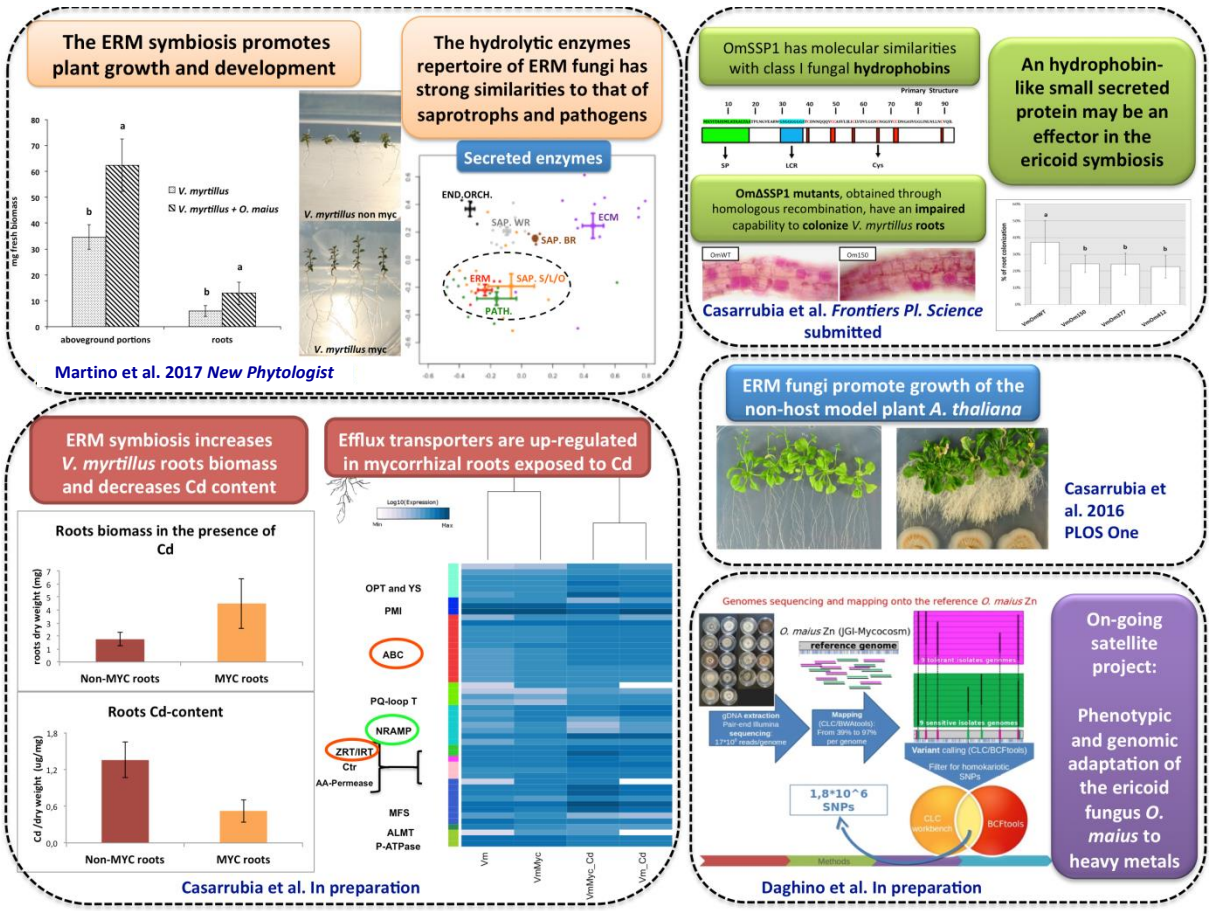
EFFECTER



Looking for effector symbiosis-related proteins in the ericoid endomycorrhizal fungus Oidiodendron maius

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Context — Ericoid mycorrhiza (ERM) is a specific endomycorrhizal type that involves symbiotic fungi mostly belonging to the Leotiomycetes (Ascomycetes) and plants in the family Ericaceae. In the harsh and stressful (heavy metals enriched) habitats where they occur, survival of ERM plants relies on nutrient mobilisation from soil organic matter (SOM) by their fungal partners as well as on ERM fungi unique abilities to withstand environmental stress and to enhance metal tolerance of their host plants.

Mutualistic and pathogenic plant-colonizing fungi use effector molecules to manipulate the host cell metabolism to allow plant tissue invasion. Some small secreted proteins (SSPs) have been identified as fungal effectors in both ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM) fungi, but it is currently unknown whether SSPs also play a role as effectors in the ERM symbiosis.

Plant growth and development can be influenced by mutualistic and non-mutualistic microorganisms. We investigated the ability of the ERM fungus *Oidiodendron maius* to influence growth and development of the non-host plant *Arabidopsis thaliana*.

Objectives — To characterize the fungal genetic machinery underpinning both symbiotic lifestyle and SOM degradation to understand ERM symbiosis functioning and evolution, and its impact on soil C turnover

To identify, through the analysis of transcriptomic data, fungal SSPs potentially involved in the molecular dialogue governing the ERM symbiosis.

Some mechanisms of heavy metal tolerance have been identified in ERM fungi, but the molecular and cellular mechanisms that help the mycorrhizal host plant to cope with toxic metals are currently unknown. We have addressed this aspect in the ERM association between the metal tolerant fungus *O. maius* Zn and *Vaccinium myrtillus*.

To investigate ERM fungi effect on growth and development of the non-host plant *Arabidopsis thaliana*.

Approach — We analysed the genomes of the ERM fungi *Meliniomyces bicolor, M. variabilis, O. maius* and *Rhizoscyphus ericae* and compared their gene repertoires to those of other fungi with different lifestyles (mycorrhizal, endophytes, saprotrophs, pathogens). We identified symbiosis-related genes by profiling fungal transcripts during symbiosis.

We analysed the transcriptomes obtained through an RNA-Seq experiment from *O. maius* Zn and from its host plant *V. myrtillus* grown under normal and Cd stressed condition, in the free living and in the mycorrhizal status.

Different experimental setups (non-compartmented and compartmented co-culture plates) were used to investigate the influence of both soluble and volatile fungal molecules on the non-host plant *A. thaliana*.

Key results —

- ERM fungi gene contents for polysaccharide-degrading enzymes, lipases, proteases, and for enzymes involved in secondary metabolism are closer to those of saprotrophs and pathogens than of ECM symbionts. Among the most highly symbiosis-upregulated genes in ERM fungi are fungal- and plant-cell wall degrading enzymes (CWDE), lipases, proteases, transporters and mycorrhiza-induced small secreted proteins (MiSSPs).
- OmSSP1, the most highly symbiosis up-regulated MiSSP in *O. maius*, was found to share some features with fungal hydrophobins, even though it lacks the Pfam hydrophobin domain. Sequence alignment with other hydrophobins and hydrophobin-like fungal proteins placed OmSSP1 within Class I hydrophobins. However, the predicted features of OmSSP1 may suggest a distinct type of hydrophobin-like proteins. The presence of a predicted signal peptide and a yeast-based signal sequence trap assay demonstrate that OmSSP1 is secreted during symbiosis. OmSSP1 null-mutants showed a reduced capacity to form ericoid mycorrhiza with *Vaccinium myrtillus* roots, suggesting a role as effectors in the ericoid mycorrhizal interaction.
- The root tissues of non-mycorrhizal plants exposed to Cd accumulated more Cd than the roots of mycorrhizal plants. The higher Cd content in non-mycorrhizal roots mirrors the higher abundance of transcripts coding for stress related proteins, such as HSPs. Regulated plant metal transporters have been identified that may be responsible for the reduced Cd content in mycorrhizal plants exposed to this metal.
- The ERM fungus *O. maius* promoted growth of the non-host plant *A. thaliana* in all experimental setups. A peculiar clumped root phenotype, characterized by shortening of the primary root and by an increase of lateral root length and number, was observed in *A. thaliana* in the non-compartmented plates, suggesting that soluble diffusible molecules are responsible for this root morphology.

Main conclusions including key points of discussion — The gene repertoire of ERM fungi reveals a capacity for a dual saprotrophic and biotrophic lifestyle. This may reflect an incomplete transition from saprotrophy to the mycorrhizal habit or, alternatively, a versatile life strategy similar to fungal endophytes.

Our data demonstrate for the first time the importance of MiSSPs in the ERM symbiosis. The peculiar features of fungus-host plant interaction in ERM, together with the distinctive features of OmSSP1 as compared to typical Class I hydrophobins, may suggest functions specific to the ERM symbiosis.

The low proportion of up-regulated stress responsive genes in mycorrhizal roots exposed to Cd may be due to the lower Cd content measured in mycorrhizal roots and to the fact that the antioxidant GSH metabolism is activated in plant roots by the symbiosis. Expression data also outlined some regulated plant metal transporters, either involved in metal uptake or in metal efflux, which may be responsible for the reduced Cd content observed especially in mycorrhizal roots.

A. thaliana is becoming a recognized model to analyse both mutualistic and non-mutualistic plant-microbe interactions, and several plant growth-promoting fungi have been shown to promote growth of this plant *in vitro*. We have confirmed this observation for ERM fungi, as well as for other mycorrhizal and non-mycorrhizal fungi. *O. maius* as well as about half of the other fungi tested induced in *A. thaliana* a peculiar clumped root phenotype, likely caused by diffusible soluble fungal compounds. Although the nature of these fungal compounds is as yet

unknown, the inability of an *O. maius* mutant impaired in its N metabolism to induce this root phenotype should help us to elucidate the mechanisms involved.

Future perspectives —

- To investigate the cellular localisation of OmSSP1 to better understand its role in symbiosis.
- To investigate the localization and the potential roles of plant transporters up-regulated upon Cd exposure in mycorrhizal roots.
- To elucidate the nature of the diffusible molecule responsible for the clumped-roots phenotype observed in *A. thaliana* plants when grown in the presence of some of the fungi tested

Valorisation —

Publications

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Presentations

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