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Comparative genomics of *Rhizophagus irregularis*, *R. cerebriforme*, *R. diaphanus* and *Gigaspora rosea* highlights specific genetic features in Glomeromycotina



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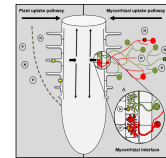


5 : Architecture et Fonction des Macromol cules Biologiques, CNRS, Aix-Marseille Universit , Marseille 13288, France

Background and data

Arbuscular mycorrhizal fungi (AMF) are members of the subphylum Glomeromycotina and are involved in a widespread symbiosis with the roots of most land plants. AMF are known to improve plant fitness through the establishment of mycorrhizal symbioses. Genetic and phenotypic variations among closely related AMF isolates can significantly affect plant growth, but are unclear.

To elucidate the genomic features underlying the high interspecific diversity of Glomeromycotina, we performed the genome assembly and gene annotation of three new AMF : *Rhizophagus cerebriforme*, *Rhizophagus diaphanus* and *Gigaspora rosea*. We compared their gene contents with an improved *Rhizophagus irregularis* assembly and annotation and four saprotrophic Mucoromycota fungi : *Mortierella elongata*, *Mucor circinelloides*, *Phycomyces blakesleeanus* and *Rhizopus microcarpus*.

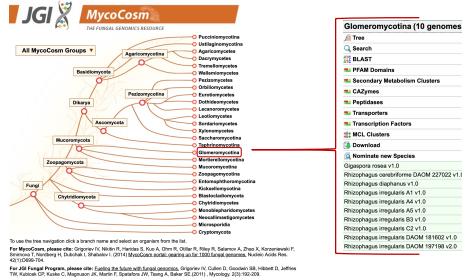


In addition to plant uptake pathway, the mycorrhizal uptake pathway comprises :
- fungal transporters in the extraradical mycelium (red or green symbols)
- transport through the hyphae (mycorrhizal interface)
- mycorrhiza-inducible plant transporters in the periarbuscular membrane (orange symbols).

Genome annotation and portals

JGI Annotation Pipeline:

- array of tools for gene prediction : ab initio, homology- and transcriptome-based
- all predicted proteins are then automatically annotated



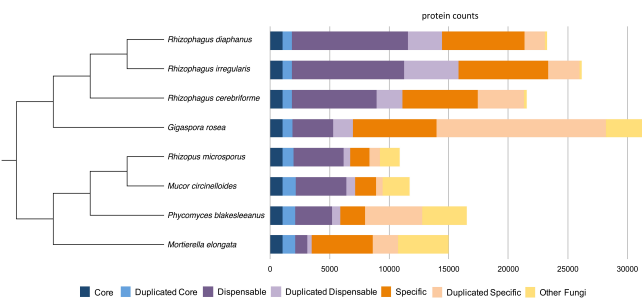
For this study, we generated a portal for each fungi, which provides different tools to browse, annotate and compare the genome to other fungal genomes.

<https://genome.jgi.doe.gov/programs/fungi>

Organismal phylogeny and gene conservation

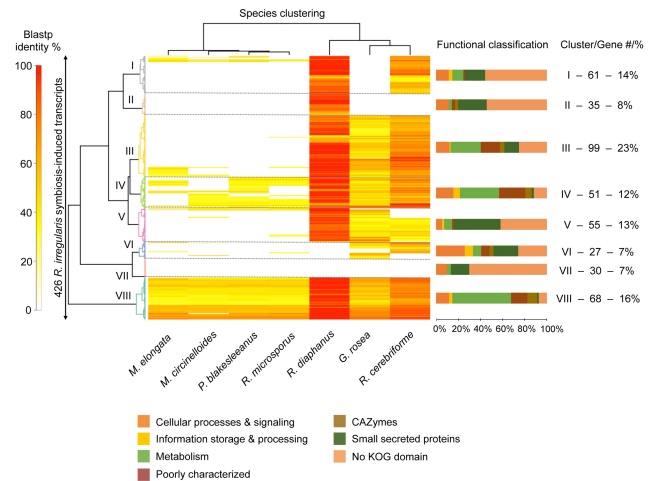
A phylogeny based on a concatenation of 784 single copy, orthologous protein sequences strongly supports the erection of Mucoromycota to unite Glomeromycotina, Mortierellomycotina and Mucoromycotina (Spatafora et al., 2016).

We compared the gene repertoires encoded by sampled Mucoromycota taxa and identified (i) sets of core genes shared by all Mucoromycota species; (ii) sets of dispensable genes shared by at least two species of Mucoromycota; (iii) sets of species-specific genes only found in a single genome. As expected for species that diverged >450 million years ago, clustering led to a very restricted core set of genes. Each AM species is characterized by a large set of species-specific genes, which are also referred to as taxonomically restricted genes



Conservation of symbiosis-upregulated genes

The expression of *Rhizophagus irregularis* genes was measured by RNA-Seq profiling in *B. distachyon* mycorrhizae; 426 *R. irregularis* transcripts (3.3% of the expressed genes) are expressed at a higher level in symbiotic roots compared to transcriptionally-active germinating hyphae. These transcripts are potentially involved in the development and physiology of the symbiotic interaction



- **Mucoromycota-conserved clusters** : only 16 % of *R. irregularis* symbiosis-upregulated genes are shared by all species of Mucoromycota (cluster VIII). Those conserved genes mainly encode for cellular and signalling processes, and pathways of the primary metabolism, and likely derived from those encoded by the saprotrophic MRCA. An other 12% (cluster IV) have orthologous sequences in one or several species of saprotrophic Mucoromycota.
- **Glomeromycotina-conserved clusters** : transcripts of clusters III and V (36%) are shared by the Glomeromycotina species, while those of cluster VI (7%) are only encoded by *G. rosea* and *R. cerebriforme*. A substantial proportion of these symbiosis-related genes have no known function
- **Rhizophagus-conserved clusters** : 7% of *R. irregularis* symbiosis-induced genes are species-specific (cluster VII), most of these genes code for proteins with unknown KOG functions and mycorrhiza-induced small secreted proteins (MiSSPs). Cluster II (8%) grouped *R. irregularis* symbiosis-induced genes, mainly coding for unknown proteins and MiSSPs, having a strong similarity with *R. diaphanus*, its closest relative.

Conclusion

In the present study, we investigated the evolutionary dynamics of key genomic traits in the subphylum Glomeromycotina of Mucoromycota. Our enhanced AM fungal taxon sampling, allows us to perform both within- and across-lineage comparisons, thus covering the different time scales at which the evolution of genome features occurred. Overall, our findings show that extant Glomeromycotina genomes have been shaped by both retention of ancestral states present in saprotrophic Mucoromycota and secondary innovations, for the multiple genomic traits investigated in the present study, namely genome size, protein domain diversity and gene content.

Morin E. et al. Comparative genomics of *Rhizophagus irregularis*, *R. cerebriforme*, *R. diaphanus* and *Gigaspora rosea* highlights specific genetic features in Glomeromycotina (2019) *New Phytologist*, doi: 10.1111/nph.15687

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