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Rice Straw-Decomposing Fungi and Their Cellulolytic and Xylanolytic Enzymes

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Filamentous fungi colonizing rice straw were collected from 11 different sites in Korea and were identified based on characterization of their morphology and molecular properties. The fungi were divided into 25 species belonging to 16 genera, including 14 ascomycetes, one zygomycete, and one basidiomycete. Fungal cellulolytic and xylanolytic enzymes were assessed through a two-step process, wherein highly active cellulase- and/or hemicellulase-producing fungi were selected in a first screening step followed by a second step to isolate the best enzyme-producer. Twenty-five fungal species were first screened for the production of total cellulase (TC), endo-β-1,4 glucanase (EG), and endo-β-1,4 xylanase (XYL) using solid-state fermentation with rice straw as substrate. From this screening, six species, namely, *Aspergillus niger* KUC5183, *A. ochraceus* KUC5204, *A. versicolor* KUC5201, *Mucor circinelloides* KUC6014, *Trichoderma harzianum* 1 KUC5182, and an unknown basidiomycete species, KUC8721, were selected. These six species were then incubated in liquid Mandels' media containing cellulose, glucose, rice straw, or xylan as the sole carbon source and the activities of six different enzymes were measured. Enzyme production was highly influenced by media conditions and in some cases significantly increased. Through this screening process, *Trichoderma harzianum* 1 KUC5182 was selected as the best enzyme producer. Rice straw and xylan were good carbon sources for the screening of cellulolytic and xylanolytic enzymes.

Keywords: Cellulase, enzymatic screening, fungal diversity, rice straw, xylanase

The cost of feedstock is one of the most important parameters affecting the economic viability of bioethanol production. It is very important to achieve a high overall ethanol yield as this is directly related to the feedstock consumption [5]. Rice straw, a renewable lignocellulosic biomass, is an attractive feedstock because it is one of the most abundant lignocellulosic waste materials in the world. In Asia, rice straw is a major agricultural by-product; it is produced in large amounts, with up to 668 million tons produced per year. Presently, rice straw is produced in Korea at a rate of about 5.5 million tons per year according to the Korean statistical information service. The rice straw totally produced could theoretically be converted to 282 billion liters of ethanol [2]. Rice straw has high cellulose and hemicellulose contents that can be hydrolyzed into fermentable sugars. The chemical composition of rice straw is cellulose (32–47%), hemicellulose (19–27%), and lignin (5–24%) [17, 24]. Hemicellulose is made up of pentose sugars, of which xylose is the most abundant (14.8–20.2%) [23].

One of the major challenges in enzymatic bioethanol production is the need for enhanced enzymatic hydrolysis with superior enzymes that can be produced at a low cost [5]. Some fungi, such as *Trichoderma*, produce a broad profile of cellulolytic and hemicellulolytic enzymes that are now important enzymes in the biological degradation of feedstock in second-generation bioethanol production [18]. Microorganisms such as fungi convert cellulose and hemicellulose into sugars, and in turn ethanol, and the fraction of monomeric sugar versus oligomeric sugar produced is essential for increasing fuel ethanol yields from lignocellulosic materials [8].

In this study, filamentous fungi colonizing rice straw were isolated and screened for further study, based on their cellulolytic and xylanolytic activities, through a two-step process. First, those fungi producing high levels of cellulases and/or xylanases were selected using solid-state fermentation. In a second step, these isolates were grown in different media to search for growth conditions that resulted in higher enzyme production.

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Materials and Methods

Fungal Isolation

Post-harvest rice straw was collected from 11 different sites in Korea: Baeksan, Jeosan, and Sangseo in Buan; Byeongcheon; Dangjin; Deokcheon, Gobu, and Jungwoo in Jeongeup; Gimje; Jincheon; and Osong. Samples were placed on 2% malt extract agar (MEA; Difco malt extract 20 g, Difco agar 15 g, and 1,000 ml distilled water) with either 100 ppm streptomycin (for general fungal flora) or 2% MEA with 4 ppm benomyl and 100 ppm streptomycin (for basidiomycetes) [17]. The plates were incubated at room temperature for several days and fungi were routinely subcultured from mycelial margins in order to obtain pure cultures. Pure cultures were preserved in the Korea University Culture Collection (KUC). After purification, most of the fungi were identified to the genus or species level by observing morphological and physiological characteristics.

Phylogenetic Analysis

To confirm our morphological identification, we sequenced the 28S (LSU) and the internal transcribed spacer (ITS) rDNA regions from the representative strains. Fungal DNA extraction, PCR, and PCR purification were performed using the techniques described by Kim *et al*. [17]. For the phylogenetic analysis, ITS sequences were initially aligned using Clustal X ver. 2.0.12 [19] and manually adjusted using MacClade ver. 4.08 [20]. The maximum parsimonius trees were constructed with PAUP* ver. 4.0b10 [31]. Heuristic searches with tree-bisection reconnection branch swapping and 10 random addition sequences were employed. Gaps were treated as missing data and branch stability was assessed by 1,000 bootstrap replications.

Enzymatic Screening

Step one: fungal screening by solid-state fermentation. For the screening of fungal strains, enzyme production was performed in 250 ml Erlenmeyer flasks containing 10 g of ground rice straw. The rice straw was moistened with 30 ml of distilled water. The flasks were autoclaved and inoculated with 1 ml of spore suspension ($1-3 \times 10^6$/ml) for ascomycetes and the zygomycete or three agar plugs with mycelium for the basidiomycete and then incubated at 27°C for 7 days. For the extraction of crude enzyme, cultures were soaked in 50 ml of citrate buffer (50 mM, pH 4.5) and extracted at 4°C for 1 h on a shaker. Total cellulase (TC) and endo-β-1,4-glucanase (EG), according to the method of Ghose [6], and xylanase (XYL), according to the method of Bailey *et al*. [1], were assayed. Reducing sugars were measured according to the dinitrosalicylic acid method [21]. One unit of TC or EG activity is defined as the amount of enzyme that releases 1 μmol of glucose equivalents from Whatman No.1 filter paper or carboxymethyl cellulose in 1 min at 50°C, respectively. One unit of XYL activity is defined as the amount of enzyme that produces 1 μmol of reducing sugars per minute by hydrolyzing xylan at 50°C. Activity data were statistically analyzed using Tukey's test. For each assay, only those fungi that had a significantly greater enzyme activity compared with others were selected for the next stage of media optimization.

Step two: selection for the best medium by liquid-state fermentation. Those fungi selected from the initial screening were analyzed again for six enzyme activities: TC, EG, β-glucosidase (BGL), cellobiohydrolase (CBH), XYL, and β-xylosidase (BXL). One ml of spore suspension (1×10^6/ml) for ascomycetes and the zygomycete or three agar plugs with mycelium for the basidiomycete was inoculated to a 100 ml Erlenmeyer flask containing 30 ml of sterile Mandels' medium [urea 0.3 g, $(NH_4)_2SO_4$ 1.4 g, KH_2PO_4 2.0 g, $CaCl_2$ 0.3 g, $MgSO_4$ 0.3 g, yeast extract 0.25 g, peptone 0.75 g, $FeSO_4 \cdot 7H_2O$ 5 mg, $CoCl_2$ 20 mg, $MnSO_4$ 1.6 mg, $ZnSO_4$ 1.4 mg, and 1,000 ml distilled water] [12] with one of four different carbon sources: cellulose powder (microcrystalline), glucose, rice straw, or birchwood xylan. The culture was incubated at 27.5°C for 7 days, and the supernatant from each culture was used as crude enzyme. The activities of TC, EG, and XYL were measured as described above. The activities of BGL, BXL, and CBH were determined using *p*-nitrophenyl-β-D-glucopyranoside (N7006), *p*-nitrophenyl-β-D-xylopyranoside (N2132), and *p*-nitrophenyl-β-D-cellobioside (N5759) as substrates, respectively, following methods by Valaskova and Baldrian [33] and Yoon *et al*. [34]. One unit of BGL, BXL, and CBH activity was expressed as the amount of enzyme required to liberate 1 μmol of *p*-nitrophenol from each substrate per minute. The protein content was determined using the Bradford method, with bovine serum albumin as the standard [3].

Results and Discussion

Fungal Identification and Phylogenetic Analysis

A total of 511 fungal isolates were obtained and identified based on their morphology and molecular characteristics. As a result, the isolates were classified into 25 species and 16 genera, 14 of which were ascomycetes: *Alternaria, Aspergillus, Bipolaris, Cladosporium, Curvularia, Dothideomycete* (unknown genus), *Epicoccum, Fusarium, Gaeumannomyces, Nigrospora, Penicillium, Phoma, Stachybotrys*, and *Trichoderma*. One genus of zygomycete, *Mucor*, and an unknown basidiomycete were also found (Table 1). Among them, more species were found in the *Aspergillus* and *Fusarium* genera than the others, including four different species. The most commonly isolated genus was *Curvularia* (77 isolates), followed by *Fusarium* (63 isolates), *Alternaria* (53 isolates), *Aspergillus* (51 isolates), and Dothideomycete (unknown genus) (51 isolates). Among these fungi, *Curvularia intermedia, Alternaria alternata,* Dothideomycete sp., *Cladosporium* sp., *Nigrospora oryzae,* and *Aspergillus niger* were the most frequently isolated species in every site and comprised approximately 61% of all the fungal isolates obtained (Table 1). *Trichoderma harzianum, Fusarium graminearum, Mucor circinelloides, Gaeumannomyces graminis* var. *graminis, Penicillium oxalicum,* and *F. proliferatum* were also frequently isolated, accounting for approximately 29% of the specimens obtained. In contrast, some fungal species, such as *Aspergillus wentii*, *Bipolaris oryzae, Bipolaris* sp., *Epicoccum nigrum,* and *Stachybotrys* sp., were isolated only at certain locations.

The sequence analysis showed that the sequences of the isolates found on rice straw were matched with diverse fungal sequences in GenBank, and the similarity of their

Table 1. Fungal species isolated from rice straw and their enzymatic activities.

ID[a]	Fungal identity	GenBank Acc. No.[b]	No. of isolates	Closest fungal match (Acc. No.)[b]	Similarity (%)[c]	Enzyme activities (U/ml)		
						Total cellulase (TC)	Endoglucanase (EG)	Xylanase (XYL)
KUC5190	*Alternaria alternata*	HQ116402	53	*Alternaria alternata* (GQ241273)	445/447 (99.6)	ND[d]	0.078 ± 0.01 defgh	1.88 ± 0.22 gh
KUC5183	*Aspergillus niger*	HQ116395	46	*Aspergillus niger* (GQ229077)	494/494 (100.0)	0.211 ± 0.01 ab[e]	0.155 ± 0.01 b	7.83 ± 0.92 d
KUC5204	*Aspergillus ochraceus*	HQ116390	2	*Aspergillus ochraceus* (FJ878631)	499/501 (99.6)	0.194 ± 0.01 bc	0.225 ± 0.01 a	17.34 ± 1.01 c
KUC5201	*Aspergillus versicolor*	HQ116387	2	*Aspergillus versicolor* (AJ937755)	504/505 (99.8)	0.173 ± 0.01 c	0.101 ± 0.01 cde	30.46 ± 2.03 a
KUC5203	*Aspergillus. wentii*	HQ116389	1	*Aspergillus wentii* (AY373884)	475/475 (100.0)	0.126 ± 0.01 d	0.063 ± 0.01 fgh	21.54 ± 1.09 b
KUC 5197	*Bipolaris oryzae*	HQ116408	1	*Cochliobolus miyabeanus* (DQ300207)	508/509 (99.8)	0.081 ± 0.01 e	0.059 ± 0.02 fgh	2.07 ± 0.67 gh
KUC5198	*Bipolaris* sp.	HQ116409	1	*Cochliobolus* sp. (FJ210544)	465/477 (97.5)	ND	0.062 ± 0.01 fgh	1.76 ± 0.15 gh
KUC5188	*Cladosporium* sp.	HQ116400	50	*Cladosporium* sp. (FJ790249)	436/436 (100.0)	ND	ND	2.06 ± 0.37 gh
KUC5194	*Curvularia intermedia*	HQ116406	77	*Cochliobolus intermedius* (AF071327)	466/475 (98.1)	ND	ND	2.52 ± 0.48 fg
KUC5191	Dothideomycete sp.	HQ116403	51	Dothideomycete sp. (EU680541)	439/452 (97.1)	ND	ND	ND
KUC5186	*Epicoccum nigrum*	HQ116398	1	*Epicoccum nigrum* (FJ903352)	441/441 (100.0)	ND	ND	ND
KUC5185	*Fusarium graminearum*	HQ116397	37	*Gibberella zeae* (DQ459832)	514/514 (100.0)	0.047 ± 0.01 g	0.055 ± 0.01 gh	1.93 ± 0.19 gh
KUC5199	*Fusarium oxysporum*	HQ116410	2	*Fusarium oxysporum* (AY928417)	526/526 (100.0)	0.071 ± 0.01 ef	0.086 ± 0.01 def	ND
KUC5195	*Fusarium proliferatum*	HQ116407	20	*Fusarium proliferatum* (HM590497)	467/468 (99.8)	ND	0.054 ± 0.01 h	1.98 ± 0.45 gh
KUC5181	*Fusarium* sp.	HQ116393	4	*Fusarium* sp. (GQ505748)	456/460 (99.1)	0.079 ± 0.01 e	0.079 ± 0.01 defgh	2.55 ± 0.31 fg
KUC5200	*Gaeumannomyces graminis* var. *graminis*	HQ116411	23	*G. graminis* var. *graminis* (AY428779)	460/465 (98.9)	ND	0.051 ± 0.00 h	1.63 ± 0.24 gh
KUC6014	*Mucor circinelloides*	HQ116391	25	*Mucor circinelloides* (GQ221218)	559/559 (100.0)	ND	0.203 ± 0.01 a	ND
KUC5189	*Nigrospora oryzae*	HQ116401	48	*Nigrospora oryzae* (FJ487918)	452/452 (100.0)	0.058 ± 0.01 ef	0.083 ± 0.01 defg	ND
KUC5184	*Penicillium oxalicum*	HQ116396	22	*Penicillium oxalicum* (GQ376104)	411/412 (99.8)	0.129 ± 0.01 d	0.097 ± 0.01 de	1.90 ± 0.53 gh
KUC5187	*Phoma sorghina*	HQ116399	6	*Phoma sorghina* (FJ427078)	478/479 (99.8)	0.048 ± 0.01 f	0.073 ± 0.01 efgh	3.63 ± 0.19 fg
KUC5202	*Stachybotrys* sp.	HQ116388	1	*Stachybotrys bisbyi* (AF081480)	387/411 (94.2)	ND	0.065 ± 0.01 fgh	2.68 ± 0.26 fg
KUC5182	*Trichoderma harzianum* 1	HQ116394	4	*Hypocrea lixii* (EF191296)	571/572 (99.8)	0.222 ± 0.02 a	0.095 ± 0.01 de	17.20 ± 1.89 c
KUC5192	*Trichoderma harzianum* 2	HQ116404	21	*Hypocrea lixii* (GU266272)	470/470 (100.0)	ND	0.106 ± 0.01 cd	3.26 ± 0.16 fg
KUC5193	*Trichoderma harzianum* 3	HQ116405	5	*Hypocrea lixii* (FJ412024)	513/519 (98.8)	0.130 ± 0.01 d	ND	4.53 ± 0.25 ef
KUC8721	Unknown basidiomycete sp.	HQ116392 *JF975409*	8	*Granulobasidium vellereum* (AY787674) *Granulobasidium vellereum* (*AY745729*)	526/597 (88.1) 508/535 (95.0)	0.117 ± 0.01 d	0.129 ± 0.01 bc	6.66 ± 1.07 de
	Total		511					

[a]KUC: Korea University Culture Collection, Seoul, Korea.
[b]Accession numbers of ITS sequences in regular font and LSU sequence in italics.
[c]% Similarity scores from pairwise alignments of sample sequences with closest BLAST match or reference strains.
[d]ND; not determined.
[e]Numbers followed by the same letter in each row are not significantly different ($\alpha = 0.05$) according to Tukey's method.

ITS sequences was between 88% and 100%. The phylogenetic analysis revealed that the ascomycetes found on rice straw were clustered into five distinct phylogenetic groups, corresponding to five taxonomic orders (Capnodiales, Eurotiales, Hypocreales, Pleosporales, and Trichosphaeriales) (Fig. 1A). Most of them were identified on a species level and this was supported by high bootstrap values. However, four species, *Bipolaris* sp., *Cladosporium* sp., Dothideomycete sp., and *Fusarium* sp., were not identified in this study. Interestingly, *Trichoderma harzianum* was divided into three different groups based on their colony morphologies and microscopic features such as the color and shape of spores (Table 1). According to Chaverri *et al*. [4], *T. harzianum* is a species complex that has variable phenotype and genotype. The phylogenetic tree showed that the three *T. harzianum* groups were monophyletic and *T. harzianum* 1 and 3 were more closely related than *T. harzianum* 2, but the bootstrap value (58%) was quite low (Fig. 1A). Fig. 1B shows the position of the unknown basidiomycete sp. which was the only basidiomycete fungus found on rice straw. It was clustered with *Granulobasidium vellereum* based on the analysis of both the ITS and LSU, but the similarity values were quite low, 88.1% and 95.0%, respectively. It was isolated from four sites (Jincheon; Backsan and Jusan in Buan; and Jeongeup) but only eight isolates were found. Although its characteristics were similar to *G. vellereum*, the identity of the basidiomycete sp. remained unknown. *Mucor circinelloides* was the sole zygomycete fungus collected from rice straw and its phylogenetic relationship is depicted in Fig. 1C.

Among the collected species, some were previously reported to be plant pathogens. *Alternaria alternata* can damage rice from cultivation to harvest [26]. *Bipolaris oryzae* is a well-known pathogen that causes brown spots in rice [13]. *Fusarium graminearum* can lead to seedling blight and brown foot rot [16]. *Phoma sorghina* can cause rice to leaf spot [27] and *F. oxysporum* can cause tomato plant wilt disease [15]. *Gaeumannomyces graminis* var. *graminis* is known to infect cereals [9]. Head blight in oats is caused by *Fusarium proliferatum* [29], and tomato and cucumber infections are caused by *Penicillium oxalicum* [32]. Accordingly, these plant pathogenic fungi have evolved numerous catalytic strategies to attack plant cell wall components, especially polysaccharides, (cellulose, hemicelluloses, and pectin) using extracellular enzymes [7].

Screening for Enzyme Production

To find fungi with high enzyme activity and substrates capable of enzyme induction, a two-step screening was carried out. The first step was performed using solid-state fermentation, because, as opposed to liquid culture, it resembles the natural environment where fungi actually proliferate [10]. The aim of the first screening step was to find fungal species that can degrade rice straw without additional nutrients. Generally, *Aspergillus* species showed higher TC, EG, and XYL activities than other genera. Moreover, *Trichoderma harzianum* strains had relatively high enzyme activities. *Curvularia intermedia, Alternaria alternata,* Dothideomycete sp., *Cladosporium* sp., and *Nigrospora oryzae* showed low enzyme activities although they were frequently isolated from rice straw. For this step, fungi meeting the selection criteria, which have significantly better enzyme activities as determined by Tukey's method ($\alpha = 0.05$), were collected into the group "a." Thus, for TC activity, both *Trichoderma harzianum* 1 KUC5182 (0.212 U/ml) and *Aspergillus niger* KUC5183 (0.211 U/ml) were selected. *Aspergillus ochraceus* KUC5204 (0.225 U/ml) and *Mucor circinelloides* KUC6014 (0.203 U/ml) were selected based on EG activity. *Aspergillus versicolor* KUC5201 (30.46 U/ml) was solely included in "a" based on XYL activity. The unknown basidiomycete sp. KUC8721 was not selected as part of the "a" group based on the activity of a particular enzyme; rather, it was selected for continued study, specifically to assess the enzyme production potential of basidiomycetes. As a result, three *Aspergillus* species (*A. niger* KUC5183, *A. ochraceus* KUC5204, and *A. versicolor* KUC5201), one *Mucor* species (*M. circinelloides* KUC6014), one *Trichoderma* species (*T. harzianum*1 KUC5182), and an unknown basidiomycete sp. (KUC8721) were chosen for the next step.

The second screening step was an analysis of the selected species for different types of cellulases and xylanases. Many reports on microbial production of cellulases have utilized submerged fermentation technology, and the widely studied fungi used in cellulase production have also been cultured mostly in liquid media [28]. For this reason, the second screening step was performed using submerged media. Each medium contained either glucose, cellulose, rice straw, or xylan as the sole carbon source.

From this two-step screening process, it was apparent that there were differences in enzyme activities between solid and liquid state fermentations. It was previously observed that there was a large difference of protein contents and the amount of each protein produced between solid-state culture and submerged culture, and solid culture have better production potential for most proteins [22]. In this study, no enzyme activities were detected for EG and XYL from *Aspergillus ochraceus* KUC5204 and *Mucor circinelloides* KUC6014, and for EG from *Aspergillus versicolor* in liquid media with rice straw even though they were selected using rice straw as a solid media in the first step (Fig. 2). Unexpectedly, TC was not detected by some species tested, although EG, CBH, and/or BGL were measured in the second step. It might be that the amount of amino-nitrosalicylic acid reduced from dinitrosalicylic acid and sugar is not exactly equivalant and very low level

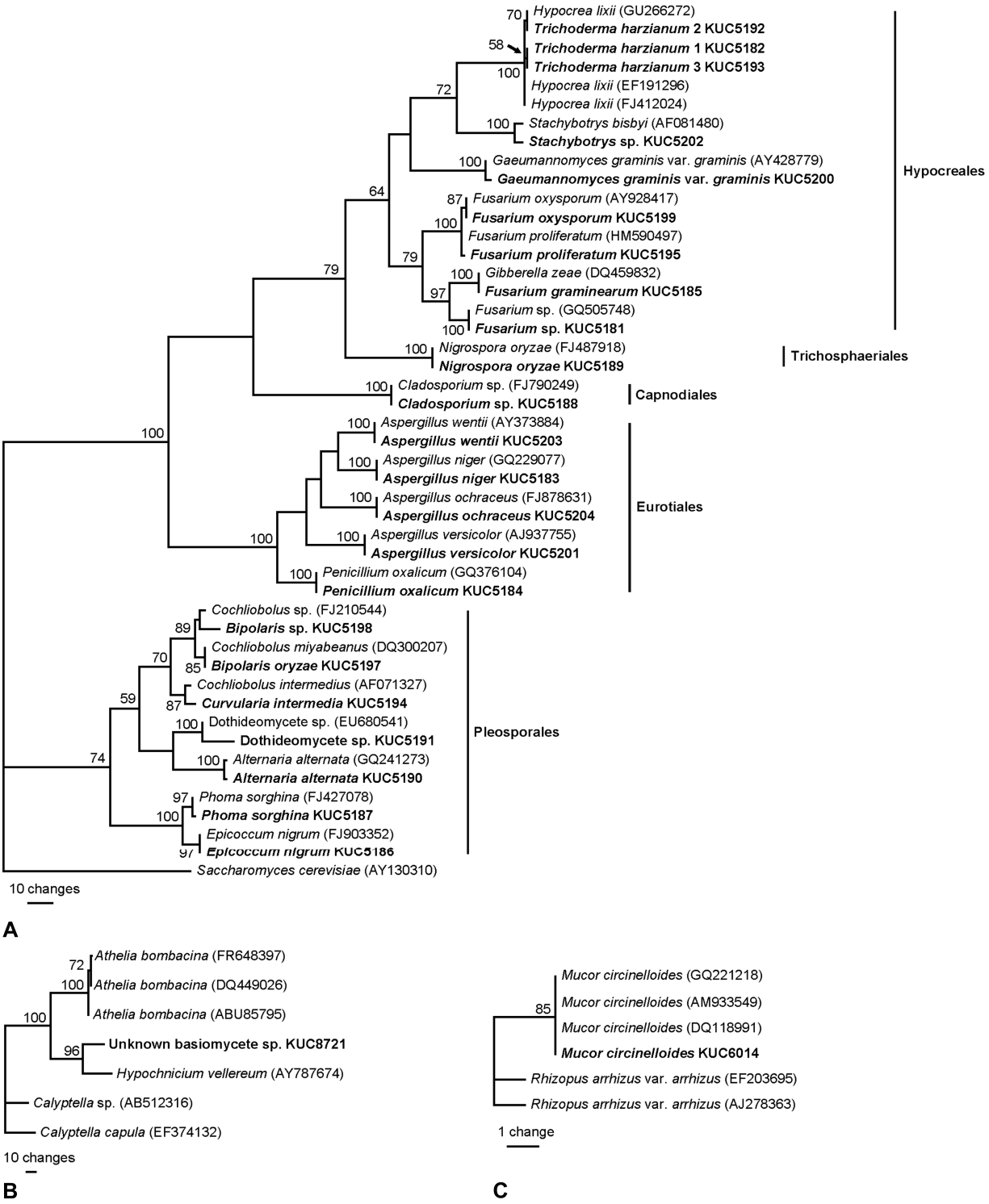

Fig. 1. Phylogenetic trees of fungal species isolated from rice straw and the reference strains from GenBank.
(**A**) Maximum parsimonious tree of ascomyceteous fungi inferred from ITS rDNA sequences. The dataset comprised 655 characters, of which 292 were parsimony informative. The tree is one of 2 most parsimonious trees with 889 steps, with a consistency index of 0.6727 and a retention index of 0.8959. (**B**) Maximum parsimonious tree based on ITS rDNA sequences of basidiomyceteous fungi. The dataset comprised 545 characters, of which 115 were parsimony informative. The tree is the most parsimonious tree with 251 steps and has a consistency index of 0.9243 and retention index of 0.8827. (**C**) Maximum parsimonious tree based on ITS rDNA sequences of zygomyceteous fungi. The dataset comprised 557 characters, of which 2 were parsimony informative. The tree is the most parsimonious tree with 4 steps, a consistency index of 1.0000 and a retention index of 1.0000. Numbers on the branches are bootstrap values ≥ 50%. Strains used in this study are in bold type. Accession numbers are indicated in parentheses.

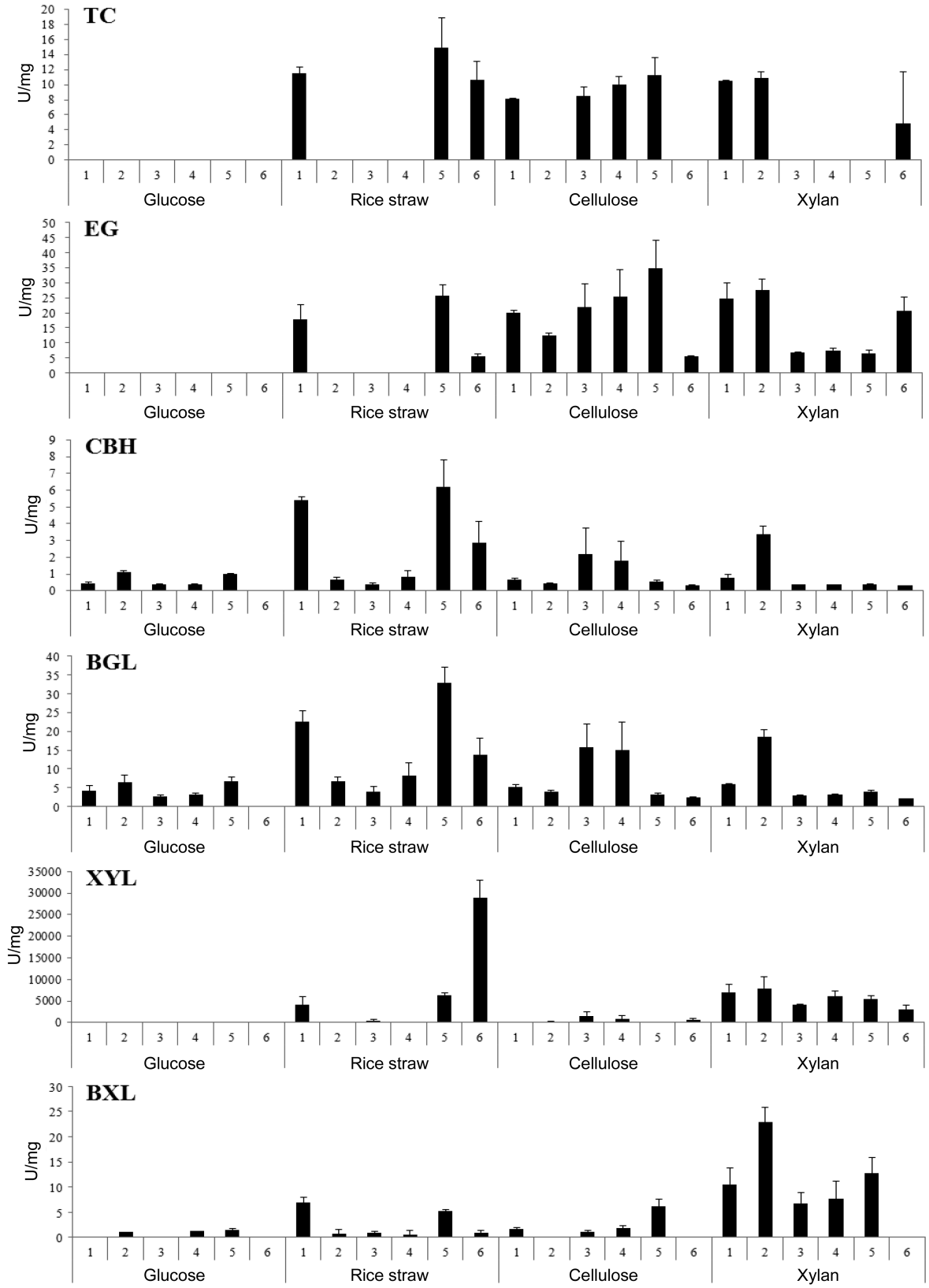

Fig. 2. Cellulolytic and xylanolytic enzyme activities from fungal species screened *via* a two-step procedure.
Isolates 1: *Aspergillus niger* KUC5183; 2: *Aspergillus ochraceus* KUC5204; 3: *Aspergillus versicolor* KUC5201; 4: *Mucor circinelloides* KUC6014; 5: *Trichoderma harzianum* 1 KUC5182; and 6: Unknown basidiomycete sp. KUC8721.

of color change might not be detected by this method [21]. Thus, TC might not be detected because of the enzyme production below the detection limit.

There were also some differences in the amount of protein produced and enzyme activity for the different substrates tested in the second screening step. Xylan was a good carbon source for fungal growth. Most fungi produced proteins effectively in Mandels' medium with xylan; in addition, enzyme activities, particularly XYL and BXL activities, were also well detected. In our study, xylan not only induces xylanases, but also cellulases as well. Similar result was also observed by other researchers [11, 25]. This result suggests that xylan might be an appropriate carbon source for the screening of fungi for xylanolytic enzymes. Cellulose induced cellulases, particularly EG, relatively well. EG showed good activity in both cellulose- and xylan-supplemented Mandels' medium. Media with glucose gave the lowest protein concentration and enzyme activity, as expected since a large amount of glucose results in catabolite repression. This phenomenon prevents fungi from synthesizing an excess amount of cellulase in circumstances where abundant, easily assimilated carbon sources such as glucose exist [30]. When rice straw was used as a substrate, interesting results were observed. Rice straw was an effective substrate for production of protein and most enzymes by fungi. It was also reported by other researchers [14]. Moreover, the unknown basidiomycete sp. KUC8721, *Trichoderma harzianum* 1 KUC5182, and *Aspergillus niger* KUC5183 showed exceptionally high enzyme activities in Mandels' medium with rice straw. The unknown basidiomycete sp. KUC8721 showed an exceptional XYL activity (29,016.05 U/mg). In addition, *Trichoderma harzianum* 1 KUC5182 showed the highest activity for TC (14.94 U/mg), CBH (6.22 U/mg), and BGL (32.84 U/mg) in rice straw amended Mandels' medium, except EG (34.86 U/ml) in Mandels' medium with cellulose. It seems that these fungi adapted well to rice straw and secreted highly active enzymes to utilize it. BXL activity in *A. ochraceus* KUC5204 was the highest in xylan-amended medium (22.88 U/mg). In this study, no general pattern was detected regarding the enzyme activity in fungi cultured in different states (solid or liquid) or on different substrates, but it might be appropriate to use the host as a substrate (in this case, rice straw), to induce enzymes for proper screening.

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