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Advanced Research on the Biology of Tree and Forest Ecosystems

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PROGRAMME

- **9h15 :** WELCOME

SESSION 1

Signalisation et mise en place de la symbiose / Signaling and symbiosis establishment

- 9h30-9h50 : Feng ZHANG
  Purification and characterization of a symbiosis-induced endocellulase from the ectomycorrhizal symbiont *Laccaria bicolor*
- 9h55-10h15 : Maira DE FREITAS PERREIRA
  Secretome analysis from the ectomycorrhizal ascomycete Cenococcum geophilum

COFFEE BREAK

SESSION 2

Approches moléculaires et intramoléculaires / Molecular and intramolecular approaches

- 10h45-11h05 : Marie GROSDIDIER
  Spatio-temporal modeling of invasive pathogen
- 11h10-11h30 : Thomas PERROT
  Study of the link between omega glutathione transferases and polyphenols: identification of ligands
- 11h35-11h55 : Agathe MAUPETIT
  Genomic consequences of a major selective event in the poplar rust
- 12h00-12h20 : Elena HEGO
  PAM mineralization by brown and white rot fungus strains

LUNCH BREAK

SESSION 3

Impact des facteurs environnementaux sur les communautés microbiennes / Impact of edaphic parameters on microbial communities

- 14h00-14h20 : Milena GONZALO
  Understanding the molecular dialogues within forest soil microbial communities and investigating their impact on plant's health and growth
- 14h25-14h45 : Na WU
  Microbial Community in the Rhizosphere of *Populus cathayana* at Chaka Salt Lake and Sex-specific Responses of Arbuscular Mycorrhizal *P. cathayana* to Salinity
- 14h25-14h45 : François MAILLARD
  Short term effects of leaf litter and dead wood harvesting on tree roots physiology and associated ectomycorrhizal corteges and on soil microbial taxonomic and functional diversity in temperate deciduous forest

SESSION 4
Exploration des génomes / Genome mining

- 14h50-15h10 : Shingo MIYAUCHI
  Visualisation of genome-wide omics data with SHIN+GO
- 15h15-15h35 : Cécile LORRAIN
  ***Melampsora larici-populina* v2.0: Re-evaluating the secretome of the poplar rust fungus combining transcriptomics and comparative genomics to target promising candidate effectors**

COFFEE BREAK

POSTERS SESSION
16h00-17h30

- Véronica BASSO
  Effector proteins from a symbiotic fungus may target phytohormone signaling to favor colonization
- Simone BELBONDO
  AscoTube: in vitro and in situ unraveling truffle sexual reproduction using Ascobolus immersus as a test tube model
- Cécile LORRAIN
  The poplar rust fungus effector biology: challenges of functional characterization of effectors in a non-model pathosystem
- Lauralie MANGEOT-PETER
  Defense-related phytohormones alter the structuring of the root microbiome in Grey poplar
- Duy Vuong NGUYEN
  Generating the mutants of *Phanerochaete chrysoporium* RP 78 resistant to wood extractives for functional characterization of the detoxification system of white rot fungi
- Océane NICOLITCH
  Exploration of the functional and taxonomic diversity of the bacterial communities occurring in the bedrock/root interface of beech trees
- Jonathan PRZYBYLA-TOSCANO
  Mitochondrial Arabidopsis thaliana NFU transfer proteins: cooperation with ISCA proteins to deliver [4Fe-4S] cluster to specific apo-targets
- Mélanie ROLAND
  The cellular maturation of iron-sulfur proteins in plants

BARBECUE

SESSION 1

Signalisation et mise en place de la symbiose
Signaling and symbiosis establishment

Purification and characterization of a symbiosis-induced endocellulase from the ectomycorrhizal symbiont *Laccaria bicolor*

Zhang F[1], Champion C[2], Haon M[2], Anasontzis G[2], Kemppainen M[3], Pardo A[3], Daguerre Y[1], Deveau A[1], Veneault-Fourrey C[1], Kohler A[1], Rosso Mn[2], Henrissat B[4], Berrin Jg[2], Martin F[1]

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In forest soils, ectomycorrhizal fungi establish a mutualistic symbiosis with tree roots. The mutualistic fungi trade host photoassimilates against soil nitrogen and phosphorus. Differentiation of symbiotic roots induces extensive cell wall architectural modifications in the host apoplastic space. The origin of enzymes involved in these cell wall modifications has been the subject of debate for several decades.
The ectomycorrhizal basidiomycete *Laccaria bicolor* has a restricted set of carbohydrate-active enzymes (CAZymes) degrading plant cell wall polysaccharides. However, several of those genes are upregulated upon symbiosis. We speculate that several of the symbiosis-induced CAZymes are involved in the remodeling of the host apoplastic space. Here, we characterize the sole GH5 endoglucanase with a cellulose-binding motif (CBM1) domain (LbGH5) identified in the genome of *L. bicolor*. We showed that the *LbGH5* gene is induced five folds in ectomycorrhizal roots using qPCR and RNA-Seq. RNAi mutants with a decreased expression of *LbGH5* have a lower ability to form ectomycorrhizal roots. Yeast secretion trap (YST) functional screen confirmed that LbGH5 is a secreted protein. We then produced and purified the recombinant protein LbGH5 with and without its CBM1 domain in *Pichia pastoris*. The recombinant LbGH5 displayed highest actitivities towards carboxymethyl cellulose (CMC) and cellulose extracted from aspen roots. In contrast, LbGH5 displayed no activities toward *L.bicolor* cell walls or aspen hemicellulose. *In situ* localization of LbGH5 in ectomycorrhizal roots by indirect immunofluorescence confocal microscopy demonstrated that the enzyme accumulates in hyphal cell walls forming the mantle and Hartig net. These data suggest that cell wall modifications within ectomycorrhizal roots arise from cell wall–modifying enzymes of fungal origin.

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Secretome analysis from the ectomycorrhizal ascomycete *Cenococcum geophilum*

Maíra de Freitas Pereira[1,3], Claire Veneault-Fourrey[1,2] Patrice Vion[1], Frédéric Guinet[1,2], Emmanuelle Morin[1], Claude Murat[1] , Stephanie Pfister[3] Simon Egli[3], Igor Grigoriev[4], Francis Martin[1] , Annegret Kohler[1] , Martina Peter[3]

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Cenococcum geophilum is an ectomycorrhizal fungus (EcM) with global distribution in numerous habitats and associate with a large range of host species including, gymnosperm and angiosperm trees. Moreover, *C. geophilum* is the unique ectomycorrhiza fungi belonging to the Dothideomycetes family, a predominantly family containing many saprotrophic and phytopathogenic fungi. The molecular dialogue between fungi and plants as well as the capacity of mycorrhizal fungi to colonize plant tissues without activating plant defense systems remains elusive. Recent studies highlight that mycorrhizal fungi, as pathogenic ones, use effectors in form of Small Secreted Proteins (SSPs) as molecular keys to promote symbiosis. Our aim is to elucidate the role of effector-like SSPs in ectomycorrhiza formation and their function during interaction of *C. geophilum* with *Pinus sylvestris* roots. Taking advantage of available *C. geophilum* genomic and transcriptomic resources, 22 candidate effectors were identified. The genome analysis from 15 re-sequenced *C. geophilum* strains allowed the detection of intra-species diversity in evolution and conservation of these sequences. For functional analysis, we cloned 22 candidate SSPs in *Nicotiana benthamiana* leaf cells to determine their subcellular localization. Confocal microscopy revealed that six candidate effectors target the endoplasmic reticulum, plasma membrane, cytosol, tonoplast or cytosol bodies. Experiments are on-going to confirm their localization and also to detect potential targets from *Populus* and *Pinus* proteins in order to understand this ectomycorrhizal association.

SESSION 2

Approches moléculaires et intramoléculaires
Molecular and intramolecular approaches

Spatio-temporal modeling of invasive pathogen

Grosdidier M., Ioos R., Marcais B., Fritsch C., Gegout-Petit A.

H. fraxineus is an invasive pathogen of *Fraxinus excelsior* and *Fraxinus angustifolia*. Introduced in Europe in the 90's, it spread rapidly and was devastating for ashes ecosystems. This heterothallic fungus disperses via air-born ascospores produced by apothecia developing in spring on ash rachises in the litter. Environmental factors favorable for the pathogen dispersal are still poorly understood. However, literature reports importance of summer temperatures above 35°C in limiting the disease. Our landscape approach showed that high humidity of the litter where rachises of previous years are deposited is very favorable to disease development while isolated trees out of forest environment offer a less conducive environment. Moreover, preliminary results suggest the presence of a significant Allee effect on *H. fraxineus* inoculum production which could influence its dispersal. Our objective is to model the airborne spread of *H. fraxineus* with reaction-diffusion spatio-temporal model using disease survey data acquired by the Forest Health Survey System in France since 2008. The model was adapted from a reaction-diffusion model realized by *Roques et al.* (2011) for pine processionary moth.

Study of the link between omega glutathione transferases and polyphenols: identification of ligands

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Fungi play a key role in the organic matter recycling and some of them; especially basidiomycetes are the most efficient microorganisms to degrade lignocellulosic materials. To perform such degradation, these organisms have developed different strategies based on oxidative processes either using specific degradation enzymes or producing hydroxyl radicals by non-enzymatic Fenton reactions. Wood decaying fungi are thus in contact with many compounds resulting from wood decay and also compounds already present in wood. Among these latters, wood extractives (flavonoids, terpenoids, stilbenes…) are potentially toxic. To cope with this potential harmful environment, fungi have developed detoxification system involving multigenic families such as cytochrome P450 monooxygenases (involved in the first oxidation step of detoxification) and glutathione transferases (acting in the second conjugaison step). It has been showed that several isoforms of fungal glutathione transferases are able to interact with extracts of various wood species. However, their roles in the fungal endogenous metabolism remain mysterious.

We have particularly worked on glutathione transferases from the white-rot *Trametes versicolor* focusing on isoforms belonging to the omega class (TvGSTO). Among the sixteen TvGSTOs identified in this fungus, we report here the biochemical and structural characterization of six isoforms. By using biochemical methods, we have found that at least one flavonoid and some benzophenones could interact with TvGSTOs.

Genomic consequences of a major selective event in the poplar rust

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Poplar is an important forestry product in Europe, both for the wood industry and for its contribution to energy production systems. The main threat to the growth of poplar is the poplar rust disease, caused by the basidiomycete *Melampsora larici-populina*. The selection of poplar cultivars expressing qualitative resistance has led to repeated failures since 1980, because of a strong selective pressure due to monoclonal fields. Several resistance breakdowns have been observed with the emergence of new virulence factors, resulting in the breakdown of all resistance types. The most spectacular breakdown occurred for resistance 7 (R7) in 1994 in Belgium and the North-East of France. *M. larici-populina* has been sampled before and after R7 breakdown.

We analyzed the population structure and the genome scan on four populations (the first sampled before the breakdown; the second sampled during and after the breakdown; the third sampled on wild poplar). The genome scan allowed to identify 20 genomic regions with potential signature of selective sweep (Persoons et al. 2017). Furthermore, a genome-wide association study highlighted a genomic region associated with the virulent/avirulent 7 *M. larici-populina* phenotype. This genomic region may contain the first avirulent gene identified for this fungus.

Key words: pathogenic fungi, genom scan, genome-wide association study (GWAS), population structure, selective sweep

PAM mineralization by brown and white rot fungus strains.

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Although acrylamide monomers are acutely toxic for organism, anionic acrylamide polymers (or polyacrylamide, PAM) are inert products, widely used for soil quality improvement, sewage disposal or industrial processes (Sojka *et al.*, 2007). Environmental legislation now raises the question of PAM evolution in treated soils, and of its biodegradation in natural conditions. Filamentous wood-decaying fungi, mainly belonging to two functional classes, white and brown-rot fungi contribute to soil mineralization processes. While the white-rot decay mechanisms are mainly based on enzymes secretion, the brown-rot results from a non-enzymatic strategy involving non-specific Fenton reaction (Lundell *et al.*, 2010). The aim of this study was to assess the ability of a brown-rot species, *Gloeophyllum trabeum* to degrade and mineralize PAM. Three other species, *Postia placenta*, *Phanerochaete chrysosporium* and *Trametes versicolor*, were also considered for exploratory assays.

As previous studies have shown that microorganisms are not able to grow on PAM as sole carbon source (Kay-Shoemake *et al.*, 1998), a co-metabolism strategy was retained. To discriminate between mineralization of chosen substrates (glucose and PAM), ^{13}C labeled substrates were used and then CO_2 production and its ^{13}C enrichment monitored. First results confirmed that PAM was not able to sustain fungal growth in liquid medium as sole C source. However, in co-metabolism experiments (PAM+glucose), an increase of both CO_2 and biomass productions was observed in comparison with cultures on glucose alone. The use of ^{12}C PAM + ^{13}C glucose indicated that PAM is poorly mineralized by fungi, glucose remaining the main consumed substrate. This weak consumption did not allow to quantify PAM mineralization. However, in presence of labelled PAM, the increase of ^{13}C enrichment of measured CO_2 was significantly higher than the natural one, demonstrating PAM mineralization by *G. trabeum*. The mineralization rate appeared dependent of glucose concentration, being higher when glucose content is low.

Taking together, those results suggest that PAM, in presence of glucose, stimulated fungal growth in a liquid synthetic medium, but the reasons remain unclear. PAM might act as additive nitrogen source, or provide a better support for fungal growth in increasing the medium viscosity. To investigate these hypothesis, additional assays have been started using sawdust, varying PAM concentrations and fragmented PAM products.

Sojka R. E., Bjorneberg D. L., Entry J. A., Lentzl R. D. and Orts W. J., 2007, Polyacrylamide in agriculture and environmental land management. *Advances in Agronomy*, *92,* 75-162.

Lundell T. K., Mäkelä M. R. and Hildén K., 2010, Lignin-modifying enzymes in filamentous basidiomycetes – ecological, functional and phylogenetic review. *Journal of Basic Microbiology*, *50*, 5-20.

Kay-Shoemakea J. L., Watwooda M. E., Lentzb R. D., Sojkab R. E., 1998, Polyacrylamide as an organic nitrogen source for soil microorganisms with potential effects on inorganic soil nitrogen in agricultural soil. *Soil Biology and Biochemistry*, *30* (8–9), 1045-1052.

SESSION 3

Impact des facteurs environnementaux sur les communautés microbiennes
Impact of edaphic parameters on microbial communities

Understanding the molecular dialogues within forest soil microbial communities and investigating their impact on plant's health and growth

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Forest tree roots are colonized by numerous microbes, such as bacteria and fungi. These microbial communities are shaped by the nutrients available and the edaphic conditions. In rhizospheres, bacteria interact with plant roots and mycorrhizal fungi, helping with the carbon, nitrogen and phosphorus cycle as well as the uptake of nutrients and water. Particularly, the ectomycorrhizosphere is a hotspot of microbial interactions since there is an exchange of secondary metabolites and signal molecules with the purpose of communication, competition, or collaboration to adapt to changing soil conditions. Despite the relevance of these compounds for plant development, little is known about their specific role and production due to the complexity of plant-microbe metabolic interconnections. The aim of this project is to understand how microbes influence tree development and how plants take part in these metabolic exchanges. To study these microbes, soil samples were extracted from a forest in Champenoux, which is characterized by oak and beech trees. In order to examine different microniches, samples such as the rhizosphere compartment (with the presence or absence of ectomycorrhiza), wood decay, and surrounding bulk soil were taken. Bacterial communities were characterized through metagenomics, classical cultivation and functional screening. First results showed a variation on bacterial and fungal communities within microniches, which might indicate that communities are specialized according to the site. In the near future, we plan to mimic soil conditions and recreate a microbial consortium to visualize plant-microbe interactions using imaging mass spectrometry and confocal microscopy.

Microbial Community in the Rhizosphere of Populus cathayana at Chaka Salt Lake and Sex-specific Responses of Arbuscular Mycorrhizal P. cathayana to Salinity

Na Wu

Gender effect may cause significant alterations in microbial community. However, little is known regarding changes in response to diecious plants. This study aimed to have a research on the microbial community in the rhizosphere of the typical dioecious plant *Populus cathayana* under salt stress and sex-specific response to AMF and salt stress at physiological, biochemical and molecular level. Results showed that gender had significant effects on microbial communities and suggested potential interaction effects between plant gender and microbial community. Meanwhile, the impact of arbuscular mycorrhizal fungi (AMF) on the physiological, biochemical and molecular level of the typical dioecious plant *Populus cathayana* exposed to salinity was investigated. Our results showed that salt ions interfered photosynthesis systems, water status, osmotic adjustment, antioxidant systems, ion balance of *P. cathayana* under salt stress. Males had a higher mycorrhizal dependency and exhibited a better salt tolerance than females. With the help of AMF, P. cathayana cuttings grown under salt stress could acquire increased tolerance to salt stress, especially in males.

Short term effects of leaf litter and dead wood harvesting on tree roots physiology and associated ectomycorrhizal fungi and on soil microbial taxonomic and functional diversity in temperate deciduous forest

François Maillard, Valentin Leduc, Chloé Viotti, Cyrille Bach, Laure Fauchery, Jacqueline Marchand, Bernhard Zeller, Laurent Saint-André, Dominique Gérant, Marc Buée

Forest ecosystem functions support the provision of ecosystem services affecting human health and wellbeing, as climate regulation, biodiversity protection, woody biomass production, etc. These forest ecosystem functions can be altered by the decrease of the soil organic matter arising from forest harvesting. But currently, impacts of intensification of forest management practices remain poorly studied in temperate forests. Soil microbiological processes and tree physiology are directly linked to the forest productivity. We conducted two distinct experiments to assess the effects of a decrease of soil organic matter on microbiological processes and tree physiology. The first experiment was to evaluate the impact of leaf litter and deadwood harvesting on soil taxonomic and functional diversity. The second experiment was focused on non-structural carbohydrate contents in tree fine roots and on ectomycorrhizae exoenzymes activities in response to organic matter removal. This study was conducted on six forest sites in France, which belong to the MOS (matière organique des sols) experimental network. At each site, leaf litter and deadwood were removed each year since three years. Manipulated plots were compared to reference plots, which are not manipulated (traditional management). We applied enzymatic profiling on soil and ectomycorrhizae and Biolog Ecoplate approach to study the functional diversity of the microbial communities. The structure of the bacterial and fungal communities was assessed by Illumina Miseq sequencing of ribosomal marker sequences. The non-structural carbon compounds of fine roots were analyzed by ion chromatography. After only three years of organic matter removing, functional and taxonomic diversity of soil bacterial and fungal communities have been significantly impacted. Bacterial carbon substrate metabolization capacities and fungal exoenzymes related to organic carbon and nitrogen mobilization decrease significantly in the manipulated plots. Structure of the fungal and bacterial communities was affected also. The decrease of fungal saprobic taxa, as the Mortierella genus, which are very sensitive to organic matter removing was counterbalanced by a significant increase of the ectomycorrhizal fungi relative abundance. Fine roots non-structural carbon compounds were not affected by the treatment but enzymatic profiling of the associated ectomycorrhizae revealed an increase of the cellulosic enzyme activities in response to leaf litter and deadwood harvesting. There results demonstrate the short-term response of both soil microbial communities and tree physiology to the soil organic matter removal. In the future, the monitoring of these functional and taxonomic indicators could anticipate potential tipping points and prevent decline of intensively harvested forests.

SESSION 4

Exploration des génomes
Genome mining

Visualisation of genome-wide omics data with SHIN+GO

Shingo Miyauchi

Genome-wide transcriptomic and secretomic activities are complex. Capturing just a single time point of fungal transcriptomic activity involves over ten thousands genes showing various transcription levels. The number of observations increases exponentially when we add the number of biological replicates, different growth conditions, and time points. The addition of secretomic information gives an extra layer of complexity.

To extract biologically meaningful patterns from such high-dimensional omics data, we have developed the multi-omics profiling platform, Self-organizing map Harboring Informative Nodes with Gene Ontology (SHIN+GO). Genome-wide omics models constructed with the platform are designed to pinpoint biological activities of interest that would otherwise be buried in the high-dimensional data.

As one of the key components of this platform, Self-organizing map (SOM) is an algorithm constructing a neural network with given input data in an unsupervised manner. SOM reduces the number of features in high-dimensional data by grouping similar items and forming clusters. It has a unique property of making two-dimensional maps suitable for large-scale data visualization. The platform has been used to generate neural networks of genome-wide genes and identify condition-specific responses in transcriptomes of newly sequenced fungal species with limited gene annotations.

Melampsora larici-populina v2.0: Re-evaluating the secretome of the poplar rust fungus combining transcriptomics and comparative genomics to target promising candidate effectors

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The poplar rust fungus *Melampsora larici-populina* causes annual epidemics damaging European poplar plantations. Understanding the molecular mechanisms underlying the infection process is essential for durable disease management. The initial sequencing and annotation of the *M. larici-populina* genome was performed 6 years ago and revealed a large repertoire of secreted proteins and most of which were species-specific. Since, several rust fungi genomes have been made available, allowing for comparative genomic studies to determine gene specificity at different taxonomical levels. Genomes of *M. larici-populina* isolates from natural populations were re-sequenced to explore the genetic diversity of the poplar rust fungus and a genetic map had been built recently. Several transcriptomic studies have been performed covering almost all of the heteroecious and macrocyclic life cycle of the poplar rust fungus (2 host plants; 5 different spore stages). All these data obtained in the frame of a Community Sequencing Program in collaboration with the Joint Genome Institute have led to the version 2 of *M. larici-populina* genome now anchored to 18 linkage groups. We re-evaluate the genome of *M. larici-populina* based on the latest annotations and compare with new rust genomes available. We also have taken advantage of the extended knowledge on the poplar rust fungus to better understand the arsenal of fungal genes promoting compatible infection of poplar leaves. We performed RNA-sequencing during compatible versus incompatible infection time-courses. Profiled transcriptomes of both poplar and poplar rust fungus will be presented from early colonization stages (0 to 12 hours post-inoculation) to uredinia formation (96 hours post-inoculation) using the new genome version.

POSTERS SESSION

Effector proteins from a symbiotic fungus may target phytohormone signaling to favor colonization

Veronica Basso

Phytohormones are master regulators of all physiological functions in the plant. Since the establishment of the mycorrhizal symbiosis requires many structural, physiological and transcriptomic changes in the root, plant hormones likely play a role in its regulation. Indeed, we previously showed that jasmonic acid (JA) and ethylene treatments inhibit ectomycorrhization[(A)]. Moreover, we showed that the ectomycorrhizal basidiomycete *Laccaria bicolor* relies on the Mycorrhiza-induced Small Secreted Protein 7 (MiSSP7) to establish the interaction. In particular, MiSSP7 interacts with the jasmonate-zim-domain protein 6 (JAZ6) of *Populus trichocarpa*, blocking JA signaling and promoting mutualism[(B)]. We aim at elucidating the structure of the JA receptor complex targeted by MiSSP7, as well as the transcriptomic consequences of this interaction. Using Yeast Two Hybrid (Y2H) screen and co-immunoprecipitation (Co-IP) in tobacco, we show that PtJAZ6 interacts with bHLH transcription factors PtMYC2 and PtJAM1. Next step will be the identification of genes targeted by the bHLH transcription factors, using ChipSeq assay and transcriptome analysis of overexpressed and silenced mutants. Furthermore, we want to assess how mycorrhization is perturbed by phytohormone treatment and, conversely, if any other fungal effector similarly targets other plant hormone pathways.

(A) Plett J.M. et al, 2014. Ethylene and jasmonic acid act as negative modulators during mutualistic symbiosis between Laccaria bicolor and Populus roots. New Phytologist 202(1) :270-286.

(B) Plett J.M. et al, 2014. Effector MiSSP7 of the mutualistic fungus Laccaria bicolor stabilizes the Populus JAZ6 protein and represses jasmonic acid (JA) responsive genes. PNAS 111(22) :8299-8304.

AscoTube: in vitro and in situ unraveling truffle sexual reproduction using Ascobolus immersus as a test tube model

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Truffles are ectomycorrhizal Ascomycota producing edible fructifications, called ascocarps, in forest or implanted orchards. Ascocarp formation face two important bottlenecks: the initiation of the sexual reproduction and the growth of ascocarps during a period of several months. It is therefore critical to better understand the mechanisms leading to sexual reproduction such as recognition between compatible strains. Using genomic resources the genes involved in *Tuber melanosporum* (the Périgord black truffle) sexual reproduction have been characterized but their role cannot be verified by genetic approach due to the absence of genetic tools. Besides, the saprophytic Ascomycete, *Ascobolus immersus,* is famous since the late 1930s as a model organism for genetic studies. *A. immersus* belongs to Pezizomycete class such as truffles and offers therefore a unique opportunity to test the functionality of truffle genes.

The aim of the AscoTube project (financed by Labex ARBRE) is to use *A. immersus* as a model species (i.e. a test tube) to address fundamental questions for other Ascomycete species such as truffles. To demonstrate the usefulness of *A. immersus* as model species, the deciphering of the truffle genetic mechanisms involved in strains recognition for sexual reproduction will be used. This *in vitro* approach to better understand truffle sexual reproduction will be complemented with *in situ* experiments aimed at detecting and following the dynamics of the different truffle strains forming both maternal and paternal tissues. AscoTube is an innovative project that will produce several outputs in term of: 1) new tools for fundamental basic research; 2) important knowledge about the sexual reproduction and strain recognition steps in truffles; 3) assessment of outcrossing occurrence between closely related truffle species such as *T. melanosporum* and *T. indicum* that could lead to new policy recommendation and 4) the identification and localisation of maternal and paternal tissues *in situ* for optimizing truffle orchards management.

The poplar rust fungus effector biology: challenges of functional characterization of effectors in a non-model pathosystem

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Rust fungi are devastating biotrophic pathogens manipulating host processes by delivering effector proteins into the plant cells. The poplar leaf rust fungus *Melampsora larici-populina* genome analysis revealed a large set of secreted proteins that some have been considered as candidate effectors. The understanding how these effector proteins function in the host cells has been the key question of effector biology for the last decade. Many efforts have been made in the field plant-microbe molecular interactions to unravel their role in the colonization of plant tissues. The poplar-poplar rust pathosystem although considered, as a genomic model in the study of tree-microbe interactions is actually non-model pathosystem when it comes to functional characterization of effectors. Absence of easy-going transformation systems for poplar and rust fungi is a major drawback. However, we combined several tools and approaches to help at elucidate the roles of *M. larici-populina* effector proteins such as heterologous *in planta* expression[1,2], recombinant protein production or structural approach. All these diverse approaches have led to partially unravelling the role of numerous *M. larici-populina* effector candidates. Summary report of the ongoing research aimed at elucidating candidate effectors functions will be presented, rising questions about the methodological limits of the study of effector biology in non-model pathosystems.

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Defense-related phytohormones alter the structuring of the root microbiome in Grey poplar

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Tree roots are colonized by ectomycorrhizal (EcM) fungi and rhizospheric/endophytic bacteria and fungi – the microbiota. EcM fungi play a key role in the host nutrition, whereas endophytic associates may modulate the plant resilience to biotic and abiotic stresses. The molecular mechanisms shaping the tree microbiota are not known. Here, we aim to determine how the plant defense signaling pathways regulate the root microbiome in Grey poplar (*Populus tremula x P. alba*). We surveyed the root microbiota from poplar lines constitutively expressing the fungal protein MiSSP7 (Mycorrhizal-induced Small Secreted Protein of 7 kDa). MiSSP7 is released by the EcM *Laccaria bicolor,* enters root nuclei and interacts with the transcriptional regulator JAZ6, the jasmonate co-receptor. By preventing JAZ6 degradation, MiSSP7 represses the triggering of the jasmonate-related defense signaling pathways. Wild type (WT) and MiSSP7-overexpressing poplar lines were grown in natural soil. Rhizospheric and bulk soils, and roots were sampled after 10 days and 6.5 weeks. Metabolite profiles of WT and MiSSP7 lines were measured by gas chromatography-mass spectrometry, whereas the root fungal and bacterial communities were surveyed by rDNA metabarcoding. We show that the fungal colonisation of MiSSP7-overexpressing roots was 30-fold higher than WT lines, suggesting that the alteration of JA signalling by MiSSP7 facilitated fungal colonization. Metabolites accumulated in roots colonized by EcM fungi were also more abundant in MiSSP7-overexpressing lines. Our results suggest that MiSSP7 may not only play a role in the formation of mycorrhizal symbiosis, but it could also influence the structuring of root-associated microbial communities.

Keywords : microbiome, jasmonic acid, DNA metabarcoding, poplar, MiSSP7

Generating the mutants of *Phanerochaete chrysoporium* RP 78 resistant to wood extractives for functional characterization of the detoxification system of white rot fungi

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During the wood degradation process, wood decaying fungi develop different strategies including stress response, extracellular degradation and intracellular detoxification, to cope with wood extractives, which contain often antifungal compounds [1]. The aim of this work is to improve our understanding of those detoxification systems. For that, we have generated mutants of the white rot fungus *Phanerochaete chrysoporium* RP 78 by UV exposure and then test their ability to grow in presence of selected wood extractives. The first tested extractives were from wood of *Bagassa guianensis* Aubl., a species found in tropical forest (French Guyana) which is well-known for its high durability [2]. Dichloromethane extracts from wood of *Prunus avium* L. also have been selected since they contain several antifungal compounds [3].

Mutations in fungi have been generated by direct exposure of fungal conidia to ultraviolet radiation. Screening of mutants was carried out by selection of conidia able to grow on Malt Agar medium mixed with wood extractives.

25 *bag* mutants and 15 *chy* mutants which are able to germinate and grow in presence of acetonic extracts of *Bagassa guianensis* Aubl. and dichloromethane extracts of *Prunus avium* L. were selected respectively. These mutants are able to germinate in the liquid medium and to grow on solid medium containing a lethal concentration of extractives for the wild type RP 78.

To pursue this work, these obtained mutants will be characterized through, in particular, a scan genomic approach in order to identify the major genes involved in the detoxification mechanism in this fungus.

Key words: *Phanerochaete chrysoporium*, wood extractive, detoxification system

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Exploration of the functional and taxonomic diversity of the bacterial communities occurring in the bedrock/root interface of beech trees

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In shallow soils, where the nutritive content of the topsoil layer is limited, trees can adopt a deep-rooting strategy to access water and nutrients from the bedrock. Despite the critical importance of microorganisms in nutrient cycling and mineral weathering processes in the topsoil horizons, their potential involvement in tree nutrient access at the bedrock/root interface was rarely addressed. In this context, we aimed at deciphering how subsoil edaphic conditions affect the taxonomic and functional diversity of the bacterial communities. By considering different interfaces (rock, rock/root, root/saprolite, saprolite), we also determined whether an enrichment of effective mineral weathering bacteria occur in the deep rhizosphere, in contact with the bedrock or not. The work was performed on a shallow soil (Calcaric Cambisol) dominated by deep-rooting beech trees (*Fagus sylvatica*). The taxonomic structure of bacterial communities was investigated using a combination of cultivation-dependent (bacterial collections) and independent (16S rRNA pyrosequencing) approaches. In this study, four different interfaces (rock, rock/root, root/saprolite, saprolite) were collected at 2m depth. The cultivation-dependent approach was completed by a functional screening using different bioassays related to nutrient mobilization. The analysis of the 16S rRNA pyrosequencing amplicons revealed that Actinobacteria, α- and β-Proteobacteria were dominant. Deep beech rhizosphere harbored taxonomically specific bacterial communities compared with the saprolite and limestone compartments without roots. Both rhizosphere compartments exhibited similar taxonomic distributions whatever the surrounding environment, but harbor different functional potentials. Indeed, effective mineral weathering bacteria were exclusively and highly enriched in the vicinity of roots penetrating inside the limestone rocks. On the contrary, organic matter decomposing bacteria were only enriched in the vicinity of the roots in contact with the saprolite. Our results evidenced that tree select specific bacterial communities in their deep rhizosphere as potential allies capable of improving nutrient availability through their ability to mobilize nutrients.

Mitochondrial *Arabidopsis thaliana* NFU transfer proteins: cooperation with ISCA proteins to deliver [4Fe-4S] cluster to specific apo-targets

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In plants, iron-sulfur (Fe-S) proteins are involved in crucial processes as photosynthesis or respiration. The Fe-S proteins are first synthesized as apoproteins and the prosthetic groups are inserted into the polypeptide through dedicated assembly machineries. Plants have three Fe-S cluster assembly machineries, namely SUF, ISC and CIA, devoted to the maturation of plastidial, mitochondrial and nuclear or cytosolic proteins, respectively [1]. While most of the ISC components involved are known, the precise molecular mechanisms controlling the late maturation steps, in particular the trafficking of Fe-S clusters achieved by transfer proteins, are still insufficiently characterized. This work aims at deciphering the roles of mitochondrial NFU transfer proteins from *Arabidopsis thaliana*, by combining molecular and genetic approaches. While plastidial NFU1/2/3 are studied and partially characterized [2,3], the *A. thaliana* genome contains two other NFU (NFU4/5), the function of which is unknown. While NFU4 is localized in mitochondria, the localization of NFU5 remains to be experimentally determined [4]. The purification of recombinant proteins coupled to Fe-S cluster reconstitution experiments show that NFU5 could incorporate a [4Fe-4S] cluster. Binary yeast two hybrid and bimolecular fluorescence complementation experiments highlighted a specific interaction with ISCA proteins among ISC machinery members and with lipoate synthase among tested mitochondrial acceptor Fe-S proteins. The follow-up of this part will be to perform Fe-S cluster transfer experiments with these partner proteins. Complementation experiments of the *nfu1* null mutant of *Saccharomyces cerevisiae* indicate that the plant mitochondrial NFU isoforms are functional orthologs. The role of NFUs in plant physiology and development is currently investigated through the study of the phenotypes of loss-of-function *Arabidopsis* mutants for these genes.

Keywords: Fe-S clusters, mitochondria, ISC machinery, NFU, *Arabidopsis*

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The cellular maturation of iron-sulfur proteins in plants

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Iron-sulfur (Fe-S) proteins exist in all living organisms. They are known to be part of crucial metabolic pathways such as respiration, photosynthesis or DNA metabolism [1]. The incorporation of Fe-S clusters is not spontaneous and assembly machineries exist in specific subcellular compartments: chloroplasts, mitochondria and cytosol, namely SUF, ISC and CIA machineries respectively [2]. These machineries are constituted of about 10 to 15 proteins, all encoded by the nuclear genome. Basically a set of proteins is required to bring electrons, sulfur and iron atoms for *de novo* assembly of Fe-S clusters on scaffold protein(s). The transfer to target proteins is mediated by transfer/carrier proteins possibly with the help of chaperones [1,2]. Among the set of Fe-S cluster transfer proteins, the function of Iba57 and IscA/SufA proteins remain relatively unknown. The yeast and human orthologs, found in mitochondria, form a complex responsible of the maturation of aconitase-type Fe-S proteins and the activation of some radical SAM proteins [3, 4]. In plants, both types of proteins are present in mitochondria and chloroplasts [2, 5] but there is no biochemical analyses about their interaction and the type of Fe-S cluster incorporated is not known either.

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