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Journées Doc/postDoc
UMR INRA/Université de Lorraine 1136 IAM

2 & 3 juillet 2015

Centre INRA de Nancy Lorraine
Site de Champenoux
Rue d'Amance - 54280 Champenoux

INRA
SCIENCE & IMPACT

iNTERACTIONS
ARBRES-MICROORGANISMES

UNIVERSITÉ
DE LORRAINE

Programme

Jeudi 2 juillet

SESSION 1 Signalisation et mise en place de la symbiose / Signalling and symbiosis establisment

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> 9h30 -10h00: Joske Ruytinx

Initial steps towards modeling of regulatory gene networks of ECM development

> 10h00 -10h30: Cora Guennoc

Study of physical interaction between the ectomycorrhizal fungi *Laccaria bicolor* S238N, mycorrhizal helper bacteria Pseudomonas

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Pause Café

> 11h00 -11h30: Yohann Daguerre

PtJAZ6 complex in *Populus trichocarpa* and its role in the ectomycorrhizal development

> 11h30 -12h00: Feng Zhang

Role of *Laccaria bicolor* symbiosis-regulated plant cell wall degrading enzymes in ectomycorrhiza development

> 12h00 -12h30: Clément Pellegrin

MiSSP8, a lectin-like protein required for the establishment of ectomycorrhizal symbiosis

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12h30 - 14h00 Pause Déjeuner

Jeudi 2 juillet

SESSION 2 Approches moléculaires et intramoléculaires / Molecular and intramolecular approaches

> 14:00 - 14h30: Nicolas Valette

Characterization of small proteins secreted by lignolytic fungi

> 14:30 - 15h00: Jonathan Przybyla-Toscano

Exploring the functions and partners of mitochondrial NFU transfer proteins from *Arabidopsis thaliana*

> 15:00- 15h30: Pierre-Alexandre Lallement

Biochemical and structural characterization of dehydroascorbate reductases from poplar (*Populus trichocarpa*), enzymes contributing to the maintenance of the ascorbate pool in plants

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Pause Café

> 16h00- 16h30: Cécile Lorrain

The poplar rust fungus *Melampsora larici-populina* candidate effectors target diverse plant cell compartments

> 16h30- 17h00: Thomas Roret

Diverse structural forms involving BolA proteins

Vendredi 3 juillet

SESSION 3 Impact des facteurs environnementaux sur les communautés microbiennes / Impact of edaphic parameters on microbial communities

> 9h00 -9h30: Erwin Sentausa

Bioinformatics analysis of forest soil fungi diversity and activity from DNA metabarcoding and metatranscriptomic data

> 9h30 -10h00: Leticia Pérez-Izquierdo

A new promising molecular marker to study the functional diversity of fungal communities: the Glycosyl Hydrolase 63 gene

> 10h00 -10h30: Giovanni Ragaglia

Studies of the role of Mycorrization Helper Bacteria in ectomycorrhizal symbiosis between *Tuber borchii Vittad*. and species of the Mediterranean flora in different forest ecosystems of Sardinia

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Pause Café

> 11h00 -11h30: Yannick Colin

Taxonomic and functional diversity of bacterial communities inhabiting *Fagus sylvatica* rhizosphere and surrounding bulk soils along a soil toposequence

> 11h30 -12h00: Océane Nicolitch

Impact of nutrient availability on the structure of the rhizospheric bacterial communities: Insights from the Montiers soil succession

> 12h00 -12h30: Marie Grosdidier

The spread of a disease, *Chalara* ash dieback

12h30 - 14h00 Pause Déjeuner

Vendredi 3 juillet

SESSION 4 Exploration des génomes / Genome mining

> 14:00 - 14h30: Antoine Persoons

Population genomics study of the poplar rust fungus *Melampsora larici-populina*

> 14:30 - 15h00: Emeric Bankole

Genetic diversity of ectomycorrhizal *Pisolithus microcarpus*: Development of a pipeline for single nucleotide polymorphisms analysis (SNPs)

> 15:00- 15h30: Clémence Marchal

Genomic and transcriptomic analyses of poplar immune receptors

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Pause Café

> 16h00- 16h30: Mathieu Lhuire

Functional and genetic characterisation of the strain *Burkholderia glathei* PML1(12) efficient to weather minerals

> 16h30- 17h00: Juan Chen

Comparative transcriptomic and proteomic analysis of symbiotic and asymbiotic seed germination in *Dendrobium officinale*

18h00 Barbecue

Acknowledgment:

We thank the LabEx ARBRE for financial support and all doc and post-doc for their participation.

SESSION 1 Signalisation et mise en place de la symbiose / Signalling and symbiosis establisment

Initial steps towards modeling of regulatory gene networks of ECM development

Joske Ruytinx, Laura Weiss, Simon Chavée, Claire Veneault-Fourrey, Annegret Kohler and Francis Martin

Ectomycorrhizae (ECM), beneficial mutualistic symbiosis between tree roots and fungal hyphae, are essential for tree health. In exchange for carbohydrates, ECM fungi offer an improved mineral supply and stress protection. To facilitate nutrient exchange, root morphology is strikingly altered and a symbiotic organ is formed. Initiation, establishment and maintenance of such a symbiotic organ require a time and spatial control of gene networks. Communication between both symbiotic partners by effector proteins and metabolites is essential for this control. However, not all signal molecules involved are yet defined and little is known about their perception and transduction. Here, we define different morphological stages of *Laccaria bicolor – Populus tremula x P. alba* ECM development and study the effect of plant diffusible molecules, rutin and quercetin on hyphal growth and development. We were not able to detect an effect of rutin nor quercetin on hyphal branching, biomass production and colonial growth at the concentrations and exposure times tested. Gene expression patterns are determined for the different stages of ECM development and for free living mycelium exposed to rutin and quercetin. Up to now we could assign a higher expression of some effector MiSSPs to specific developmental stages and a higher expression of two transcription factors to the presence of the host plant. Finally, integration of the data sets will allow the modeling of *L. bicolor* regulatory networks in ECM development and identify master switches of these gene networks.

Study of physical interaction between the ectomycorrhizal fungi *Laccaria bicolor* S238N, mycorrhizal helper bacteria Pseudomonas

Cora Guennoc1, 2, Aurelie Deveau1, Jessy Labbé2, Gerald A. Tuskan2, Francis Martin1

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In forest soils, ectomycorrhyzal fungi (ECM) associate with roots of trees to form a symbiosis that contributes to the growth and the health of the trees. In natural environments mycorrhizal fungi are surrounded by and shape complex bacterial communities. From these communities, Mycorrhiza Helper Bacteria (MHB) promote the formation and/or the functioning of the ectomycorrhizal symbiosis between tree roots and ectomycorrhizal fungi. Despite the high relevance of MHB for forestry and for sustainable tree production in tree nurseries, little is known on the mechanisms of the interaction between ectomycorrhizal fungi and helper bacteria. In order to extend our knowledge about these mechanisms, my PhD project focus on the interactions between the model organisms Laccaria bicolor S238N, an ectomycorrhizal fungal strain and Pseudomonas fluorescens BBc6R8, a MHB strain. Previous study having shown that BBc6R8 can form a biofilm like structure on L. bicolor, my studies are focusing on this physical interaction, in order to know whether or not, this biofilm formation is necessary to the helper effect. For my studies I use BBc6R8 mutants having lost their helper effect and analyze them for biofilm formation on abiotic surface and on L. bicolor, by combining several approaches for biofilm quantification (microscopic analysis, crystal violet staining and direct enumeration).

PtJAZ6 complex in *Populus trichocarpa* and its role in the ectomycorrhizal development.

Yohann Daguerre[(1)], Romain Schellenberger[(1)], Sebastian Wittulsky[(1)], Jonathan Plett[(2)], Annegret Kohler[(1)], Claire Veneault-Fourrey[(1)], Francis Martin[(1)]

(1)Lab of Excellence ARBRE, « Tree-Microbe Interactions » Department, INRA-Nancy, Lorraine University, Champenoux, France

(2)Hawkesbury Institute for the Environment, University of Western Sydney, Richmond, NSW, Australia

Roots of most trees form symbiosis with mutualistic soil-borne fungi. The crosstalk between the two partners is fundamental for the timing, establishment and maintenance of beneficial relationships. However, very little is known about how symbiosis is initiated by both partners. We previously showed that the ectomycorrhizal basidiomycete *Laccaria bicolor* (Maire) P.D. Orton relies on Mycorrhizal-induced Small Secreted Proteins (MiSSP) to establish the interaction (Plett *et al.*, 2011). In particular MiSSP7 interacts with the jasmonic acid (JA) co-receptor PtJAZ6 of *P. trichocarpa,* blocking JA signaling and promoting mutualism (Plett *et al.*, 2014). JAZ proteins are known to interact with NINJA and TOPLESS proteins as well as bHLH transcriptional factor in leaves of *Arabidopsis*. We aim identifying the proteins interacting with PtJAZ6 in roots of *P. trichocarpa*. Using Yeast Two Hybrid (Y2H) screen, we show that PtJAZ6 interacts with PtNINJA proteins and bHLH transcription factors, in particular PtJAM1. Next step will be the validation of these interactions by Bimolecular Fluorescence Complementation (BiFC) and the identification of genes targeted by the bHLH transcription factor PtJAM1, using *in silico* prediction and ChipSeq assay in order to fully understand mechanism underlying ectomycorrhizal ontogenesis.

References:

Plett *et al.*, **A secreted effector protein of Laccaria bicolor is required for symbiosis development.** *Current Biology* (2011), 21(14):1197-203.

Plett *et al.*, **Effector MiSSP7 of the mutualistic fungus Laccaria bicolor stabilizes the Populus JAZ6 protein and represses jasmonic acid (JA) responsive genes.** *PNAS* (2014), 111(22):8299-304.

Role of Laccaria bicolor symbiosis-regulated plant cell wall degrading enzymes in ectomycorrhiza development

ZHANG F[1], CHAMPION C[2], HAON M[2], FOURREY-VENEAULT C[1], KOHLER A[1], BERRIN JG[2], MARTIN F[1]

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Plant-associated fungi have evolved enzymatic toolboxes to adapt to diverse host plants and lignocellulosic substrates during the host colonisation. Ectomycorrhizal fungi have reduced complements of plant cell wall degrading enzymes (PCWDEs). It has been suggested that they are used to penetrate host cells (Kohler et al., 2015). Using comparative analyses of available genomics and transcriptomics data, we have identified the sets of enzymes that are produced by Laccaria bicolor during symbiosis development. The few retained genes coding for PCWDEs acting on pectin (GH28, GH88 and CE8), hemicellulose (GH30) and cellulose (GH5_5 with a CBM1 domain and LPMOs) are upregulated in ECM root tips and they likely modify the plant cell wall during colonization of the host root apoplastic space. To characterize the enzyme activity and substrates of the symbiosis-induced PCWDEs, we are producing the recombinant proteins for the sole glycosyl hydrolase 5 (GH5) with a carbohydrate-binding motif CBM1, an expansin-like protein and the polygalacturonase GH28. As of today, the GH5-CBM1, its CBM1 motif and expansin-like protein are well produced in Pichia pastoris. The recombinant proteins will be used for assaying the enzyme activity, their 3D structure and to elicit antibodies for further protein immunolocalization in ectomycorrhizal roots. This project will elucidate how symbiotic fungi modify plant cell walls to successfully establish within host tissues. In addition, it may generate new enzymatic tools for green chemistry.

KOHLER A, KUO A, NAGY LG, MORIN E, BARRY KW, BUSCOT F, CANBÄCK B, CHOI C, CICHOCKI N, CLUM A et al. (2015) Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. Nature Genetic**s 47**, 410–415.

Acknowledgements. This project is funded by ANR (project FUNTUNE) and the Laboratory of excellence ARBRE (project SYMWOOD)

MiSSP8, a lectin-like protein required for the establishment of ectomycorrhizal symbiosis.

Clément Pellegrin[1,2]*, Yohann Daguerre[1,2], Minna Kemppainen[3], Alejandro Pardo[3], Claire Veneault-Fourey[1,2] and Francis Martin[1]

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Ectomycorrhizal (ECM) symbioses are mutualistic interactions occuring between soil-born fungi and roots of trees. Sequencing of mycorrhizal fungal genomes sheds the light on hundreds of Mycorrhizal induced Small Secreted Proteins (MiSSPs) in the fungus *Laccaria bicolor*. (Martin et al, 2008; Kohler et al., 2015). In *Laccaria bicolor-Populus trichocarpa* ECM, the MiSSP8 gene encoding a 8-kDa protein, is over-expressed during symbiosis, but also expressed in fruiting bodies. RNAi knockdown of MiSSP8-encoding gene impairs *Laccaria bicolor*'s mycorrhization ability. Localization of synthetic MiSSP8 fused to fluorescein as well as transient expression in *Nicotiana benthamiana* leaves of MiSSP8:GFP fusion shows cell-wall and plasma membrane localization of MiSSP8. Using yeast-two hybrid screen, we identified several interacting proteins. However, none of them was confirmed using BiFC assay, suggesting an interaction of MiSSP8 with a non-protein partner. Lectin, antimicrobial and lipid-binding activity have been tested in order to determine MiSSP8's function. Collectively, our results suggest that MiSSP8 is lectin-like protein participating to the establishment of the ectomycorrhizal symbiosis.

References

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Kohler A, Kuo A, Nagy LG, Morin E, Barry KW, Buscot F, Canbäck B, Choi C, Cichocki N, Clum A, *et al.* 2015. Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. *Nature genetics* 47: 410–415.

SESSION 2 Approches moléculaires et intramoléculaires / Molecular and intramolecular approaches

Characterization of small proteins secreted by lignolytic fungi

Nicolas Valette, Eric Gelhaye and Mélanie Morel-Rouhier

UMR 1136 : Interactions arbre/microorganisme Equipe Réponses aux stress et régulation redox
Université de Lorraine Nancy

The mechanisms of wood degradation by saprophytic fungi are currently the focus of many research projects, especially because of the potential of these microorganisms in biomass valorization. While most of the studies concern the extracellular enzymes, very few concern the associated processes, which enable fungi to resist to the oxidative and toxic environment arising from wood degradation. In particular, the first step of wood breakdown is the release of aromatic compounds known as extractives, which could be toxic for the cells. Interestingly, beside the classical genes involved in stress responses, small secreted proteins (SSP) seem to be involved in the cell response to oak molecules. Their exact role has not been determined yet however, first results clearly showed a relationship between wood specificity and secretion of SSP. The main objective of this project is to precise the role of SSP in fungal physiology (wood modification, cell signaling, stress response or detoxification....) during wood decomposition. For this, a recombinant SSP has been produced and purified in *Escherichia.coli*. First results show that the SSP, which is rich in cysteine residues is completly oxidized and difficult to reduce. Moreover, it forms soluble aggregates similar to a hydrogel and seems to chemically modify oak extracts. To better understand the role of this protein, we are currently developing a method of genetic transformation for *Phanerochaete chrysosporium* allowing to study the effect of the overexpression or the repression of the SSP of interest.

Exploring the functions and partners of mitochondrial NFU transfer proteins from *Arabidopsis thaliana*

Jonathan Przybyla-Toscano[1,2], Thomas Roret[1,2,] Tiphaine Dhalleine[1,2], Cyril Magno[3], Brigitte Touraine[3], Frédéric Gaymard[3], Jérémy Couturier[1,2], Florence Vignols[3], and Nicolas Rouhier[1,2]

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In plants, iron-sulfur (Fe-S) proteins are involved in crucial processes as photosynthesis or respiration. The Fe-S clusters are inserted into apoproteins through specific maturation systems. 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. While most ISC components involved are identified, the precise molecular mechanisms, in particular those involved in 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. Whereas plastidial NFU1 to 3 have been partially characterized, the *A. thaliana* genome contains two other NFU (NFU4 and 5) the function of which is unknown. Both proteins should be expressed in mitochondria although this remains to be firmly established for NFU5. By producing in *E. coli* and purifying recombinant proteins and by performing *in vitro* Fe-S cluster reconstitution experiments, we showed that NFU5 could indeed incorporate an Fe-S cluster. Characterizing the nature of the incorporated cluster(s) will be the basis before achieving Fe-S cluster transfer experiment with selected recipient target apoproteins. Besides, binary yeast two-hybrid and bimolecular fluorescence complementation experiments performed with other ISC components or putative target proteins have highlighted the existence of specific interactions with some ISCA members and with the lipoate synthase. Together with the study of loss-of-function *Arabidopsis* mutants, these results will help elucidating the functions of mitochondrial NFU for plant physiology and development.

Biochemical and structural characterization of dehydroascorbate reductases from poplar (*Populus trichocarpa*), enzymes contributing to the maintenance of the ascorbate pool in plants

Pierre-Alexandre Lallement[1,2], Thomas Roret[1,2], Pascale Tsan[3,4], José M. Gualberto[5], Jean-Michel Girardet[1,2], Nicolas Rouhier[1,2] and Arnaud Hecker[1,2]

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Glutathione transferases (GSTs) constitute a ubiquitous multigenic superfamily of enzymes involved in cellular detoxification processes and secondary metabolism. These enzymes mainly catalyze the conjugation of glutathione (GSH) onto various target compounds. While GSH-conjugation reactions are typically catalyzed by GSTs exhibiting a catalytic serine or tyrosine, other GSTs rather possess a catalytic cysteine (Cys-GSTs). Among them, dehydroascorbate reductases (DHARs), which are specific to plants, are key enzymes involved in the ascorbate-glutathione cycle and participate to the recycling of ascorbate in the cells. To understand the respective roles of the three poplar *DHAR* genes, named DHAR2, 3 and 4 in accordance to *A. thaliana* numbering, we have analyzed their functional, catalytic and structural properties. Our results showed that DHAR transcripts are mainly found in female flowers, fruits, petioles, leaves and buds. Transient expression of GFP-fusion proteins in *Nicotiana benthamiana* young leaves revealed that DHAR2 is localized in chloroplasts whereas DHAR3 and 4 are found both in the cytosol and nucleus. The biochemical characterization of recombinant DHARs suggested that the enzymes employ different catalytic mechanisms to reduce dehydroascorbate (DHA) into ascorbate. According to the presence of one or two cysteine residues in the active site signature, they use respectively a monothiol or a dithiol mechanism similar to some glutaredoxins. Finally, structural studies performed by NMR showed that DHAR4 adopts a canonical GST fold being structurally closer to CLIC (chloride intracellular channel) proteins and allowed identifying residues contributing to glutathione and DHA binding.

THE POPLAR RUST FUNGUS MELAMPSROA LARICI-POPULINA CANDIDATE EFFECTORS TARGET DIVERSE PLANT CELL COMPARTMENTS

Cécile Lorrain[1,2,3], Benjamin Petre[1,2,3], Diane G.O. Saunders[1,4,5], Jan Sklenar[1], Joe Win[1], Sébastien Duplessis[2,3], Sophien Kamoun[1]

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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. We developed an effectoromic pipeline to select, clone and expressed 20 candidate effectors in *Nicotiana benthamiana* leaf cells to (i) determine their subcellular localisation and (ii) to identify plant proteins interactors. We performed coimmunoprecipitation and mass spectrometry to identify plant proteins associating with 5 candidate effectors. Using confocal microscopy, we report 6 candidate effectors localizing in particular cell compartments such as nucleus, nucleoli, chloroplasts and mitochondria. In particular, a 143 amino acid effector candidate targets plant chloroplasts and the predicted cTP is cleaved *in planta*, and is sufficient to translocate GFP into chloroplasts. Plus, this candidate effector named CTP1 is part of a *Melampsoracae*-specific family of polymorphic and modular small-secreted proteins that also localise in chloroplasts. We hypothesize that the CTP1 family has evolved recently in *Melampsoracae* species to manipulate host chloroplasts functions. Ongoing research aimed at elucidating CTP1 function will be presented.

Diverse structural forms involving BolA proteins

Thomas Roret[1], Pascale Tsan[2], Jérémy Couturier[1], Bo Zhang[3], Michael K. Johnson[3], Claude Didierjean[2], Nicolas Rouhier[1]

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BolAs were initially defined as stress-responsive transcriptional regulators whose overexpression in *Escherichia coli* modified bacterial cell shape and induced biofilm formation [1, 2]. It was thus surprising that a BolA, referred to as Fra2 (Fe repressor of activation-2), and monothiol glutaredoxins 3/4 contributed to the regulation of iron homeostasis by forming stable [2Fe-2S] cluster-bridged heterodimers controlling the nuclear translocation of the *Saccharomyces cerevisiae* Aft1/Aft2 transcription factors in response to a mitochondrial signal [3,4,5]. On the other hand, it was reported that Grx-BolA couples from various sources could also form apo-heterodimers [5, 6]. This is consistent with the observation that an *in vivo* Grx3/4-Fra2 interaction is found both in iron-replete and iron-depleted yeast cells [3]. Besides, a homodimeric disulfide-bridged form of *Arabidopsis thaliana* BolA2 can be preferentially reduced by the nucleo-cytoplasmic GrxS17 indicating that a redox regulation mechanism, disconnected from their capacity to form [2Fe-2S] cluster-bridged heterodimers, may be physiologically relevant for BolA2 [7]. Finally, the observed capacity of some BolAs to bind diverse metals and to confer metal resistance in yeast points to a link with metal homeostasis [8, 9, 10]. The structural characterization of BolA-BolA dimers, BolA-Grx apo/holo-heterodimers and BolA-metal complexes will be presented.

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SESSION 3 Impact des facteurs environnementaux sur les communautés microbiennes / Impact of edaphic parameters on microbial communities

Bioinformatics analysis of forest soil fungi diversity and activity from DNA metabarcoding and metatranscriptomic data

Erwin Sentausa, Annegret Kohler, Francis Martin, Marc Buée, Joint Genome Institute, 'Metatranscriptomics of Forest Soil Ecosystems' consortium

In forest ecosystems, soil fungi play essential roles in tree nutrition and biogeochemical cycles as primary decomposers. However, little is known about the relative contributions of different fungal species to in situ organic matter decomposition. This study aims to explore fungal diversity and associated transcriptional activities—especially in relation to organic matter degradation processes—from diverse forest soil ecosystems submitted to different environmental constraints. We analyzed soil DNA metabarcoding data using fungal internal transcribed spacer (ITS1) amplicons and metatranscriptomic data of both total rRNA and polyadenylated mRNA from Illumina high-throughput sequencers. The ITS analyses were done using an adapted UPARSE pipeline, wherein the amplicon reads are clustered into operational taxonomic units (OTUs) using the UNITE database as fungal taxonomic reference. So far, we identified 35007 fungal OTUs from 259 samples that come from ten different sites in Europe and the United States. Multivariate analyses as non-metric multidimensional scaling (NMDS) and principal component analysis (PCA) will be performed to explore the fungal community assemblage and the relationship between environmental variables. Furthermore, the mRNA metatranscriptome was analyzed in several steps that consist of rRNA filtering using SortMeRNA, read assembly using Trinity, and functional annotation by comparison to carbohydrate active enzyme (CAZyme) databases. Preliminary analyses showed that SortMeRNA could detect between 3.77% to 15.86% rRNA reads in the enriched mRNA data from one study site. The mapping and annotation strategy of these metatranscriptomic data using fungal genomic resources of 1000FungalGenomes project will be discussed.

A new promising molecular marker to study the functional diversity of fungal communities: the *GLYCOSYL HYDROLASE* 63 gene

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Fungal communities are a key component of forest ecosystems, involved in biogeochemical cycling and tree productivity. Forest resilience response to environmental changes depends on ecosystem services provided by fungi. Our main goal is to assess the effects of tree genotype(s) and fire recurrence(s) on the structure and functions of fungal communities in Mediterranean pine forests, as well as developing new functional diagnostic molecular markers to identify fungi involved in carbon cycling and sequestration. We developed a series of primers to amplify the single-copy *GLYCOSYL-HYDROLASE GH63* gene, encoding α-glucosidases, in basidiomycetes. These *GH63* primers were validated by PCR-amplification in 125 different fungal genomic DNAs, and the assay compared with the efficiency of already published markers targeting genes coding for laccases, N-acetylhexosaminidases (GH18), cellobiohydrolases (GH7), or classII Peroxidases (AA2). The success rate of *GH63* amplification was strikingly higher than with the other functional genes. Specific amplicons were recovered for 95% of the species tested. We then downloaded the *GH63* sequences from the 483 fungal genomes publicly available at the JGI MycoCosm database. *GH63* is present in 461 fungal genomes (with a single-copy gene in 86 % of them) belonging to all phyla, except Microsporidia. By comparing the phylogenetic trees constructed using either GH63, Rpb1 or Rpb2 protein sequences, we showed that the new GH63 could also be a potential barcoding and phylogenetic gene. Finally, a high proportion of GH63 proteins was predicted as secreted, especially within *Basidiomycota*. The added-value of this new molecular tool will be discussed in terms of functional diversity and as potential indicator of fungal secretome.

Studies of the role of Mycorrization Helper Bacteria in ectomycorrhizal symbiosis between *Tuber borchii* Vittad. and species of the Mediterranean flora in different forest ecosystems of Sardinia

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The work that I'm going to perform during my PhD is focused on the study and the identification of Mycorrhization Helper Bacteria (MHB) linked to to the ectomycorrhizal symbiosis between *Tuber borchii* Vittad. and some forestry species of Sardinia, especially *Quercus ilex* L. and *Quercus suber* L., in soils with a different pH level.The study of this particular symbiosis is very important for the Mediterranean areas. Indeed, the difficult conditions of growth that provides this habitat lead to high mortality rate during afforestation. We hypothesize that the use of controlled mycorrhized seedlings and their co-inoculation with MHB could reduce this mortality and have a positive influence on the performance of new afforestation.During a sampling carried out at the beginning of 2015, we have collected several samples of *T. borchii* and forest soils in two areas of the island characterized by different pH values. On these samples will be performed some ecological analyses of the bacterial communities, in order to evaluate their biodiversity and their metabolic fingerprints. Furthermore, I will make a bacterial library that I will characterize at the taxonomic and functional level.Bacterial isolates will be subsequently tested by inoculation of seedlings of various species of Mediterranean trees mycorrhized exclusively with *T. borchii* on sterile soil. Finally, during the last year of my work, we will evaluate the results by measuring various physiological and dendrometical parameters including rate and time of mycorrhization, root-collar diameter, height, dry weight and volume of the root system and the apical part, and the evaluation of the amount of nitrogen and phosphorus in the various plant parts.

Taxonomic and functional diversity of bacterial communities inhabiting *Fagus sylvatica* rhizosphere and surrounding bulk soils along a soil toposequence

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In forest ecosystems, soils microorganisms have been demonstrated to be involved in mineral weathering and were suggested to play a crucial role in the sustainability of non-fertilized, acidic and nutrient-poor soils. Accumulating evidences reported that belowground bacterial communities are shaped by edaphic factors but also by specific interactions in tree-rhizosphere. Using cultivation-independent molecular methods, we aimed to investigate how soil conditions impacted bacterial communities inhabiting beech *(Fagus sylvatica)* rhizosphere and surrounding bulk soils at the taxonomic and functional levels. The study site of Montiers-sur-Saulx is characterized by a succession of acidic nutrients-poor soils to nutrient-rich soils with neutral pH. Based on pyrosequencing analysis of the 16S rRNA amplicons, a significant decrease of the bacterial diversity was reported in acidic soils and was related to a reduction of nutrients availability. However, the relative abundance of the *Actinobacteria*, *Alphaproteobacteria* and *Gammaproteobacteria* remained stable at the vicinity of beech roots whatever the soil conditions. Among them, specific taxa were identified and could play a potential key role in tree nutrition. In addition, functional array-based analysis suggested that beech roots impacted the functional structure of bacterial communities. The abundance of genes involved in carbon degradation, cations transport, nitrogen cycle and stress response varied significantly along the soil toposequence and between the rhizosphere and bulk soil compartments.

Impact of nutrient availability on the structure of the rhizospheric bacterial communities: Insights from the Montiers soil succession

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Temperate forest ecosystems are often developed on nutrient-poor, non-fertilized and acidic soils. In this context, understanding the relative role of microbial communities in the releasing of nutrients from soil minerals and consequently their ability to help trees to access to these nutrients, in relation to soil nutrient availability, becomes essential. To answer such questioning, we studied the impact of the availability of inorganic nutrients on the functional and taxonomic structure of forest bacterial communities. Particularly we have focused on the rhizosphere compartment to determine if the trees recruit specific communities and functions in relation with nutrient availability compared to the surrounding bulk soil. We test this hypothesis on the experimental site of Montiers, which is caracterized by a homogeneous landcover of beech (*Fagus sylvatica*) developed along a soil succession with different nutritive potentials. Bacterial communities have been studied through global (metabolic tests), cultivation-dependent (functional screening) and -independent (metagenomics) approaches related to soil types. Our first results showed an impact of the soil characteristics on the functional structuration of bacterial communities, particularly in the rhizosphere. The weathering function appeared to be significantly more frequent and more effective in the rhizosphere compared to the bulk soil in the poorest soils. These results suggest that the soil conditions impact the structure of the soil bacterial communities and that the tree select effective mineral weathering bacteria according to the soil condition. We hypothesize that the enrichment of effective weathering bacterial communities in rhizosphere may facilitate the trees to access limiting nutrient.

The spread of a disease, Chalara ash dieback

Marie Grosdidier

Chalara fraxinea, an invasive alien fungal pathogen of ash native to East Asia was discovered for the first time in Poland in the 1990s. It causes severe damages that can lead to tree death. It quickly expanded throughout Europe with a dispersal of 50 km per year. My PhD work aims at modelling the spread of the pathogen at the scale of France based on visual symptom data recorded in the large "Département de la Santé des Forêts" database. I will seek to study the long-distance spore dispersal patterns and to determine the influence of environment on the severity of the disease, and finally optimize a spores capture method to monitor an ongoing epidemic. An effective method to trap and quantify spores is essential to study the long-distance dispersal patterns of the disease. The practical aspect, with effective and practical spores traps, and the PCR detection efficiency can further be improved. Indeed, the detection of dispersion / progression of the disease based in part on the measures / quantification of the *H. fraxineus* inoculum. The qPCR assay used is able to detect small amounts of inoculum, but like any screening test, it has limits of detection and quantification. It must be able to verify that the values generated by the test are reliable, especially in case of very low load target to be detected. The sensitivity and specificity of the qPCR method was assessed and an adequate threshold was defined with ROC methods.

SESSION 4 Exploration des génomes / Genome mining

Population genomics study of the poplar rust fungus Melampsora larici-populina

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The outcome of host-pathogen interactions depends on a complex molecular dialogue betweenthe protagonists. Among the molecules involved, effectors released by the pathogen are critical for the success of infection, as they interfere with host metabolism, signaling and defense responses and allow expression of the disease. Effector proteins reported so far in rust fungi exhibit common features (e.g. secreted, small, cysteine-rich) and candidate effectors most likely reside among fungal secreted proteins. *Melampsora larici-populina* is a fungal pathogen responsible for the foliar rust disease on poplar trees, causing severe damage in plantations. Almost all the resistances (R) released so far have been overcome, with the latest major breakdown event in 1994 (R7). The genome of the virulent 7 isolate 98AG31 has been sequence dusing a whole genome shotgun strategy, revealing a large genome of 101 megabases containing 16,399 predicted genes including 1184 small secreted proteins. A population genetics study based on 600 isolates was performed to finely determine the impact of this breakdown on the demographic history of *M. larici-populina*. The genomes of 80 poplar rust isolates, distributed among three genetic groups, were sequenced using Illumina technology to understand the effectof the R7 breakdown at the genetic scale. More than 300,000 polymorphic sites (SNPs) were uncovered across isolates, indicating a remarkable level of polymorphism. In order to understand the emergence of the virulence 7, we performed a genome scan analysis based on SNP data using differentiation and selection indices, taking into account the demographic history. We found several genomic regions related to the virulence 7 that bear genes encoding small secreted proteins. This study demonstrates the benefit of population genomics in the search for candidate effector genes.

Genetic diversity of ectomycorrhizal Pisolithus microcarpus: Development of a pipeline for single nucleotide polymorphisms analysis (SNPs)

Emeric Bankole, Emmanuelle Morin, Annegret Kohler& Francis Martin

Ectomycorrhiza fungi have an important role in the forest ecosystems due to their mode of associative life with the roots of trees. With the progress of the tools of sequencing and the development of the bioinformatics tools for analyzing of data sequencing, study the genetic diversity based on genetic markers as single nucleotide polymorphism is possible. Pisolithus microcarpus is the model fungi which is studied here. This diversity study was made on six isolates of Pisolithus micorcarpus of which Pisolithus albus and with as reference the genome of Pisolithus microcarpus 441. The work made during my internship allowed to develop a pipeline including several bioinformtics tools for calling these single nucleotide polymorphism. The pipeline highlighted the genetic variations which exist between the various genomics and the reference genome, or still the genes polymorphics which contibute the most to this genetic variability.

Keys words : Pipeline, SNPs, ectomycorrhiza fungi, diversity

Functional and genetic characterisation of the strain *Burkholderia glathei* PML1(12) efficient to weather minerals

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Forests growing on acidic and nutrient-poor soils are usually characterised by an important stock of inorganic nutrients entrapped in soil minerals. However, plant roots cannot directly exploit these nutrients. Mineral weathering, by abiotic or biotic factors allows the release of these nutrients, making them accessible to tree roots. Biotic factors involve plants and soil microorganisms. Among these microorganisms, several bacteria genera (such as *Burkholderia* and *Collimonas*) are known to be able to weather minerals, and in some cases to improve tree nutrition. The genus *Burkholderia* is one of the most abundant genus detected in the tree root vicinity and on mineral surfaces in forest soils. The bacterial strain *Burkholderia glathei* PML1(12) has been chosen as a model organism to study mineral weathering by bacteria. The strain PML1(12) is known to be able to weather biotite and to improve pine growth in microcosms conditions. Its genome has been sequenced and an assembly was performed. This presentation will give you a broad view of my PhD thesis focused on the bacterial strain *Burkholderia glathei* PML1(12). After a study of the genome structure of this strain, two approaches of mutagenesis, with or without *a priori*, will be used to identify the genes and mechanisms allowing this strain to weather minerals.

Comparative transcriptomic and proteomic analysis of symbiotic and asymbiotic seed germination in *Dendrobium officinale*

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Mycorrhizal fungi induce and colonize orchid seed called symbiotic germination is a critical developmental process for whole lifecycle of all orchids. To date, mycorrhizal fungi diversity and specificity associated with orchid seed and adult plants have been well documented and the interaction between orchid root and mycorrhizal fungi was also investigated by recent studies. However, Little is known about the molecular change during the seed symbiotic germination. Dendrobium officinale is endangered epiphytic orchid and widely used in traditional Chinese medicine with abundant polysaccharide and alkaloids for its special pharmacological effective on gastritis infection, cancer and aging. To better understand the molecular mechanism of orchid seed germination and development, we performed comparative proteomic and transcriptomic analysis to explore genes and proteins expression profiling at the three different development stages of both asymbiotic and symbiotic situation and identify the key candidate genes or proteins involved in symbiotic germination. A total 82318 unigenes were identified in transcriptomic level. Among of them, 79 core genes were differentially expressed across whole development stage both asymbiotic and symbiotic germination. 1516 proteins were detected in proteomic level and117 common proteins displayed differentially expression in more than one stage of asmbiotic and symbiotic assays, and 335 proteins were specific expressed in symbiotic germination. These genes or proteins were involved in plant hormone signal transduction (GA or ABA), carbohydrate metabolism, amino acid metabolism, seed maturation, stress response, and secondary biosynthesis et al. Moreover, the expression pattern of 23 genes induced by fungi was validated by qRT-PCR. Our results provide fundamental molecular information for orchid seed germination and also provide some potential proteins for further genetic function analyses for orchid mycorrhizal symbiosis.