Chapter 7
The Contribution of New Technologies Toward Understanding Plant–Fungus Symbioses

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Abstract Symbiotic associations between beneficial soil fungi and the roots of about 90 % of land plants, commonly known as mycorrhizae, exist in a wide range of terrestrial ecosystems. During the interaction, both the plant and the fungus benefit from the relationship. Complete genome sequences give useful information to deeper understanding of the molecular mechanisms underlying the symbiotic lifestyle and several genome sequencing projects on mycorrhizal fungi have been launched. Genomic projects are currently coupled to transcriptome analysis, which represents the starting point for the post-genomic activities, in which research is focused to ascribe function to genes. The introduction of new sequencing techniques (next-generation sequencing, NGS), which produce short-read sequences in large quantity, has been accompanied by advances in bioinformatics. In this chapter we will review recent advances in plant/fungus symbiotic interactions, focusing on the recent fungal genome projects and on the NGS application in this field.

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Introduction

In their natural habitats, plants interact with a large number of microbes, and among these many are fungi. While some microbes colonize the plant for their own benefit, others can directly cooperate with plants in a mutually beneficial manner. In addition, microbes can indirectly affect plants by altering their environments (Schenk et al. 2012). Plant-fungi associations play an important role in terrestrial ecosystem and vary from pathogenic interactions to mutualistic associations (Cox et al. 2010). Symbiotic associations between beneficial soil fungi and the roots of about 90 % of land plants, commonly known as mycorrhizae, exist in a wide range of terrestrial ecosystems. During the interaction, both the plant and the fungus benefit from the relationship: the fungus supplies the plant with nutrients, such as phosphate and nitrogen, while the plant supplies the fungus with carbohydrates. Mycorrhizal fungi play a central role in the capturing of nutrients in natural as well as in agricultural systems, in which they can contribute to plant health and productivity, increasing the tolerance to biotic and abiotic stress (Smith and Read 2008; Balestrini et al. 2012a). According to their ability to penetrate the roots cells, mycorrhizae can be classified as two main types: endomycorrhizae and ectomycorrhizae (ECM) (Balestrini et al. 2012a). Recent research has focused on soil organisms involved in symbioses, which play a key role in plant/microbial communities and in ecosystem processes (Finlay 2008). While the researches, originated from the several plant genome projects, have pointed out the plant genes involved in symbiosis (Güimil et al. 2005; Güther et al. 2009; Dermatsev et al. 2010; Hogekamp et al. 2011), less information is available on the fungal side (Bonfante and Genre 2010). Understanding how fungi with different nutritional strategies achieve their lifestyle is crucial to understand their ecological functions, their interactions with other organisms, and their impact on plant communities and productivity (Martin et al. 2011).

Complete genome sequences are seen as valuable tools to help obtain a deeper understanding of the molecular mechanisms that underlie the symbiotic lifestyle. Generally, genomic projects are associated with transcriptome analysis, which represent a good starting point for post-genomic activities, in which research is focused on the assignment of a function to the gene dataset discovered in an organism. Bioinformatics can provide powerful tools to identify and evaluate candidate genes through database searches and through the analyses of expression profiling. The knowledge of expressed genes is essential to interpret the functional elements of the genome and to reveal the molecular determinants of physiological processes, i.e., during the development and life cycle of an organism, as well as the processes that occur during interaction with other organisms. The transcriptomics approaches that are commonly applied are large-scale approaches, i.e., microarrays (Breakspear and Momany 2007) and next-generation sequencing (NGS) (Wang et al. 2009; Nagalakshmi et al. 2010). Recent papers have shown the great potential of transcriptome analysis in fungi (Tisserant et al. 2011, 2012; Zuccaro et al. 2011), as it unravels the biological

processes that occur in fungi life. In the last few years, with the advancements in NGS technologies (Zhang et al. 2011), the sequencing of fungal genomes/ transcriptomes has become simpler and less expensive, becoming a relatively routine approach to data collection for all areas of mycology. A deeper understanding of the evolutionary history of the Fungi Kingdom is necessary to complement and expand our knowledge of the natural evolution of ecosystems and to enhance the development of tools that will possibly allow the recognition of undescribed species (Hibbett et al. 2007).

JGI Fungal Genomics Program

About 80,000 described species belonging to the Fungi Kingdom, but the diversity in the group has been estimated to involve about 1.5 million species, and it thus represents one of the largest branches of the Tree of Life. In the early 2000s, the Assembling the Fungal Tree of Life project (AFTOL; http://aftol.org/) was launched to increase the understanding of the evolution of the Fungi Kingdom (Hibbett et al. 2007). Recently, thanks to the advances in massive-scale DNA sequencing and analysis capabilities, the Joint Genome Institute (JGI) of the US Department of Energy has launched the Fungal Genomics Program (FGP; http://genome.jgi.doe.gov/ programs/fungi/index.jsf), with the aim of exploring fungal diversity for energy and environmental sciences and applications (Grigoriev et al. 2011). They started with the sequencing of a few single fungal genomes (Martinez et al. 2004, 2008, 2009; Jeffries et al. 2007; Martin et al. 2008a) and then moved onto higher-scale system-level genomics.

In the frame of FGP, two main research lines are currently under way:

1. The Genomic Encyclopedia of Fungi (http://genome.jgi.doe.gov/programs/ fungi/GE_Fungi.jsf)
2. The 1000 Fungal Genomes Project (F1000) (http://genome.jgi.doe.gov/programs/ fungi/1000fungalgenomes.jsf).

The former focuses on three areas of research related to bioenergy: (1) Plant Feedstock Health, which encompasses symbiosis, plant pathogenicity, and biocontrol; (2) Biorefinery, which involves the analysis of lignocellulose degradation, sugar fermentation, and industrial organisms; and (3) Fungal Diversity.

The latter, in collaboration with an international research team, has the goal of filling the gaps in the Fungal Tree of Life by sequencing at least two reference genomes from more than 500 recognized families of fungi (Spatafora 2007).

In the DOE JGI framework, fungi (as symbionts, pathogens, and biocontrol agents) are considered key organisms that can exert an impact on the maintenance of plant health and on the sustainable growth of biofuel feedstock. In this perspective, the optimization of bioenergy feedstock plant growth and productivity depends on the understanding of the molecular mechanisms that are involved in the interactions between plants and mycorrhizal fungi.

In this contest, JGI has started the Mycorrhizal Genomics Initiative, which targets several fungal species from different orders (Table 7.1) (Grigoriev et al. 2011; Plett and Martin 2011; http://mycor.nancy.inra.fr/IMGC/MycoGenomes/). At the moment, there are 72 ongoing or completed genome/transcriptome projects in the frame of the "Exploring the Genome Diversity of Mycorrhizal Fungi to Understand the Evolution and Functioning of Symbiosis in Woody Shrubs and Trees" proposal, which is coordinated by Francis Martin (INRA). On the basis of previous genome projects conducted on *Laccaria bicolor* (Martin et al. 2008a) and *Tuber melanosporum* (Martin et al. 2010), the main goal of this project is to explore the genomics sequence of a phylogenetically and ecologically diverse suite of mycorrhizal fungi (Basidiomycota and Ascomycota), which includes the major clades of symbiotic species associating with trees and shrubs, including endo- and ectomycorrhiza.

The JGI Genome Portal (http://genome.jgi.doe.gov/) offers an access point to all the sequencing genome project managed by the DOE JGI. A specialized tool for the analysis and exploration of fungal genomes, named MycoCosm (http://genome.jgi.doe.gov/fungi), was released in March 2010, in response to a request from the fungal community to integrate all fungal genomes and interactive analytic tools in one place (Grigoriev et al. 2012). Newly sequenced and annotated fungal genomes from JGI and elsewhere (e.g., *T. melanosporum* sequenced and annotated by Genoscope) are available to the public, and new annotated genomes are being added to this resource upon completion of their annotation. MycoCosm offers useful web-based genome analysis tools that can be used to search through sequenced genomes and explore them in different contexts. Genome-centric tools offer the Genome Browser, BLAST, and the possibility of searching within the data for a single genome. Predicted gene models and annotations are displayed within the Genome Browser along with different lines of evidence in support of these predictions (e.g., gene and protein expression profiles). The Genome Browser also displays other types of data mapped to a genome assembly, G+C profiles, and annotation features, including regions of homology, domains, repeats, and noncoding genes. The functional profiles of genomes are based on summaries of predicted gene annotations, according to the GO (The Gene Ontology Consortium 2000), KEGG (Kanehisa et al. 2008), and KOG (Koonin et al. 2004) classifications. Genome conservation and synteny can be explored using the *VISTA* tool, which has been designed for the visualization and analysis of pairwise and multiple DNA alignments and which makes the analysis of whole-genome alignments, functional profiles, and gene clusters possible. The cluster analysis enables the exploration of gene families within a given group of organisms. Clusters are built using the Markov clustering algorithm MCL (Enright et al. 2002) and all-against-all BLAST alignments of the proteins from the entire dataset. Registered users participating in a particular genome project can validate and improve predicted gene models and annotations.

The JGI initiative has the main goal of providing new useful information that, through a comparative analysis, can be used to improve the understanding of fungal lifestyles, their interaction with plants, and their evolution. An example of this is the work by Eastwood and colleagues (2011). They have conducted, through the sequencing of the brown rot wood decay fungus *Serpula lacrymans*, a genome comparison with sequenced fungal species that represent several functional niches and

Table 7.1 List of fungal species sequenced in the frame of JGI proposal "Exploring the Genome Diversity of Mycorrhizal Fungi to Understand the Evolution and Functioning of Symbiosis in Woody Shrubs and Trees" and related genome projects of interest in mycorrhizal research (updated May 2013). Modified after Martin and Bonito (2012)

Fungal sequencing project	Genome release
Tier 1 – 2011 [14 species]	
Basidiomycotina:	
Laccaria amethystina (Agaricales, Hydnangiaceae)	v.1.0
Hebeloma cylindrosporum (Agaricales, Cortinariaceae)	v.2.0
Paxillus involutus (Boletales, Paxilineae)	v.1.0
Paxillus rubicundulus (Boletales, Paxilineae)	v.1.0
Pisolithus microcarpus (Boletales, Sclerodermatineae, Pisolithaceae)	v.1.0
Pisolithus tinctorius (Boletales, Sclerodermatineae, Pisolithaceae)	v.1.0
Piloderma croceum (Atheliales)	v.1.0
Scleroderma citrinum (Boletales, Sclerodermataceae)	v.1.0
Sebacina vermifera (Sebacinales, forms endomycorrhiza [orchid])	v.1.0
Tricholoma matsutake (Agaricales, Tricholomataceae)	v.3.0
Tulasnella calospora (Cantharellales, Tulasnellaceae)	v.1.0
Ascomycotina:	
Cenococcum geophilum (Dothideomycetes)	v.2.0
Oidiodendron maius (Leotiomycetes)	v.1.0
Terfezia boudieri (Pezizales, Pezizaceae)	v.1.0
Tier 2 – 2012 [13 species]	
Basidiomycotina:	
Amanita muscaria (Agaricales, Amanitaceae)	v.1.0
Boletus edulis (Boletales, Boletineae)	v.1.0
Cantharellus cibarius (Cantharellales)	In progress
Cortinarius glaucopus (Agaricales, Cortinariaceae)	In progress
Gymnomyces xanthosporus (Russulales)	
Lactarius quietus (Russulales)	In progress
Gyrodon lividus (Boletales)	In progress
Suillus luteus (Boletales)	v.1.0
Thelephora terrestris (Thelephorales)	In progress
Tomentella sublilacina (Thelephorales)	In progress
Ascomycotina:	
Meliniomyces bicolor (Helotiales)	v.2.0
Meliniomyces variabilis (Helotiales)	v.1.0
Rhizoscyphus ericae (Helotiales)	In progress
Others	
Laccaria bicolor (Agaricales, Hydnangiaceae)[a]	v.2.0
Tuber melanosporum (Pezizales, Tuberaceae; Genoscope)[b]	v.1.0
Tuber magnatum (Pezizales, Tuberaceae)	In progress
Rhizophagus irregularis (Glomeromycota)	v.1.0
Piriformospora indica (Sebacinales)[c]	v.1.0
Suillus brevipes (Boletales)	v.1.0

[a]Martin et al. (2008a)
[b]Martin et al. (2010)
[c]Zuccaro et al. (2011)

have demonstrated that the evolution of ectomycorrhizal biotrophy and brown root saprotrophy is accompanied by a reduction and losses in specific gene families, which would suggest an adaptation to intercellular colonization of the plant tissue.

Early Findings in Mycorrhizal Genomics

It is still unknown whether several mycorrhizal fungi have a common core set of genes that are necessary for the symbiosis development or whether the mechanisms required for the symbiotic interaction changed during the evolution (Plett and Martin 2011). The first sequenced mycorrhizal fungi were the basidiomycete *Laccaria bicolor* (Martin et al. 2008a) and the ascomycete *Tuber melanosporum* (Martin et al. 2010), and other ECM genome sequencing projects are currently under way (Table 7.1). *L. bicolor* has a genome of 64.9 Mb in size with ~19,000 estimated protein-coding genes, while *T. melanosporum* has a 125 Mb genome and only ~7,500 predicted protein-coding genes, showing a relatively small complement of predicted proteins in comparison with other sequenced filamentous fungal genomes (Martin et al. 2010). This expansion in truffle genome size results from a proliferation of repeated transposable elements, which account for ~58 % of the genome. Although both fungi are ectomycorrhizal species and form similar symbiotic structures, they encode different proteomes: large with many expanded multigene families in *Laccaria versus* compact with very few multigene families in *Tuber*. Differences can be seen in symbiosis-regulated genes. Both genomes reveal a reduced set of plant cell wall (PCW) degrading enzymes, but there are significant differences in the enzyme repertoire in the two fungi and in the expression during the symbiosis (Martin et al. 2010). Moreover, the effector-like proteins expressed in *Laccaria* (i.e., MiSSPs) are not expressed in *T. melanosporum* ectomycorrhizae. Looking at the different symbiosis-related toolboxes in the two genomes, the evolution of the ectomycorrhizal lifestyle seems to be quite divergent (Plett and Martin 2011). The results of genome sequencing of more ECM fungi that are currently being sequenced will allow the symbiotic fungal strategies developed by different ECM fungal lineages to be compared. As far as endomycorrhizal fungi are concerned, *Oidiodendron maius* (ericoid symbiont) and *Tulasnella calospora* (orchid symbiont) genome sequencing has recently been released, thus providing the possibility of comparing different mycorrhizal strategies.

A sequencing project of an arbuscular mycorrhizal (AM) fungus genome (*Glomus intraradices* DAOM197198, now named *Rhizophagus irregularis*) was started in 2004 and is currently under way (Martin et al. 2008b; Lanfranco and Young 2012). The first sequencing data provided an estimate of the genome size of about 150 Mb (Martin et al. 2008b), and this value has recently been confirmed experimentally (Sedzielewska et al. 2011). On the other hand, the *G. intraradices* mitochondrial genome and the mitochondrial genome of two *Gigaspora* isolates have already been completed (Lee and Young 2009; Formey et al. 2012; Pelin et al. 2012; Nadimi et al. 2012). In the absence of a complete genome sequence, the

knowledge of the *G. intraradices* DAOM197198 genome has recently been expanded through the publication of genome-wide transcriptomic data (Tisserant et al. 2012). The expression of genes encoding membrane transporters and small secreted proteins has been found in the intraradical mycelium, along with a lack of expression of hydrolytic enzymes acting on PCW polysaccharides, which would suggest that *G. intraradices* shares some features with obligate biotrophic pathogens (Spanu 2012; Kemen et al. 2011) and ECM symbionts (Plett and Martin 2011). However, the obligate biotrophy of *G. intraradices* does not seem to be associated with a large reduction in metabolic complexity, as observed in many obligate biotrophic pathogens; in this way, the ability to interact with the soil environment, regarding the nutrient uptake, is maintained in the symbiotic fungus. The work of Tisserant et al. (2012) on *G. intraradices* is the first comprehensive gene inventory of a Glomeromycotan fungus and can be considered a keystone for accessing symbiosis-related functional features in other members of this unique phylum.

The Microarray Era

The transcriptome, i.e., the mRNA pool of a cell at any one moment, has long been analyzed using methods such as expressed sequence tag (EST) sequencing (through cDNA libraries construction) and cDNA (macro) microarrays, in which gene-specific oligonucleotides are spotted on a solid surface (Wilkes et al. 2007). These techniques have led to the rapid identification of expressed genes in several organisms, thus providing data for the large-scale analysis of thousands of genes. In the first work using cDNA arrays to study mycorrhizal symbiosis, gene expression was analyzed during the ECM symbiosis between *Eucalyptus globulus* and *Pisolithus tinctorius*. A comparison of signals from the free-living partners and symbiotic tissues has led to the identification of many plant/fungus symbiosis-regulated genes, thereby demonstrating the utility of this technique in the study of gene expression changes during symbiosis development (Voiblet et al. 2001). Liu et al. (2003) then used cDNA arrays to examine transcript profiles in *M. truncatula* roots during interaction with the AM fungus *Glomus versiforme* and during growth under different phosphorous concentrations. Interestingly, most genes showing increased transcript levels in AM roots did not change in response to high phosphorus level, suggesting that the changes in transcript levels during symbiosis were a consequence of the AM fungus rather than a secondary effect due to the improved phosphorus nutrition (Liu et al. 2003). To date, thanks to the increase in plant genome sequencing, genome-wide cDNA arrays are available for several mycorrhizal plants such as rice, tomato, grapevine, *Populus trichocarpa*, *Lotus japonicus*, and *Medicago truncatul*a (Rensink and Buell 2005). Over the last 10 years, major changes in gene expression that accompany the establishment of symbiosis and a wide spectrum of genes involved have been revealed, providing insight into the molecular mechanism that underlie symbiosis both for AM (Hohnjec et al. 2005; Küster et al. 2007; Güther et al. 2009; Dermatsev et al. 2010) and ECM symbiosis (Le Quéré et al. 2005; Duplessis et al.

2005; Heller et al. 2008). Microarrays have also been applied, in the frame of genome projects, to symbiotic fungi such as *L. bicolor* and *T. melanosporum* (Martin et al. 2008a, 2010). This approach has been used to verify changes in gene expression during the three stages of the complex life cycle of a symbiotic ectomycorrhizal fungus: free-living mycelium, ectomycorrhiza, and fruiting bodies. A global characterization of the endophytic fungus *Piriformospora indica* transcriptional responses has recently been performed (Zuccaro et al. 2011) during the colonization of living and dead barley roots using microarrays (60-mer probes) containing also 265 barley genes (including genes related to defense and transport). Microarrays, constructed using the nonredundant virtual transcripts obtained with the Sanger and 454 sequencing technologies from germinated spores, extraradical mycelium, and symbiotic roots, have been developed to study gene expression in several life cycle stages of the AM fungus *Glomus intraradices*, including RNA extracted from arbuscule-containing cells collected using laser microdissection (LMD). Over the last few years, LMD has been used to study cell specificity in AM symbiosis, and particular attention has been paid to the cortical cells containing the main feature of the symbiosis: the arbuscules. Several works on AM symbiosis have been focused on verifying the expression of specific plant-fungal genes, which appeared previously regulated in microarrays, in different cell-type populations (Güther et al. 2009; Gomez et al. 2009; Hogekamp et al. 2011). In addition, in order to obtain insight into cell-specific reprogramming in AM symbiosis, transcriptome analyses of several cell types have been performed using an LMD approach combined with microarray hybridization (Gaude et al. 2011).

Despite the wide use of this approach to detect differential gene expression in symbiotic interactions, the microarray construction (probe design and synthesis) remains limited to organisms with a sufficient level of information on gene sequences.

The Advantages of High-Throughput Sequencing

With the improvements in the fields of microfluidics, nanotechnology, and informatics, alternative technologies have recently emerged that can increase the large-scale DNA/RNA sequencing (Margulies et al. 2005; Branton et al. 2008; Wang et al. 2009). The term NGS is commonly used to describe technologies other than Sanger sequencing that have the ability to produce an enormous volume of data cheaply.

There are several commercially available NGS platforms, and, of these, the Roche/454 (http://www.454.com/), Solexa/Illumina (http://www.illumina.com/), and Life Technology/SOLiD (http://www.appliedbiosystems.com/absite/us/en/home/applications-technologies/solid-next-generation-sequencing.html) are currently dominating the market.

Microarrays, although still an accurate and useful tool in gene expression studies, are now being replaced by seq-based methods, which can identify and quantify

rare transcripts without prior knowledge of the gene sequences and can provide information on alternative splicing and sequence variation in identified genes (Malone and Oliver 2011). Whole-transcriptome sequencing using NGS technologies, also known as RNA sequencing (RNA-seq), has started to reveal the complex landscape and dynamics of a transcriptome. However, the manipulation and the interpretation of the millions of short-read sequences produced by a typical NGS experiment still present significant computational challenges (Zhang et al. 2011; Martin and Wang 2011).

The obtained short reads can be (1) aligned to a reference genome, to obtain a quantitative measure of the transcript expression level, which is measured as read coverage (Mortazavi et al. 2008; Wilhelm and Landry 2009); and (2) de novo assembled, without an existing genome reference (Martin and Wang 2011).

To date, high-throughput transcriptome sequencing has been performed on only a few symbiotic (*L. bicolor*, *T. melanosporum*, *G. intraradices*) and two endophytic fungi (*Epichloe festucae*, *P. indica*) with the aim of improving the genome annotation as well as of identifying specific genes expressed during symbiosis (Larsen et al. 2010; Martin et al. 2010; Tisserant et al. 2012; Eaton et al. 2010; Zuccaro et al. 2011).

Larsen and colleagues (2010), using the RNA-seq approach in *L. bicolor*, have corrected most of the gene models that in the previous oligoarray analysis resulted to be differentially expressed during symbiosis, including genes related to carbon metabolism, membrane permeability and transport, and intracellular signaling (Martin et al. 2008a). Moreover, RNA-seq data obtained from fully developed *L. bicolor/Populus tremuloides* ECMs have been used to predict metabolomic models of mycorrhizal systems (Larsen et al. 2011). The deep RNA sequencing short reads have been used to identify significantly expressed gene models belonging to specific metabolic pathways. This approach allows the transcript profiles of the plant and its symbiotic fungus to be simultaneously determined, providing information on how the two partners cooperate to form this important symbiotic association. The mycorrhizal metabolome model suggests that *L. bicolor* can synthesize nitrogen compounds (i.e., glycine, glutamate, allantoin) via pathways not expressed in *P. tremuloides* roots, and these compounds might be exchanged with the photosynthetically derived sugars of the plant (Larsen et al. 2011).

In 2011, Tisserant and colleague deep sequenced the *T. melanosporum* transcriptome at three different developmental stages (free-living mycelium, fruiting body, ectomycorrhiza). These data have improved the *T. melanosporum* genomic structural annotation and led to the identification of 91 previously unidentified transcripts, exons, untranslated regions (UTRs) that extended in silico gene models, and alternative splicing events. In addition, RNA-seq transcript profiling, which provides a global view on the transcriptome complexity, has been used for detailed analyses of specific groups of genes (Balestrini et al. 2012b; Ceccaroli et al. 2011; Montanini et al. 2011; Rubini et al. 2011). To date, among the 72 JGI proposals on mycorrhizal fungi, ten projects have been aimed at fungal transcriptome and annotation.

Perspectives

Democratization of genome sequencing and the low cost and high quantities of the data being produced by new sequencing technologies will surely result in an avalanche of new sequenced genomes. This will enable the ECM/AM research community to go beyond the sequencing of new single genomes and allow already sequenced organisms to be "resequenced" (e.g., resequencing of a dozen strains of the ectomycorrhizal model species *L. bicolor*; Plett and Martin 2011), with the aim of verifying the intraspecific variability. In addition to elucidating the role of the mycorrhizal symbioses in nutrient cycling and plant health, genomic and transcriptomic sequencing projects have the goal of identifying the common core of symbiosis-related genes, as determinants of the symbiotic lifestyle. However, genome sequencing is only the first step toward knowledge of an organism and its capabilities to interact with the environment and with other organisms. The integration of functional, structural, molecular, cellular, and bioinformatics approaches is still required to obtain a deep understanding of the function of genes/proteins and the multiplicity of processes that occur inside an organism. Laser microdissection, for instance, is a powerful tool that can be used to isolate selected tissues/cell types from sectioned specimens, which allows DNA, RNA, proteins, and even metabolites to be extracted. It represents a useful and innovative technique that can be used to study plant-fungus interactions and in the analysis of gene expression in specific target cells/fungal compartments (Fig. 7.1) (Balestrini et al. 2009 and references therein; Hogekamp et al. 2011; Hacquard et al. 2013).

The metagenomics and metatranscriptomics studies that are currently under way, coupled with microarray construction, can provide a powerful approach to the analysis of environmental microbial transcriptomes, in order to uncover the functions encoded in the genomes of thousands of soil fungal species that cannot be cultured and sequenced directly (Martin and Martin 2010).
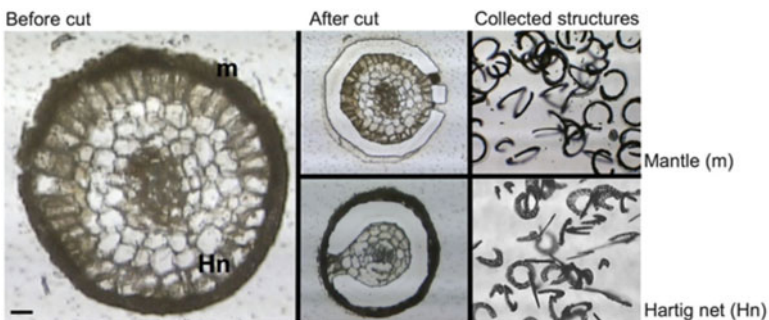
Fig. 7.1 Laser microdissection of the two compartments presents in an ectomycorrhiza from paraffin sections (mantle and Hartig net). *Bar* corresponds to 25 μm

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