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Short communication

Screening of selective lignin-degrading basidiomycetes and biological pretreatment for enzymatic hydrolysis of bamboo culms

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ABSTRACT

Fifty-one fungal isolates were screened for their biological pretreatment of bamboo culms (*Phyllostachys pubescence*) by enzymatic saccharification. These fungi were isolated from the interior of decayed bamboo culms or from basidiomata on bamboo culms. The white rot basidiomycete *Punctularia* sp. TUFC20056 and an unidentified basidiomycete TUFC20057 showed high lignin degradation (>50%) and high lignin/holocellulose loss ratios (>6) after 12 weeks of treatment. Other *Punctularia* sp. strains also showed high lignin/holocellulose ratios (>4). In residual component of *Punctularia* sp. TUFC20056-treated bamboo, glucose and total sugar were increased (42.9 ± 10.9 and 60.3 ± 16.1%, respectively). Pretreatment of bamboo culms with *Punctularia* sp. TUFC20056 improved the effect of enzymatic hydrolysis. Total reducing sugar yields reached 10.1 ± 1.2% with 1 month of treatment, and higher than that of two famous white rot fungi. *Punctularia* sp. TUFC20056 may be suitable for the biological pretreatment of bamboo for bioethanol production.

1. Introduction

Lignocellulosic biomass is one of the more important potential sources of bioethanol because it does not compete with food sources required for humans (Lee, 1997; Hayes, 2009). Bamboos are perennial woody grasses in family Gramineae, subfamily Bambuseae, and are distributed widely across Asia (Bystriakova et al., 2003). They are important forest biomass because of the large quantities of growing stock and their rapid growth rate. In Japan, 3.3 Tg y^{-1} of bamboo is produced annually, most of which is not harvested or used commercially (Yoshioka et al., 2005; Tokunaga and Araki, 2007). The chemical composition of bamboo culms (stems) is similar to that of hardwoods (Fengel and Shao, 1984). Some studies have indicated that bamboo culms could be exploited as the raw material for biomass energy, including in ethanol production (Tsuda et al., 1998; Kobayashi et al., 2004). However, it is necessary to degrade lignin by pretreatment to enable energy production from bamboo culms, as the bamboo lignin network prevents enzymatic saccharification and fermentation. Some studies have indicated biological pretreatment, especially by white-rot fungi, can enhance the enzymatic hydrolysis of lignocellulose with the advantage of very limited energy consumption (Lee, 1997; Sun and Cheng, 2002; Itoh et al., 2003; Hakala et al., 2004, 2005; Shi et al., 2009). Selective lignin-degrading fungi are considered especially good candidates for biological pretreatment (Itoh et al., 2003; Taniguchi et al., 2005; Baba et al., 2011). Bamboo culms are different from wood and grass in their chemical constitution and fiber characteristics (Scurlock et al., 2000), and very few studies have been reported on biological treatment of bamboo culms with white rot fungi (Zhang et al., 2007a,b).

The purpose of this work was to isolate and characterize the relevant enzymatic capability of fungi that selectively degrade lignin in bamboo for use in ethanol production.

2. Materials and methods

2.1. Materials

Bamboo was purchased from Kabushikikaisha OMC (Tottori, Japan) as bamboo chip.

2.2. Microorganisms

To obtain fungal isolates that had selective lignin-degradation ability for bamboo, Moso bamboo culm chips (approximately

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5 × 5 × 1 cm in size) were spread directly over forest floor (mixture of soil, leaf-litter and humus) of the Field Science Center, Faculty of Agriculture, Tottori University (Tottori City, Japan) open to environmental air. Size of the test area was 2 × 2 m^2 and 15 cm deep. Bamboo chips were covered with 5 cm square mesh bird-net and allowed to decay from April 2007 to October 2009.

Fungal isolates were obtained from spore print of basidiomata on malt extract agar [MA: 15 g malt extract (Difco, Detroit, MI, USA) and 15 g Bacto agar (Difco) per l], or obtained by placing small piece of decayed bamboo culm on MA containing benomyl (50 mg per l) collected from this examination area. Species were identified by morphological observation of basidiomata using a stereomicroscope (Nikon Eclipse 80i, Nikon, Tokyo), and by genetic analyses of isolates. For microscopic observation, pieces of dried fungal material were mounted in 3% (w/v) KOH or Melzer's reagent (Weresub, 1953). Twenty measurements were made per element (spore, basidia, cystidia, and other tissue features) for each specimen. All fungal isolates were deposited in the Fungus/Mushroom Resource and Research Center (TUFC), Tottori University, Japan. Species names and strain numbers are shown with the experimental results in Table 1.

2.3. Genetic analysis

Genetic analysis was carried out on the internal transcribed spacer region (ITS) of ribosomal DNA using ITS1-F and ITS4-B primers (Gardes and Bruns, 1993). The detailed procedure of genetic analysis has been reported previously (Suhara et al., 2002). Sequences were subjected to a BLAST search using the International Nucleotide Sequence Database Collaboration (INSDC), to infer phylogenetic position. Some sequences were also compared with our authentic DNA data (data not shown).

2.4. First screening

As initial screening, 51 isolates were inoculated onto MA containing 0.02% (v/v) guaiacol (Wako, Shiga, Japan). Guaiacol is turned reddish brown by lignin-degrading fungi (Tamai and Miura, 1991; Morisaki et al., 2001). After two weeks incubation, isolates exhibiting diffusible guaiacol-browning (over 1 cm in radius) were selected for the pretreatment test. For this observation one guaiacol plate was used for each isolate.

2.5. Secondary screening

The fungal strains selected above were pre-cultured on MA plates at 21 °C. Distilled water (1400 ml) and malt extract (4 g) were added to bamboo chips (1000 g), and slant media were prepared using approximately 10 g of this mixture. The solid medium was sterilized at 121 °C for 21 min. Two mycelial disks (5 mm in diameter), punched from colony edges on MA, were inoculated to the surface of the prepared medium then incubated at 21 °C for 12 weeks. Uninoculated bamboo was used as a control. Lignin degradation causes bleaching (brightness) of lignocellulose substances, generally called white-rot. And, bleaching of lignocellulose substances and lignin degrading ability are well linked in previous report (Li et al., 2002). The bleaching degree of bamboo chip was evaluated by comparing non-inoculated controls and the most bleached inoculum. Degree of bleaching was classified using the following five ratings: −, none; +, weak; ++, medium; +++, strong; ++++, very strong.

2.6. Chemical analysis of bamboo culms

Residual lignin and holocellulose, the total polysaccharide of wood, were estimated using pretreated samples that showed medium (++) or greater bleaching. Oven-dried samples of pretreated bamboo culms were extracted with ethanol-*n*-hexane (1:2 v/v) 8 times, to remove extractive content. Klason lignin was quantified using the TAPPI method (TAPPI, 1996; Canettieri et al., 2007) with slight modifications, and holocellulose was quantified following Wise et al. (1946). The Klason lignin/holocellulose loss ratio (*L*/*C*) in each fungal isolate was calculated using the following equation:

$$L/C = \text{mass loss of Klason lignin}(\%\text{ of non-inoculated Klason lignin mass})/\text{mass loss of holocellulose}(\%\text{ of non-inoculated holocellulose mass})$$

Table 1
Fungal isolates used in this study: their radius of halo (guaiacol-browning) in the first screening.

Family[a]	Species	Strain	Radius of halo (cm)
Auriculariaceae	*Exidiopsis sublivida*	TUFC20068	2.6
	Exidiopsis sublivida	TUFC20069	3
	Exidiopsis sublivida	TUFC20070	3.4
	Exidiopsis sublivida	TUFC20071	3
Corticiaceae	*Punctularia* sp. 1	TUFC20056	3.4
	Punctularia sp. 1	TUFC20076	3.6
	Punctularia sp. 1	TUFC20077	3.7
	Punctularia sp. 1	TUFC20078	3
	Punctularia sp. 1	TUFC20079	3.2
	Punctularia sp. 1	TUFC20080	2.7
	Punctularia sp. 1	TUFC20081	2.5
	Punctularia sp. 1	TUFC20083	3
Cyphellaceae	*Cylindrobasidium evolvens*	TUFC20093	–
Marasmiaceae	*Henningsomyces* sp. 1	TUFC20075	0.9
	Henningsomyces sp. 2	TUFC20086	–
Meruliaceae	*Hyphoderma argillaceum*	TUFC20087	–
	Hyphoderma argillaceum	TUFC20088	–
Mycenaceae	*Mycena* sp.	TUFC20089	–
	Dictyopanus sp.	TUFC20090	2.9
Pezizaceae	*Peziza* sp.	TUFC20092	1.3
Rickenellaceae	*Resinicium friabile*	TUFC20062	1.5
	Resinicium friabile	TUFC20063	1
	Resinicium friabile	TUFC20064	2
	Resinicium friabile	TUFC20065	1.1
	Resinicium friabile	TUFC20066	–
	Resinicium friabile	TUFC20067	3
Schizophyllaceae	*Schizophyllum commune*	TUFC20094	–
Sphaerobolaceae	*Sphaerobolus* sp. 1[b]	TUFC20084	2.7
	Sphaerobolus sp. 1[b]	TUFC20085	0.8
Strophariaceae	*Agrocybe* sp.1[b]	TUFC20095	1.8
	Agrocybe sp.1[b]	TUFC20096	2
Unidentified		TUFC11782	–
		TUFC11783	–
		TUFC11872	–
		TUFC12137	–
		TUFC12138	3.6
		TUFC12139	–
		TUFC12140	–
		TUFC12141	–
		TUFC12142	2.7
		TUFC12143	2.5
		TUFC12144	–
		TUFC12152	2.8
		TUFC12159	–
		TUFC12733	–
		TUFC13820	–
		TUFC20072	2
		TUFC20073	–
		TUFC20074	–
		TUFC20057	3.4
		TUFC20097	–

[a] Classification of the family is according to the MycoBank (http://www.mycobank.org).
[b] Identified by ITS sequence homology. Strains which showed over 98% homology to the data deposited in INSDC were tentatively identified as same genus.