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## Sequencing the Fungal Tree of Life

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### **Sequencing the Fungal Tree of Life**

Terrestrial ecosystems host a complex array of interacting communities, with thousands of species of animals, plants, fungi and bacteria. In soils, this complex web of life is responsible for the cycling of carbon, water and nutrients, soil quality, and plant nutrition and health. To predict future changes of these threatened ecosystems and to fully grasp the biological and chemical workings of these complex interactions, one must not only regard organisms as individuals, but as members of a larger community, considering the interplay and communication between individuals within these entangled populations, i.e. their extended phenotype (Whitham *et al.*, 2010). One emerging model for such studies is the interaction between soil-borne fungi and plant communities.

Fungi are one of the largest and most diverse kingdoms of eukaryotes and function as important biological components of all terrestrial ecosystems. They are central to the global carbon cycle, constitute the major group of plant pathogens in managed and natural ecosystems, serve as symbionts with heterotrophic and autotrophic organisms alike, and play an integral and growing role in the development and production of renewable bio-based fuels and chemicals. The success and importance of Fungi to life on Earth are directly attributable to the remarkable diversity of enzymes and metabolites that they produce which afford them a broad range of nutritional modes and grant them access to an amazing breadth of carbon sources and ecological niches.

Understanding how saprotrophic, symbiotic and pathogenic fungi achieve their lifestyle is crucial to understand their ecological functions, and their subsequent impact on the fate of plant communities. The inconspicuous nature of soil fungi, the inaccessibility of their habitats and our inability to culture many of them have made them difficult to study. Advances in large scale DNA barcoding surveys have circumvented some of these limitations and allowed us to determine the composition and dynamics of several fungal soil communities, including mycorrhizal fungi (Buée *et al.*, 2009; Opik *et al.*, 2009; Jumpponen *et al.*, 2010). Several hundred species are active in soils and on-going metagenomics and metatranscriptomics studies will uncover the functions encoded in their genomes as well as their expressed transcripts (Martin & Martin, 2010). Many of the fungi whose genomes have been sequenced are residents of soil and plants (Martinez *et al.*, 2004, 2009; Martin *et al.*, 2008, 2010; Ohm *et al.*, 2010; Spanu *et al.*, 2010; Stajich *et al.*, 2010) and these sequences will provide baseline genomic information that enables scientists to explore the genomes and functions of thousands of soil fungal species which cannot be cultured and sequenced directly.

Unfortunately, reference fungal genomes sequenced to date and those in progress show significant bias towards fungi of medical importance (Cuomo & Birren, 2010). The availability of genome sequences from ecologically and taxonomically diverse fungi not only would allow ongoing research on those species, but enhances the value of other sequences through comparative studies of gene evolution, genome structure, metabolic and regulatory pathways, and saprotrophism/symbiosis/pathogenesis lifestyles. Recently, the US Department of Energy Joint Genome Institute (JGI) launched the Fungal Genomics Program (FGP) aimed at exploration of fungal diversity for energy and environmental sciences and applications through scale up of sequencing and analysis (Grigoriev *et al.*, 2011).

### **Fungal Genomics for Energy and Environment**

The JGI FGP's Genomic Encyclopedia of Fungi ([www.jgi.doe.gov/fungi](http://www.jgi.doe.gov/fungi)) targets fungal genomes in three areas: plant health, biorefinery and fungal diversity. Plant health depends on plant interactions with both fungal mutualists and parasites. While on a surface, effects of both on a host are orthogonal, they may share common characteristics to escape plant defense system and acquire host nutrients (e.g., the role of effector-like secreted proteins in *Laccaria bicolor*, *Melampsora larici-populina*), live within the host (e.g., the semibiotroph *Mycosphaerella graminicola*), and balance between saprobic and parasitic (or mutualistic) lifestyles (e.g., *Heterobasidion annosum*).

Comparative genomics should lead us to understanding these mechanisms critical for sustainable growth of forest trees and bioenergy feedstocks like poplars, pines and eucalypts. *Biorefinery*, includes industrial hosts like *Trichoderma reesei* and *Aspergillus niger*. Novel “modules” or “parts” – metabolic processes, enzymes, and regulatory elements with desired properties from other fungi – will be plugged into these established hosts to accelerate the development of new bioprocesses that efficiently convert biomass into biofuels and renewable chemicals. Finally, *Exploration of fungal ecological and phylogenetic diversity* – should bring us more useful “modules” or “parts” such as thermostable enzymes or thermotolerant hosts from extreme environments as well as give clues to fungal evolution. Dramatic improvements in genome sequencing technologies enable us to go beyond sequencing a single reference genome allowing “resequencing” of several accessions (e.g., resequencing of a dozen of strains of the ectomycorrhizal model species *L. bicolor*). Through comparative analysis of gene expression, genomic variation across the diversity of Fungi and metagenomics of complex systems like soil we will reach a higher level of understanding of fungal lifestyles, their interactions with plants, and their evolution, laying a strong foundation upon which to build practical applications for energy and environment.

### Sequencing Saprotrophic Agaricomycotina

Diverse Fungi obtain nutrition from primary plant tissues, exudates, or phloem sap, but a relatively small number are able to efficiently degrade wood. The majority of the wood decaying Fungi are in the Agaricomycotina (Basidiomycota), which also includes mycorrhizal and pathogenic forms.

Within the Agaricomycotina, a polyphyletic assemblage known as white-rot fungi have the unique ability to depolymerize and mineralize the recalcitrant lignin in order to gain access to cell wall carbohydrates as carbon and energy sources. Another assemblage, the so-called brown-rot fungi, rapidly depolymerize cellulose but do not extensively degrade the lignin, which remains in situ as a modified polymeric residue that is highly resistant to further microbial decay. Both types of wood decay fungi are common inhabitants of forest ecosystems and play an important, if not pivotal, role in the carbon cycle.

Genomes of three wood-decaying Agaricomycotina, *Phanerochaete chrysosporium*, *Postia placenta*, and *Schizophyllum commune*, have been published (Martinez *et al.* 2004, 2009; Ohm *et al.*, 2010). *Phanerochaete chrysosporium* produces a white rot whereas *Postia placenta* is a brown rot species. They are both members of the Polyporales, but they were found to have radically different decay chemistries. For example, *Phanerochaete* has fifteen genes encoding class II fungal peroxidases, 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. In contrast, *S. commune* has one of the largest set of cellulolytic enzymes. These findings from three closely related taxa suggest that there has been considerable diversification in decay mechanisms in Agaricomycotina, warranting a broad sampling of this clade.

The JGI initiative has targeted thirty wood decay fungi representing twelve orders. Fourteen first-tier species, nine white rot and five brown rot, are designated for sequencing within the first year. Five of the first-tier species represent major clades of Agaricomycotina that include widespread wood decay species for which there are not yet any published genomes or (to our knowledge) active genome projects (i.e, Auriculariales, Dacrymycetales, Hymenochaetales, Corticiales). The second tier species further broaden the phylogenetic diversity of wood decay genomes (including the as-yet unsampled Atheliales, Amylocorticiales, Jaapiales, and Phallomycetidae). Comparative analyses of these annotated genomes will also integrate gene expression profiles determined by high-throughput Illumina RNA-Seq and by protein mass spectrometry from twelve fungal species. For five of them (*P. chrysosporium*, *P. placenta*,

*Pleurotus ostreatus*, *Serpula lacrymans* and *H. annosum*) the genome sequence is already available, and for the other seven (*Gloeophyllum trabeum*, *Fistulina hepatica*, *Fomitiporia mediterranea*, *Dacryopinax spathularia*, *Fomitopsis pinicola*, *Auricularia auricular-judae* and *Punctularia strigosozonata*) the genome sequence will be produced in the saprotrophic Agaricomycotina project described above. The set of selected species are representatives of the two classes of ligninolytic basidiomycetes, and cover as much as possible the taxonomic distribution of the genomes sequenced (nine out of eighteen major clades of Agaricomycotina). Comparative whole transcriptome analyses of different decayer types and clades will facilitate the long range understanding of their biological lifestyles and lignocellulolytic strategies.

### **Sequencing Mycorrhizal Genomes**

In soils of most forests, hundreds of species of ectomycorrhizal fungi establish a mutualistic symbioses with lateral roots of trees (Buée *et al.*, 2009; Martin & Nehls, 2009). Genomic-based analyses of the ectomycorrhizal symbiosis in environmental settings are in their infancy (Courty *et al.*, 2008), but research in environmental genomics is rapidly developing and is crucial for understanding the role of the mycorrhizal symbiosis in nutrient cycling and plant health. One key step for the future of mycorrhizal research is production of additional sequenced genomes, both for a variety of mycorrhizal fungi and also their plant hosts. To date, genomes of two symbionts, the basidiomycete *L. bicolor* (Martin *et al.*, 2008) and the ascomycete *Tuber melanosporum* (Martin *et al.*, 2010), have been sequenced. Based on their symbiosis-related gene networks, evolution of the ectomycorrhizal lifestyle appears to be quite divergent (Plett & Martin, 2011). To better understand the differences between symbiotic clades and types of symbiosis, the JGI initiative has targeted twenty-five mycorrhizal fungi from different orders (Plett & Martin, 2011). Genomic DNA from *Amanita muscaria*, *Cenococcum geophilum*, *Hebeloma cylindrosporum*, *L. amethystina*, *Oidiodendron maius*, *Piloderma croceum*, *Paxillus involutus*, *Pisolithus microcarpus* and *P. tinctorius* is currently being sequenced using next generation sequencing platforms. Sequencing of *Boletus edulis*, *Cantharellus cibarius*, *Coltricia cinnamomea*, *Cortinarius glaucopus*, *Gymnomyces xanthosporus*, *Lactarius quietus*, *Meliniomyces bicolor*, *Paxillus rubicundulus*, *Ramaria formosa*, *Rhizoscyphus ericeae*, *Scleroderma citrinum*, *Suillus luteus*, *Sebacina vermifera*, *Tomentella sublilacina*, *Tricholoma matsutake*, *Tulasnella calospora* and *Terfezia boudieri* will soon follow. The sequenced mycorrhizal species were selected for (i) their ability to promote plant growth and health, (ii) their phylogenetic novelty, (iii) their ability to establish different types of mycorrhizal symbiosis (ectomycorrhizas, ericoid and orchid endomycorrhizas), and (iv) their

ecological niche and host specificity. The comparative genomics of the available *L. bicolor* and *T. melanosporum* genomes (Martin *et al.*, 2008, 2010) and the mycorrhizal genomes that are currently sequenced will provide new insights including but not limited to: A better understanding of the complex interactions between trees and mycorrhizal symbionts; Comparative genomics with the other economically important saprobic and pathogenic fungi; Bioinformatics identification of important symbiosis-related genes; and molecular markers for investigating adaptation signatures of symbiotic fungi in various ecosystems and environmental conditions. The fact that mycorrhizal fungi appear to be independently derived from multiple saprobic lineages means that genomic data will provide independent assessments of the genetic underpinnings of mycorrhizal competence (Plett & Martin, 2011).

### **Exploring the Fungal Tree of Life**

The fungal tree of life (FTOL) serves as the foundation for comparative fungal biology and it is the focus of considerable phylogenetic research (Blackwell *et al.*, 2006). The early diverging lineages of the FTOL comprise species that possess a smooth posterior flagellum. Flagellated fungi were previously classified in Chytridiomycota, but are now recognized as members of multiple paraphyletic lineages. The remaining early diverging lineages of Fungi include the zygomycetous fungi and the arbuscular mycorrhizae, which are characterized by aseptate filamentous growth and lack of sporocarp formation. They were classified in Zygomycota, but like Chytridiomycota may comprise multiple phylogenetic lineages. Dikarya is the most derived clade of Fungi and consist of Ascomycota (e.g., yeasts, molds) and Basidiomycota (e.g., mushrooms, shelf-fungi).

Our increased understanding of the FTOL has resulted in refinement of concepts of morphological homologies, evolution of ecologies and physiologies, and a more accurate assessment of previously unappreciated phylogenetic diversity. Importantly, the FTOL informs our selection of taxa for genomic sequencing. As stressed above, the sequencing of Dikarya is focused on lineages of current economic and medicinal importance (Cuomo & Birren, 2010). While these data have advanced insights into fungal biology, a more complete understanding of fungi and their potential application and benefit to human society is limited by current gaps in our sampling of genomes throughout the FTOL.

As an initial step to advance a more complete genomic coverage of the FTOL, scientists associated with the Assembly the Fungal Tree of Life project proposed to sequence ten target species from unsampled lineages of the FTOL through the JGI Community Sequencing Program.

These species collectively represent over one billion years of eukaryotic evolution and occupy diverse ecological niches (e.g., insect endosymbionts, osmophilic fungi) and exhibit novel metabolic capabilities (e.g., detoxification of noxious plant compounds, colonization of extreme environments). Fungi have been used to the benefit of humans for over 5000 yrs, from fermentation to antibiotics, yet we have only harnessed a fraction of their metabolic potential. Sequencing of these genomes will significantly improve our understanding of early fungal evolution through phylogenomic analyses and it will result in a more complete assessment of the genomic and metabolic diversity of Fungi that is needed to more fully apply them to the human endeavor.

### **Outlook**

Genomic analyses have so far been restricted to a limited set of ecologically-relevant fungal species. Within the next two years, we will see a massive shift towards the inclusion of the environmental and evolutionary perspectives in genome sequencing initiatives. Environmental genomics of the communities of soil-borne fungi will lead to a better understanding of the biological and ecological roles of these eukaryotic microbes (Kyrpides, 2009). Several fungal species selected in the present JGI projects are amongst the most abundant species found in large-scale 454 pyrosequencing of soil fungal ribosomal DNA and are known to interact with plants (Buée *et al.*, 2009; Jumpponen *et al.*, 2010). The sequenced genomes from across the tree of life will therefore serve as anchors for sorting through thousands of metagenomic repertoires and categorizing them. This will undoubtedly lead to a better understanding of plant-microbe interactions.

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