Community Sequencing Program: Project Proposal

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Project Title: Community proposal to sequence a diverse assemblage of saprotrophic Basidiomycota (Agaricomycotina)

Proposal ID:

A) Brief description:

Abstract:

We propose a community-based sequencing project for whole-genome sequencing of a suite of saprotrophic (decayer) Fungi in the Basidiomycota, subphylum Agaricomycotina. The proposed organisms are of central relevance to the DOE mission with regard to lignocellulose bioconversion, biofuel production, feedstock improvement, and carbon cycle functioning. This proposal is derived from the basidiomycete focus group of the JGI Fungal Genomics Program, which commenced in October 2009. The organisms targeted in this proposal are particularly relevant to the "Biorefinery" section of the Genomic Encyclopedia of Fungi that the FGP seeks to create.

Scope of Work:

We propose a suite of 30 species for genome sequencing, divided into two Tiers of 13 and 17 species each. This set of taxa includes all of the 24 saprotrophic species identified by the Basidiomycota community in response to the 2009 FGP. The Tier 1 taxa are already being processed as a "pilot project" of the FGP. We therefore propose the remaining seventeen species in Tier 2 for sequencing as a follow-up to the current pilot project. The taxa have been selected on the basis of five major criteria: 1) phylogenetic diversity, 2) functional diversity and ecological importance, 3) utility as experimental systems, and community interest, 4) complementarity and relevance to other CSP proposals, and 5) availability of starting materials (monokaryons) and experimental tractability.

The chosen taxa represent fourteen of the approximately eighteen major clades (orders and subclasses) of Agaricomycotina, including five independent brown rot lineages. This selection has been developed in coordination with a parallel proposal by Francis Martin and colleagues to sequence the genomes of twenty-three ectomycorrhizal (ECM) taxa, including 18 Agaricomycotina and 5 Ascomycota. Together, the genomes of the saprotrophic taxa proposed here and the ECM taxa being proposed by Martin et al. will provide insight into the functional diversity of Agaricomycotina, including the genetic bases of transitions between saprotrophic and ECM lifestyles, as well as between white rot and brown rot. A third parallel proposal by Joseph Spatafora and colleagues proposes to sequence an assemblage of diverse Fungi representing undersampled branches of the fungal Tree of Life, with an emphasis on basal fungal lineages (the paraphyletic "chytrids" and "zygomycetes"). Several Basidiomycota are targeted in the Tree of Life proposal, but none are in the Agaricomycotina.

The primary goals of the proposed genome sequencing effort are to describe gene content and facilitate functional (expression) and evolutionary analyses. It is understood that the sequencing technology is rapidly evolving. We therefore defer decisions regarding the optimal approach to WGS of these haploid monokaryons to the JGI staff. Illumina-based transcriptome analysis, specifically focused on colonized wood, is also being requested in this proposal. Augmenting the genome and transcriptome data, the PIs and collaborators will perform mass spectroscopy-based secretome analysis, wood compositional analysis and microscopy, all under standardized conditions. Finally, we will perform comparative phylogenetic analyses to assess the evolution of decay mechanisms in Agaricomycotina.

B) Background information (Limit 3 pages)

Technical Information:

Direct information about genome size, G+C content, polymorphism level, and repeat structure is not available for the target taxa. Genomes for Agaricomycotina range from 19 Mbp in *Cryptococcus neoformans* (a pathogenic yeast in the Tremellomycetes) to 65 Mbp in *Laccaria bicolor* (an ectomycorrhizal mushroom in the Agaricales). The genome sizes for wood decay fungi have ranged from 35 Mbp (*Phanerochaete chrysosporium*, Polyporales) to 47 Mbp (*Serpula lacrymans*, Boletales). Based on comparisons with these fungi, the species proposed here are unlikely to have a problematic G+C content and the haploid genome sizes probably range from 30 to 50 Mb. Similarly, substantial amounts of repetitive DNA in the form of class I and II transposons and their remnants are expected. Up to 10% of the genome might be repetitive, although this is no longer particularly daunting for the JGI assembly team. (Another basidiomycete successfully sequenced by the JGI, *Laccaria bicolor* (Martin et al. 2008), was found to contain over 20% repeats and further complicated by a substantial amount of endobacterial DNA 'contamination'.) Polymorphisms related to ploidy, which were problematic in the JGI *Postia placenta* genome project (Martinez et al. 2009), will be circumvented by sequencing monokaryons exclusively.

Available Resources:

Most of the taxa targeted here have not been used as model experimental systems, so there is little preliminary information about genome structure (e.g., physical maps, genetic maps, fingerprinted BAC libraries, etc). However, seven species (*Bjerkandera adusta, Dichomitus squalens, Coniophora puteana, Fomitiporia mediterranea, Gloeophyllum trabeum, Phlebia brevispora, Trametes versicolor*) have been used in various studies of decay chemistry, and there is published information on decay capabilities (e.g., ability to degrade crystalline cellulose by *Gloeophyllum* and *Coniophora*) and selected enzyme families (e.g., class II peroxidases and laccases in *Bjerkandera adusta, Fomitiporia mediterranea, Phlebia brevispora,* and *Trametes versicolor*) (Baldrian and Valaskova 2008, Lundell et al. 2010, Morgenstern et al. 2008). Genetic transformations systems are available for *T. versicolor*. Additionally, two species proposed here, *Phanerochaete velutina* and *Phanerochaete flavido-alba*, are closely related to the model white rot species *Phanerochate chrysosporium*, for which a complete genome sequence is available (Martinez et al. 2004).

Technical Challenges:

One of the Tier 2 target species, *Hygrocybe sp*., has yet to be successfully cultured on standard mycological media (e.g., malt-extract agar, potato-dextrose agar). We will attempt to obtain this organism as a monokaryotic culture using various enhanced media, such as Melins-Norkrans medium, vitamin-enhanced media, etc. Another Tier 2 target species, *Sphaerobolus stellatus*, is culturable, but attempts to obtain monokaryons have failed (it is possible that this species produces dikaryotic spores). We have identified several alternative taxa that are known to be culturable; *Hydnomerulius pinastri* or *Marasmius* spp. are alternatives for *Hygrocybe* sp., and *Lentaria michneri* is an alternative for *Sphaerobolus stellatus*. We will pursue monokaryotic

cultures of the alternative taxa at the same time that we attempt to obtain monokaryotic cultures of *Hygrocybe sp*. and *Sphaerobolus stellatus*.

Starting Materials:

Saprotrophic Agaricomycotina are relatively easy to culture in both dikaryotic and monokaryotic forms and excellent culture collections exist in the United States and abroad. As noted, nucleic acids of thirteen monokaryotic strains have already been submitted and are in various stages of genome sequencing as a pilot project. Monokaryotic cultures for eight of the remaining 17 species have been located in the culture collections of the USDA Forest Products Laboratory (FPL), the University of Tennessee (TENN; laboratory of Ronald H. Petersen), and the Belgian Coordinated Collections of Microorganisms/Mycothèque de l'Universite catholique de Louvain (BCCM/MUCL; one species). Monokaryons of *Phanerochaete velutina* and *Phanerochaete flavido-alba* have not been located, but monokaryons of other closely related *Phanerochaete* species are present in FPL and could be substituted if necessary. Monokaryons of seven species will need to be located elsewhere or obtained from fruiting bodies collected from nature. Isolates in culture collections are occasionally misidentified. We will confirm identities of all cultures using sequences of the internal transcribed spacers (ITS1-2) of nuclear ribosomal genes.

The PIs of the present proposal will obtain monokaryotic cultures of all of the target taxa from established culture collections and from new collections in nature, beginning in the summer of 2010. We anticipate that new DNA and RNA preparations will begin to be available for submission to JGI by the end of 2010.

To aid annotation, we will continue to provide total RNA from various complex and defined media. For quantitative transcriptome analysis, total RNA and mRNA is isolated from wood using a system devised in Bob Blanchette's laboratory (letter attached) (Vanden Wymelenberg et al. 2006). This method substantially reduces sample variation and involves thin wood wafers placed directly on actively growing mycelia. Wood wafers (1 cm X 1 cm X 2 mm) are cut from freshly harvested sapwood, sterilized and inoculated by contact with mycelium growing on malt extract agar (1.5% Difco malt extract and 1.5% agar liter^{-1}) in Petri plates. Up to 100 ug high quality total RNA can be purified by simple modifications of the Qiagen RNEasy system. If Illumina transcriptome analysis is approved, mRNA may be preferred. If so, we can easily and efficiently purify mRNA from the colonized wafers by magnetic capture techniques. The wood-derived mRNA is an excellent template for cDNA synthesis. Ultimately, colonized wafers permit an integrated view of transcript patterns (Vanden Wymelenberg et al. 2006), microscopic assessments of decay, and chemical composition over time. Secretome characterization is performed on an Orbitrap instrument as recently described (Vanden Wymelenberg et al. 2010).

Project Description:

Lignocellulosic plant materials comprise the most abundant biopolymers in terrestrial ecosystems. Fungal-mediated degradation of lignocellulose is thus a critical link in the carbon cycle, and is of great interest for its potential applications in the production of biomaterials, including biofuels, enzymes, and other commodities (e.g., chitin). Saprotrophic Agaricomycotina are of particular interest as they are active and abundant degraders of all classes of plant tissues. Two principal modes of decay occur in the Agaricomycotina, termed white rot and brown rot. White rot fungi are capable of efficiently degrading all components of plant cell walls, including the highly recalcitrant lignin fraction. Brown rot fungi modify but do not appreciably remove the lignin, which remains as a polymeric residue following removal of cellulose and hemicellulose. Brown rot residues are highly resistant to further decay and contribute to the fixed carbon pool in humic soils, particularly in cool-temperate and boreal, conifer-dominated ecosystems. Brown rot fungi thus play a significant role in terrestrial carbon sequestration.

White rot fungi are distributed across most major clades of Agaricomycotina, and may represent the primitive nutritional mode for the Agaricomycotina as a whole (Hibbett and Donoghue 2001). The Agaricomycotina is a group of great antiquity, estimated to be roughly 400 million years old (Taylor and Berbee 2006), and it is likely that the ancestral white rot decay apparatus has been extensively modified through evolution in the diverse clades of Agaricomycotina. Nested within the white rot groups are multiple clades of brown rot fungi, which are thought to have convergently evolved mechanisms for circumventing lignin while degrading cellulose and hemicellulose. Ectomycorrhizal fungi, which play important ecological roles as tree symbionts, are also polyphyletically derived within the Agaricomycotina (Hibbett et al. 2000). We propose to sequence the genomes of a diverse suite of saprotrophic Agaricomycotina, including representatives of several independent origins of brown rot.

Lessons from *Phanerochaete* and *Postia*. Genomes of two wood-decaying Agaricomycotina, *Phanerochaete* and *Postia* (Polyporales) have been published (Martinez et al. 2004, 2009). *Postia* is a brown rot fungus whereas *Phanerochaete* produces a white rot. Even though they are members of the same order, *Phanerochaete* and *Postia* were found to have radically different decay chemistries. For example, *Phanerochaete* has fifteen class II fungal peroxidase, which function in lignin degradation, whereas *Postia* has only one low redox potential peroxidase, of unknown function. In addition, *Postia* lacks exocellobiohydrolases and cellulose binding modules, which are previously known from all cellulolytic fungi. These findings in two closely related taxa suggest that there has been considerable diversification in the mechanisms of lignocellulose deconstruciton in Agaricomycotina, warranting a broad sampling of this clade.

Criteria for selection. The five major criteria for target selection include:

1. Phylogenetic diversity. By sampling a phylogenetically broad set of taxa, we will maximize the diversity of decay mechanisms (and other biological attributes) that we will discover. The proposed taxa include representatives of fourteen out of sixteen of the major clades (orders or subclasses) of Agaricomycotina (Binder et al. 2010, Hibbett 2006, Hibbett et al. 2007, James et al. 2006) that contain saprotrophic taxa (**Fig. 1**). Eight species represent major clades for which no genome sequences are currently available. An added benefit of this strategy is that the resulting genome sequences will improve resolution of the backbone of the Agaricomycotina phylogeny, including the backbone of the Polyporales, which remains problematical in some regards (Matheny et al. 2007).

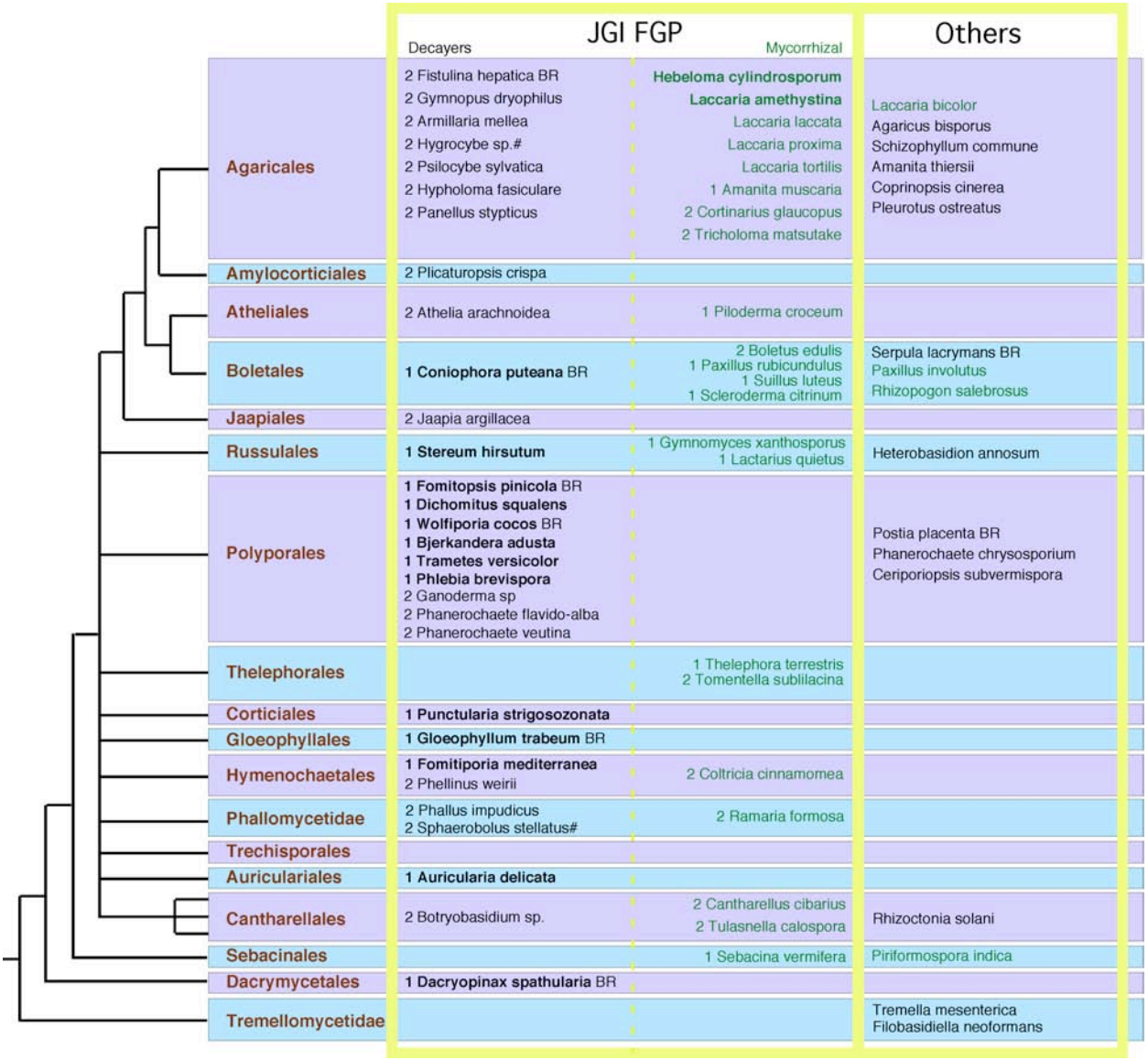

Fig. 1. Phylogenetic distribution of proposed targets. Black font indicates saprotrophs. Green font indicates ectomycorrhiza-formers. The "JGI FGP" box contains the taxa proposed for CSP. Saprotrophic taxa proposed here are left of the dashed line; ectomycorrhizal taxa proposed separately by F. Martin and colleagues are right of the dashed line. Taxa in bold font: Tier 1 taxa with DNA/RNA already submitted and being processed as a "pilot project". Numbers before names indicate proposed Tier. # indicates problematical taxa that may need to be replaced. BR indicates brown rot species. See **Table 1** and notes below for additional details. The "Others" box contains other genome projects completed or in progress.

2. Functional diversity and ecological importance. The target taxa include both white-rot and brown-rot wood decomposers, as well as members of the litter decay guild. Thus, they embrace diverse functional classes of decomposers. The target organisms also include representatives of many of the most commonly encountered lineages that are responsible for a large fraction of fungal decomposition of lignocellulose in nature.

3. Experimental systems and community interest. The target taxa include organisms that have long been the focus of mechanistic studies of lignocellulose decomposition (e.g., *Gloeophyllum trabeum, Coniophora puteana, Trametes versicolor, Bjerkandera adusta*). Most research on these fungi has related to the biochemistry of decay processes although, at least in the case of *T. versicolor*, basic tools for genetic analyses, e.g. transformation, are available (Yeo et al. 2007). The existence of active research groups presently working with these organisms insures that there will be broad community support for the project, including annotation and post-genome experiments.

4. Coordination with other current CSP endeavors and complementarity to other genome projects. The taxa nominated for sequencing here complement those selected by the mycorrhizal community (see the separate proposal by F. Martin and colleagues), and include many saprotrophic species that are related to ectomycorrhizal species. The availability of both saprotrophic and ectomycorrhizal genomes will enable studies into the mechanistic bases of transitions between saprotrophy and ectomycorrhizal symbiosis. In addition, the focus on Agaricomycotina will complement the sampling of the other subphyla of Basidiomycota (Pucciniomycotina, Ustilaginomycotina, and the unplaced *Wallemia*) that is being proposed by the Fungal Tree of Life community (proposal by J. Spatafora and colleagues). This proposal also complements the CSP application entitled 'Comparative transcriptomics pipeline for saprophytic Basidiomycota' submitted by co-PI Antonio Pisabarro. That project seeks to systematically examine the transcript profiles of these fungi when grown under a range of carbon sources and culture conditions. Although distinct from the wood wafers proposed here, the two CSPs would provide a comprehensive view of transcriptomes across a diverse set of defined and complex.

5. Availability of monokaryotic cultures and experimental tractability. Saprotrophic Agaricomycotina are generally amenable to culturing under laboratory conditions. Several culture collections contain large numbers of monokaryotic (haploid) isolated, principally derived from prior studies on genetics and species limits. We have benefitted from close working relationships with the managers of the culture collections of the USDA Forest Products Laboratory and the University of Tennessee (laboratory of Dr. Ronald H. Petersen). Most of the target taxa (and all those in Tier 1 proposed for sequencing in FY2010) are available as monokaryotic isolates at FPL or TENN. Many others are common species from which new monokaryons should be relatively simple to obtain.

Number, identity, and status of target organisms. We propose a suite of 30 species for genome sequencing, divided into two Tiers of 13 and 17 species (**Fig. 1, Table 1**). This set includes all of the 24 saprotrophic species identified by the Basidiomycota community in response to the 2009 FGP. All of the 13 Tier 1 taxa are already being processed as a pilot project of the FGP. We therefore propose the remaining seventeen taxa in Tier 2 for sequencing as a follow-up to the current pilot project. Two taxa identified by the Basidiomycota group in 2009 have proven to be challenging. These taxa have been moved to Tier 2 and alternate taxa have been identified in the event that these species prove to be impossible to obtain. Specific information on the status of each target taxon is presented in **Table 1**. Summaries by Tier follow, along with notes on the rationale for sampling the seventeen Tier 2 species that have not yet been submitted as part of the pilot project:

Tier 1: Five white rot and eight brown rot species, representing eight major clades of Agaricomycotina. Material for all of these species was obtained in response to the 2009 JGI FGP

and has been submitted to JGI. As of this writing, two species, *Gloeophyllum trabeum* and *Fomitiporia mediterranea*, are "in production" and the rest are in "library construction".

Tier 2: Sixteen white rot and one brown rot species, representing seven major clades of Agaricomycotina (**Figs. 2-3**). Monokaryotic cultures of two species, *Ganoderma* sp., and *Botryobasidium* sp., are in the laboratory of DSH and are being prepared for DNA/RNA isolation. Monokaryons for eight other species have been located in the FPL, TENN, or MUCL (Belgium) culture collections. In several cases, the exact species identified as a target is not known to be available, but a closely related congener is available. The remaining seven species will need to be isolated from nature, or located in other culture collections. Two species, *Sphaerobolus stellatus* and *Hygrocybe* spp., may not be tractable (attempts to obtain monokaryons by fruiting *Sphaerobolus* dikaryons in culture have failed, and *Hygrocybe* is not known to be culturable) and may need to be replaced. Candidates for replacement have been identified (**Table 1**). Notes on individual species follow.

Fig. 2. Selected Tier 2 species (and close relatives). A: *Ganoderma oregonense*. B: *Phallus hadriani*. C: *Fistulina hepatica*, D; *Gymnopus dryophilus*. From www.mykoweb.com A, B, D by Mike Wood. C by Harry Stevens.

Notes on Tier 2 species:

- *Ganoderma sp.* This is a large, cosmopolitan genus of white rot polypores in the "core polyporoid clade" (Binder et al. 2005), which includes decayers of conifers and hardwoods. *Ganoderma* will provide a useful comparison to *Trametes versicolor*, which is also in the core polyporoid clade.
- *Botryobasidium sp.* This is the only representative of the Cantharellales in the target list. The Cantharellales is an early-diverging clade of Agaricomycotina that is dominated by

mycorrhizal taxa. The companion proposal on ECM by Martin and colleagues includes two Cantharellales, *Cantharellus cibarius* and *Tulasnella calospora*; comparison with *Botryobasidium* will elucidate transitions between decayer and mycorrhizal lifestyles.

- *Athelia arachnoidea*. The Atheliales is phylogenetically significant as the sister group of the Boletales (which includes brown rot species and mycorrhizal species) (Binder et al. 2010). This is the only member of the Atheliales in the target list.
- *Phallus impudicus*. This is the only representative of the Phallales on the target list. The Phallales is in the Phallomycetidae, which is an early-diverging lineage of Agaricomycotina (Hosaka et al. 2006). At present, there are no genome sequences of any Phallomycetidae. *Sphaerobolus stellatus* is also in the Phallomycetidae (Geastrales), but obtaining monokaryons has proven problematical. A mycorrhizal member of the Phallomycetidae, *Ramaria formosa*, is being proposed by Martin and colleagues.
- *Fistulina hepatica*. This unusual species in the Agaricales produces a brown rot. It closely related to the white rot genome target *Schizophyllum commune* (not part of this proposal) (Matheny et al. 2006). Among white rot basidiomycetes, *Schizophyllum* is unusual in that it does not appear to produce class II peroxidases. The combination of *Fistulina* and *Schizophyllum* provides an opportunity to study what appears to be a novel and variable mode of decay.
- *Gymnopus dryophilus*. This member of the Agaricales represents the ecologically important guild of leaf litter decayers. (*Collybia* is a synonym for *Gymnopus*.)
- *Plicaturopsis crispa*. This is a representative of the Amylocorticiales, which is the sister group of the Agaricales. No other genome species for this order exist.
- *Jaapia argillacea*. This species is one of two species in the newly recognized order Jaapiales (Binder et al. 2010). The Jaapiales is the sister group to the Agaricomycetidae, including Agaricales, Boletales, Atheliales, and Amylocorticiales. Thus, this species represents a very ancient lineage that could provide clues to the diversity and evolution of decay mechanisms in the largest clade of Agaricomycotina.
- *Armillaria mellea*. This is the famous "humongous fungus", which is able to colonize vast areas of forest via rhizomorphs. Presumably, it has extremely efficient mechanisms for hyphal transport. *Armillaria* is in the Physalacariaceae, which appears to be the sister group of the Schizophyllaceae, containing *Schizophyllum* and *Fistulina* (Matheny et al. 2006b).
- *Hypholoma fasciculare*. This is a white rot member of the Agaricales that has been used in experimental studies on co-occurrence and competition of fungi and prokaryotes in wood (Folman et al. 2007).
- *Phanerochaete flavido-alba* and *Phanerochaete velutina*. These are close relatives of the well-characterized *Phanerochaete chrysosporium*. These species, or alternatives, will provide opportunities to study functional diversity of wood decay systems over relatively short evolutionary timescales.
- *Phellinus weirii.* Hymenochaetales includes vigorous. widespread white rot fungi. This would be only the second genome sequence from the Hymenochaetales, after *Fomitiporia mediterranea*. *Phellinus weirii* is a timber pathogen that causes root rot in Douglas fir.
- *Psilocybe silvatica*. This is a member of the Hymenogastraceae (Agaricales), which also includes mycorrhizal taxa, such as *Hebeloma cylindrosporum*, which is being sequenced as part of the ECM project led by F. Martin. *Psilocybe* will provide another opportunity to study the transition between decayer and mycorrhizal lifestyles.

- *Panellus stypticus*. This is a very widespread white rot member of the Agaricales. It is a member of the Mycenaceae, which includes many litter decayers. (It is also of interest because it is bioluminescent.)

Fig. 3. Selected Tier 2 species (and close relatives), continued. A: *Armillaria mellea*. B: *Hypholoma fasiculare*. C: *Phellinus igniarius*, D: *Phanerochaete flava*. E: *Sphaerobolus stellatus.* F: *Hygrocybe coccinea*. A-C, D, E From www.mykoweb.com A, B, E by Fred Stevens, C by Darvin DeShazer, F by Mike Wood. D by David Hibbett.

Notes on Tier 2 species, continued:

- *Sphaerobolus stellatus*. See comments above regarding *Phallus impudicus* (Tier 2). *Lentaria michneri* is another saprotrophic member of the Phallomycetidae that would be an appropriate alternative for this taxon.
- *Hygrocybe sp.* The nutritional model of this enigmatic member of the Agaricales is uncertain. Prior attempts to culture the fungus have been unsuccessful, but we will make additional attempts this summer. Alternatives for which monokaryons are available include *Hydnomerulius pinastri* (a saprotrophic member of Boletales; Binder and Hibbett 2006) or *Marasmius* sp. (litter-decaying Agaricales).

Table 1. Target taxa and status. Species 1-13 (Tier 1) are already being processed as a pilot project. Species 14-30 (Tier 2) are proposed as new targets.

Species	Tier	Clade	Ecology	Status as of 5/18/2010*
1. *Fomitiporia mediterranea*	**1**	Hymenochaetales	white rot wood decay	**Production**
2. *Gloeophyllum trabeum*	**1**	Gloeophyllales	brown rot wood decay	**Production**
3. *Auricularia auricula-judae*	**1**	Auriculariales	white rot wood decay	**Library construction**
4. *Dacryopinax spathularia*	**1**	Dacrymycetes	brown rot wood decay	**Library construction**
5. *Stereum hirsutum*	**1**	Russulales	white rot wood decay	**Library construction**
6. *Coniophora puteana*	**1**	Boletales	brown rot wood decay	**Library construction**
7. *Punctularia strigoso-zonata*	**1**	Corticiales	white rot wood decay	**Library construction**
8. *Dichomitus squalens*	**1**	Polyporales	white rot wood decay	**Library construction**
9. *Fomitopsis pinicola*	**1**	Polyporales	brown rot wood decay	**Library construction**
10. *Wolfiporia cocos*	**1**	Polyporales	brown rot wood decay	**Library construction**
11. *Trametes versicolor*	**1**	Polyporales	white rot wood decay	**Library construction**
12. *Bjerkandera adusta*	**1**	Polyporales	white rot wood decay	**Library construction**
13. *Phlebia brevispora*	**1**	Polyporales	white rot wood decay	**Library construction**
14. *Ganoderma spp*	**2**	Polyporales	white rot wood decay	**DNA/RNA in preparation**
15. *Botryobasidium sp*	**2**	Cantharellales	white rot? wood decay?	**DNA/RNA in preparation**
16. *Athelia arachnoidea*	**2**	Atheliales	white rot? pathogen	**monokaryon needed**
17. *Phallus impudicus*	**2**	Phallales	white rot soil/litter decay	**monokaryon needed**
18. *Fistulina hepatica*	**2**	Polyporales	brown rot wood decay	**monokaryon needed**
19. *Gymnopus dryophilus*	**2**	Agaricales	white rot litter decayer	***Collybia* sp.: monokaryon available at FPL, TENN** ***Collybia earleae*: monokaryon available at TENN**
20. *Plicaturopsis crispa*	**2**	Amylocorticiales	white rot wood decay	**monokaryon needed**
21. *Jaapia argillacea*	**2**	Jaapiales	white rot wood decay?	**monokaryon available at BCCM/MUCL (Belgium)**
22. *Armillaria mellea*	**2**	Agaricales	white rot timber pathogen	**FPL, TNN**
23. *Hypholoma fasciculare*	**2**	Agaricales	white rot wood decay	***H. subviride*: monokaryon available at FPL, TENN**
24. *Phanerochaete flavido-alba*	**2**	Polyporales	white rot wood decay	**monokaryon needed** **other *Phanerochaete* spp: monokaryon available at FPL**

25. *Phanerochaete velutina*	**2**	Polyporales	white rot wood decay	**monokaryon needed** **other *Phanerochaete* spp: monokaryon available at FPL**
26. *Phellinus weirii*	**2**	Hymenochaetales	white rot timber pathogen	**monokaryon available at FPL, TENN**
27. *Psilocybe silvatica*	**2**	Agaricales	white rot litter decay	**monokaryon needed**
28. *Panellus stypticus*	**2**	Agaricales	white rot wood decay	***P. stypticus*: monokaryon available at FPL** ***P. serotinus*: monokaryon available at TENN**
29. *Sphaerobolus stellatus*	**2**	Phallomycetidae	white rot litter decay	**monokaryon is difficult** **Alternative: *Lentaria michneri***
30. *Hygrocybe spp*	**2**	Agaricales	uncertain	**unculturable?** **Alternatives: *Hydnomerulius pinastri* or *Marasmius* spp.**

*FPL = USDA Forest Products Laboratory, Madison; TENN = University of Tennessee, Knoxville; BCCM/MUCL = Belgian Coordinated Collections of Microorganisms/Mycothèque de l'Universite catholique de Louvain.

Technical description of the sequencing project.

With the exception of a few genomes already within the pipeline, no Sanger sequencing is expected. We defer to the JGI staff for the appropriate balance of Illumina and 454 Titanium runs. Similarly, the need for ESTs and the appropriate platforms are best judged by the JGI, and in any case we will continue to provide RNA as needed. For Illumina transcriptome analyses, we presume that 36 cycle paired runs would be more than adequate for the genomes under consideration. The depth of coverage should, at least initially, be high enough to identify rare transcripts assuming 4-5 orders of magnitude difference.

Genome assemblies and gene predictions will be carried out by JGI using training parameters previously used for fungal genomes. Approaches for analyzing RNA-seq data are rapidly evolving (Haas and Zody 2010). The PIs have full access to DNASTAR QSeq and Partek's Genome Suite, although we would expect to work closely with the JGI as the project moves forward. Of some relevance, an ongoing research project at FPL and the UW Biotech Center is comparing Roche NimbleGen expression microarrays with Illumina RNA-seq for *Ceriporiopsis subvermispora* (2008 JGI CSP). Ideally, ESTs and perhaps cDNA tags would be mapped to their respective browsers.

Utilization, timetable, and deliverables

Tier 1 pilot project species 1-13

Assuming that library construction is successful, sequencing and annotation of the thirteen Tier 1 species currently being processed as a pilot project may commence at JGI's discretion. We are prepared to undertake manual annotation and comparative analyses of these species beginning in late summer-fall 2010. During this time, mass spectroscopy analysis of the secretomes will be conducted at the University of Wisconsin Biotechnology Center as described (Vanden Wymelenberg et al. 2010). Growth characteristics and decay patterns of the sequenced strains will be determined in Bob Blanchette's lab, and a subset of representative samples will be

subjected to compositional analysis (e.g. lignin) and microscopy. (Hardwood and softwood substrates would be examined.) If the Illumina-based RNA-seq aspects of this proposal are accepted, this same colonized material will serve as our source of mRNA. Some members of our group are particularly interested in analyses based on gene content information, which may begin prior to completion of finished genome sequences, while others are interested in analyses of transposon distribution and other issues that require completed genomes; the extent of genome polishing is likely to be minimal but determined in consultation with JGI and other project partners. Some RT-PCR amplification and cDNA sequencing is expected to confirm or correct particularly important models. We envision the preparation of a manuscript early in 2011.

Tier 2 new target species 14-30

Cultures of the remaining 17 Tier 2 taxa will be obtained in summer 2010 from culture collections (Table 1) or from nature. Identities of all cultures will be confirmed by sequencing the internal transcribed spacers of nuclear ribosomal RNA genes. DNA and RNA should be available to submit to JGI beginning in January 2011. Depending on the rate of progress in sequencing and automated annotation, manual annotation and functional and comparative analyses of Tier 2 species could begin by summer-fall 2011. Similar wood colonization analysis would be conducted on the Tier 2 taxa.

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