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NOTE

Comparative Enzyme Production by Fungi from Diverse Lignocellulosic Substrates

Marie K. W. Sin, Kevin D. Hyde, and Stephen B. Pointing*

Centre for Research in Fungal Diversity, Department of Ecology and Biodiversity, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, China

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Fungi commonly encountered on monocotyledonous substrates were evaluated for their *in vitro* ability to produce enzymes involved in lignocellulose breakdown. Most were capable of structural polysaccharide utilization, but few produced enzymes associated with lignin breakdown. None of the monocotyledon-inhabiting fungi produced reactions as strongly as wood decay fungi.

***Key words*: monocotyledons, cellulase, xylanase, lignin-modifying enzymes**

There has been considerable research into the biodiversity of tropical microfungi, and the most frequently studied are those inhabiting lignocellulose substrates (Hyde 1997). Recent research has included detailed investigations of saprobic fungal biodiversity on various non-wood lignocellulose substrates such as bananas (Photita *et al.*, 2001), bamboo (Hyde *et al.*, 2001), grasses (Wong and Hyde, 2001) and palms (Yanna *et al.*, 2001). Relatively little, however, is known about the physiology of substrate utilization by such fungi. All lignocellulosic materials are formed predominantly of three components: cellulose, a structural carbohydrate responsible for strength and flexibility; lignin, a polyaromatic heteropolymer conferring decay resistance and hardness; and hemicellulose, a structural carbohydrate intimately associated with lignin (Eaton and Hale, 1993). The composition of bananas, bamboo, grasses and palms, differs only slightly from wood in both morphology and association of these components (Fengel and Wegener, 1989). The only major chemical difference lies in the incorporation of coumaryl, sinapyl and vanilyl monomers in the lignin of grasses and gymnosperm/angiosperm wood.

Most of our knowledge on lignocellulose substrate utilization is from studies of those species involved in the decay of commercially important timber in temperate regions (Eaton and Hale, 1993). The degradation of lignocellulose by such fungi is well understood. Cellulose is attacked predominantly by hydrolytic cellulases although oxidative enzymes may also be involved in cellobiose and glucose utilization. Hemicellulose breakdown is less well understood but is thought to involve hydrolytic endo-type hemicellulases. The mineralization of lignin is achieved by a group of peroxidase and phenoloxidase enzymes known as lignin-modifying enzymes (LME's). They produce highly reactive radicals that oxidize phenolic and non-phenolic lignin components (Pointing, 2001).

Three decay types are recognised in terrestrial fungi (Eaton and Hale, 1993). Soft rot, in which superficial enzymic decay of cellulose and hemicellulose is accompanied by little or no lignin degradation. This is characteristic of many ascomycete and mitosporic genera, where softening of surface layers in wood and characteristic soft-rot cavities are observed due to decay. The other types of decay are white-rot and brown-rot, found only among basidiomycetes and a few higher ascomycete genera among the xylariaceae.

The aim of this study was to determine the relative ability among fungi commonly encountered on diverse non-wood lignocellulosic substrates to produce enzymes involved in lignocellulose decay. These substrates include bananas, bamboo, grasses and palms, which vary in their composition and have received very little attention in terms of investigations of their decay. The result may provide some insight into the substrate utilization capabilities of these fungi. It may also shed some light on why certain fungi are host-specific or recurrent (*sense* Zhou and Hyde, 2001), since substrate-utilizing ability (or lack of it) may restrict some saprobic fungi to certain hosts.

All fungi used in this study were selected as the most

* To whom correspondence should be addressed.
(Tel) 852-2299-0677; (Fax) 852-2517-6082
(E-mail) pointing@hkucc.hku.hk

frequently encountered saprobes isolated from decaying monocotyledonous substrates in tropical and sub-tropical regions, based on previous research (Hyde *et al.*, 2001; Photita *et al.*, 2001; Wong and Hyde, 2001; Yanna *et al.*, 2001; Zhou and Hyde, 2001). All cultures were maintained on potato-dextrose agar (Difco) at 25°C in darkness. Discs of inoculum cut from the actively growing colony margin of 7-14 day old cultures were used for all experiments. The following growth conditions were used to test for lignocellulolytic enzyme production, with

Table 1. Comparative enzyme production by fungi from diverse lignocellulosic substrates

Substrate	Fungus	Culture collection number	Cellulolytic	Xylanolytic	Poly R (LMEs)	Azure B (peroxidase)	Syringaldazine (laccase)
Wood	*Trametes versicolor* (simultaneous white-rot)	HKUCC 4063	5	5	5	5	5
	Phanerochaete chrysosporium (selective white-rot)	IMI 284010	5	5	5	5	5
	Gloeophyllum trabeum (brown rot)	CBS 318.50	5	5	0	0	0
	Chaetomium globosum (soft rot)	HKUCC 4097	4	4	0	0	0
Bamboo	*Roussoella hysterioides*	HKUCC 2933	4	4	3	0	1
	Roussoella pustulans	HKUC 3016	3	4	0	0	0
	Gilmaniella bambusae	HKUCC 3411	5	2	0	0	0
	Phaeoisaria clematidis	HKUCC 3412	3	4	0	0	0
	Podosporium nilgirense	HKUCC 3471	4	4	0	0	0
	Apiospora sinensis	HKUCC 3990	2	1	1	3	3
	Anthostomella flagellariae	HKUCC 4484	4	4	0	0	0
	Anthostomella bruneiensis	HKUCC 4529	3	3	0	0	0
	Arecophila bambusae	HKUCC 4794	4	5	0	0	0
	Podosporium elongatum	HKUCC 5951	4	4	0	0	0
Palm	*Anthostomella* sp.	HKUCC 6046	N	5	1	0	0
	Appendicospora hongkongensis	HKUCC 6015	1	5	2	0	0
	Arecomyces sp. nov.	HKUCC 6132	2	3	0	0	0
	Helicomyces sp.	HKUCC 6214	4	2	0	0	0
	Lachnum palmae	HKUCC 6210	N	N	N	N	N
	Linocarpon sp. nov.	HKUCC 6128	2	3	0	0	0
	Massarina sp.	HKUCC 6045	2	4	0	P	0
	Hyphomycetes sp.	HKUCC 6016	3	5	0	0	0
	Spacicoides sp.	HKUCC 6213	N	2	0	0	0
	Sporoschisma sp.	HKUCC 6428	5	2	0	0	0
Grass	*Annulatascus* sp.	HKUCC 5809	2	4	0	P	0
	Ascotaiwania sp.	HKUCC 6458	3	5	0	0	0
	Coelomycete sp. 3	HKUCC 5714	1	1	0	0	0
	Curvularia senegalensis	HKUCC 5702	N	5	0	0	0
	Massarina phragmiticola	HKUCC 6446	3	2	0	0	0
	Periconia sp. 1	HKUCC I29	3	3	1	0	0
	Periconia minmibissia	HKUCC GR13	3	2	0	0	0
	Piricauda sp.	HKUCC GR8	3	1	1	0	0
	Roussoella sp.	HKUCC I30	2	2	0	0	0
	Tetraploa aristata	HKUCC 5798	3	3	0	0	0
Banana	*Dictyosporium heptasporum*	HKUCC HK32	4	5	2	0	0
	Coryresporopsis inaequiseptata	HKUCC HK37	4	1	0	0	0
	Hansfordia ovalispora	HKUCC HK23	4	4	0	0	0
	Tetraploa aristata	HKUCC HK33	4	5	0	0	0
	Massarina rubi	HKUCC HK43	4	1	0	0	0
	Diaporthe sp. 2	HKUCC HK44	4	3	1	0	0
	Phaeosphaeria sp.	HKUCC HK27	4	4	0	0	0
	Glomerella sp.	HKUCC 5694	4	1	0	0	0
	Hansfordia sp. 1	HKUCC CM23	1	5	0	0	0
	Diaporthe sp. 1	HKUCC 44A	4	1	1	0	0

0 denotes no reaction, 5 denotes reaction equivalent to that of *T.versicolor* on a graduated scale; N=no growth recorded; P=partial decolorization; CBS=Centraal Bureau voor Schimmelcultures; HKUCC=The University of Hong Kong Culture Collection; IMI=CABI Bioscience UK, formerly IMI.

assessment of results carried out as previously described (Pointing, 1999). Cellulose-azure agar contains 1% (w/v) cellulose-azure (Sigma), 0.1% (w/v) mycological peptone (Oxoid), 0.01% (w/v) yeast extract (Difco), and 1.6% (w/v) agar (Difco). Cellulolysis was assessed by monitoring release of azure dye from cellulose-dye complex and diffusion into clear agar not containing cellulose-azure. Ligninolytic peroxidase production was indicated by subsequent decolorization of azure dye. Xylan agar contains 1% (w/v) birchwood xylan (Sigma), 0.1% (w/v) mycological peptone, 0.01% (w/v) yeast extract, and 1.6% (w/v) agar. Zones of xylanolysis were visualized after flooding petri dishes with 0.25% (w/v) I_2 and KI solution. Xylan is the major component of hemicellulose and its hydrolysis in this study is used to indicate hemicellulolytic ability. Poly R agar contains 0.2% (w/v) glucose (Sigma), 0.02% (w/v) Poly R 478 (Sigma), 0.1% (w/v) mycological peptone, 0.01% (w/v) yeast extract, and 1.6% (w/v) agar. Production of LME was recorded as clearance of Poly R agar. Syringaldazine agar contains 0.2% (w/v) glucose, 0.1% (w/v) mycological peptone, 0.01% (w/v) yeast extract, and 1.6% (w/v) agar. Formation of pink-purple zones around wells in the agar flooded with 0.1% (w/v) syringaldazine (Sigma) indicated laccase (phenoloxidase) production. Intensity of reaction for each test was recorded over a 14 day period (except cellulose azure decolorization, 30 days), and a comparative score between 0 and 5 given with 0 representing a negative reaction and 5 representing that observed for *Trametes versicolor*. Each treatment was repeated in triplicate, with incubations carried out at 25°C in darkness.

The results of the enzyme assays are displayed in Table 1. The control group representing simultaneous white, preferential white, brown and soft rot fungi grew rapidly and displayed typical responses to the various agar enzyme assays. Test fungi from bananas, bamboo, grasses and palms grew well on PDA and on test agar growth substrates, although weak/negative reactions to cellulose-azure and xylan agar tests were accompanied by poor or zero colony growth. This was expected since such enzyme assays rely on utilization of the substrate as C source for growth. None of the test fungi produced more intense reactions to enzyme tests than the control (wood decay) fungi. Results obtained for wood decay fungi in this study are consistent with those achieved for other wood decay taxa in separate studies (de Koker *et al.*, 2000).

Isolates obtained from bamboo are from woody culms, so this group of fungi were expected to display similar lignocellulolytic ability to wood-inhabiting fungi, particularly in light of the known *in vitro* soft-rot capability on bamboo of *Chaetomium globosum* (Sulaiman and Murphy, 1995). All of the ten species tested displayed moderate cellulolytic and xylanolytic activity, supporting the lignocellulose-degrading saprobic nature assumed for these isolates. Conversely, only two of the ten isolates decolorized Poly R, which is a general indicator of LME production. *Roussoella hysteroides* and *Apiospora sinensis* decolorized Poly R (moderately and weakly, respectively) and oxidized syringalazine weakly, suggesting laccase is produced as part of the ligninolytic enzyme system. *Apiospora sinensis* was also capable of decolorizing azure B, a substrate only for lignin peroxidase, thus suggesting peroxidase and laccase type LME's are produced. The use of different combinations of LME to efficiently degrade lignin is well documented among white-rot basidiomycetes (Hatakka, 1994), and this study indicates that lignin degradation by bamboo saprobes may also be achieved via different combinations of LME among various taxa.

The fungi from grasses were generally obtained from stout grass species, which had large amounts of sclerochymatous (i.e. woody) tissues and were similar in structure to the bamboo culms. One would therefore expect that some of the saprobes from grasses would also be capable of ligninolytic enzyme production. This was the case, with *Periconia* sp. 1 and *Piricaudia* sp. which weakly decolorized Poly R, indicating some production of LMEs by these fungi. One isolate, *Ammulelosum* sp., also partially decolorized azure B (from blue to pink) suggesting some partial breakdown of this compound, although the mechanism by which this was achieved is not known. The other taxa however, appeared to be capable of carbohydrate degradation only.

Fungal isolates from palms were obtained from petioles, which are less lignified than woody tissues. Two isolates from palms, i.e. *Anthostomella* sp. and *Appendicospora* sp., weakly decolorized Poly R, indicating some production of LME's by these fungi. *Massarina* sp. also partially decolorized azure B (from blue to pink) but all other taxa appeared to be capable of carbohydrate degradation only.

Fungi from bananas were obtained from herbaceous leaves and thus are expected to be capable of carbohydrate degradation only. Three isolates (*Dictyosporim* sp., *Diaporthe* sp. 1 and 2), however, also weakly decolorized Poly R, suggesting some production of LMEs by these fungi. *Dictyosporium* sp. is a common saprobe of submerged lignocellulose substances, while *Diaporthe* spp. are commonly found on terrestrial woody substrates. The remaining isolates appeared to be capable of carbohydrate degradation only and have only been reported from decaying herbaceous substrates.

The ability to produce cellulase and xylanase enzymes suggests that many of the saprobes tested in this study are capable of soft rot type decay. This could be confirmed by observations of decay morphology within the substrate. The results here indicate that there is little potential for ligninolytic ability among these banana- bamboo-, grass- and palm-inhabiting fungi. This is not surprising, since most known LME producing (ligninolytic) fungi are

basidiomycetes (Pointing, 2001). Nonetheless, lignin within such plant material must be recycled, and this may depend upon other soil-inhabiting mycoflora that have access to decaying material. Alternatively, the inherent bias in currently used isolation techniques (inducing sporulation from the substrate in moist chamber incubations) towards readily sporulating taxa may account for the lack of basidiomycetes encountered, when they may be important in later stages of decay involving lignin breakdown. Based on the results of this experiment, fungi commonly encountered on monocotyledonous substrates appear less aggressive producers of lignocellulolytic enzymes than wood decay fungi. Direct studies of mass loss and component utilization in native substrates are required to confirm this.

The generally similar pattern of substrate-utilizing enzyme (i.e. producing cellulases and hemicellulases but not ligninolytic enzymes) production among the taxa from different hosts suggests that ability to utilize the substrate is unlikely to play any role in determining specificty or recurrence among these fungi on their respective hosts. Saprobic fungi have, however, been shown to be host-specific or recurrent (Photita *et al.*, 2001; Yanna *et al.*, 2001; Zhou and Hyde, 2001) but the mechanisms for this specificity/ recurrence are not understood.

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