Exploring the Genome Diversity of Mycorrhizal Fungi – A JGI Sequencing Project Update

Annegret Kohler[1], Joshua Herr[1,2], Emmanuelle Morin[1], Claude Murat[1], Annick Brun[1], Claire Venault-Fourrey[1], David Hibbett[3], Igor Grigoriev[4], and Francis Martin[1]

[1] Tree-Microbe Interactions Research Group, INRA-Nancy, UMR 1136, Champenoux, FRANCE
[2] Schatz Center for Tree Molecular Genetics, Pennsylvania State University, University Park, PA 16802 USA,
[3] Biology Department, Clark University, Worcester, MA 01610 USA
[4] Eukaryotic Genomics Group, Joint Genome Institute, Walnut Creek, CA 94598 USA

Abstract

Mycorrhizal fungi are responsible for plant and ecosystem health by providing an array of benefits. Mycorrhizal fungi help plants (including woody trees and shrubs, forbs, grasses, etc.) to acquire nutrients - notably nitrogen and phosphorus – and aid in water acquisition. Additionally, mycorrhizal fungi provide both a physical barrier to pathogens and initiate gene expression to aid in responses to be biotic and abiotic stresses. In ecosystems, mycorrhizal fungi contribute to nutrient cycling and may possess novel enzymes for the breakdown of a wide array of natural and synthetic chemicals.

The goal of this sequencing initiative is to identify and compare genome determinants shaping evolution across the disparate fungal taxa in symbiosis with plants. Sequencing mycorrhizal fungal genomes will contribute to, which includes but is not limited to, the better understanding of interactions with host-trees; the basis for understanding phenotypic differences in fruit body structure and development; the description and understanding of organic secondary metabolites typical of antibiotics and volatile compounds; the comparative genomics of additional fungal parasites and saprobes; and the development of molecular markers to be used for the tractability of fungi in various environments.

Sequencing the Genomes of Differing Types of Mycorrhizal Fungi

- Although Smith & Read (2008) describe seven types of mycorrhizae, mycorrhizal fungi can be described in terms of ecto- and endomycorrhizae, which may form an infection gradient from plant to plant. Describing mycorrhizal fungi in this way is an artificial classification that probably owes as much to the plant host as the fungal symbiont.
- Ectomycorrhizae interact with a plant symbiont by forming a sheath around a plant root but do not penetrate the plant apoplast. This form of mycorrhizae is common with trees and scrubs and accounts for approximately 10% of plant species, which cover large temperate and tropical ecosystems.

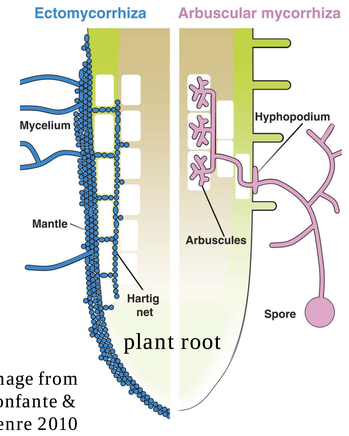
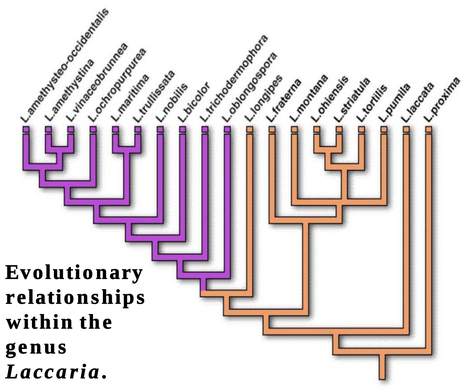


Image from Bonfante & Genre 2010

- Endomycorrhizae are characterized by the penetration of the fungal tissue into the plant apoplast, but typically do not contribute a large amount of fungal hyphae to cover a plant root. Mycorrhizal types such as Ericoid and Orchid mycorrhizae, for the sake of simplicity, can be put into this category.

The *Laccaria* Pan-Genome

- *Laccaria* is a cosmopolitan genus of mycorrhizal fungi found in temperate regions throughout the world.
- *Laccaria* are model mycorrhizal taxa because, unlike many fungi, they can be cultured and manipulated in the laboratory. Additionally, copious amounts of ecosystem information concerning their natural history is available.
- Numerous *Laccaria* species are being sequenced using the Illumina GA platform for both intra-genus and population genomic studies. The *Laccaria bicolor* S238N-H82 genome will be used for genome assembly (Martin et al 2008).
- Sequencing these genomes will allow the comparison of adaptive mutations to influences of environment, geography, and host specificity that have been noted across the genus.
- The analysis will consist of 'ingroup' *L. bicolor* (annotation 2.0) and *L. amethystina* (draft genome) with 'outgroup' *L. laccata* and *L. proxima*.



Evolutionary relationships within the genus *Laccaria*.

Image courtesy of G. Mueller (www.fieldmuseum.org).

- The analysis will also consist of 20 European, American, and Asian strains of *L. bicolor* and *L. amethystina* for the purpose of determining geographic, environmental, and host influence on *Laccaria* genome architecture.

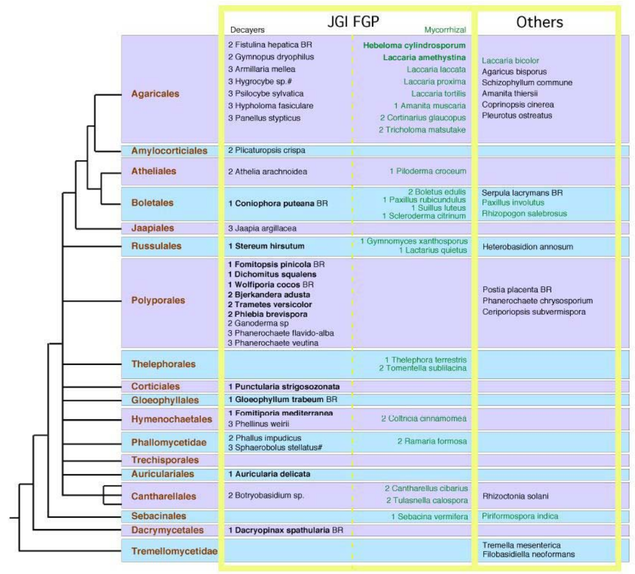
Status of Mycorrhizal Fungal Genome Sequencing

- Along with completed genomes and those in the sequencing, assembly, and annotation stages (red and orange boxes, respectively), two tiers of genome sequencing efforts have been planned through JGI for mycorrhizal fungi.
- These sequencing efforts, planned for the next two years, will yield upwards of 30 fungal genomes from all fungal phyla and sub-phyla exhibiting the mycorrhizal habit. These fungal genomes will contribute to our knowledge of biochemical evolution and adaptation as well as ecosystem functioning.

	Species Name	Taxonomic Position	Mycorrhizal Type	Status
Completed Genomes	*Laccaria bicolor* S238N-H82	Basidiomycota, Agaricales, Hydnangiaceae	Ecto	Completed (Martin et al 2008), Version 2.0 soon to be released
	Tuber melansporum	Ascomycota, Pezizales, Tuberaceae	Ecto	Completed (Martin et al 2010), Version 1.0
Genomes in Sequencing, Assembly, and Annotation.	*Cenococcum geophilum* 1.58	Ascomycota, Dothideomycetes	Ecto	In Sequencing Queue
	Glomus intraradices	Glomerulomycota, Glomales	Endo	Illumina, 454 & Fosmid Sequencing In Progress
	Hebeloma cylindrosporum h7	Basidiomycota, Agaricales, Cortinariaceae	Ecto	Illumina, 454 & Fosmid Sequencing Complete, Assembly Near Completion
	Paxillus involutus	Basidiomycota, Boletales	Ecto	Illumina, 454 & Fosmid Sequencing Complete, Assembly In Progress
	Pisolithus microcarpus 441	Basidiomycota, Boletales	Ecto	Illumina, 454 & Fosmid Sequencing Complete, Assembly In Progress
	Pisolithus tinctorius Marx 270	Basidiomycota, Boletales	Ecto	On Hold Pending Pisolithus microcarpus 441 Assembly
	Rhizopogon salebrosus	Basdiomycota, Boletales, Suillineae (?)	Ecto	Illumina, 454 & Fosmid Sequencing Complete, Assembly In Progress
	Tuber aestivum	Ascomycota, Pezizales, Tuberaceae	Ecto	Sequencing started by Genoscope Genome Institute, France
Tier 1 – Mycorrhizal Fungal Genomes Scheduled for Completion in 2011.	*Amanita muscaria* BX008	Basidiomycota, Agaricales, Aminitaceae	Ecto	In Sequencing Queue
	Laccaria amethystina	Basidiomycota, Agaricales, Hydnangiaceae	Ecto	454 Sequencing completed through INRA
	Lactarius quietus	Basidiomycota, Russulales	Ecto	Soon to be shipped to JGI
	Paxillus rubicundulus	Basidiomycota, Boletales, Paxilineae	Ecto	Soon to be shipped to JGI
	Piloderma croceum F1598	Basidiomycota, Atheliales	Ecto	Illumina & Fosmids Completed, 454 In Progress
	Suillus luteus	Basidiomycota, Boletales, Suillineae	Ecto	Soon to be shipped to JGI
	Thelephora terrestris	Basidiomycota, Thelephorales	Ecto	Soon to be shipped to JGI
	Sebacina vermifera MAFF 305830	Basidiomycota, Sebacinales	Endo/Orchid	Sequencing In-Progress, No Fosmids
	Meliniomyces bicolor	Ascomycota, Helotiales	Ecto/Ericoid	Soon to be shipped to JGI
	Oidiodendron maius Zn	Ascomycota, Leotiomycetales	Endo	Illumina Completed, INRA 454 Completed, JGI 454 In Progress
	Rhyzoscyphus ericeae	Ascomycota, Helotiales	Ecto/Endo	Soon to be shipped to JGI
	Terfezia boudieri	Ascomycota, Pezizales	Ecto/Endo	DNA ready to be shipped to JGI
Tier 2 – Mycorrhizal Fungal Genomes Scheduled for Completion in 2012.	*Boletus edulis*	Basidiomycota, Boletales, Boleineae	Ecto	DNA ready to be shipped to JGI
	Cantharellus cibarius	Basidiomycota, Cantharellales	Ecto	DNA in production
	Coltricia cinnamomea	Basidiomycota, Hymenochaetales	Ecto	DNA in production
	Cortinarius glaucopus	Basidiomycota, Agaricales, Cortinariaceae	Ecto	DNA in production
	Gymnomyces xanthosporus	Basidiomycota, Russulales	Ecto	DNA in production
	Ramaria formosa	Basidiomycota, Gomphales	Ecto	DNA in production
	Scleroderma citrinum	Basidiomycota, Boletales, Sclerodermataceae	Ecto	DNA in production
	Tomentella sublilacina	Basidiomycota, Thelephorales	Ecto	DNA in production
	Tricholoma matsutake	Basidiomycota, Tricholomataceae	Ecto	DNA in production
	Tulasnella calospora	Basidiomycota, Cantharellales, Tulasnellaceae	Endo/Orchid	DNA in production
	Meliniomyces variabilis	Ascomycota, Helotiales	Ericoid, Endophytic	DNA in production

Mycorrhizal Basidiomycetes in the Joint Genome Institute's Fungal Genomics Program

- The sequencing of mycorrhizal genomes by JGI is part of the larger Fungal Genome Project (JGI-FGP).
- Basidiomycete representatives of both mycorrhizal and saprophytic genomes will be compared to elucidate how ecological roles have shaped genome architecture in the Basidiomycetes (Martin & Nehls 2009).



Phylogenetic distribution of Basidiomycete representatives of the JGI-FGP with in progress or pending genome sequencing. Green text represents mycorrhizal forming fungi and black text represents saprophytic or parasitic fungi. The box with 'other' taxa represent completed Basidiomycete genomes sequenced by JGI or other organizations which will be used for the purposes of comparative genomics of the Basidiomycota and Eukaryotes.

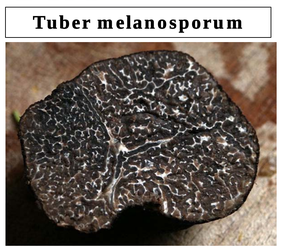
A Tale of Two Symbionts: *Laccaria bicolor* vs. *Tuber melanosporum*

- Sequencing the genomes of *Laccaria bicolor* (Martin et al 2008) and *Tuber melanosporum* (Martin et al 2010) have very different genomes, but exhibit a very similar suite of symbiosis-induced nutrient cycling enzymes (Plett & Martin 2011).

Laccaria bicolor

- *Laccaria bicolor* appears to be less dependent on the host and more active at acquiring carbon from the soil substrates and, as a result, may act as a weak saprotroph in the environment (Martin et al 2008).

Tuber melanosporum



- *Tuber melansporum* is more aggressive in its colonization of plant roots and does not appear to be able to acquire carbon from the soil and therefore is more dependent on the host for its survival (Martin et al 2010).

References Cited

- Bonfante & Genre (2010) Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis. *Nature Communications* 1:48.
- Martin et al (2008) Symbiosis insights from the genome of the mycorrhizal basidiomycete *Laccaria bicolor*. *Nature* 452: 88-92.
- Martin et al (2010) Périgord Black Truffle genome uncovers evolutionary origins and mechanisms of symbiosis. *Nature* 464, 1033-1038.
- Martin & Nehls (2009) Harnessing ectomycorrhizal genomics for ecological insights. *Curr Op Plant Biol* 12: 1–8.
- Plett & Martin (2011) Blurred boundaries: lifestyle lessons from ectomycorrhizal fungal genomes. *Trends in Genetics*, in press.
- Smith & Read (2008) *Mycorrhizal Symbiosis*. Academic Press.

Contact Information

Francis Martin
(fmartin@nancy.inra.fr)

Tree-Microbe Interactions Research Group, UMR 1136,
INRA-Nancy, Champenoux, FRANCE

Acknowledgements

Funding was provided for genome sequencing by the Joint Genome Institute, (U.S. Department of Energy) for sequencing the *Laccaria bicolor* genome and pending mycorrhizal fungi and by Genoscope France for the sequencing of *Tuber melanosporum*.