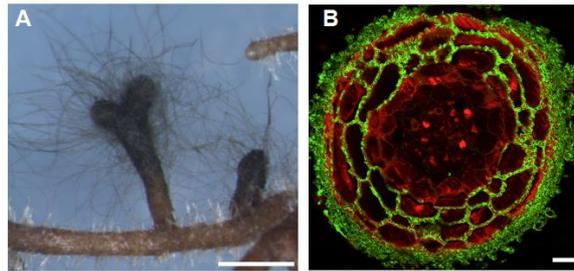
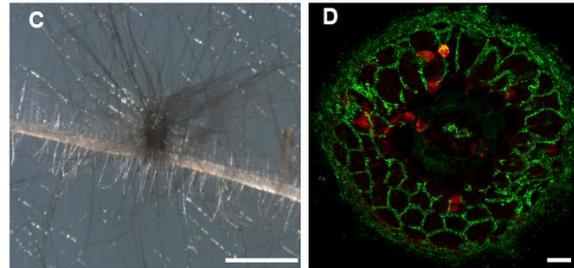


BLACKSECRET

Pinus sylvestris



Populus tremula × *Populus alba*



Blacksecret 2

Principle investigator: Annegret Kohler (& Martina Peter Forest Dynamics WSL)

Partners: WSL Birmensdorf/Suisse

Collaboration : Claire Veneault-Fourrey (IAM), Maira de Freitas Pereira (postdoc IAM INRA/ Forest Dynamics WSL)

Context — In nature, soil-born fungi associate with roots of trees to form ectomycorrhizas (ECM). In exchange for carbohydrates, ECM fungi improve mineral supply of host trees, protect them against stresses such as drought and directly contribute to the exclusion of competitive microbes. As mycorrhizal fungi form intimate intracellular contacts with living plant cells they need to manipulate host defenses and metabolism. Recent studies highlight that mycorrhizal fungi, similar to pathogens, use mycorrhiza induced small-secreted proteins (MiSSPs) as molecular keys to promote symbiosis.

Objectives — The main goal of this project was to elucidate the role of effector-like SSPs in the mycorrhiza formation and functioning of the ecologically very important species *Cenococcum geophilum* by taking advantage of available genomic and transcriptomic resources.

Approach — We combined different approaches such as gene expression profiling, genome localization and conservation of MiSSP genes in different *C.geophilum* strains and closely related species as well as protein localization studies of potential targets of MiSSPs in interacting plants using tobacco leaf cells.

Key results —

- Gene expression analyses of *C. geophilum* interacting with the gymnosperm *Pinus sylvestris* (pine) and the angiosperm *Populus tremulaxPopulus alba* (poplar) showed that similar sets of genes coding for secreted proteins were up-regulated and only very few were specific to each host; whereas pine induced more carbohydrate active enzymes (CAZymes), the interaction with poplar was enriched with SSPs. From these data sets we identified 22 candidate effector proteins (MiSSPs) for further characterization.

- We used 15 re-sequenced *C. geophilum* strains from different European countries and closely related dothideomycetous genomes to study intra- and interspecific diversity in the sequences of our candidate effectors. Interestingly, some MiSSPs are conserved in all genomes while others are absent in several of the studied re-sequenced genomes. When looking at similarities in presence–absence of the 22 MiSSPs among the *C. geophilum* isolates, clade affiliation explained best the polymorphism, whereas country origin marginally and the forest type did not significantly explain these patterns.
- Finally, we showed that six CgMiSSPs target four distinct sub-cellular compartments such as endoplasmic reticulum, plasma membrane, cytosol and tonoplast in a heterologous tobacco leaf system.

Main conclusions including key points of discussion — Overall, this work presents a first comprehensive analysis of secreted proteins and MiSSPs in *C. geophilum*. We could show that *C. geophilum* use a very similar arsenal to communicate with Pine and Poplar roots, with nevertheless differences in expression level for CAZymes or SSPs, respectively. Some of these MiSSPs are well conserved among *C. geophilum* strains from different geographic origin while others are absent in numerous strains. These differences in conservation among strains could reflect different roles of these small-secreted proteins, either as effectors, in signaling or in plant-microbe interface forming.

Future perspectives — Since an efficient protocol for *C. geophilum* transformation has not been developed yet, it would be interesting to use alternative methods to decrease SSP transcript expression in order to demonstrate their essential role for ectomycorrhiza formation. One promising method could be the use of double stranded interfering (dsi) RNA to knock down transcription of SSPs. This method has been successfully used for different plant-fungus systems, such as *Botrytis cinerea*, an aggressive fungal pathogen or *Pisolithus albus*, an ectomycorrhizal fungus of *Eucalyptus* sp. We propose to test it for a selection of the above studies *C. geophilum* MiSSPs.

Valorisation —

The first BLACKSECRET publication will be submitted End of November 2017 to the Research Topic “Mycorrhizosphere Communication: Mycorrhizal Fungi and Endophytic Fungus-Plant Interactions” in *Frontiers in Microbiology*. A second publication is in preparation and submission is planned for January 2018.

The post-doctoral fellow (Maira de Freitas Pereira) attended several conferences and presented her results by poster or oral presentations. One of these conferences was the French consortium “Effectome” where she met national and international researchers working on effectors of plant-associated microorganisms. She attended the international Molecular Mycorrhiza Meeting (iMMM) in Toulouse as well as Swiss conferences such as, Zürich Mycology Symposium 2017 (ETH Zürich 27.1.2017), Host-Microbes Genomics 2017 (Neuchâtel 8.9.2017), good opportunities to present BLACKSECRET and to discuss with Swiss researchers.

In addition, we presented our project and LABEX Arbre to a broad community via television (<http://www.nancy.inra.fr/Toutes-les-actualites/Le-reseau-NFZ-au-caeur-d-un-reportage-de-France-3-Lorraine>) and radio (<http://www.radiocrystal.org/podcasts/l-invite.html>).