbon, nitrogen, and the C/N ratio during biodegradation of rice straw

The degradation of rice straw showed a decreasing trend in total carbon and C/N ratio whereas total nitrogen content increased throughout the biodegradation period (Table 7). During the biodegradation process organic carbon is converted to energy and CO_2 as metabolic end products. Thus, the total carbon content of substrate decreases as degradation proceeds. In our experiment, the maximum carbon mineralization rate in the substrate occurred during the first three weeks of incubation. This might be due to the high concentration of easily degradable organic carbon in the substrate, which enhanced growth of the microbial population during biodegradation. Solano et al. (2001) found a faster degradation rate during the initial stage of composting of straw amended with sheep manure. A continuous decline in total organic carbon was also reported during the composting of sawdust and cattle manure (Huang et al. 2006). However, others have reported that there is an initial increase followed by a gradual decrease in the total organic carbon during composting of organic wastes (Hsu and Lo 1999). The result of this experiment was in line with Dashtban et al. (2009), who reported enhanced carbon mineralization by fungi during the composting of rice straw. The total nitrogen content both in the inoculated and non-inoculated substrates increased throughout the biodegradation process. The increase of total nitrogen content might be due to the formation of new cell structure, enzymes, and hormones, as well as nitrification by the microorganisms (Zhu 2007). The increase of total nitrogen content during the biodegradation process was in agreement with the studies of Veeken et al. (2001), who showed that the amount of total nitrogen increased with the incorporation of lignocellulosic materials during the composting of sewage sludge and chicken manure. The C/N ratio is a reliable indicator in the composting process and is used as an index of compost maturity. The changes in the C/N ratio of the substrate reflect the organic matter degradation and stabilization during biodegradation of rice straw. The fungal consortium (*A. niger–T. viride*) was found to reduce the C/N ratio significantly ($p \leq 0.05$) compared to the control throughout the biodegradation period of rice straw. *Trichoderma spp.* mineralizes the lignocellulosic waste of high C/N ratio and promotes the composting process. A C/N ratio less than or equal to 20 is considered a satisfactory value for maturity when the initial value of composting substrates is between 25 and 30 (Goyal et al. 2005). After three weeks, the C/N ratio of inoculated rice straw substrate was 19.5, indicating it was sufficiently mature for field application. Results of the present study revealed that incorporation of the lignocellulolytic consortium (*A.niger–T. viride*) could be an efficient way to achieve the rapid biodegradation of rice straw.

Table 6
The content of cellulose, hemicelluloses, and lignin (as a percentage) during six weeks biodegradation of rice straw.

Isolates	Rice straw properties (%)					
	Three weeks			Six weeks		
	Cellulose	Hemicelluloses	Lignin	Cellulose	Hemicelluloses	Lignin
F44 & F26	13.01 a	10.15 a	9.54 a	9.15 a	7.68 a	10.46a
Control	20.97 b	16.88 b	10.67b	19.02 b	14.65 b	10.80a
Significance level	**	**	**	**	**	NS
$LSD_{0.05}$	1.81	1.87	0.73	1.57	1.33	0.79

Values having the same letter(s) in a column do not differ significantly at the 5% level of probability.
** Significant at the 1% level of probability.

Table 7
The content of carbon and nitrogen (as a percentage) and the C/N ratio during six weeks biodegradation of rice straw.

Isolates	Rice straw properties (%)					
	Three weeks			Six weeks		
	Carbon	Nitrogen	C/N	Carbon	Nitrogen	C/N
F44 & F26	33.18 a	1.67 a	19.47a	31.07 a	2.03 a	15.33 a
Control	37.01b	1.38b	26.16 b	35.29 b	1.65 b	19.83 b
Significance level	**	**	**	**	**	**
$LSD_{0.05}$	2.06	0.24	4.89	2.13	0.37	2.79

Values having the same letter(s) in a column do not differ significantly at the 5% level of probability.
** Significant at the 1% level of probability.

4. Conclusions

A total of 49 fungal isolates were isolated from several rice straw compost sources. Ten isolates were selected based on their enzymatic degradation of lignin and cellulose and further screened on rice-straw-powder-amended media. Four isolates (F26, F28, F29, and F44) showing optimum lignocellulolytic activities based on their adaptability and ability to degrade lignin and cellulose in rice-straw-powder-amended media were evaluated for their in-vitro compatibility. A consortium of *A. niger* (F44) and *T. viride* (F26), which gave a partial compatible interaction, has the potential to be developed as a lignocellulolytic consortium for rapid and efficient composting of rice straw into a value-added product of agro waste materials.

Acknowledgements

We thank Universiti Putra Malaysia and the Ministry of Science, Technology and Innovation for providing the financial support for this Ph.D. project.

References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. Journal of Molecular Biology 215, 403–410.
Chefetz, B.P., Hatcher, G., Hader, Y., Chen, Y., 1996. Chemical and biological characterization of organic matter during composting of municipal solid waste. Journal of Environmental Quality 25, 776–785.
Clarkson, J.P., Mead, A., Payne, T., Whipps, J.M., 2004. Effect of environmental factors and *Sclerotium cepivorum* isolate on sclerotial degradation and biological control of white rot by *Trichoderma*. Plant Pathology 53, 353–362.
Conesa, A., van den Hondel, C.A.M.J.J., Punt, P.J., 2000. Studies on the production of fungal peroxidases in *Aspergillus niger*. Applied and Environmental Microbiology 66, 3016–3023.
Dashtban, M., Schraft, H., Qin, W., 2009. Fungal bioconversion of lignocellulosic residues; opportunities and perspectives. International Journal of Biological Sciences 5, 578–595.
Dinis, M.J., Bezerra, R.M.F., Nunes, F., Dias, A.A., Guedes, C.V., Ferreira, L.M.M., Cone, J.W., Marques, G.S.M., Barros, A.R.N., Rodrigues, M.A.M., 2009. Modification of wheat straw lignin by solid state fermentation with white-rot fungi. Bioresource Technology 100, 4829–4835.
Edwards, K., Johnstone, C., Thompson, C., 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Research 19, 1349.
Elisashvili, V., Penninckx, M., Kachlishvili, E., Tsiklauri, N., Metreveli, E., Kharziani, T., Kvesitadze, G., 2008. *Lentinus edodes* and *Pleurotus* species lignocellulolytic enzymes activity in submerged and solid-state fermentation of lignocellulosic wastes of different composition. Bioresource Technology 99, 457–462.
Gadde, B., Bonnet, S., Menke, C., Garivait, S., 2009. Air pollutant emissions from rice straw open field burning in India, Thailand and the Philippines. Environmental Pollution 157, 1554–1558.
Goyal, S., Dhull, S.K., Kapoor, K.K., 2005. Chemical and biological changes during composting of different organic wastes and assessment of compost maturity. Bioresource Technology 96, 1584–1591.
Gutierrez-Correa, M., Tengerdy, R.P., 1997. Production of cellulase on sugar cane bagasse by fungal mixed culture solid substrate fermentation. Biotechnology Letters 19, 665–667.
Hart, T.D., De Leij, F.A.A.M., Kinsey, G., Kelley, J., Lynch, J.M., 2002. Strategies for the isolation of cellulolytic fungi for composting of wheat straw. World Journal of Microbiology and Biotechnology 18, 471–480.
Hsu, J.H., Lo, S.L., 1999. Chemical and spectroscopic analysis of organic matter transformations during composting of pig mature. Environmental Pollution 104, 189–196.
Huang, G.F., Wu, Q.T., Wong, J.W.C., Nagar, B.B., 2006. Transformation of organic matter during co-composting of pig manure with sawdust. Bioresource Technology 97, 1834–1842.
Lynd, L.R., Weimer, P.J., van Zyl, W.H., Pretorius, I.S., 2002. Microbial cellulose utilization: fundamentals and biotechnology. Microbiology and Molecular Biology Reviews 66, 506–577.
Molla, A.H., Fakhrul-Razi, A., Abd-Aziz, S., Hanafi, M.M., Alam, M.Z., 2001. *In vitro* compatibility evaluation of fungal mixed culture for bioconversion of domestic wastewater sludge. World Journal of Microbiology and Biotechnology 17, 849–856.
Molla, A.H., Fakhrul-Razi, A., Abd-Aziz, S., Hanafi, M.M., Roychoudhury, P.K., Alam, M.Z., 2002. A potential resource for bioconversion of domestic wastewater sludge. Bioresource Technology 85, 263–272.
Mylavarapu, R.S., Zinati, G.M., 2009. Improvement of soil properties using compost for optimum parsley production in sandy soils. Scientia Horticulturae 120, 426–430.
Panagiotou, G., Olavarria, R., Olsson, L., 2007. *Penicillium brasilianum* as an enzyme factory; the essential role of feruloyl esterases for the hydrolysis of the plant cell wall. Journal of Biotechnology 130, 219–228.
Pandey, A.K., Gaind, S., Ali, A., Nain, L., 2009. Effect of bioaugmentation and nitrogen supplementation on composting of paddy straw. Biodegradation 20, 293–306.
Perez-Piqueres, A., Edel-Hermann, V., Alabouvette, C., Steinberg, C., 2006. Response of soil microbial communities to compost amendments. Soil Biology and Biochemistry 38, 460–470.
Raper, K.B., Fennell, D.I., Austwick, P.K.C., 1965. The Genus *Aspergillus*. Williams and Wilkins, Baltimore, pp. 293–344.
Rasool, R., Kukal, S.S., Hira, G.S., 2008. Soil organic carbon and physical properties as affected by long-term application of FYM and inorganic fertilizers in maize–-wheat system. Soil and Tillage Research 101, 31–36.
Rifai, M.A., 1969. A revision of the genus *Trichoderma*. Mycological Paper 116, 1–56.
Rodrigues, M.A.M., Pinto, P., Bezerra, R.M.F., Dias, A.A., Guedes, C.V.M., Cardoso, V.M.G., Cone, J.W., Ferreira, L.M.M., Colaço, J., Sequeira, C.A., 2008. Effect of enzyme extracts isolated from white-rot fungi on chemical composition and *in vitro* digestibility of wheat straw. Animal Feed Science and Technology 141, 326–338.
Said-Pullicino, D., Erriquens, F.G., Gigliotti, G., 2007. Changes in the chemical characteristics of water-extractable organic matter during composting and their influence on compost stability and maturity. Bioresource Technology 98, 1822–1831.
Sanchez, J.G., Tsuchii, A., Tokiwa, Y., 2000. Degradation of polycaprolactone at 50 °C by a thermotolerant *Aspergillus* sp. Biotechnology Letters 22, 849–853.
Shearer, C.A., Zare-Maivan, H., 1988. *In vitro* hyphal interactions among wood- and leaf-inhabiting ascomycetes and fungi imperfect from freshwater habitats. Mycologia 80, 31–37.
Solano, M.L., Iriarte, F., Ciria, P., Negro, M.J., 2001. SE-Structure and Environment: Performance characteristics of three aeration systems in the composting of sheep manure and straw. Journal of Agricultural Engineering Research 79, 317–329.
Stahl, P.D., Christensen, M., 1992. *In vitro* mycelial interactions among members of a soil microfungal community. Soil Biology and Biochemistry 24, 309–316.
Struyf, A., Hubert, M., Rousseeuw, P.J., 1997. Integrating robust clustering techniques in S-PLUS. Computational Statistics and Data Analysis 26, 17–37.
Thormann, M.N., Currah, R.S., Bayley, S.E., 2002. The relative ability of fungi from *Sphagnum fuscum* to decompose selected carbon substrates. Canadian Journal of Microbiology 48, 204–211.
USDA, 2009. Rice Production, Area and Yield. IRRI World Rice Statistics (WRS). Online at: http://www.irri.org/science/ricestat.
Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods of dietary fiber, neutral detergent fiber and non starch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74, 3583–3597.
Veeken, A.H.M., Adani, F., Nierop, K.G.J., de Jager, P.A., Hamelers, H.V.M., 2001. Degradation of bio-macromolecules during high rate composting of wheat straw-amended feces. Journal of Environmental Quality 30, 1675–1684.
Webber, J.F., Hedger, J.N., 1986. Comparison of interactions between *Ceratocystis ulmi* and Elm bark saprobes in vitro and *in vivo*. Transaction and Journal of British Mycological Society 86, 93–101.
White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR protocols: a guide to methods and applications. Academic Press, San Diego, California, pp. 315–322.
Zayed, G., Abdel-Motaal, H., 2005. Bio-active composts from rice straw enriched with rock phosphate and their effect on the phosphorous nutrition and microbial community in rhizosphere of cowpea. Bioresource Technology 96, 929–935.
Zhang, L., Li, D., Wang, L., Wang, T., Zhang, L., Chen, X.D., Mao, Z., 2008. Effect of steam explosion on biodegradation of lignin in wheat straw. Bioresource Technology 99, 8512–8515.
Zhu, N., 2007. Effect of low initial C/N ratio on aerobic composting of swine manure with rice straw. Bioresource Technology 98, 9–13.