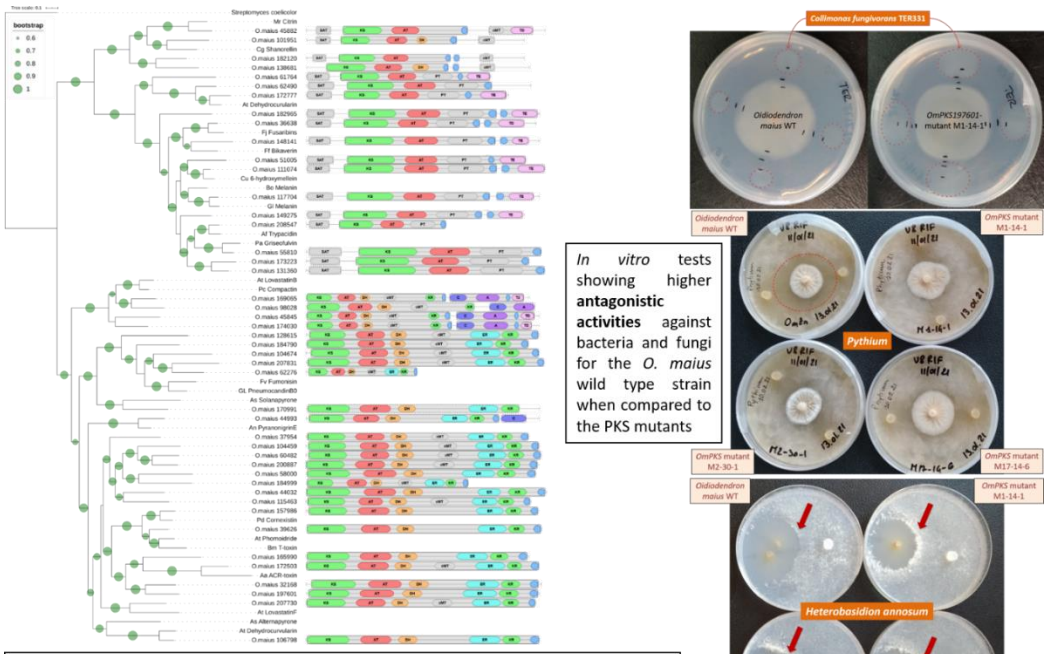
# MycorPKS




*In vitro* tests showing higher **antagonistic activities** against bacteria and fungi for the *O. maius* wild type strain when compared to the PKS mutants

**Maximum Likelihood phylogenetic tree of *O. maius* PKS and sequences as well as protein sequences from well-characterized PKS genes from other species.** Genealogy of PKSs and PKS-NRPSs was inferred by Maximum Likelihood (PhyML) of the aligned amino acid sequences of the KS domains. Products of each well-characteized PKS enzyme are indicated on the tree. Branch length indicates number of inferred amino acid changes. Only branch nodes with >50% support are shown. PKS and PKS-NRPS domains from *O. maius* were annotated using the anti-SMASH 5.0 tools

## *Isolation, identification, structural and functional characterization of new polyketides produced by the mycorrhizal fungus Oidiodendron maius*

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*Action thématique concernée : WP1*

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***Context*** — Fungi produce a wide variety of biologically active secondary metabolites, a large proportion of them being polyketides, they are produced by polyketide synthases (PKSs). Fungal polyketides play a role in the ecological and evolutionary adaptation of fungi: they can be toxin precursors, pigments important for virulence or for resistance to abiotic stresses, they are also important for growth, sexual development and spore production. So far, the study of fungal polyketides has been limited by the difficulty of identifying and characterizing the polyketide itself. Only recently a more in-depth analysis of the genetic potential of fungi for polyketide production became possible thanks to the rapid increase of the fungal genome sequencing projects. Now selective cloning of genes encoding fungal PKSs may precede the identification of the produced metabolite, contributing to the diversity and functional analysis of fungal polyketides.

While there is an extensive literature on the role of PKS in pathogenic interactions or in biological control, no data are available concerning the role of PKSs and the produced polyketides in the symbiotic interactions. A comparative genomic study of 60 fungi with different taxonomy and ecology showed that *Oidiodendron maius*, an endomycorrhizal symbiotic fungus, contains so far the largest number of genes encoding PKSs. In addition, the available transcriptomic data provided a list of the most induced genes during the symbiosis between *O. maius* and its host plant, including several genes encoding PKSs.

***Objectives*** — The general objective of the project was to characterize some polyketide synthases of the fungus *O. maius* in order to understand their role in the molecular signaling between the fungus and the host plant, and to identify new molecules with a potential interest for pharmacology, ecological engineering, biocontrol and stress response. In particular, the aims of the project included: i) in silico prediction of Biosynthetic Gene Clusters in the *O. maius* genome and analysis of the expression in symbiosis; ii) domain prediction, phylogenetic analysis and comparison with other fungal functionally characterized enzymes of *O. maius* PKSs and iii) generation of mutants lacking PKSs genes highly regulated in symbiosis, in order to analyse their mycorrhizal phenotype, their response to abiotic stresses and their antagonistic activities when compared with the wild type strain

***Approaches*** — Construction of the OmPKS197601-Disruption Vector and *Agrobacterium*-Mediated Transformation: in order to obtain knock-out mutants, plasmids were designed for three selected PKS genes and cloned in the pCAMBIA0380_HYG vector. The pCAMBIA0380_HYG_PKS197601 vector was cloned into *Agrobacterium tumefaciens* LBA1100, and used to transform ungerminated *O. maius* conidia according to the protocol described in Abbà et al. (2009). Phylogenetic and Bioinformatic Analyses - Prediction of biosynthetic gene clusters: antiSMASH 4.1 was used for prediction of biosynthetic gene clusters performed on the INRAE-Nancy computing cluster. Global up/down regulated gene clusters were determined and a hierarchical clustering analysis was made with the normalized log2 reads of the genes using a custom R script. Integration of multi-omics data and visualization: differential expression of genes was calculated from RNA-seq data. Transcript read counts from biological replicates from the mycorrhizal roots were used to calculate log2 fold changes against the free-living mycelia with DESeq2. Secreted proteins were predicted using methods described previously (Pellegrin et al., 2015). CAZy annotations were provided from CAZy team (www.cazy.org). Transposable element (TE) identification was performed with Transposon Identification Nominative Genome Overview (TINGO; Morin et al., 2019). Finally, output files obtained from the various analyses and functional annotations from JGI Mycocosm were cleaned, sorted, combined and visualised with R package karyoploteR using a set of custom R scripts, Visually Integrated Numerous Genres of Omics (VINGO). PKS domain description and Phylogenetic Analyses: a phylogenetic tree was constructed with 47 KS protein domain sequences from *O. maius*, as well as the protein sequences of some well-characterized PKS enzymes from other fungal species: the phylogenetic tree was reconstructed using the maximum likelihood method implemented in the PhyML program (v3.1/3.0 aLRT). iTOL tree of life was used to visualise the phyylogenetic tree.

***Key results*** —

- In silico prediction of Biosynthetic Gene Clusters (BGCs) in the genome of *O. maius* and analysis of the expression in symbiosis: using antiSMASH a total of 59 gene clusters were predicted on the genome of *O. maius*. Out of these, 25 PKS and 11 PKS-NRPS hybrids were identified. A total of 3 gene clusters co-up/down-regulated were identified under the conditions of mycorrhizal formation according to the RNA-seq data set analysed.
- Global genomic view of predicted gene clusters: differential transcription of genes between two conditions (Mycorrhizal roots vs Free living mycelia) was calculated and showed using hierarchical clustering analysis. A total of 47 PKS genes were combined with the normalized reads of the genes using DESeq2. Three PKS coding genes were highly regulated under the mycorrhizal condition: PKS 197601 and PKS200887 were upregulated and PKS174030 was highly downregulated in the mycorrhizal condition compared to the free-living mycelium.
- Domain prediction and phylogenetic analysis: Blastp analysis was done for all of the *O. maius* predicted PKSs and PKS-NRPS hybrids protein sequences, using the NCBI database. A phylogenetic tree was constructed with all of the KS protein domain sequences from *O. maius*, as well as the protein sequences of some well-characterized PKS enzymes from other fungal species.

- Among the three PKS coding genes highly regulated under the mycorrhizal condition, mutants lacking the 197601 PKSs gene were generated in order to analyze their mycorrhizal phenotype. 700 mutants were collected in single culture and a PCR-screening was performed. The negative result of the PCR confirmed the knock-out of the PKS 197601 gene in three *O. maius* PKS 197601 knock-out mutants. A Southern blot analysis was performed to validate the mutants obtained. The three *O. maius* candidate mutants were used for *in vitro* mycorrhizal syntheses, for growth tests in the presence of Cd and for biocontrol experiments. No significant differences were observed among the wild type and the PKSs mutants while performing mycorrhization and Cd growth tests, while bacterial and fungal biocontrol tests showed promising results.
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***Main conclusions including key points of discussion —***

- Mutants lacking PKSs genes highly expressed in symbiosis did not show a significant different phenotype when compared to the wild type strain
- Likewise, PKS mutants didn't showed higher impaired ability to grow on Cd enriched media when compared to the wild type strain
- On the other hand, PKS mutants seems to have lower biocontrol ability against selected bacterial and fungal strains when compared to the wild type strain. More *in vitro* tests to confirm these observations are being implemented
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***Perspectives —***

In order to identify some (new) polyketides produced by *O. maius* the heterologous expression of selected *O. maius* PKSs in yeast is envisaged. No data are currently available on the polyketides produced by this symbiotic fungus, and future investigations should provide a better picture of its genetic potential in the polyketide biosynthetic pathway, as well as the characterization of some of these compounds, their potential role and the possible identification of new molecules with a potential interest for biocontrol, pharmacology and ecological engineering

**Valorization —**

**Belmondo S., Daghino S., Miyauchi S., Wu G., Meloni D., Aiello C., Collin S., Kohler A., Perotto S., Martino E., Jacob C**. Polyketide synthases in the ericoid endomycorrhizal fungus *Oidiodendron maius*. 15th European Conference on Fungal Genetics (ECFG15); 17 – 20 February **2020**, Rome, Italy. Poster presentation

**Belmondo S., Daghino S., Miyauchi S., Wu G., Meloni D., Aiello C., Collin S., Kohler A., Perotto S., Martino E., Jacob C.** Characterization of polyketide synthases in an ericoid endomycorrhizal fungus using a molecular approach. 1st Conference for Young Botanists (CYBO); 6 – 7 February **2020**, Genova, Italy. Oral presentation

**Martino E., Daghino S., Belmondo S., Meloni D., Miyauchi S., Collin S., Kohler A., Jacob C., Perotto S.** Polyketide synthases in the ericoid endomycorrhizal fungus *Oidiodendron maius*. 114° Congresso S.B.I. (IPSC); 4 – 7 September **2019**, Padova, Italy. Poster presentation.

## Leveraging effect of the project—

Contract for Simone Belmondo, post-doc from the Turin University group, working at the project (01/06/2019 – 31/05/2020); new contract for Marco Chiapello, post-doc from the Turin University group, working at the project (01/12/2021-30/11/2022)