Mycol Progress (2014) 13:1049–1056
DOI 10.1007/s11557-014-0992-0

ORIGINAL ARTICLE

# Several fungi from fire-prone forests of southern India can utilize furaldehydes

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Received: 18 February 2014 / Revised: 1 May 2014 / Accepted: 16 May 2014 / Published online: 7 June 2014


**Abstract** Furfural and 5-hydroxymethylfurfural (HMF), released during thermo-chemical degradation of lignocellulosic biomass, inhibit microbial fermentation of sugars to biofuels. One approach to circumvent this roadblock is through microbial degradation of furaldehydes in biomass hydrolysates. Since these furaldehydes are the most common and abundant volatile organic compounds in plant litter and are released during biomass burning, we investigated endophytic and litter fungi of fire-prone forests for their ability to utilize furaldehydes. Of the 23 (11 endophytic and 12 litter) fungi we tested, 10 grew on furfural, 21 on HMF, and nine on both substrates as the sole carbon source. These fungi initially grew slower on furaldehydes than on sucrose, but their growth increased on subsequent sub-culturing on the same furaldehyde medium, suggesting an innate-adaptation competence. The ability of endophytic and litter fungi of fire-prone forests to metabolize furaldehydes is more common than previously anticipated and helps rationalize their unusual ecological fitness in specific niches. Our findings should also motivate a closer examination of all locales of biomass (including crop residue) burning for identifying furaldehyde-utilizing fungi.

**Keywords** Biofuel · Agricultural waste · Volatile organic compounds · Lignocellulosic biomass hydrolysates · Bioabatement

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## Introduction

The lignocellulosic biomass of agricultural wastes has drawn attention as a feedstock for producing transportation fuels and bio-based chemicals through microbial processes. Lignocellulose in the plant cell wall is a complex structure made of crystalline cellulose enmeshed in lignin and hemicellulose, and it is recalcitrant to enzymatic hydrolysis. Therefore, a pre-treatment involving high temperature and dilute acid is essential to hydrolyze the hemicellulose network and disrupt the lignin polymers to expose the cellulose microfibrils for enzymatic degradation to glucose. This pre-treatment generates a semi-solid fraction of amorphous cellulose and lignin, and a supernatant with soluble sugars, as well as weak organic acids (acetic, formic, and levulinic acids), phenolic derivatives (vanillin and 4-hydroxybenzaldehyde), and furaldehydes [furfural and 5-hydroxymethylfurfural (HMF) (Fig. 1)]. Of all these compounds, furaldehydes are the most toxic (Modig et al. 2002) and hinder efficient biomass utilization by decreasing cellulolytic saccharification (Jing et al. 2009), as well as microbial growth and fermentation efficiency (Almeida et al. 2009; Mills et al. 2009). Several fungi including *Kluyveromyces marxianus*, *Pichia stipitis*, and *Saccharomyces cerevisiae*, and bacteria such as *Escherichia coli* and *Zymomonas mobilis* (Almeida et al. 2009) are adversely affected by furaldehydes, likely due to oxidative damage and inhibition of key enzymes in pathways that catabolize sugars (Allen et al. 2010).

Although furaldehydes could be removed from the biomass hydrolysates by washing and alkali treatment or by ion exchange (Almeida et al. 2009), these processes are expensive

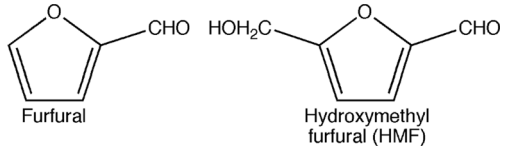


**Fig. 1** Structure of furfural and HMF

and entail large volumes of waste water and loss of fermentable sugars (Nilvebrant et al. 2001). In contrast, microbial detoxification of furaldehydes affords an environmentally attractive and cost-effective approach (Dong and Bao 2010). A few fungal species including *Coniochaeta ligniaria* (Lopez et al. 2004), *Aspergillus niger* and *Trichoderma reesei* (Rumbold et al. 2009), *Amorphotheca resinae* ZN1 (Zhang et al. 2010; Ran et al. 2014), and *Chaetomium globosum*, *Cunninghamella elegans*, *Mortierella isabellina*, and *Mucor plumbeus* (Zheng et al. 2012) have been examined in this context, although the molecular basis for their bioabatement potential remains unclear. Recent advances in understanding the catabolism of furfural and HMF in aerobic bacteria such as *Cupriavidus basilensis* have renewed the interest in bioabatement of these toxic compounds (Koopman et al. 2010; Wierckx et al. 2010). Since this bacterial pathway is not readily amenable for industrial applications requiring low pH and anaerobic conditions (Wierckx et al. 2011), identifying microbes which can catabolize furaldehydes through new routes will fulfil an unmet need. Here, we have used a directed approach to identify fungi that can utilize furaldehydes.

We screened endophytic and litter fungi isolated from the dry tropical forests of the Western Ghats, southern India, based on the following arguments. First, since biomass burning produces furaldehydes (Pandit and Maheshwari 1996; Yokelson et al. 2007), we focused on the fungi isolated from forests experiencing periodic ground fires (Kodandapani et al. 2008; Kodandapani 2013). Second, foliar endophytes continue their existence in leaf litter by switching to a saprotrophic mode of life (Kumaresan and Suryanarayanan 2002; Porras-Alfaro and Bayman 2011; Zuccaro et al. 2011) and so, along with litter fungi, would be exposed to furaldehydes which are the most common and abundant volatile organic compounds present in leaf litter (Isidorov and Jdanova 2002). Third, endophytes possess a vast repertoire of enzymes, and thus are likely to be primed for utilizing different carbon sources (Suryanarayanan et al. 2012). Hence, we postulated that at least a few fungi inhabiting these fire-prone forests of the Western Ghats might have evolved to metabolize furaldehydes. In this study, we demonstrate that indeed several endophytic and litter fungal species can metabolize furfural, HMF or both, and speculate on the possible catabolic enzymes based on recent insights from bacterial studies. Our results reveal a wider choice of organisms for bioabatement strategies to remove key fermentation inhibitors from biomass hydrolysates.

## Materials and methods

### Isolation and identification of endophytic and litter fungi

We screened 11 endophytes isolated from leaves or outer bark, and 12 litter fungi (including three producing heat-tolerant spores, Suryanarayanan et al. 2011b) isolated from decaying leaves in forests situated in the Nilgiri Biosphere Reserve (latitude 11°32' and 11°43'N, longitude 76°22' and 76° 45'E) of the Western Ghats. The fungi were identified primarily based on spore characteristics (Ellis 1971; Subramanian 1971; Nag Raj 1993), with seven of them being further corroborated by 5.8S rRNA analysis (Tables 1 and 2; Suryanarayanan et al. 2011b). Six of the isolates have been deposited with either the Microbial Type Culture Collection (MTCC) in Chandigarh or the National Fungal Culture Collection of India (NFCCI) in Pune, India – *Curvularia lunata* (MTCC 10334), *Sordaria* sp. (MTCC 10342), *Pithomyces* sp. (MTCC 8657), *Spegazzinia sundara* (MTCC 9597), *Bartalinia* sp. (NFCCI 2307), and *Pestalotiopsis* sp. 2 (NFCCI 2309).

### DNA extraction, amplification and sequencing of ribosomal internal transcribed spacer (ITS) DNA

Using a standard phenol-chloroform extraction method, genomic DNA was isolated from mycelia grown on Czapek Dox Agar (CDA) medium for a period of 7–10 days. The ITS regions of fungal 5.8S rDNA were amplified by PCR using the fungal-specific primers ITS4 and ITS5 (White et al. 1990) to yield a 500–600 bp product. The amplicons were electrophoresed on a 1 % (w/v) agarose gel, excised, extracted, and sequenced using ITS4 in an automated sequencer (ABI 3130 Genetic Analyzer). The sequences obtained were used as queries in BLASTn (default settings) to find the closest match in GenBank and MycoBank (www.mycobank.org). The sequences were submitted to GenBank and their accession numbers are listed in Tables 1 and 2.

### Test for furaldehyde utilization

Each isolate was grown on a solid modified CDA (mCDA) medium [made with 1.5 % (w/v) agarose *but without sucrose*] for 7 days at 27 °C to deplete its endogenous food reserves before each growth experiment. Mycelial plugs (5-mm diameter) excised with a sterile cork borer from the margin of these colonies were used as the subsequent inoculum. Each plug was placed, mycelium surface down, at the center of a 9-cm Petri dish containing 20-mL mCDA (control) or 20-mL mCDA supplemented with 5 mM furfural (purity, ~99 %; AVRA Synthesis, Hyderabad, India) or 5 mM HMF (purity, ≥99 %; Sigma-Aldrich, St. Louis,

**Table 1** Growth of endophytic fungi on either 5 mM furfural or 5 mM HMF as the carbon source

| Fungus[a] | Accession numbers for ITS sequences | Isolate from tree species[b] | 5 mM furfural | 5 mM HMF | Cellulase activity |
|---|---|---|---|---|---|
| *Alternaria* sp. 1 (L) | - | *Terminalia paniculata* (MD) | - | + | ND |
| *Arthrinium* sp. (B) | - | *Elaeocarpus serratus* (EG) | - | - | ✓ |
| *Curvularia lunata* (B) | - | *Ilex wightiana* (EG) | - | + | ✓ |
| *Fusarium* sp. 1 (L) | - | *Trema orientalis* (MD) | - | + | ND |
| *Pestalotiopsis* sp. 1 (L) | - | *Terminalia paniculata* (MD) | + | + | ND |
| *Phomopsis* sp. 1 (L) | - | *Terminalia chebula* (DT) | + | + | ✓ |
| *Phomopsis* sp. 2 (L) | KJ398147 | *Acacia suma* (DT) | + | + | ✓ |
| *Phomopsis* sp. 3 (L) | - | *Viburnum dentatum* (MD) | - | + | ✓ |
| *Phomopsis* sp. 4 (L) | KJ398149 | *Gmelina asiatica* (DT) | + | + | ✓ |
| *Pithomyces* sp. (B) | KJ398148 | *Eurya nitida* (EG) | - | + | ✓ |
| *Sordaria* sp. (L) | - | *Eurya nitida* (EG) | + | - | ✓ |

+ = growth, − = no growth, *ND*, not determined, x = no activity, ✓ = activity observed

[a] *L* = leaves, *B* = bark

[b] *DT*, dry thorn forest, *MD*, moist deciduous forest, *EG*, evergreen forest

USA). Four replicates were incubated for 15 to 30 days at 27 °C and the colony diameter was measured every 24 hours.

### Test for cellulase activity

Each isolate was grown on yeast peptone agar medium [0.01 % (w/v) yeast extract, 0.05 % (w/v) peptone] supplemented with 0.5 % (w/v) carboxymethyl cellulose. After 5 days at 27 °C, the plates were stained with 0.2 % (w/v) aqueous congo red solution and destained with 1 M NaCl. The appearance of a yellow zone around the colony indicated cellulase activity (Rohrmann and Molitoris 1992).

## Results

### More fungi can grow on HMF than furfural

Of the 23 isolates tested, only *Arthrinium* sp. failed to grow on either furaldehyde (Tables 1 and 2). Nine isolates grew on both substrates, 12 only on HMF, and *Sordaria* sp. only on furfural. It was unexpected that many more fungal isolates can grow on HMF than furfural given additional enzymes are needed for HMF utilization in *C. basilensis* and other related bacteria (Koopman et al. 2010). In light of this finding, the *Sordaria* sp. is quite unique in that it grew only on furfural. Therefore, we compared its growth on 5 mM furfural with that on 1.5 % (w/v) sucrose as the sole carbon source. Although

**Table 2** Growth of litter fungi on either 5 mM furfural or 5 mM HMF as the carbon source

| Fungus | Accession numbers for ITS sequences | Isolate from tree species | 5 mM furfural | 5 mM HMF | Cellulase activity |
|---|---|---|---|---|---|
| *Alternaria* sp. 2 | KJ398150 | *Holoptelea integrifolia* | + | + | ND |
| *Alternaria* sp. 3 | – | *Bridelia retusa* | – | + | × |
| *Bartalinia* sp. 1 | – | *Pterocarpus marsupium* | – | + | ✓ |
| *Bartalinia* sp. 2 | HQ909075 | *Bridelia retusa* | + | + | ✓ |
| *Chaetomium* sp. | – | *Butea monosperma* | – | + | ✓ |
| *Colletotrichum* sp. 1 | – | *Bridelia retusa* | – | + | ✓ |
| *Curvularia* sp. 1 | HQ909079 | *Holoptelea integrifolia* | + | + | ✓ |
| *Curvularia* sp. 2 | – | *Butea monosperma* | – | + | × |
| *Drechslera australiensis* | – | *Butea monosperma* | – | + | ✓ |
| *Fusarium* sp. 2 | – | *Zizyphus xylocarpus* | + | + | ✓ |
| *Pestalotiopsis* sp. 2 | HQ909077 | *Maytenus emarginatus* | + | + | ✓ |
| *Spegazzinia sundara* | – | *Randia dumetorum* | – | + | ✓ |

+ = growth, − = no growth, *ND*, not determined, x = no activity, ✓ = activity observed

there was an initial lag in growth on furfural, after 7 days it achieved a final colony diameter close to that on sucrose (Fig. 2).

## The furaldehyde-dependent growth is an adaptive response

As yeasts exhibit an adaptive response to furaldehyde exposure (Almeida et al. 2009), we tested our isolates for such a trait by the following method. *Drechslera australiensis*, *Fusarium* sp. 1, *Pestalotiopsis* sp. 1 and 2, and *Phomopsis* sp. 2 were grown on mCDA or mCDA supplemented with 5 mM HMF for 30 days at 27 °C. Plugs of mycelium excised from both these media were transferred to a fresh plate containing mCDA supplemented with 5 mM HMF and the colony diameter was measured daily. This experiment was also performed using 5 mM furfural in place of HMF and with *Alternaria* sp. 2, *Pestalotiopsis* sp. 1 and 2, *Phomopsis* sp. 1 and 2. Indeed, we observed an adaptive response as isolates subjected to an initial exposure to a furaldehyde exhibited significantly faster growth and a shorter lag phase when transferred to a medium having the same amount of that furaldehyde. We have depicted this attribute in *Phomopsis* sp. 2 and *Pestalotiopsis* sp. 2 in Fig. 3.

## Most of these isolates also have a cellulase activity

To determine if a correlation exists between furaldehyde utilization and biomass degradation, we screened 19 isolates (eight endophytic and 11 litter fungi) for cellulase activity. A qualitative assay revealed that 17 of these isolates have appreciable cellulase activity while *Alternaria* sp. 3 and *Curvularia* sp. 2 did not. However, we could not find any correlation between the ability to produce cellulase and the ability to utilize either furfural or HMF (Tables 1 and 2). The lack of a positive staining in this assay could stem from either unfavorable conditions for expression of cellulase or inability of the secreted enzyme to diffuse in the agar. However, results from our ongoing in vitro assays of cellulolytic (endoglucanase, cellobiosidase, and ß-glucosidase) activities in crude protein secretomes from endophytes grown in liquid media largely mirror the trend from these agar plate assays (unpublished data).

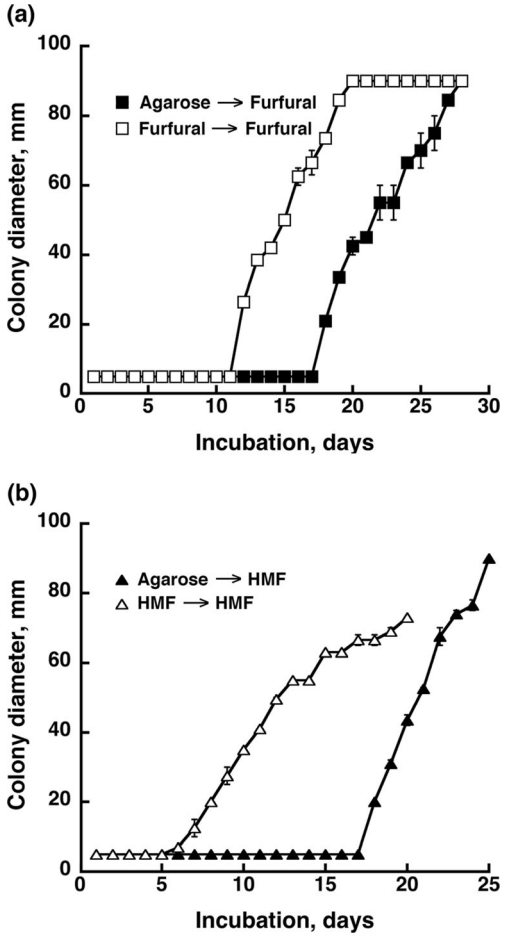


**Fig. 3** Adaptive tolerance of endophytes on furfural and HMF. *Pestalotiopsis* sp. 2 was tested with furfural (**a**) and *Phomopsis* sp. 2 with HMF (**b**). In both instances, inoculum (5-mm mycelial plug obtained from a 30-day-old colony grown on either mCDA or mCDA+5 mM respective furaldehyde) was transferred to a fresh mCDA plate containing 5 mM furaldehyde as the carbon source. The mean and error from two independent measurements are reported. In many cases, the error bars are smaller than the symbols, and thus not seen in this depiction

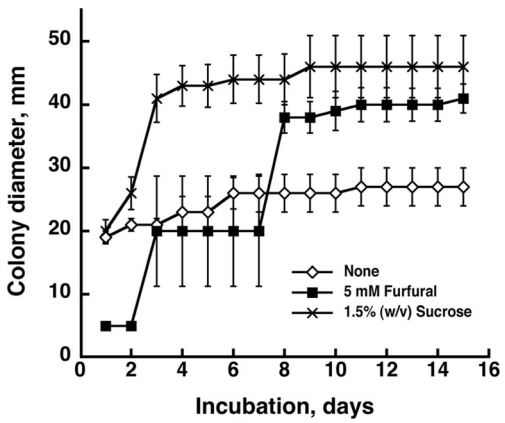


**Fig. 2** Growth of *Sordaria* sp. at 27 °C on mCDA medium without or with 5 mM furfural or 1.5 % (w/v) sucrose as the sole carbon source. The colony diameter on day 0 was 5 mm. The mean was calculated from four independent measurements; bars depict standard error. Note that the sparse growth under some of these conditions resulted in limited variability

## Homologs of the bacterial Trudgill pathway enzymes are present in many fungi

Biochemical and genetic studies in *Pseudomonas putida* and *C. basilensis* HMF14, respectively, have helped elucidate the enzymatic steps in the bacterial Trudgill pathway, which converts furfural and HMF to α-ketoglutarate, a TCA cycle intermediate (Fig. 4) (Koenig and Andreesen 1990;

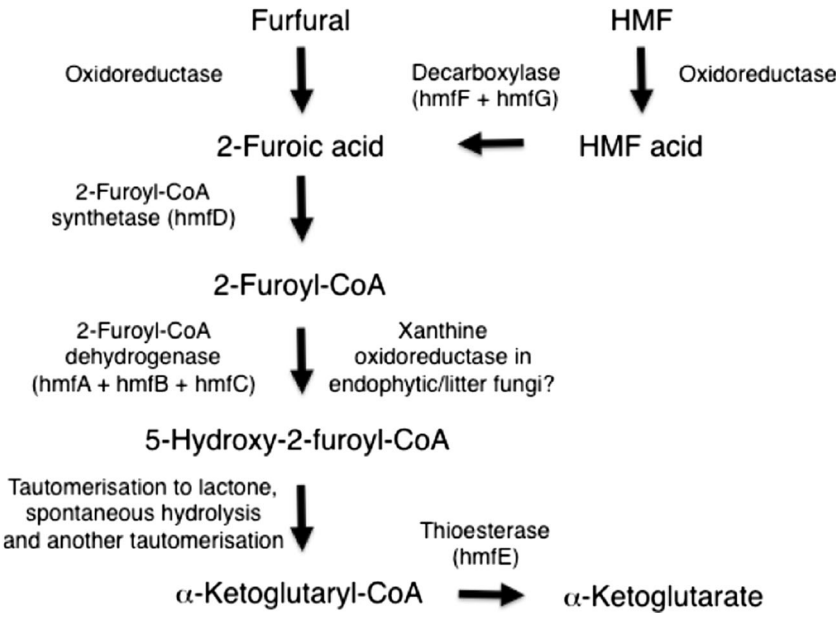


**Fig. 4** The Trudgill pathway for utilization of furfural and HMF characterized in bacteria

Koopman et al. 2010; Trudgill 1969). In this pathway, the first step in furfural utilization is an oxidoreductase-mediated conversion of furfural to 2-furoic acid, while a similar catalytic activity first converts HMF to 2, 5-furan dicarboxylic acid before decarboxylation to 2-furoic acid (Koopman et al. 2010). Thus, the metabolism of furfural and HMF converges at furoic acid, after which a shared pathway is employed (Fig. 4).

Two gene clusters code for enzymes necessary for these catabolic routes in the bacteria: while *hmfABCDE* encodes three enzymes responsible for converting 2-furoic acid to α-ketoglutarate in the shared pathway, *hmfFG* encodes the 2, 5-furan dicarboxylic acid decarboxylase that is unique to HMF metabolism. In the shared pathway, furoyl-CoA synthetase HmfD converts 2-furoic acid to the 2-furoyl-CoA thioester, which is then used to generate 5-hydroxy-2-furoyl-CoA by a molybdenum-dependent, three-subunit 2-furoyl-CoA dehydrogenase (HmfABC). Keto-enol tautomerism of 5-hydroxy-2-furoyl-CoA leads to formation of a lactone, whose spontaneous hydrolysis generates α-ketoglutaryl-CoA, setting the stage for the final thioesterase HmfE step.

Using BLASTp or tBLASTn and the bacterial protein sequences as queries, we searched fungal genomes for homologs of bacterial *hmfA-G* genes. The lack of genome sequences for the 23 isolates that we studied led us to examine 13 genomes that represent five of the genera that we studied here (*Alternaria*, *Chaetomium*, *Colletotrichum*, *Fusarium*, and *Sordaria*). We highlight the salient findings from our BLAST searches of these genomes (Table 3). First, we found homologs for nearly all the bacterial *hmf* genes in most of the genomes. Second, instead of three separate genes (*hmfABC*) encoding the three subunits of the Mo-dependent 2-furoyl-CoA dehydrogenase as in bacteria, we found a single gene encoding xanthine oxidoreductase (XOR) in all 13 fungal genomes when each of the HmfA-C sequences was used as the query. Notably, when one of these 13 XOR sequences was used as a query to search for orthologs in FUNGIpath v3.0, a web-based tool that examines presence of metabolic pathways

**Table 3** Homologs identified using BLASTp or tBLASTn and the protein sequences of the *Cupriavidus basilensis* HMF14 furfural (HmfABCDE) and HMF (HmfFG) degradation pathways as queries

| Fungal endophytes | ABC[a] | D[b] | E[b] | F[c] | G[c] |
|---|---|---|---|---|---|
| *Alternaria arborescens EGS 39–128* | 2 | ✓ | ✓ | 0 | 0 |
| *Alternaria brassicicola ATCC 96836* | 2 | ✓ | ✓ | 1 | 0 |
| **Chaetomium globosum CBS 148.51* | 1 | ✓ | ✓ | 0 | 0 |
| **Chaetomium thermophilum var. thermophilum DSM 1495* | 1 | ✓ | ✓ | 0 | 0 |
| *Colletotrichum gloeosporioides Nara gc5* | 2 | ✓ | ✓ | 3 | 3 |
| *Colletotrichum higginsianum IMI 349063* | 1 | ✓ | ✓ | 1 | 1 |
| *Colletotrichum orbiculare MAFF 240422* | 1 | ✓ | ✓ | 1 | 0 |
| *Fusarium fujikuroi B14* | 2 | ✓ | ✓ | 4 | 4 |
| *Fusarium graminearum PH-1 strain PH-1* | 1 | ✓ | ✓ | 2 | 2 |
| **Fusarium oxysporum Fo5176* | 2 | ✓ | ✓ | 2 | 2 |
| *Fusarium pseudograminearum CS3096* | 1 | ✓ | ✓ | 1 | 1 |
| *Fusarium verticillioides 7600* | 2 | ✓ | ✓ | 4 | 4 |
| **Sordaria macrospora k-hell* | 1 | ✓ | ✓ | 0 | 0 |

[a] The same protein/gene was identified when *C. basilensis* HmfA-C sequences individually were used as queries

[b] Multiple hits (✓) were obtained, with the E (expect) values for the best hits ranging from $7\times10^{-17}$ to $2\times10^{-43}$

[c] In all cases, both F and G homologs are adjacent to each other when both are present

* The protein databases were used for these species, and the nucleotide databases for the remainder

in fungi (Grossetête et al. 2010), hits were found for all 21 species in the subphylum Pezizomycotina, while there were either no or few hits in other subphyla of Ascomycota. Coincidentally, XOR had been found to act on furoyl-CoA as one of its substrates (Koenig and Andreesen 1989; Koenig and Andreesen 1990). Third, as acyl-CoA synthetases and enoyl-CoA hydratases are large protein families, we were not surprised to find several *hmfD* and *hmfE* homologs, respectively, in all genomes. Finally, in a few fungal genomes (seven out of 13) where both *hmfF* and *hmfG* homologs were identified, for each *hmfF* homolog, an *hmfG* homolog is found adjacent to it, akin to the arrangement in bacteria. In two additional genomes, only one *hmfF* homolog is present without any *hmfG* homolog.

We next sought to establish correlations between growth with a given furaldehyde and the presence of specific genes, as previously shown in bacteria (Koopman et al. 2010). Some relationships were more obvious than others. For example, the *Sordaria* species could utilize furfural but not HMF. Indeed, the *Sordaria macrosopora* genome does not possess any *hmfF* or *hmfG* gene needed for the unique upstream step in HMF metabolism. The litter fungus *Fusarium* sp. 2 utilized both furfural and HMF, in agreement with the finding that all five *Fusarium* genomes we examined have the entire suite of genes (*hmfA-G*). However, not all findings were rationalizable. All three *Alternaria* isolates we studied were able to grow on HMF, but of the two *Alternaria* genomes available, we found only one *hmfF* and no *hmfG* homolog in one of them and none in the other. This finding could also indicate the inability of BLAST searches to identify homologs that have diverged evolutionarily.

## Discussion

Although furaldehydes are toxic to most fungi (Jung et al. 2007), we observed that endophytic and litter fungi belonging to Botryosphaeriales, Diaporthales, Glomerellales, Hypocreales, Pleosporales, Sordariales, and Xylariales tolerate and grow on these compounds. Since the inoculum was obtained from a 7-day-old colony growing on a carbon-deficient medium (mCDA), the growth observed on furaldehyde-containing media was not due to utilization of reserves carried over by the inoculum. However, growth on furaldehydes was sparse and devoid of the cottony aerial mycelium when compared to the robust growth obtained with sucrose as the carbon source. Fungi exhibit cryptic growth under unfavourable conditions where they grow to a limited extent by garnering carbon generated through hyphal autolysis (Trinci and Righelato 1970; Richie and Askew 2008). In contrast, the adaptive response in our isolates (Fig. 3) clearly shows that the growth on furaldehydes was not due to such cryptic growth. While the underpinnings for this adaptation is unclear, the remarkable potential of a single mycelium to host genetically different nuclei may be responsible; such a heterokaryotic arrangement is believed to underlie fungal diversification and ecological success (Roper et al. 2011). We postulate that a few nuclei in the multinucleate mycelium possess the genotype associated with the furaldehyde-utilization phenotype—upon exposure to furfural or HMF, only hyphae housing such nuclei can grow, resulting in development of sparse colonies.

Our computational efforts to determine if bacterial-like furfural/HMF catabolic pathways exist in fungi yielded interesting findings. As outlined earlier, there are three enzymes that are common to metabolism of furfural and HMF (2-furoyl-CoA synthetase, 2-furoyl-CoA dehydrogenase, and α-ketoglutaryl-CoA thioesterase), and one enzyme unique to HMF utilization (2,5-furan dicarboxylic acid decarboxylase) (Fig. 4). In many fungi, we were able to identify genes encoding homologs of these enzymes. Interestingly, 2-furoyl-CoA dehydrogenase, which in bacteria comprises three subunits (the large subunit, HmfA; the FAD-binding subunit, HmfB; the 2Fe-2S iron sulfur subunit, HmfC), is encoded by a single fungal gene. XOR is a ~1300 aa polypeptide in contrast to 1015, 271, and 192 aa for HmfA-C, respectively. Albeit speculative, these findings inform how endophytic and litter fungi might metabolize furfural and HMF.

XOR, found in all domains of life, is a molybdenum-dependent hydroxylase whose central role in purine metabolism is to catalyze the oxidation of hypoxanthine to xanthine and then to uric acid. The broad substrate specificity of XOR for purines and other heterocyclics includes 2-furoyl-CoA; in fact, HMF was recently shown to be an inhibitor of XOR (Lin et al. 2012). In *P. putida* Fu1, the inducible 2-furoyl-CoA dehydrogenase and the constitutive xanthine dehydrogenase (XDH, an XOR relative), which are structurally related as judged by shared immunological reactivity, can both act on 2-furoyl-CoA but only XDH can additionally convert xanthine to uric acid (Koenig and Andreesen 1989; Koenig and Andreesen 1990). Thus, it is possible that furfural catabolism in fungi partly entails XOR participation.

Given the Trudgill pathway (Fig. 4), it is not surprising that bacteria such as *C. basilensis* which possess both *hmfABCDE* and *hmfFG* clusters could utilize both furfural and HMF, while others like *Burkholderia xenovorans* LB400, which do not have the *hmfFG* genes, could metabolize only furfural (Koopman et al. 2010). Of the 10 Gram-negative bacteria (from seven genera) tested, no examples were found where growth was supported by HMF, but not furfural. Thus, we were intrigued to find several fungi which could utilize HMF, but not furfural; this finding might either reflect selectivity in transport of HMF over furfural or the absence of an enzyme to specifically convert furfural to furoic acid. Although we have identified some fungal enzymes that can perform various steps

of the bacterial Trudgill pathway for catabolism of these furaldehydes, an entirely different path which lends selectivity for HMF over furfural cannot be ruled out in fungi. If indeed endophytic fungi harbor such a novel furfural/HMF catabolic pathway, it might provide an alternative to the oxygen-requiring Trudgill pathway that would not work in anaerobic fermenting microorganisms (Wierckx et al. 2011).

Our results help explain a long-standing conundrum. Despite the expectation of high diversity of endophytes in the tropics mirroring that of their plant hosts (Arnold and Lutzoni 2007; Hyde and Soytong 2008; Rodriguez et al. 2009), only a few endophyte species have been found even among taxonomically-unrelated tree species in the fire-prone dry forests of the Western Ghats (Suryanarayanan et al. 2002; Pandey et al. 2003; Murali et al. 2006; Suryanarayanan et al. 2011a). The answer might in part be due to the ability of select fungi to catabolize furaldehydes generated by frequent forest fires (Pandit and Maheshwari 1996; Yokelson et al. 2007). Since the spores of several litter and endophytic fungi from such forests are heat resistant and survive exposure to more than 100 $^{o}$C (Suryanarayanan et al. 2011b), a few foliar endophytes may have evolved mechanisms for tolerating or even using furaldehydes as carbon sources. Also, with the ability to shift from an endophytic to a litter fungal lifestyle (Porras-Alfaro and Bayman 2011; Zuccaro et al. 2011) and to degrade and utilize cellulose, they would be ideally placed to outcompete other litter-degrading organisms in the forest floor. It is possible that only the fungal species with these collective traits can survive in this unique forest environment, and they evolved to become polyphagous endophytes capable of infecting tree species of unrelated taxa, thus accounting for the low endophyte diversity.

Although the concentrations of the furaldehyde inhibitors we used were not as high as those of a few earlier studies (Heer and Sauer 2008; Rumbold et al. 2009), our results should motivate a wider screening of endophytic and litter fungi, especially from litter accumulating and fire-prone locales, for their capability to utilize furaldehydes. If indeed the presence of XOR is indicative of such ability, it might be profitable to focus on members of the subphylum Pezizomycotina, which have already drawn attention for their capacity to degrade biomass to sugars for utilization as an energy source (Arvas et al. 2007). Enrichment-based selections could then be used to identify fungi capable of growth in even higher concentrations of furfural/HMF (Heer and Sauer 2008; Lopez et al. 2004; Wierckx et al. 2010). Moreover, if these newly identified fungi are to be used for selective removal of furaldehydes from lignocellulosic hydrolysates, design of sugar transport mutants will be critical to ensure that feedstock sugars for microbial fermentation are not depleted.

**Acknowledgments** We are grateful to Dr. Sreenivasulu Guntha [AAP Pharma Technologies India (P) Ltd., Hyderabad, India] for the kind gift of furfural and 5-hydroxymethylfurfural, and Dr. T. Ezeji (OSU) for comments on the manuscript. TSS thanks the Department of Biotechnology, Government of India for funding the Indo-German Research Project BT/IN/FRG/09/TSS/2007 on endophyte enzymes, and the United States-India Educational Foundation (USIEF), New Delhi and the Fulbright Scholar Program (USA) for the award of a Fulbright-Nehru Senior Researcher grant to characterize fungal endophyte enzymes in VG's laboratory at OSU. VG acknowledges funding support from the Ohio Plant Biotechnology Consortium (awarded to T. Ezeji and VG).

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