The Genome of *Laccaria bicolor* Provides Insights into Mycorrhizal Symbiosis

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**Mycorrhizal symbioses** -- the union of roots and soil fungi -- are universal in terrestrial ecosystems and may have been fundamental to land colonization by plants1-2. Boreal, temperate, and montane forests all depend upon *ectomycorrhizae*3. Identification of the primary factors that regulate symbiotic development and metabolic activity will therefore open the door to understanding the role of
ectomycorrhizae in plant development and physiology, allowing the full ecological significance of this symbiosis to be explored. Here, we report the genome sequence of the ectomycorrhizal basidiomycete *Laccaria bicolor* (Fig. 1) and highlight gene sets involved in rhizosphere colonization and symbiosis. This 65-million-base genome assembly contains ~20,000 predicted protein-encoding genes and a very large number of transposons and repeated sequences. We detected unexpected genomic features most notably a battery of effector-type small secreted proteins (SSP) with unknown function, several of which are only expressed in symbiotic tissues. The most highly expressed SSP accumulates in the proliferating hyphae colonizing the host root. The ectomycorrhizae-specific SSP likely play a decisive role in the establishment of the symbiosis. The unexpected observation that the genome of *L. bicolor* lacks carbohydrate-active enzymes involved in degradation of plant cell walls, but maintains the ability to degrade non-plant cell walls, reveals the dual saprotrophic and biotrophic lifestyle of the mycorrhizal fungus which enables it to grow within both soil and living plant roots. The predicted gene inventory of the *L. bicolor* genome, therefore, points to previously unknown mechanisms of symbiosis operating in biotrophic mycorrhizal fungi. The availability of this genome provides an unparalleled opportunity to develop a deeper understanding of the processes by which symbionts interact with plants within their ecosystem in order to perform vital functions in the carbon and nitrogen cycles that are fundamental to sustainable plant productivity.

The 65 million base pairs genome of *Laccaria bicolor* (Maire) P.D. Orton (hereafter referred to as *Laccaria*) is the largest sequenced fungal genome published so far (Table 1). While no evidence for large scale duplications was observed within the *Laccaria* genome, tandem duplication occurred within multigene families (Supplementary Fig. 4). Transposable elements (TE) comprised a higher proportion (21%) than that identified in the other sequenced fungal genomes and may therefore account for the relatively large genome of *Laccaria* (Supplementary Table 3). Approximately 20,000 protein-coding genes were identified by combined gene predictions (Supplementary Information Section 2). Expression of nearly 80% (ca. 16,114) of the predicted genes was detected in either free-living mycelium, ectomycorrhizal root tips or fruiting bodies (Supplementary Table 4) using NimbleGen custom-oligoarrays (Supplementary Information Section 9). Most genes are activated in almost all tissues, whereas other more specialized genes were only activated in some specific developmental stages, such as free-living mycelium, ectomycorrhizae or fruiting body (Supplementary Table 5).

Only 14,464 of *Laccaria* proteins (70%) showed significant sequence similarity to documented proteins. Most homologs were found in the sequenced basidiomycetes Phanerochaete chrysosporium⁴, Cryptococcus neoformans⁴, Ustilago maydis⁶, and Coprinopsis cinerea⁷ (Supplementary Table 6). The percentage of proteins found in multigene families was related to genome size and was the largest in
**Laccaria** (Fig. 2). This was mainly due to the expansion of protein family size, but also due to the larger number of protein families in **Laccaria** when compared to the other basidiomycetes (Supplementary Table 7). Expansion of protein family sizes in **Laccaria** was prominent in the lineage-specific multigene families. Striking gene family expansions occurred in those genes predicted to have roles in protein-protein interactions (e.g. WD40) and signal transduction mechanisms (Supplementary Table 7). Two new classes of Gα genes were found and may be candidates for the complex communication that must occur between the mycobiont and its host-plant during mycorrhizae establishment (Supplementary Table 8). Several transcripts coding for expanded and lineage-specific gene families were upregulated in symbiotic and fruiting body tissues, suggesting a role in tissue differentiation (Supplementary Tables 5 & 9).

In our analysis of annotated genes, and in particular paralogous gene families, we highlighted processes which may be related to the biotrophic and saprotrophic lifestyles of **Laccaria**. Twelve predicted proteins showed a similarity to known haustoria-expressed secreted proteins (HESP) of the basidiomycetous rusts, *Uromyces fabae* and *Melampsora lini*, which are involved in pathogenesis (Supplementary Table 10). Of the 2,931 proteins predicted to be secreted by **Laccaria**, most (67%) cannot be ascribed a function and 82% of these predicted proteins are specific to **Laccaria**. Within this set, we found a large number of genes that encode cysteine-rich products with a predicted size of <300 amino acids. Of these 278 small secreted proteins (SSP), 69% belong to multigene families, but only nine groups comprising a total of 33 SSP co-localized in the genome (Supplementary Fig. 5). The structure of two of these clusters is shown in Supplementary Fig. 6. Other SSP are scattered all over the genome and we found no correlation between SSP and TE genome localization (Supplementary Fig. 5). Transcript profiling revealed that the expression of several SSP genes is specifically induced upon in the symbiotic interaction (Table 1, Supplementary Fig. 10). Five of the 20 most highly upregulated fungal transcripts in ectomycorrhizal root tips code for SSP (Supplementary Table 5). These mycorrhiza-induced cysteine-rich SSP (MISSP) belong to **Laccaria**-specific orphan gene families. Within the MISSP, we found a family of secreted proteins with a CFEM domain (IPR014005) (Supplementary Fig. 7 & 8), as previously identified in the plant pathogenic fungi *M. lini* and *M. grisea* (Supplementary Table 10), and proteins with a gonadotropin- (IPR0001545) or snake toxin-like (SSF57302) domains related to the cysteine-knot domain. Expression of several SSP were downregulated in ectomycorrhizal root tips (cluster E in Supplementary Fig. 10) suggesting a complex interplay between these secreted proteins in symbiosis interaction.

The rich assortment of MISSP may therefore act as effector proteins to manipulate host cell signalling or suppress defence pathways during infection, as suggested for pathogenic rusts*8,9*, smuts*6* (*U.
maydis) and Phytophthora species. To play a role in symbiosis development, MISSP should be expressed in Laccaria hyphae colonizing the root tips. To test this assertion, we determined the tissue distribution of the MISSP7 protein (ID 298595) showing the highest induction in ectomycorrhizal tips (Table 1, Supplementary Table 5). Two peptides located in the N-terminal and C-terminal parts of the mature protein were selected as antigens for the production of anti-MISSP7 antibodies. The selected peptides were not found in the deduced protein sequences of other Laccaria gene models nor in the Populus trichocarpa genome. MISSP7 localization in Laccaria/Populus ectomycorrhizal root tips by indirect immunofluorescence is illustrated in Fig. 1 and Supplementary Fig. 1. Control images in which the ectomycorrhiza sections were obtained replacing primary anti-MISSP7 antibodies by pre-immune IgG are shown in Supplementary Fig. 12. Where ectomycorrhizae were treated with anti-MISSP7 antibody followed by fluorescent-labeled secondary antibody, fluorescence was localized in the hyphae colonizing short roots (Fig. 1, Supplementary Fig. 11) and not detected in the free-living mycelium (Supplementary Fig. 12). Although MISSP7 was detected in the hyphal mantle layers ensheating the root tips, the protein mainly accumulated in the finger-like, labyrinthine branch hyphal system (Hartig net) which provides a very large area of contact between cells of the two symbionts. It accumulated in the cytosol and cell wall of the fungal cells. The MISSP7 protein could thus interact with the plant components after secretion. MISSP7 shares no sequence similarity or protein motif with other SSP. Comparison of the MISSP sequences did not reveal a specific conserved motif, such as the RXLR motif of phytopathogenic Phytophthora or the malaria parasite, that could potentially contribute to their function or to targeting to the host cell. Those SSP with an upregulated expression in fruiting body (Supplementary Table 5, Supplementary Fig. 10) may play a role in the differentiation of the sexual tissues and/or aggregation of sporophore tissues. Interestingly, they are a large set of SSP genes showing significant changes in gene expression in both ectomycorrhizal root tips and fruiting body (cluster A in Supplementary Fig. 10) suggesting that both developmental processes recruit similar gene networks (e.g., those involved in hyphal aggregation).

Host trees are able to harness the formidable web of mycorrhizal hyphae, that permeates the soil and leaf litter, for their nutritional benefit. A process that is pivotal to the success of ectomycorrhizal interactions is thus the equitable exchange of nutrients between the symbiont and its host-plant. A comparison with other basidiomycetes (Supplementary Table 12) revealed that the total number of predicted transporters has been expanded in Laccaria compared to C. cinerea and P. chrysosporium. Interestingly, Laccaria has multiple ammonia transporters although it encodes a single nitrate permease. Ammonia is arguably the most important inorganic nitrogen source for ectomycorrhizal fungi. One of the
ammonia transporters (*LbAMT2.2*), for instance, is greatly upregulated in ectomycorrhizae (Supplementary Table 5). *Laccaria*, thus, shows an increased genetic potential in terms of nitrogen uptake when compared to other basidiomycetes. These capabilities are consistent with *Laccaria* being exposed to a range of nitrogen sources from organic matter decay.

Although the *Laccaria* genome contains numerous genes coding for key hydrolytic enzymes, such as proteases and lipases, we observed an extreme reduction in the number of enzymes involved in the degradation of plant cell wall (PCW) oligo- and polysaccharides. Glycoside hydrolases (GH), glycosyltransferases (GT), polysaccharide lyases (PL), carbohydrate esterases (CE) and their ancillary carbohydrate-binding modules (CBM) were identified using the carbohydrate-active enzyme (CAZyme) classification (http://www.cazy.org/). A comparison of the *Laccaria* candidate CAZymes with fungal phytopathogens confirms the adaptation of its enzyme repertoire to symbiosis and reveals the strategy used for the interaction with the host (Supplementary Tables 13 and 14). The reduction in PCW CAZymes affects almost all GH families culminating in the complete absence of several key families. For instance, there is only one candidate cellulase (GH5) appended to the sole fungal cellulose-binding module (CBM1) found in the genome and no cellulases from families GH6 and GH7 (Supplementary Table 14). Similar reductions or loss of hemicellulose and pectin degrading enzymes were also noted. These observations suggest that the inventory of *Laccaria* PCW degrading enzymes underwent massive gene loss as a result of its adaptation to a symbiotic lifestyle and that this species is now unable to use many PCW polysaccharides as a carbon source, including those found in soil and leaf litter. The remaining small set of secreted CAZymes with potential action on plant polysaccharides (e.g. GH28-polygalacturonases) is probably required for cell wall remodeling during fungal tissue differentiation as their expression was upregulated in both fruiting body and ectomycorrhizae (Supplementary Table 15, Supplementary Fig. 13). In contrast, transcripts coding for proteins with expansin domain were only induced in ectomycorrhizae suggesting they may be used by *Laccaria* for penetrating into the root apoplastics space. To survive before its mycorrhizal association with its host, *Laccaria* appears to have developed a capacity to degrade non-plant (e.g. animal, bacterial) oligo- and polysaccharides which is suggested by retention of CAZymes from families GH79, PL8, PL14 and GH88 (Supplementary Table 14). Interestingly, there is no invertase gene in the *Laccaria* genome, implying that this fungus is unable to directly use sucrose from the plant. This is consistent with earlier observations that *Laccaria* depends on its host plant to provide glucose in exchange for nitrogen. We also noticed an expansion of CAZymes involved in the fungal cell wall biosynthesis and rearrangement, almost entirely due to an increased number of putative chitin synthases.
and enzymes acting on β-glucans (Supplementary Table 14). Several of the corresponding genes are up- or downregulated upon developmental processes requiring cell wall alterations such as formation of fruiting bodies or mycorrhizae (Supplementary Table 15, Supplementary Fig. 13).

Ectomycorrhizal fungi play a significant role in mobilizing N from well-decomposed organic matter\textsuperscript{2,15}. The hyphal network permeating the soil might therefore be expected to express a wide diversity of proteolytic enzymes. The total number of secreted proteases (116 members) identified (Supplementary Fig. S14) is relatively large compared with other sequenced saprotrophic basidiomycetes, such as \textit{C. cinerea} and \textit{P. chrysosporium}. Secreted aspartyl-, metallo- and serine-proteases may play a role in degradation of decomposing litter\textsuperscript{15} confirming that \textit{Laccaria} has also the ability to use nitrogen of animal-origin, as suggested previously\textsuperscript{17}. They may also play a role in developmental processes as the expression of several secreted proteases is up- or downregulated in fruiting bodies and ectomycorrhizal root tips (Supplementary Table 16). Mycelial mats formed by \textit{Laccaria} hyphae colonizing organic matter therefore possess the ability to degrade decomposing leaf litter.

Our analysis of the gene space reveals a multi-faceted mutualistic biotroph equipped to take advantage of transient occurrences of high-nutrient niches (living host roots and decaying soil organic matter) within a heterogeneous, low-nutrient environment. The availability of genomes from mutualistic, saprotrophic\textsuperscript{4}, and pathogenic\textsuperscript{6} fungi, but also from the mycorrhizal tree \textit{Populus trichocarpa}\textsuperscript{12}, now provides an unparalleled opportunity to develop a deeper understanding of the processes by which fungi colonize wood and soil litter, and also interact with living plants within their ecosystem in order to perform vital functions in the carbon and nitrogen cycles\textsuperscript{2} that are fundamental to sustainable plant productivity.

**METHODS SUMMARY**

The Methods are described in Supplementary Information (www.nature.com/nature). The sections of the Supplementary Methods are arranged in the same order as the manuscript to facilitate cross-referencing. Here, we describe the datasets generated by this project and their availability.

**Genomic sequence.** The WGS project has been deposited at GenBank/EMBL/DDJB under project accession ABFE00000000. The version described in this paper including assembly and annotation is the first version ABFE01000000. Scaffolds and assemblies for all genomic sequence generated by this project are also available from the JGI portal (http://genome.jgi-psf.org/Lacbi1/Lacbi1.download.ftp.html). A genome browser is available from JGI (www.jgi.doe.gov/laccaria). BLAST search of the genome is
predicted gene models. Consensus gene predictions, produced by combining several different gene predictors, are available from JGI (www.jgi.doe.gov/laccaria) as GFF files. These gene models can also be accessed from the Genome Browser in JGI Laccaria portal (http://genome.jgi-psf.org/cgi-bin/browserLoad/46b9a4360b37752a766008cb).

Gene annotations. Tables compiling KEGG, PFAM, KOG, and best BLAST hits for predicted gene models, transposable element and CAZyme data, and Tribe-MCL gene families are available from INRA LaccariaDB (http://mycor.nancy.inra.fr/IMGC/LaccariaGenome/index.html).

Array data. The complete expression dataset is available as series (accession number # GSE9784) at the Gene Expression Omnibus at NCBI (http://www.ncbi.nlm.nih.gov/geo/).

References

1. Smith, S. E. & Read, D. J. Mycorrhizal Symbiosis (2nd edition, Academic Press, London) (1996).
2. Read, D. J. & Perez-Moreno, J. Mycorrhizas and nutrient cycling in ecosystems - a journey towards relevance? New Phytol. 157, 475-492 (2003).
3. Galagan, J. E., Henn, M. R., Ma, L. J., Cuomo, C. A., Birren, B. Genomics of the fungal kingdom: insights into eukaryotic biology. Genome Res. 15, 1620-1631 (2005).
4. Martinez, D. et al. Genome sequence of the lignocellulose degrading fungus *Phanerochaete chrysosporium* strain RP78. Nature Biotech. 22, 695-700 (2004).
5. Loftus, B. J. et al. The genome of the basidiomycetous yeast and human pathogen *Cryptococcus neoformans*. Science 307, 1321-1324 (2005).
6. Kämper, J. et al. Insights from the genome of the biotrophic fungal plant pathogen *Ustilago maydis*. Nature 444, 97-101 (2006).
7. *Coprinus cinereus* Database: http://www.broad.mit.edu/annotation/genome/coprinus_cinereus/Home.html
8. Wirsel, S. G. R., Voegele, R. T., Mendgen, K. W. Differential regulation of gene expression in the obligate biotrophic interaction of *Uromyces fabae* with its host *Vicia faba*. Mol. Plant Microb. Int. 14, 1319-1326 (2001).
9. Catanzariti, A. M., Dodds, P. N., Lawrence, G. J., Ayliffe, M. A., Ellis, J. G. Haustorially expressed secreted proteins from flax rust are highly enriched for avirulence elicitors. Plant Cell 18, 243-256 (2006).
10. Kulkarni, R. D., Kelkar, H. S., Dean, R. A. An eight-cysteine-containing CFEM domain unique to a group of fungal membrane proteins. Trends Bioch. Sci. 28, 118-118 (2003).
11. Kamoun, S. A. Catalogue of the effector secretome of plant pathogenic oomycetes. Annu. Rev. Phytopathol. 44, 41-60 (2006).
12. Tuskan, G. A. et al. The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). *Science* **313**, 1596-1604 (2006).

13. Martin, F., Kohler, A., Duplessis, S. Living in harmony in the wood underground: ectomycorrhizal genomics. *Curr. Opin. Plant Biol.* **10**, 204-210 (2007).

14. Chalot, M., Blaudez, D., Brun, A. Ammonia: a candidate for nitrogen transfer at the mycorrhizal interface. *Trends Plant Sci.* **11**, 263-266 (2006).

15. Lindahl, B. D., Ihrmark, K., Boberg, J., Trumbore, S. E., Högberg, P., Stenlid, J., Finlay, R. D. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. *New Phytol.* **173**, 611-620 (2007).

16. Nehls, U., Grunze, N., Willmann, M., Reich, M., Küster, H. Sugar for my honey: Carbohydrate partitioning in ectomycorrhizal symbiosis. *Phytochem.* **68**, 82-91 (2007).

17. Klironomos, J. N. & Hart, M. M. Animal nitrogen swap for plant carbon. *Nature* **410**, 651 (2001).

**Supplementary Information** is linked to the online version of the paper at www.nature.com/nature.

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**Author Contributions** is described in Supplementary data section 11.

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Table 1. Genome characteristics of *Laccaria bicolor* and other basidiomycetes

| Genome characteristics | Laccaria | Coprinopsis | Phanerochaete | Cryptococcus | Ustilago |
|------------------------|----------|-------------|---------------|--------------|---------|
| Strain                 | S238N-HB2| Okayama7#130| RP78          | H99          | 521     |
| Sequencing institution | JGI      | Broad-MIT   | JGI           | Broad-MIT    | Broad-MIT |
| Genome assembly (Mbp)  | 64.9     | 37.5        | 35.1          | 19.5         | 19.7    |
| GC content (%)         | 46.6     | 51.6        | 53.2          | 48.2         | 54      |
| Protein coding genes   | 20,614   | 13,544      | 10,048        | 7,302        | 6,522   |
| CDS<300 bp             | 2,191    | 838         | 163           | 313          | 58      |
| Average gene length (bp)| 1,533   | 1,679       | 1,667         | 1,828        | 1,935   |
| Average CDS length (bp)| 1,134   | 1,352       | 1,366         | 1,502        | 1,840   |
| Average exon length    | 210.1    | 251         | 232           | 253          | 1,051   |
| Average intron length  | 92.7     | 75          | 117           | 66           | 127     |
Table 2. Changes in the expression of transcripts coding for mycorrhizae-induced cysteine-rich small secreted proteins

| Protein ID | Size | Length (AA) | Transcript Concentration (FLM) | *Pseudotsuga* ECM/FLM Ratio (fold) | *Populus* ECM/FLM Ratio (fold) | Features |
|------------|------|-------------|---------------------------------|----------------------------------|-------------------------------|----------|
| 298595     | sc   | 68          | nd                              | 21877                            | 12913                         | MISSP7   |
| 333839     | 5    | 129         | nd                              | 7844                             | 1931                          | GPI-anchored |
| 298667     | 2    | 70          | nd                              | 1906                             | 1407                          |          |
| 332226     | 8    | 181         | 43                              | 847                              | 780                           | CFEM domain (IPR014005) |
| 311468     | 2    | 59          | nd                              | 191                              | nd                            |          |
| 295737     | 8    | 288         | 131                             | 171                              | 252                           |          |
| 334759     | sc   | 101         | nd                              | 109                              | 18                            |          |
| 395403     | 4    | 121         | 24                              | 103                              | 93                            |          |
| 333423     | 9    | 120         | 6                               | 102                              | 72                            | Gonadotropin domain (IPR0001545) |
| 312262     | 4    | 106         | 85                              | 69                               | 53                            |          |
| 295625     | 4    | 199         | 325                             | 66                               | 48                            |          |
| 325402     | 8    | 238         | 310                             | 49                               | 74                            | Snake toxin-like (SSF57302) |
| 316998     | sc   | 56          | 137                             | 29                               | 57                            |          |
| 333197     | 3    | 148         | 266                             | 17                               | 8                             |          |
| 327918     | 2    | 154         | 763                             | 13                               | 4                             | Homolog in *Coprinopsis cinerea* |
| 307956     | sc   | 74          | 336                             | 13                               | 90                            | Whey acidic domain (IPR008197) |
| 327246     | sc   | 194         | 1025                            | 10                               | 18                            | Homolog in *Coprinopsis cinerea* |
| 303550     | 5    | 98          | 1365                            | 10                               | 14                            |          |
| 300377     | 2    | 291         | 5499                            | 10                               | 8                             |          |
| 293250     | sc   | 224         | 127                             | 9                                | 10                            | Homolog in *Coprinopsis cinerea* |
| 298648     | sc   | 64          | 1108                            | 8                                | 12                            |          |
| 298646     | 2    | 73          | 1028                            | 7                                | 14                            |          |
| 293729     | 3    | 210         | 3000                            | 7                                | 7                             |          |

Transcript profiling was performed on free-living mycelium (FLM), and ectomycorrhizal root tips (ECM) of poplar (*Populus trichocarpa*) and Douglas fir (*Pseudotsuga menziesii*). See Supplementary Information section 9 for details. Abbreviations: AA, amino acids; nd, not detected; sc, single copy.
METHODS

Genome sequencing. The haploid genome of the strain S238N-H82 from *L. bicolor* (Maire) P.D. Orton was sequenced with the use of a whole-genome shotgun (WGS) strategy. All data were generated by paired-end sequencing of cloned inserts using Sanger technology on ABI3730xl sequencers. Supplementary Table 1 gives the number of reads obtained per library.

Genome assembly. The data was assembled using release 1.0.1b of JAZZ, a JGI WGS assembler. Based on the number of alignments per read, the main genome scaffolds were at a depth of 9.88. The amount of sequence in the unplaced reads was 6.5 Mbp, which is sufficient to cover the main-genome gaps to a mean depth of 9.9. A total of 64.9 Mbp are captured in the scaffold assembly (Supplementary Table 2).

Genome annotation. Gene models were predicted using FgenesH\(^17\), homology-based FgenesH+\(^18\), Genewise\(^19\), as well as EuGène\(^20\) and TwinScan\(^21\), and alignments of several cDNA resources (Supplementary Information section 3). The JGI pipeline selected a best representative gene model for each locus based on EST support and similarity to known proteins from other organisms, and predicted 20,614 protein-coding gene models. All predicted genes were annotated using Gene Ontology\(^21\), eukaryotic clusters of orthologous groups\(^22\), and KEGG pathways\(^23\). Protein domains were predicted using InterProScan\(^24\). Signal peptides were predicted in 2,931 Laccaria proteins by both the hidden Markov and the neural network algorithms of SignalP\(^25\). After eliminating predicted transmembrane proteins and removal of transposable element fragments, we selected 278 cysteine-rich secreted proteins with a size <300 AA. Gene families were built from proteins in *Laccaria, C. cinerea, P. chrysosporium, C. neoformans* and *U. maydis* using Tribe-MCL tools\(^26\) with default settings.

Indirect immunofluorescent localization of MISSP7. The peptides LRALGQASQGDDLHR and GPIPNVFRRVPPEPFN located in the N-terminal and C-terminal parts of the MISSP7 sequence (without the signal peptide) were synthesized and used as antigens for the generation of antibodies in rabbits according to the manufacturer’s procedures (Eurogentec, Seraing, Belgium). The anti-MISSP7 IgG fraction was purified using MAbTrap kit (GE Healthcare) according to the manufacturer’s recommendations. Subsequently, IgG-containing fraction was desalted using a HiTrap™ desalting column (GE Healthcare). The concentration of purified IgG from pre-immune serum was determined by Bradford assay using a Bio-
Rad protein assay. Final concentration of anti-MISSP7 IgG was 0.16 mg/ml. Immunolocalization was performed essentially as described by 27,28 with slight modifications (Supplementary section 10).

**Gene expression.** Average expression levels of genes in different tissues and conditions (SOM) were analyzed using CyberT statistical framework (http://www.igb.uci.edu/servers/cybert/) and hierarchical clustering with EPCLUST (http://ep.ebi.ac.uk/EP/EPCLUST/) (Supplementary section 8).

**References**

18. Salamov, A., Solovyev, V. *Ab initio* gene finding in *Drosophila* genomic DNA. Genome Res **10**: 516-522 (2000).
19. Birney, E., Clamp, M., Durbin, R. GeneWise and genomewise. Genome Res **14**: 988-995 (2004).
20. Schiex, T., Moisan, A., Rouzé, P. EuGène: an eukaryotic gene finder that combines several sources of evidence. In: *Computational Biology*, Gascuel O, Sagot MF, eds, LNCS 2066, pp. 111-125 (2001).
21. Tenney, A.E. et al. Gene prediction and verification in a compact genome with numerous small introns. Genome Res **14**: 2330-2335 (2004).
22. Ashburner, M. et al. Gene ontology: tool for the unification of biology. Nature Genetics **25**: 25-29 (2000).
23. Koonin, E. V. et al. A comprehensive evolutionary classification of proteins encoded in complete eukaryotic genomes. Genome Biology **5**: R7 (2004).
24. Kanehisa, M, Goto, S, Kawashima, S, Okuno, Y, Hattori, M. The KEGG resource for deciphering the genome. Nucleic Acids Research **32**: D277-D280 (2004).
25. Zdobnov, EM, Apweiler, R. InterProScan – an integration platform for the signature-recognition methods in InterPro. Bioinformatics **17**: 847-848 (2001).
26. Emanuelsson, O., Brunak, S., von Heijne, G., Nielsen, H. Locating proteins in the cell using TargetP, SignalP, and related tools. Nature Protocols **2**: 953-971 (2007).
27. Blancaflor, E.B., Zhao, L., Harrison, M.J. Microtubule organization in root cells of *Medicago truncatula* during development of an arbuscular mycorrhizal symbiosis with *Glomus versiforme*. Protoplasma **217**: 154–165 (2001).
28. Harrison, M.J., Dewbre, G.R., and Liu, J.Y. A phosphate transporter from *Medicago truncatula* involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. Plant Cell **14**: 2413–2429 (2002).
Legends of Figures

Figure 1 The *Laccaria bicolor* ectomycorrhizal symbiosis and the immunofluorescent localization of the small secreted protein MISSP7 in ectomycorrhizae.

a, Fruiting bodies of *L. bicolor* colonizing seedlings of Douglas fir (*Pseudotsuga menziesii*). The subterranean mycelial web has developed symbiotic ectomycorrhizal tissues on host root tips and has produced fruiting bodies above ground (Photograph courtesy of D. Vairelles, INRA-Nancy). b, Laser scanning confocal microscopy image of a transverse section of *P. menziesii–L. bicolor* ectomycorrhizal root tips showing extramatrical mycelium (em), aggregated hyphae of the mantle sheath (m), hyphae proliferating between the epidermal (ec), tannin (tc) and cortical (cc) of the host root to form the symbiotic Hartig net (hn). Bar = 10 μm. c-f, Indirect immunofluorescent localization of MISSP7. Transverse (c, e) and longitudinal (d, f) sections of *Populus trichocarpa-L. bicolor* ectomycorrhizal tips. MISSP7 was detected with anti-MISSP7 IgG and secondary antibody conjugated with AlexaFluor 488 in the hyphae of the mantle (m) and the uniseriate Hartig net (hn) ensheathing the epidermal cells (ec) of the colonized roots. Rectangle in panels (d) and (f) show the finger-like, labyrinthine hyphal system accumulating large amount of MISSP7. (e) and (f), phase contrast images. Bar = 10 μm.

Figure 2 Expansion of protein families in *Laccaria bicolor*.

a, Relationship between genome size and number of protein families. b, Relationship between genome size and protein family sizes in five sequenced basidiomycetes. Protein sequences predicted from the genome sequences of *Laccaria bicolor*, *Coprinopsis cinerea*, *Phanerochaete chrysosporium*, *Cryptococcus neoformans* and *Ustilago maydis* were clustered into families using the TRIBE-MCL algorithm (see Supplementary Information section 5 for details).
Figure 2.