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Citation	Microbes and Environments , 26 (3) : 220 - 227
Issue Date	2011
DOI	10.1264/jsme2.ME10210
Self DOI	
URL	http://ir.lib.hiroshima-u.ac.jp/00034739
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Relation	

Microbes Environ. Vol. 26, No. 3, 220–227, 2011
http://wwwsoc.nii.ac.jp/jsme2/ doi:10.1264/jsme2.ME10210

MICROBES AND ENVIRONMENTS

Evaluation of Cellulolytic and Hemicellulolytic Abilities of Fungi Isolated from Coffee Residue and Sawdust Composts

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(Received December 14, 2010—Accepted April 20, 2011—Published online May 11, 2011)

This study focused on the evaluation of cellulolytic and hemicellulolytic fungi isolated from sawdust compost (SDC) and coffee residue compost (CRC). To identify fungal isolates, the ITS region of fungal rRNA was amplified and sequenced. To evaluate enzyme production, isolates were inoculated onto wheat bran agar plates, and enzymes were extracted and tested for cellulase, xylanase, β-glucanase, mannanase, and protease activities using different azurine cross-linked (AZCL) substrates. In total, 18 isolates from SDC and 29 isolates from CRC were identified and evaluated. Four genera (*Aspergillus*, *Galactomyces*, *Mucor*, and *Penicillium*) and five genera (*Aspergillus*, *Coniochaeta*, *Fusarium*, *Penicillium*, and *Trichoderma/Hypocrea*) were dominant in SDC and CRC, respectively. *Penicillium* sp., *Trichoderma* sp., and *Aspergillus* sp. displayed high cellulolytic and hemicellulolytic activities, while *Mucor* isolates exhibited the highest β-glucanase and mannanase activities. The enzyme analyses revealed that *Penicillium*, *Aspergillus*, and *Mucor* isolates significantly contributed to the degradation of SDC, whereas *Penicillium*, *Aspergillus*, and *Trichoderma* isolates had a dominant role in the degradation of CRC. Notably, isolates SDCF5 (*P. crustosum*), CRCF6 (*P. verruculosum*), and CRCF2 and CRCF16 (*T. harzianum/H. lixii*) displayed high activity regarding cellulose and hemicellulose degradation, which indicates that these species could be beneficial for the improvement of biodegradation processes involving lignocellulosic materials.

Key words: fungi, cellulolytic activity, hemicellulolytic activity, AZCL-substrates, compost

Composting organic wastes represents an important pathway for carbon flow and nutrient cycling in both developed and developing countries. However, knowledge of the microbiology of this process is limited, particularly concerning the fungi associated with degradation of organic materials (11, 43).

Lignocellulosic materials, which are the major constituent of plant matter produced by photosynthesis, represent the most abundant renewable organic resource. These materials are composed of three types of polymers, namely cellulose, hemicellulose, and lignin, which are strongly engaged and chemically bonded (25, 38, 58). For example, lignocellulosic materials of coffee residue are composed of 35% cellulose, 46.3% hemicellulose, and 18.8% lignin (42), whereas those of sawdust consist of 40–50% cellulose, 25–35% hemicellulose, and 20–30% lignin (45). Due to their complexity, time-consuming processes are required to degrade these polymers and complete compost maturation.

Numerous microorganisms are capable of decomposing cellulose, although only a few of these microorganisms produce significant quantities of extracellular enzymes to decompose cellulose *in vitro* (47). Fungi are the main cellulase-producing microorganisms, but a few species of bacteria and actinomycetes have also been reported to yield cellulase (47, 56). Fungi, which are capable of degrading a wide variety of materials and compounds, are among the major decomposers of plant polymers, including lignocellulosic materials, in any ecosystem. Fungi can degrade mixtures of heterogeneous substrates, such as municipal solid waste, cattle manure, and agricultural and industrial wastes (15, 46).

Cellulolytic and hemicellulolytic enzymes play vital roles in biodegradation processes by fungi, bacteria, actinomycetes, and protozoa (13, 36). The efficient degradation of cellulolytic and hemicellulolytic plant materials requires a complex set of extracellular enzymes. All organisms known to efficiently degrade cellulose and hemicelluloses produce different degradative enzymes of varying specificities, which act synergistically (9, 54). For example, extracellular hydrolases and oxidoreductases produced by many microorganisms are involved in the degradation of lignocelluloses (53). Cellulose is the major polysaccharide in plant biomass (7), whereas xylans, β-glucans, and mannans are the most important constituents of hemicelluloses in plant cell walls (14). Consequently, determination of cellulase, xylanase, β-glucanase, and mannanase activities may indicate the cellulolytic and hemicellulolytic degradative potential of an organism.

Cellulolytic fungi are of great importance in our ecosystem, as they catalyze the enzymatic decay of lignocellulosic material. Notably, extracellular cellulases are produced at high levels by several fungi, including *Trichoderma*, *Penicillium*, *Aspergillus*, and *Fusarium* (19, 29). Filamentous fungi play critical roles in the biodegradation of numerous types of organic waste materials by releasing exoenzymes involved in oxidation and hydrolysis (15). Therefore, the study of fungal biodegradation processes must include determination of an organism's catalytic capability.

The application of screening techniques that are rapid and easy to evaluate allows the testing of a large number of

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isolates for the selection of highly active strains (24). However, the qualitative and semi-quantitative screening of microorganisms which are able to produce cellulases and xylanases using classical screening methods does not typically provide conclusive results due to difficulties in the evaluation of substrate consumption during cultivation on agar media with cellulose or xylan (20). For the detection of highly active strains during primary screening, a few recent studies have described effective methods based on the utilization of insoluble chromogenic substrates that provide results with greater fidelity (21). A number of different plate screening methods to identify polysaccharide-degrading microorganisms have been described (49), which are typically based on either the formation of complexes between polysaccharides and dyes, the solubility and gel-forming characteristics of polysaccharides, or the use of soluble and/or insoluble dye-labeled polysaccharides (50, 58). During hydrolysis, cross-linked macromolecular substrates labeled with colorant particles are attacked, resulting in the solubilization of the colorant particles and formation of a colored zone around microorganisms which are able to produce extracellular enzymes. The intensity of the colored zone depends on several factors, including substrate and enzyme concentrations, and the catalytic properties of enzymes (16). As chromogenic substrates, commercially available insoluble azurine cross-linked (AZCL) substrates, which contain a blue azurine copper compound bound to the target polysaccharide, have proven effective for these assays (37). Using these substrates, a clear correlation between the diameters of the formed blue haloes and levels of enzymes produced was demonstrated (50).

Traditional fungal identification methods are technically difficult and time consuming. Recently, several improved techniques that use DNA sequencing for microbial identification have been developed (30). For example, variations in ribosomal-RNA (rRNA) internal transcribed spacer (ITS) regions have been used for the molecular identification of fungi to the species level. The non-coding ITS region is suitable for fungal identification because of its high copy number in fungal genomes and fast rate of evolution, which results in high sequence variation among even closely related species (27, 32). Therefore, DNA sequences in the ITS region generally provide superior taxonomic characters than those from coding regions (5, 31).

In this study, sawdust compost (SDC) and coffee residue compost (CRC) were selected for the isolation and characterization of cellulolytic and hemicellulolytic fungi due to their high cellulose and hemicellulose content, and also their low degradation rates. In addition, most previous papers have only examined limited enzymatic activities of fungal groups, and we were unable to find any reports describing multiple activities of different fungal groups isolated from compost. Therefore, several fungi were isolated from SDC and CRC, identified, and screened for cellulase, xylanase, β-glucanase, mannanase, and protease activities.

Materials and Methods

Fungal isolation

The SDC produced from residue of a culture medium for mushroom was obtained from the Agricultural Cooperative Association of Saitama prefecture, while CRC was collected from a composting center located in Hiroshima prefecture. Basically, they were produced by composting heaps that were turned periodically during 2–3 months until they reached maturity. The chemical and biological properties of SDC and CRC are presented in Table 1.

Fungi were isolated from SDC and CRC using a dilution plate method. The primary suspensions were prepared by suspending 10 g of each compost type in 90 mL of sterile distilled water and were shaken (150 rpm) for 30 min at room temperature. Then, ten-fold serial dilutions were prepared in sterilized distilled water. One hundred microliters of the 10^{-5} dilution was spread on Martin's medium (glucose, 10 g L^{-1}; peptone, 5 g L^{-1}; KH_2PO_4, 1 g L^{-1}; $MgSO_4 \cdot 7H_2O$, 0.5 g L^{-1}; Rose Bengal, 0.033 g L^{-1}; and streptomycin, 0.03 g L^{-1}; pH 5.5–6.0) and plates were then incubated at 30°C for 5–7 days. Five plates were prepared for each compost type. All the colonies of the most typical plate were selected. The colonies were transferred several times on the same medium and further incubated at 30°C for 7 days to obtain pure cultures. All fungal isolates were maintained on potato dextrose agar (PDA, Nissui Pharmaceutical, Tokyo, Japan) slants at 4°C. Fungal isolates were identified conventionally according to their morphological features to determine their genera (2, 8, 17, 18, 52).

Enzyme preparation

For enzyme production, all fungal isolates were inoculated onto wheat bran agar (WBA) plates (wheat bran, 30 g L^{-1}; agar, 15 g L^{-1}; $ZnSO_4 \cdot 7H_2O$, 0.01 g L^{-1}; and $CuSO_4 \cdot 5H_2O$, 0.005 g L^{-1}; pH 7.4) (37). The plates were then incubated for 7 days at 25°C in darkness in perforated plastic bags.

Enzyme complexes of the isolated fungi were extracted following the method described by Pedersen *et al.* (37). Briefly, enzymes were extracted from 7-day-old cultures grown on WBA plates. Twenty agar plugs (6 mm in diameter) with mycelia were excised from the plate of each isolate. The plugs were transferred to a 14-mL vial, 2.5 mL of distilled water was added, and the vial was then shaken at 150 rpm for 2 h on a Yamato SA-31 shaker (Yamato Scientific, Tokyo, Japan) at room temperature. The extracted solution was filtered through a sterile 0.45-μm membrane filter (25 mm in diameter, Toyo Roshi, Tokyo, Japan). The extracted enzyme solutions were kept at −30°C until used.

Enzyme assays

Five different AZCL substrates (AZCL-HE-cellulose, -arabinoxylan, -barley β-glucan, -galactomannan, and -casein [Megazyme, Bray, Ireland]) were used for enzyme assays. Assays were performed according to the method described by Pedersen *et al.* (37). Briefly, after the assay suspension containing agarose and each AZCL substrate was solidified in Petri dishes, 15 wells with a 6-mm diameter were made in the agarose plates using a cork borer, and 15 μL of enzyme extract was added to each well. The plates were

Table 1. Chemical and biological properties of SDC and CRC

Compost used	Dry matter (g kg^{-1})	Total (g kg^{-1})*			C/N ratio	Fungal population (×10^6 cfu g^{-1})*
		C	N	P		
SDC	538	456	14.7	12.3	30.0	2.3
CRC	390	448	21.4	0.7	20.9	3.0

* Values expressed on an oven-dry basis.

incubated for 24 h at 30°C in the case of HE-cellulose, while for all other substrates the incubation time was 6 h. Enzymatic activities were determined by measuring the diameter of the blue zone formed around each well, which was then converted to area (mm^2) and subtracted from the area of the well.

DNA extraction and PCR amplification

For DNA extraction, all fungal isolates were grown using PDA medium and incubated at 30°C for 7 days. DNA was then extracted with an Isoplant Kit (Nippon Gene, Toyama, Japan) according to the manufacturer's instructions.

PCR was performed using a GeneAmp PCR System 2700 (Applied Biosystems, Foster City, CA, USA). ITS regions of rRNA were amplified using ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) as forward and reverse primers, respectively, according to White *et al.* (57). All reactions were carried out in 50-μL volumes containing genomic DNA (1.0 μL 20 ng μL^{-1}), 5.0 μL of 10×Buffer (including 20 mM $MgCl_2$), 5.0 μL of dNTP mixture (2.5 mM each), 2.5 U of Ex Taq (Takara Bio, Otsu, Japan) and 1.0 μL (20 μM) of each primer (ITS1 and ITS4). The thermal cycling program was as follows: 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 10 s, annealing at 63°C for 1 min, and extension at 72°C for 1 min, with a final extension step at 72°C for 5 min. The annealing temperature was increased to 65°C to obtain a single band from isolates SDCF1, SDCF10, SDCF17, and CRCF27. The PCR products were analyzed by agarose gel electrophoresis, stained with ethidium bromide, and visualized by UV transillumination with an AE-6932GXCF printgraph (Atto, Tokyo, Japan). The amplified PCR products were purified using a QIAquick PCR purification Kit (Qiagen, Hilden, Germany) according to the protocol provided by the manufacturer.

Sequencing and data analysis

Sequencing of the amplified ITS regions of the fungal rRNA was performed using a BigDyeR Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA) on an Applied Biosystems 3730xl DNA Analyzer. Similarities of the determined fungal DNA sequences with other known species were investigated by comparisons with sequence data in the National Center for Biotechnology Information (NCBI) database using the BLASTN 2.2.24 program (59). A phylogenetic tree based on the ITS regions of partial rRNA sequences was constructed using the neighbor-joining method contained within the Clustal X program (51) and MEGA4 software (48).

Nucleotide sequence accession numbers

The nucleotide sequences determined in this study have been deposited with GenBank under the nucleotide accession numbers HQ657273–HQ657319.

Results

Fungal isolation from compost

Two compost types, SDC and CRC, were subjected to screening for the isolation and characterization of cellulolytic and hemicellulolytic fungi. A total of 18 isolates were obtained from SDC, while 29 were isolated from CRC (Table 2).

Identification and phylogenetic analysis of fungal isolates

The morphological characteristics of the fungi showed that the isolates were species of the genera *Mucor*, *Trichoderma*, *Penicillium*, *Coniochaeta*, *Galactomyces* (*Geotrichum*), *Aspergillus*, and *Fusarium*.

Although the analysis of fungal ITS sequences revealed a wide diversity of fungal taxa in both SDC and CRC, BLAST searches demonstrated that four genera (*Mucor*, *Penicillium*, *Galactomyces*, and *Aspergillus*) were dominant in SDC (Table 2 and Fig. 1). The phylogenetic tree constructed based on ITS sequences of SDC isolates indicated that nine isolates were close to *M. circinelloides* with 99–100% similarity, while five isolates appeared to be members of the genus *Penicillium*, as they shared 100% similarity with *P. crustosum*. In addition, three isolates were close (98–100% similarity) to *G. geotrichum*, whereas one isolate (SDCF16) shared 100% similarity with *A. fumigatus*, according to BLAST searches of sequences in the NCBI database, as summarized in Table 2.

For the fungal isolates from CRC, data derived from BLAST analyses and the phylogenetic tree of ITS sequences revealed that five genera (*Aspergillus*, *Penicillium*, *Coniochaeta*, *Fusarium* and *Trichoderma*/*Hypocrea*) were dominant, with similarities ranging from 98 to 100% (Fig. 2). Among the 29 isolates in total, 14 were closely related to the genus *Trichoderma*/*Hypocrea*, with three of these isolates matching *H. virens* (*T. virens*) with 99–100% maximum identity, and the other 11 displaying 99–100% identity with *H. lixii* (*T. harzianum*). Eleven isolates were matched with two different species of the genus *Coniochaeta*; nine of these displayed 99% similarity to *C. ligniaria*, while two isolates were closely related to *C. velutina* (98% similarity). In addition, two isolates (CRCF6 and CRCF12) exhibited 99% similarity with *P. verruculosum*, while CRCF11 and CRCF27 matched 100% with *A. fumigatus* and *F. acuminatum*, respectively (Table 2).

Characterization of SDC fungal isolates

In SDC compost, *Mucor* sp. was the most dominant fungal genus, representing 50% (9/18) of isolates. Although the *Mucor* isolates all showed higher β-glucanase and mannanase activities than the other SDC isolates, they did not produce cellulase or xylanase when grown on WBA medium. In addition, only three *Mucor* isolates (SDCF19, SDCF21, and

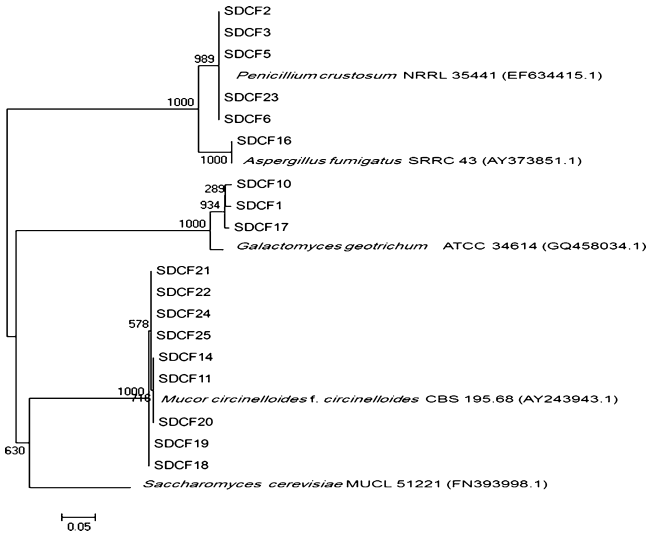

Fig. 1. Neighbor-joining tree showing the relationship between ITS sequences from fungi isolated from sawdust compost (SDC). Bootstrap values of the neighbor-joining analysis with 1,000 replications are shown on the branches. The scale bar represents the number of changes per nucleotide position (substitution/site).

Table 2. Top BLAST results for the ITS region of fungal rRNA and GenBank sequence accession numbers of fungi isolated from SDC and CRC

Isolate No.	GenBank accession No.	Top BLAST hit GenBank	Percentage of similarity
SDCF1	HQ657273	*Galactomyces geotrichum* (DQ907937.1)	100
SDCF2	HQ657274	*Penicillium crustosum* (FJ228197.1)	100
SDCF3	HQ657275	*Penicillium crustosum* (EF634415.1)	100
SDCF5	HQ657276	*Penicillium crustosum* (HM037943.1)	100
SDCF6	HQ657277	*Penicillium crustosum* (FJ228197.1)	100
SDCF10	HQ657278	*Galactomyces geotrichum* (DQ681363.2)	98.8
SDCF11	HQ657279	*Mucor circinelloides* (DQ118991.1)	100
SDCF14	HQ657280	*Mucor circinelloides* f. *circinelloides* (DQ118991.1)	100
SDCF16	HQ657281	*Aspergillus fumigatus* (EF134624.1)	100
SDCF17	HQ657282	*Galactomyces geotrichum* (DQ907937.1)	99.1
SDCF18	HQ657283	*Mucor circinelloides* (DQ118989.1)	99.8
SDCF19	HQ657284	*Mucor circinelloides* (DQ118989.1)	100
SDCF20	HQ657285	*Mucor circinelloides* f. *circinelloides* (EU484236.1)	100
SDCF21	HQ657286	*Mucor circinelloides* (AY213658.1)	100
SDCF22	HQ657287	*Mucor circinelloides* (AY213658.1)	99.8
SDCF23	HQ657288	*Penicillium crustosum* (FJ228197.1)	100
SDCF24	HQ657289	*Mucor circinelloides* f. *circinelloides* (EU484236.1)	99.8
SDCF25	HQ657290	*Mucor circinelloides* f. *circinelloides* (EU484236.1)	99.8
CRCF1	HQ657291	*Coniochaeta ligniaria* (AY198390.1)	99.8
CRCF2	HQ657292	*Hypocrea lixii* (HM176572.1)	100
CRCF3	HQ657293	*Hypocrea lixii* (HM176572.1)	100
CRCF4	HQ657294	*Hypocrea virens* (GU066623.1)	100
CRCF5	HQ657295	*Hypocrea virens* (GU066623.1)	100
CRCF6	HQ657296	*Penicillium verruculosum* (HM049911.1)	99.2
CRCF7	HQ657297	*Coniochaeta ligniaria* (AY198390.1)	99.8
CRCF8	HQ657298	*Hypocrea lixii* (HM176572.1)	100
CRCF9	HQ657299	*Hypocrea lixii* (HM176572.1)	100
CRCF10	HQ657300	*Hypocrea lixii* (HM176572.1)	100
CRCF11	HQ657301	*Aspergillus fumigatus* (EF134624.1)	100
CRCF12	HQ657302	*Penicillium verruculosum* (HM049911.1)	99.6
CRCF13	HQ657303	*Hypocrea lixii* (HM176572.1)	100
CRCF14	HQ657304	*Hypocrea lixii* (FJ517550.1)	99.6
CRCF15	HQ657305	*Hypocrea lixii* (FJ442646.1)	99.8
CRCF16	HQ657306	*Hypocrea lixii* (HM176572.1)	99.8
CRCF17	HQ657307	*Coniochaeta ligniaria* (AY198390.1)	99.8
CRCF18	HQ657308	*Hypocrea lixii* (HM176572.1)	100
CRCF20	HQ657309	*Coniochaeta ligniaria* (AY198390.1)	99.8
CRCF21	HQ657310	*Coniochaeta ligniaria* (AY198390.1)	99.8
CRCF22	HQ657311	*Coniochaeta ligniaria* (AY198390.1)	99.8
CRCF23	HQ657312	*Coniochaeta ligniaria* (AY198390.1)	99.8
CRCF24	HQ657313	*Coniochaeta velutina* (GQ154545.1)	98.9
CRCF26	HQ657314	*Hypocrea virens* (GU066623.1)	99.8
CRCF27	HQ657315	*Fusarium acuminatum* (HM068325.1)	100
CRCF28	HQ657316	*Hypocrea lixii* (HM176572.1)	99.7
CRCF29	HQ657317	*Coniochaeta ligniaria* (AY198390.1)	99.8
CRCF30	HQ657318	*Coniochaeta velutina* (GQ154545.1)	98.5
CRCF31	HQ657319	*Coniochaeta ligniaria* (AY198390.1)	99.8

SDCF indicates fungi that were isolated from sawdust compost, and CRCF indicates fungi that were isolated from coffee residue compost, while the numbers represent the isolate number.

SDCF22) produced protease (Table 3).

The five isolates identified as *P. crustosum* (100% similarity) possessed cellulase, xylanase, β-glucanase, and mannanase activities, and did not exhibit protease activity, with the exception of SDCF6, which produced proteases, but not cellulases. Among the SDC isolates, *Penicillium* isolates displayed the highest xylanase activity and relatively high cellulase activity. Notably, SDCF5 exhibited the highest cellulase activity and comparatively high xylanase activity among the *Penicillium* isolates (Table 3).

The three *Galactomyces* isolates did not show any activities towards the different AZCL substrates. Finally, the single isolate identified as *A. fumigatus* (SDCF16) exhibited positive activity in all assays. SDCF16 showed the highest cellulase activity among SDC isolates, in addition to relatively high β-glucanase and mannanase activities (Table 3).

Characterization of CRC fungal isolates

Members of the genus *Trichoderma*/*Hypocrea* were the most abundant fungi isolated from CRC (14/29), with the 14 identified isolates belonging to only one of two species, either *T. harzianum*/*H. lixii* or *T. virens*. All *Trichoderma*

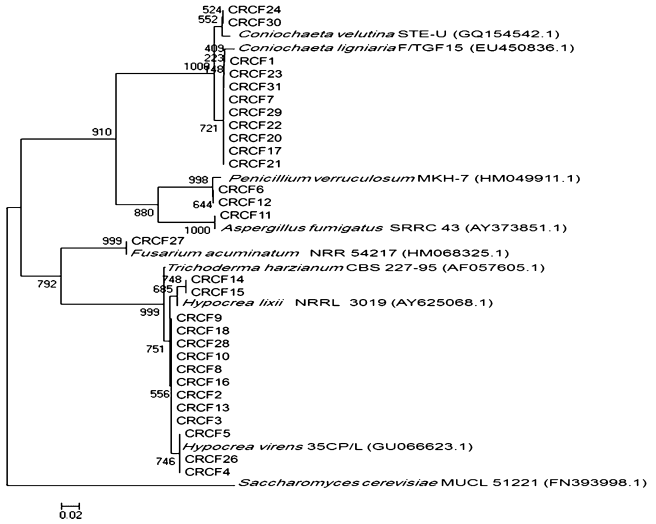

Fig. 2. Neighbor-joining tree showing the relationship between ITS sequences from fungi isolated from coffee residue compost (CRC). Bootstrap values of the neighbor-joining analysis with 1,000 replications are shown on the branches. The scale bar represents the number of changes per nucleotide position (substitution/site).

isolates, with the exception of CRCF3, CRCF5, and CRCF15, exhibited cellulase activity. CRCF2 and CRCF16 (*T. harzianum*/*H. lixii*) produced the highest cellulase activity among *Trichoderma* isolates. The xylanase activity of *Trichoderma* isolates exhibited a similar trend with cellulase activity (Table 4), as all isolates except CRCF15 had xylanase activity. *T. virens* isolates (CRCF4, CRCF5, and CRCF26) did not possess β-glucanase activity, while *T. harzianum* isolates displayed the highest β-glucanase activity among CRC isolates. In addition, while all isolates except CRCF15 had mannanase activity, only three isolates (CRCF14, CRCF15 and CRCF16) could degrade AZCL-casein as a substrate in the protease assay (Table 4).

Coniochaeta sp. was the second most abundant genus in CRC (11/29); however, only one isolate (CRCF23) showed cellulase activity, which was the lowest activity among the CRC isolates. Seven *Coniochaeta* isolates produced xylanase, with CRCF21 having the highest activity among *Coniochaeta* isolates. All *Coniochaeta* isolates displayed β-glucanase, mannanase, and protease activities, with the exception of CRCF24 and CRCF30, which did not possess mannanase activity (Table 4).

Notably, *P. verruculosum* (CRCF6) showed the highest cellulase activity of all CRC isolates, while the other *P. verruculosum* isolate (CRCF12) did not produce cellulase under the assay conditions. Both isolates exhibited xylanase, β-glucanase, and mannanase activities, although no protease activity was detected. In addition to high cellulase activity, CRCF6 showed moderately high β-glucanase and mannanase activities (Table 4). Similar to isolate SDCF16, *A. fumigatus* (CRCF11) displayed cellulase, xylanase, β-glucanase, mannanase, and protease activities, while the *F. acuminatum* isolate (CRCF27) showed only relatively low xylanase and β-glucanase activities (Table 4).

Discussion

In this study, we investigated the fungi present in SDC and CRC in an attempt to clarify the role of isolated fungal groups in the degradation of lignocellulosic materials through the characterization of enzymatic activities. Although our results suggest that different fungi are associated with cellulolytic and hemicellulolytic activities in the two compost types, *Penicillium* and *Aspergillus* were dominant in both SDC and CRC. The variance in fungal community between SDC and CRC may reflect the differences in the raw material components available for composting such as differences in

Table 3. Enzymatic activities of fungi isolated from SDC

Genus	Isolate No.	Area of the blue halo zone (mm^2)				
		Cellulase	Xylanase	β-Glucanase	Mannanase	Protease
Mucor	SDCF11	N.D.	N.D.	287 ± 27.1	150 ± 15.1	N.D.
	SDCF14	N.D.	N.D.	301 ± 35.5	170 ± 14.2	N.D.
	SDCF18	N.D.	N.D.	285 ± 30.3	158 ± 18.5	N.D.
	SDCF19	N.D.	N.D.	270 ± 23.7	140 ± 12.8	20 ± 11.6
	SDCF20	N.D.	N.D.	303 ± 25.1	144 ± 21.6	N.D.
	SDCF21	N.D.	N.D.	275 ± 22.2	182 ± 15.7	59 ± 8.4
	SDCF22	N.D.	N.D.	286 ± 26.3	**202 ± 18.8**	36 ± 9.0
	SDCF24	N.D.	N.D.	212 ± 39.4	180 ± 16.5	N.D.
	SDCF25	N.D.	N.D.	**323 ± 27.1**	187 ± 23.3	N.D.
Penicillium	SDCF2	44 ± 15.7	**315 ± 27.8**	151 ± 14.4	70 ± 11.3	N.D.
	SDCF3	60 ± 19.1	298 ± 35.0	162 ± 16.7	49 ± 9.5	N.D.
	SDCF5	94 ± 19.0	314 ± 35.6	153 ± 15.4	48 ± 10.7	N.D.
	SDCF6	N.D.	212 ± 1.2	215 ± 22.1	129 ± 20.6	**45 ± 9.4**
	SDCF23	73 ± 17.7	280 ± 26.6	115 ± 15.5	20 ± 8.7	N.D.
Galactomyces	SDCF1	N.D.	N.D.	N.D.	N.D.	N.D.
	SDCF10	N.D.	N.D.	N.D.	N.D.	N.D.
	SDCF17	N.D.	N.D.	N.D.	N.D.	N.D.
Aspergillus	SDCF16	**117 ± 14.6**	99 ± 11.9	223 ± 20.4	147 ± 15.0	47 ± 8.7

Enzymatic activities are reported as the area (mm^2) of the blue halo surrounding wells (±SD) containing fungal enzyme extracts for five assays using AZCL-HE-cellulose (cellulase), AZCL-arabinoxylan (xylanase), AZCL-barley β-glucan (β-glucanase), AZCL-galactomannan (mannanase), and AZCL-casein (protease) as substrates. The values in bold represent the isolate with the highest activity for each assay. N.D., not detected.

Table 4. Enzymatic activities of fungi isolated from CRC

Genus	Isolate No.	Area of the blue halo zone (mm^2)				
		Cellulase	Xylanase	β-Glucanase	Mannanase	Protease
Trichoderma/*Hypocrea*	CRCF2	172 ± 21.4	172 ± 20.5	171 ± 16.6	110 ± 11.8	N.D.
	CRCF3	N.D.	156 ± 29.1	193 ± 20.4	95 ± 11.1	N.D.
	CRCF8	99 ± 11.7	196 ± 18.0	204 ± 16.5	131 ± 13.9	N.D.
	CRCF9	119 ± 18.9	194 ± 17.6	217 ± 16.6	146 ± 11.7	N.D.
	CRCF10	73 ± 11.9	192 ± 22.6	**220 ± 17.5**	110 ± 13.0	N.D.
	CRCF13	62 ± 9.9	174 ± 19.2	201 ± 20.0	124 ± 10.4	N.D.
	CRCF14	99 ± 10.4	**255 ± 22.6**	147 ± 15.0	142 ± 20.4	**75 ± 11.4**
	CRCF15	N.D.	N.D.	17 ± 13.2	N.D.	56 ± 7.9
	CFRCF16	131 ± 21.3	172 ± 19.2	205 ± 18.3	**153 ± 14.5**	36 ± 10.2
	CRCF18	67 ± 12.6	193 ± 15.0	189 ± 16.2	139 ± 18.8	N.D.
	CRCF28	128 ± 18.8	163 ± 17.8	188 ± 18.9	142 ± 13.5	N.D.
	CRCF4	77 ± 13.4	182 ± 14.6	N.D.	77 ± 13.9	N.D.
	CRCF5	N.D.	39 ± 13.9	N.D.	47 ± 9.7	N.D.
	CRCF26	73 ± 13.7	100 ± 12.6	N.D.	58 ± 10.3	N.D.
Coniochaeta	CRCF1	N.D.	96 ± 9.9	79 ± 10.0	47 ± 11.7	31 ± 14.4
	CRCF7	N.D.	69 ± 16.1	51 ± 9.2	30 ± 6.7	29 ± 12.3
	CRCF17	N.D.	N.D.	21 ± 10.6	27 ± 6.7	38 ± 7.1
	CRCF20	N.D.	61 ± 20.1	104 ± 15.1	51 ± 12.9	49 ± 7.0
	CRCF21	N.D.	150 ± 20.8	61 ± 9.2	69 ± 11.5	38 ± 6.1
	CRCF22	N.D.	N.D.	78 ± 10.2	20 ± 9.1	26 ± 9.2
	CRCF23	39 ± 13.8	48 ± 12.1	120 ± 15.1	67 ± 13.6	46 ± 8.1
	CRCF29	N.D.	77 ± 14.7	119 ± 13.9	58 ± 14.3	62 ± 7.4
	CRCF31	N.D.	44 ± 10.2	47 ± 11.1	81 ± 13.4	23 ± 9.6
	CRCF24	N.D.	N.D.	95 ± 11.4	N.D.	26 ± 9.0
	CRCF30	N.D.	N.D.	183 ± 18.6	N.D.	60 ± 12.2
Penicillium	CRCF6	**194 ± 16.1**	83 ± 12.4	182 ± 17.2	91 ± 11.5	N.D.
	CRCF12	N.D.	116 ± 20.0	170 ± 18.3	38 ± 10.2	N.D.
Aspergillus	CRCF11	116 ± 13.3	90 ± 15.9	201 ± 18.7	78 ± 11.2	69 ± 10.0
Fusarium	CRCF27	N.D.	41 ± 13.4	54 ± 12.3	N.D.	N.D.

Enzymatic activities are reported as the area (mm^2) of the blue halo surrounding wells (±SD) containing fungal enzyme extracts for five assays using AZCL-HE-cellulose (cellulase), AZCL-arabinoxylan (xylanase), AZCL-barley β-glucan (β-glucanase), AZCL-galactomannan (mannanase), and AZCL-casein (protease) as substrates. The values in bold represent the isolate with the highest activity for each assay. N.D., not detected.

cellulose, hemicellulose, lignin, protein, ash, fibers, lipids, caffeine, and tannins (4, 6, 12, 35). Jing-Chun *et al.* (28) demonstrated that during composting, different raw materials are the main factors affecting microbial community structure. Our analyses revealed that a number of isolates, including *P. crustosum* (SDCF5), *P. verruculosum* (CRCF6), and *T. harzianum*/*H. lixii* (CRCF2 and CRCF16), possess high cellulolytic and hemicellulolytic activities, and likely play a dominant role in degrading lignocellulosic materials in compost.

The genera isolated from SDC were *Mucor* (9 isolates), *Penicillium* (5 isolates), *Galactomyces* (3 isolates) and *Aspergillus* (1 isolate), while *Trichoderma*/*Hypocrea* (14 isolates), *Coniochaeta* (11 isolates), *Penicillium* (2 isolates), *Aspergillus* (1 isolate), and *Fusarium* (1 isolate) were isolated from CRC. Our results are consistent with previous reports that have found that *Aspergillus*, *Trichoderma*, *Penicillium*, *Mucor*, and *Fusarium* fungi are commonly present in compost (4, 6, 36). In addition, Purnomo *et al.* (40) isolated *M. circinelloides* and *G. geotrichum* from cattle manure compost, while Hatamoto *et al.* (23) reported that *Coniochaeta* is a key eukaryote associated with the decomposition of rice straw compost in Japanese rice paddy fields. Sánchez (42) also reported the isolation of filamentous fungi species of *Penicillium*, *Trichoderma*, *Aspergillus*, and *Fusarium* from lignocellulosic residues. Notably, our results showed that *Penicillium* and *Aspergillus* are dominant in both SDC and CRC, a conclusion which is supported by several reports that have demonstrated the ubiquity of *Aspergillus* and *Penicillium* fungi in environmental samples, which are typically found as saprophytes in compost and soil (4, 6, 10, 39).

In this study, we evaluated cellulase, xylanase, β-glucanase, mannanase, and protease activities of several fungal groups isolated from compost. The enzymatic activities of the isolated fungi grown on wheat bran medium clearly varied not only between genera and species, but also between individual strains in each species. This result is in accordance with those of Pederson *et al.* (37), who found that enzymatic activities of isolates from the genus *Ulocladium* varied among species and also individual strains of the same species. In addition, they determined that the source of isolation affected enzyme production in individual strains.

Among the SDC isolates, *G. geotrichum* strains did not exhibit any activities towards cellulose, xylan, β-glucan, mannan, or casein. In contrast, *M. circinelloides* isolates showed the highest β-glucanase and mannanase activities, although they did not display cellulase or xylanase activity.

This result is consistent with Noots *et al.* (34), who reported that β-glucan was intensively degraded by *Mucor* sp., although no cellulase or xylanase activity was detected (26). Three of the 9 (33%) *Mucor* strains isolated here displayed protease activity, which is considerably lower than that found among *Mucor* sp. isolates (82%) reported by Alves *et al.* (3). However, the *P. crustosum* and *A. fumigatus* isolates from SDC showed high cellulase and xylanase activities, and relatively high β-glucanase and mannanase activities. Our results are supported by Jahangeer *et al.* (26), who reported cellulase production by *Penicillium* sp. and *Aspergillus* sp., while the production of xylanase by these fungi was demonstrated by Chávez *et al.* (13) and Gawande and Kamat (22), respectively. Furthermore, β-glucanase activity of *Penicillium* sp. and *Aspergillus* sp. was reported by Santos *et al.* (44) and Noots *et al.* (34), respectively. The results of the present study suggest that during the composting of sawdust, the degradation of cellulose and xylan is mainly due to *Penicillium* and *Aspergillus*, whereas the bioconversion of β-glucans and mannans is performed by *Mucor* and *Aspergillus* isolates. Therefore, *Penicillium*, *Aspergillus*, and *Mucor* appear to play a major role in the degradation of SDC.

Regarding the CRC isolates, *Coniochaeta* and *Fusarium* spp. showed low activity for the degradation of xylan, β-glucan, and mannan, and did not possess cellulase activity. Although all *Coniochaeta* isolates exhibited protease activity, few other fungal isolates possessed such activity, which may reflect the low protein content of the waste materials present in the CRC. In contrast, nearly all *Trichoderma*/*Hypocrea*, *Penicillium*, and *Aspergillus* isolates exhibited high cellulase, xylanase, β-glucanase, and mannanase activities when grown on WBA medium. *Trichoderma* spp., which have been reported to produce cellulase (19), xylanase (1), β-glucanase (33), and mannanase (41), have been shown by Vitikainen *et al.* (55) to be the main industrial producers of cellulase and hemicellulase. In addition, de Siqueira *et al.* (19) reported the production of cellulase by *Penicillium* and *Aspergillus* spp. Taken together, these results indicate that *Trichoderma*/*Hypocrea*, *Penicillium*, and *Aspergillus* isolates play a predominant role in the degradation of lignocellulosic materials present in CRC.

Penicillium and *Aspergillus* were commonly found in SDC and CRC, with isolates of both genera having high cellulolytic and hemicellulolytic activities. In particular, *P. crustosum* (SDCF5) and *A. fumigatus* (SDCF16) showed the highest cellulase and xylanase activities among SDC isolates, which suggests they play a significant role in the biodegradation of cellulose and hemicellulose. Among CRC isolates, *P. verruculosum* (CRCF 6) and *T. harzianum/H. lixii* (CRCF2 and CRCF16) were found to have the highest cellulolytic and hemicellulolytic activities, indicating their importance for the degradation of cellulose and hemicellulose during the composting of coffee residue.

Therefore, the results of the current study could be useful for determining the possible contribution of each characterized fungus in the degradation of lignocellulosic waste materials during the composting process.

The results revealed that *Penicillium*, *Aspergillus*, and *Mucor* isolates made a major contribution to the degradation of SDC, while *Penicillium*, *Aspergillus*, and *Trichoderma* isolates had a large role in the degradation of CRC. A number of isolates, such as *P. crustosum* (SDCF5), *P. verruculosum* (CRCF6), and *T. harzianum*/*H. lixii* (CRCF2 and CRCF16), displayed high cellulolytic and hemicellulolytic activities, and could be useful for improving biodegradation processes involving lignocellulosic waste materials.

Acknowledgements

We are extremely grateful to the Egyptian government for providing financial assistance to the first author.

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