Preamble:

Dear all,

Each year, the unit IAM UMR 1136 organizes a team meeting which includes a seminar day to give upcoming scientists an opportunity to give short presentations on their work. The main goal of this project is to discuss once a year all of the scientific work done over the past year together as a team (this is open for all IAM members), to share new ideas and to exchange scientific feedback on the variety of different topics we are working on: wood decaying, litter decomposition, mineralization, physiology (bacteria, fungi, and plants), genomics, transcriptomics, ecology, pathology and so on. During this seminar, master, PhD students and post-docs are encouraged to present their achieved data to the IAM-team audience. This year, we are happy to announce a high number of fascinating and promising talks. Four of our five teams submitted their abstracts to allow a preview of their work. Unfortunately all young scientists working on theme 4 (Ecology of forest tree diseases in the context of global change) are not available on the June 27th.

The sessions will be divided among these four teams:

Session I	Theme 1: Stress response and redox regulation
Session II	Theme 2: Molecular mechanisms of the interactions between soil bacteria, fungi and trees
Session III	Theme 3: Ecology and role of forest microbial communities
Session IV	Theme 5: Mechanisms and evolution of poplar-poplar rust interactions

Master students are asked to present their data in 10 minutes while PhD students and post-docs will have 15 minutes for their presentations.

We are sure that this day will be interesting filled with complex science direct from the labs. Please refer to the timetable below for an overview of the entire day or if you would like to choose specific talks to attend that day. To give everyone a chance to discuss and exchange, we will have a coffee break in the morning (after the first few talks), a second coffee break in the afternoon and dinner together that evening. At the end of this marathon, the Unit would like to thank all, presenters and the audience alike, by holding a barbeque for the whole IAM unit.

We hope you all will come join this discussion about new data and or drawbacks these young scientists have experienced over the last research period - you are all welcome!!

THANK YOU, ALL OF THE PRESENTERS!

The organizers – Aurelie Deveau and Sebastian Wittulsky

Ecogenomics Team
UMR1136 INRA Université de Lorraine "Interactions Arbres/Micro-organismes" Centre INRA de Nancy, 54280 Champenoux, France

Timetable Postdoc Postgrad Day 2014

Date: June 27th 2014; 9h00 - 16h00

9h00 – 9h15 Welcome

9h15 – 10h00 Session I - Theme 1: Stress response and redox regulation

9h15 - 9h30 Pierre-Alexandre Lallement
Structure-function analysis of Lambda glutathione transferases from *Populus trichocarpa*
Status: PhD student

9h30 - 9h45 Desirée Gütle
Redox regulation in plastids via the FTR system
Status: PhD student

9h45 - 10h00 Jonathan Przybyla-Toscano
Fe-S cluster maturation in *Arabidopsis thaliana* mitochondria : the ISC machinery
Status: PhD student

10h00 – 13h55 Session II – Theme 2: Molecular mechanisms of the interactions between soil bacteria, fungi and trees

10h00 - 10h25 Salvatore Casarrubia[1] and Héma Fritz[2]
Looking for effector symbiosis-related proteins in the ericoid endomycorrhizal fungus *Oidiodendron maius*
Status: [1]Phd student and [2]master student

10h25 – 10h55 Coffee break

10h55 - 11h10 Cora Guennoc
Study of interaction mechanisms between the ectomycorrhizal fungi *Laccaria bicolor* S238N, mycorrhizal helper bacteria *Pseudomonas* and the *Populus*
Status: PhD student

11h10 – 11h35 Sebastian Wittulsky
Let's play JAZ! The *Laccaria* MiSSP7 effector interacts with *Populus* JAZ Co-receptors
Status: Post-doc

11h35 - 11h45 Romain Schellenberger

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Search for jasmonic acid through immunolocalisation on the mutualistic ectomycorrhizal interaction between *Laccaria bicolor* and poplar
Status: Master student

11h45 – 12h00 Clement Pellegrin
Functional characterization of MiSSP8, a mutualistic effector required for ectomycorrhizal symbiosis
Status: PhD student

12h00 – 12h10 François Jobert
Seeking for new fungal symbiosis effectors and MiSSP8 interacting proteins
Status: Master student

12h10 - 12h25 Maíra de Freitas Pereira
The transcriptional landscape of the *Pisolithus microcarpus* basidiocarp
Status: PhD student

12h25 – 13h40 Lunch at the restaurant

13h40 - 13h55 Thibaut Payen
Comparative genomics reveals repeated sequences as main diversity source for black truffle
Status: PhD student

13h55 – 15h00 Session III – Theme 3: Ecology and role of forest microbial communities

13h55 - 14h10 Herminia De la Varga
Spatial genetic structure of *Tuber melanosporum* in a productive orchard determined by mating type genes and microsatellites analysis
Status: Post-doc

14h10 - 14h20 Océane Nicolitch
Functional characterization of bacterial communities associated with black Perigord truffle during its life cycle
Status: Master student

14h20- 14h35 Marta Torres
"Bacteria are speaking!" Intercellular communication systems
Status: PhD student

14h35 - 14h50 Emila Akroume
MOS long-term monitoring network: elaboration and characterization of initial state of MOS network by NIRS/MIRS
Status: PhD student

14h50 - 15h00 Pierrick Royer

Impact of massive forest remnant removal on fungal richness and community structure
Status: Master student

15h00 – 15h15 **Session IV – Theme 5: Mechanisms and evolution of poplar-poplar rust interactions**

15h00 – 15h15 **Antoine Persoons**
Evolution of the determinants of gene-for-gene interactions: the case of poplar rust *Melampsora larici-populina* during a major resistance breakdown
Status: PhD student

15h15 – 15h30 **Natalya Saveleva**
Identification of candidate effectors of *Melampsora larici-populina*
Status: Post-doc

15h30 – 16h00 **Conclusion & Discussions with coffee**

End **TEAM – BBQ**

ABSTRACTS:

Theme 1: Stress response and redox regulation

Structure-function analysis of Lambda glutathione transferases from *Populus trichocarpa*

Pierre-Alexandre Lallement[1,2], Edgar Meux[1,2], José Gualberto[3], Pascalita Prosper[4], Claude Didierjean[4], Ahmed Haouz[5], Frederick Saul[5], Nicolas Rouhier[1,2] and Arnaud Hecker[1,2]

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Glutathione transferases (GSTs) represent a widespread superfamily of multifunctional proteins with essential roles in cellular detoxification processes and secondary metabolism (**1**). Instead of promoting the conjugation of glutathione (GSH) to a molecule acceptor as most GSTs, Lambda GSTs (GSTLs) rather catalyze deglutathionylation reactions *via* a conserved catalytic cysteine residue (**2**), but their physiological role is still unclear. By combining molecular, cellular, biochemical and structural approaches, we analysed the GSTL family from *Populus trichocarpa*. Three genes have been found but two transcripts were identified for *Pt-GSTL3*. Surprisingly, transcripts for all these genes were primarily found in flowers, fruits, petioles and buds, but not leaves and roots. From the expression of GFP fusion proteins in tobacco, Pt-GSTL1 is localized in plastids whereas Pt-GSTL2 and Pt-GSTL3A and B are found in both cytoplasm and nucleus. By using recombinant proteins, we determined that PtGSTLs exhibit thiol-transferase, DHA reductase as well as quercetin deglutathionylation activities and that they can bind ligands, including coumarins and cyclic terpenes, to their catalytic site. Finally, Pt-GSTL1 and Pt-GSTL3 structures solved by x-ray crystallography indicated that these monomeric proteins adopt a canonical GST fold quite similar to the one found in dimeric Omega GSTs (**3**), their counterparts in fungi, insects and animals. Although PtGSTLs do not share the quaternary structure of Omega GSTs, they exhibit quite similar biochemical properties. The finding that GSTLs are able to bind aromatic/cyclic compounds and are expressed in specific compartments, notably in reproductive organs, suggests that they are involved in secondary metabolism and/or in the protection of plants towards stress conditions in the case of antioxidant molecules.

References:

1. Jacquot *et al*., 2013, *In Oxidative Stress and Redox Regulation* (Springer), 213-231.
2. Dixon et al., 2002, J. Biol. Chem. 277, 30859-30869.
3. Board et al., 2000, J. Biol. Chem. 275, 24798-24806.

Redox regulation in plastids via the FTR system

Desirée Gütle[1,2], Stefanie Müller[1], Jeremy Couturier[2], Markus Schwarzländer[3], Andreas Meyer[3], Ralf Reski[1,4,5,6] and Jean-Pierre Jacquot[2]

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The regulation of enzymes by redox mechanisms is an important process taking place in nearly all organisms. For photosynthetic organisms, it is particularly important to regulate their energy production processes in accordance to the light regime and intensity. As a strategy for the realization of this light-regulation the use of regulatory thiols has developed early in plant evolution. One exemplary regulation system in chloroplasts is the ferredoxin- thioredoxin reductase system (FTR system).
The first step in this project is the generation of transgenic plants lacking the catalytic subunit of the FTR. In order to investigate the alteration of the redox state in chloroplasts of these mutants, will be generated in the background of wild-type as well as plastid targeted redox- sensitive GFP (cproGFP) reporter lines. The redox state will be measured under different growth conditions such as light, darkness or stress treatments. The target proteins of the FTR will be tested for their activity and substrate affinity. Furthermore it would be of interest to investigate the photosynthesis and the CO2 fixation rates to see if the FTR mutation is sufficient to influence these important energy pathways. Another part of the project will be in cooperation with Prof. Einsle from the University of Freiburg and involve the structural analysis of the SBPase from *P. patens* via X ray crystallography.
General and specific roles of different redox regulation systems in the chloroplast will be investigated. It is expected to detect a shift in the redox ratio to an oxidized range in the FTR mutants, due to the missing electron transfer from the light reactions to FTR target proteins.

Fe-S cluster maturation in *Arabidopsis thaliana* mitochondria: the ISC machinery

Jonathan Przybyla-Toscano, Nicolas Rouhier and Jérémy Couturier

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Iron is an essential micronutrient for plant growth and development. At the cellular level, several crucial processes such as nitrogen and sulfur assimilation, photosynthesis, respiration or DNA replication or repair require metalloproteins and more particularly iron-sulfur (Fe-S) proteins. Fe-S proteins fulfill many functions being involved in electron transfer reactions, enzyme catalysis or regulation of gene expression. In prokaryotes and eukaryotes, Fe-S proteins are first synthesized as apoproteins and the prosthetic groups are inserted in the polypeptide through dedicated assembly machineries. Plants have three different Fe-S cluster assembly machineries, namely SUF (sulfur mobilization), ISC (iron-sulfur cluster) and CIA (cytosolic iron-sulfur cluster assembly), devoted to the maturation of plastidial, mitochondrial and nuclear or cytosolic proteins respectively. Basically, Fe-S cluster biogenesis requires sulfur provided by cysteine through the action of cysteine desulfurases, iron whose origin is yet unidentified and electrons. These elements are first assembled as Fe-S clusters on scaffold proteins. Then the Fe-S cluster is transferred to acceptor proteins through the action of carrier proteins and eventually of chaperones. To date, the current knowledge on Fe-S cluster biogenesis in plant mitochondria mainly concerns the identification of the different components involved but the precise molecular mechanisms controlling this process are still largely uncharacterized. Using a functional genomic approach, this work aims at deciphering the roles of different members of the ISC assembly machinery focusing primarily of two families of carrier proteins, Nfu and A-type carriers.

Theme 2: Molecular mechanisms of the interactions between soil bacteria, fungi and trees

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

Salvatore Casarrubia, **Héma Fritz,** Claire Veneault-Fourrey, Annegret Kohler and Elena Martino.

Lab of Excellence ARBRE, Tree-Microbe Interactions Department, INRA - Nancy, Champenoux, France

Ericoid mycorrhizal fungi (ERM) are soil-born fungi forming endomycorrhizae with plants within the family Ericaceae. This plant family is widespread in a diverse range of heathland and open forest communities. These habitats are characterised by very poor nutrient status and considerable edaphic stress and it is thought that ERM fungal partners enhance stress tolerance in their host plants (Bradley et al. 1982. New Phytol 91,197). *Oidiodendron maius* Barron (Ascomycota, class Leotiomycetes), is a common ericoid fungus that establish symbiotic association with *Vaccinium myrtillus*. Studies on genes involved in pathogenicity and symbiosis have highlighted a particular class of effectors corresponding to small secreted proteins (SSPs). While SSPs have been reported for AM and ECM fungi (Kloppholz et al. 2011. Curr. Biol. 21,1204; Plett et al. 2011. Curr. Biol. 21,1197), no similar studies have been reported for ERM fungi. *O. maius* genome sequence is available, as well as RNA-Seq transcriptomic data from free living mycelium and mycorrhizal roots. Aims of our research are the analysis of these data searching for the most highly regulated symbiosis genes, the qPCR validation of their expression level and the functional characterization of the most highly regulated gene (GFP-fusion protein and ectopic expression in tobacco leaves). *O. maius* wild-type and random mutants are being used to study the interaction with a non-host plant.

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

Cora Guennoc[1, 2], Jessy Labbé[2], Aurelie Deveau[1], Gerald A. Tuskan[2], Francis Martin[1]

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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. This symbiosis has already been well studied, and the knowledge gathered allows today applications in sylviculture, in the context of controlled mycorrhization. During the 90's, the existence of bacteria that stimulate the establishment and the functioning of this symbiosis has been demonstrated. These bacteria were then called "Mycorrhiza Helper Bacteria" (MHB). The mechanisms by which these bacteria influence mycorrhization are still poorly known, that is why their utilization in the context of controlled mycorrhization is not yet possible.
In order to extend our knowledge about the molecular mechanisms of the helper effect, my PhD project focus the interactions between the model organisms *Laccaria bicolor* S238N, an ECM strain; *Pseudomonas fluorescens* BBc6R8, *Pseudomonas sp.* GM41 and GM18*, three* MHB strains and the *Populus*.
For my studies I use an *a priori* approach based on previous results on BBc6R8 in which BBc6R8 mutants lost their helper effect, and an approach with no *a priori* concerning the molecular interaction during a tripartite interaction plant/fungi/bacteria.

Let's play JAZ! The *Laccaria* MiSSP7 effector interacts with *Populus* JAZ Co-receptors

Sebastian Wittulsky, Yohann Daguerre, Jonathan Plett, Claire Veneault-Fourrey, Aurelie Deveau, Alice Vayssieres and Francis Martin

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Mutualistic ectomycorrhizal interactions (ECM) that exist between soil fungi and tree fine roots are essential to forest sustainability. The molecular cross-talk taking place between the symbiotic partners is fundamental for the timing, establishment and maintenance of beneficial mutualistic relationships. However, very little is known on how the ECM symbiosis is initiated in both partners. The colonization of the root rhizodermis and cortex by the fungal hyphae is precisely tuned and tightly controlled to allow the massive proliferation of hyphae in the host roots and avoid plant defense reactions. The ECM fungus *Laccaria bicolor* secretes the mycorrhiza-induced

small secreted protein MiSSP7 which is required for the intraradicular Hartig net development and subsequent symbiosis development (Plett et al., 2011). In the present study, we aimed to characterize the targets of MiSSP7 in planta. Using yeast two hybrid (Y2H) assays, we identified PtJAZ6 and PtJAZ5, two Jasmonate-ZIM-domain proteins (JAZ; also known as TIFY domain proteins) as direct interactors of MiSSP7. JAZ proteins are involved in hormonal homeostasis, and more specifically can act as jasmonic acid co-receptors. JAZ proteins mediate signaling pathways in response to biotic and abiotic stresses and act to modulate the development of plant organs, such as roots. Thus, these two JAZ proteins are likely targets of *Laccaria* MiSSP7 for manipulating poplar root cells to allow fungal colonization. Here, we present the results of protein-protein interaction studies using bimolecular fluorescence complementation (BIFC) assays, as well as Y2H assays, to decipher the roles of both MiSSP7-targeted JAZ proteins in root development, i.e. the identification of their interaction partners within the JA-signaling cascade. Further, we will discuss the effects of RNAi knock-down and overexpression of those proteins in poplar.

Plett JM, Kemppainen M, Kale S, Kohler A, Legué V, Brun A, Tyler B, Pardo A, Martin F, (2011). A secreted effector protein of Laccaria bicolor is required for symbiosis Development. Curr Biol, 21, pp 1197-1203

Search for jasmonic acid through immunolocalisation on the mutualistic ectomycorrhizal interaction between *Laccaria bicolor* and poplar

Romain Schellenberger, Sebastian Wittulsky, Francis Martin and Claire Veneault-Fourrey

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Mycorrhizae are mutualistic interactions between soil-born fungi and plant roots. Ectomycorrhizal (ECM) associations are widespread in temperate and borea forests. These associations are crucial to promote the optimal growth of trees. However, the molecular dialogue between both partners is still unclear. In the association between *Laccaria bicolor* and poplar, compounds on root exudate such as rutine and quercitin are able to induce the secretion of at least MiSSP7 (Mycorrhizal-induced Small Secreted Protein), a symbiosis effector of *Laccaria bicolor* (J.M.Plett and F.Martin, 2012). MiSSP7 after secretion is able to enter into the plant cell nucleus and interacts with two poplar JASMONATE ZIM DOMAIN proteins (JAZ), JAZ 5 and JAZ 6 (J.M.Plett and al, 2014). In *Arabidopsis thaliana*, JAZ proteins are negative regulators of jasmonic acid (JA) signaling pathway. JA, a defense and developmental phytohormone, is already known to be involved in endomycorrhizae and root nodule symbiosis (C.Wasternack and B.Hause, 2013). In ECM, MiSSP7 was shown to block JA-responsive gene transcription by inhibiting JAZ protein degradation. To further test the effect of *Laccaria bicolor* colonization on root poplar JA homeostasis, immunolocalisation with JA-specific antibodies was performed. Here I will present the method in detail and its application in ECM research.

Plett, J.M., Daguerre, Y., Wittulsky, S., Vayssières, A., Deveau, A., Melton, S.J., Kohler, A., Morrell-Falvey, J.L., Brun, A., Veneault-Fourrey, C., Martin, F., 2014. Effector MiSSP7 of the mutualistic fungus Laccaria bicolor stabilizes the Populus JAZ6 protein and represses jasmonic acid (JA) responsive genes. PNAS 111, 8299–8304. doi:10.1073/pnas.1322671111
Plett, J.M., Martin, F., 2012. Poplar root exudates contain compounds that induce the expression of MiSSP7 in Laccaria bicolor. Plant Signal Behav 7, 12–15. doi:10.4161/psb.7.1.18357
Wasternack, C., Hause, B., 2013. Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany. Ann. Bot. 111, 1021–1058. doi:10.1093/aob/mct067

Functional characterization of MiSSP8, a mutualistic effector required for ectomycorrhizal symbiosis

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

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Ectomycorrhizal symbioses are mutualistic interactions between soilborn fungi and roots of trees. Sequencing of mycorrhizal fungal genomes (Martin et al, 2008) sheds the light on hundreds of Mycorrhizal induced Small Secreted Proteins (MiSSPs). Among them, MiSSP8 is a 8-kDa protein highly expressed during symbiosis. RNAi knockdown of MiSSP8-encoding gene impairs *Laccaria bicolor*'s mycorrhization ability. Localization of synthetic MiSSP8 fused to fluorescein shows an apoplastic localization. The fungal Cap64-like protein is an putative interacting protein of MiSSP8 using a yeast-two hybrid assay. In addition, we showed using a polysaccharides precipitation assay that MiSSP8 is able to bind both fungal and plant polysaccharides, likely through an unknown repetitive motif. Collectively, our results suggest that MiSSP8 is a lectin-like protein participating to the building of the symbiotic interface during the ectomycorrhizal symbiosis.

Seeking for new fungal symbiosis effectors and MiSSP8 interacting proteins

François Jobert, Clement Pellegrin, Claire Veneault-Fourrey and Francis Martin.

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During the ectomycorhizal symbiosis with *P. trichocarpa* the fungus *L.* bicolor express and secretes small proteins called MiSSPs (Mycorrhiza-small Secreted Proteins). Among these proteins, MiSSP7 localizes in the nucleus of the plant cells and interacts with a master negative regulator of the jasmonic acid pathway (Plett *et al.,* 2014). We propose that other fungal small-secreted proteins (called effectors) could target master regulators proteins of hormonal pathways in *Populus*. The first aim of this study was to initiate a yeast two-hybrid assay in order to identify potential fungal effectors interacting with the poplar proteins *Pt*GAI1 and *Pt*NPR1, two regulators of the gibberellic acid and salicylic acid pathway, respectively. The autoactivation tests revealed that *Pt*GAI1 cannot be used with this yeast-two hybrid screens, in contrast to *Pt*NPR1. MiSSP8 and MiSSP17, two MiSSPs highly up-regulated during the ectomycorhizal symbiosis between *L. bicolor* and *P. trichocarpa* (Martin *et al.*, 2008) are required for symbiosis development as demonstrated by *L. bicolor* RNAi lines unable to establish a symbiosis with poplar roots. Candidate proteins interacting with either MiSSP8 or MiSSP17 were identified by yeast two-hybrid (Yohann Daguerre PhD). These interactions were tested in a recent BiFC system (Grefen et Blatt, 2012) in order to confirm or not them. Using transient transformation of tobacco leaves, first, validate the system used, and second, show that three MiSSP8-interacting proteins are not valid. Further investigations are in progress for other interacting proteins of MiSSP8 and MiSSP17.

Grefen C. & Blatt M. R. A 2in1 cloning system enables ratiometric bimolecular fluorescence complementation (rBiFC). *Biotechniques* **53,** 311–14 (2012).
Martin F. *et al.* The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. *Nature* **452,** 88–92 (2008).
Plett J. M., Daguerre Y., Wittulsky S., Vayssières A., Deveau A., Melton S. J., Kohler A., Morrel-Falvey J. L., Brun A., Veneault-Fourrey C. & Martin F. Effector MiSSP7 of the mutualistic fungus *Laccaria bicolor* stabilizes the *Populus* JAZ6 protein and represses jasmonic acid (JA) responsive genes. *PNAS* vol. **111** no. 22 8299-8304 (2014).

The transcriptional landscape of the *Pisolithus microcarpus* basidiocarp

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Pisolithus microcarpus is a gasteromycete of wide distribution in eucalypt plantations worldwide. This fungus produces a complex basidiocarp composed of peridioles at different developmental stages. Inside the basidiocarps, a large number of spores is produced that can be routinely used for seedling inoculation in eucalypt nurseries. Despite the progress achieved in the understanding of basidiosporogenesis in this species, nothing is known about the transcriptional patterns that occur along basidiocarp development. Taking advantage of the recently sequenced *P. microcarpus* genome and by using RNA-Seq technology we analyzed the transcriptome of different compartments of the *P. microcarpus* basidiocarp: unconsolidated, young and mature peridioles and as well as internal and mature spores.
A set of 737 transcripts was significantly regulated in minimum one of the compartments. Several genes involved in cell cycle, replication, transcription, and sugar transporters were strongly regulated in all the peridioles. However, in internal spores and mature spores, genes related to energy production were expressed. These results will be important for the understanding of *Pisolithus* biology and ecology and for its use in forestry production.

This project was funded by a LABEX ARBRE grant. We also would like to thank the Joint Genome Institute (JGI) for the access to unpublished data.

Comparative genomics reveals repeated sequences as main diversity source for black truffle

Thibaut Payen and Claude Murat

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Keywords: black truffle, genome plasticity, genome resequencing, SNPs, transposable elements
The Périgord Black truffle (*Tuber melanosporum*) is an ectomycorrhizal filamentous fungus found naturally in French, Italian and Spanish forests and is considered as a gastronomy delicacy. The genetic diversity of these fungi is questioned since 15 years. The aim in this work is to assess the overall genetic diversity and the factors driving genome plasticity of this species by resequencing six *T. melanosporum* isolates. These strains were chosen in different populations covering the whole natural distribution of this species. These isolates were sequenced using Illumina technology single end reads of 76 bp for a coverage of ~ 20 X. Considering the mapping of the reads against the reference genome previously sequenced, the *T. melanosporum* core genome was estimated to ~110 Mbp. Most of the regions without mapped reads corresponded to repeated sequences. A total of 442,326 SNPs corresponding to 3,540 SNPs/Mbp were identified among the seven genomes. *T. melanosporum* presented a genetic diversity similar to other filamentous fungi. The SNPs were more frequent in repeated sequences (85 %) although 4,716 SNPs were also identified in the coding region of 2,655 genes. Differences in insertion point for *gypsy* retrotransposons as well as high frequencies of SNPs suggested that transposable elements are among the main plasticity factor for the black truffle. In conclusion, this study proposed a set of SNPs that could be used in the future for analysing black truffle population genetic. To our knowledge this study presents the first large-scale identification of polymorphisms for a mycorrhizal species by genome resequencing.

Theme 3: Ecology and role of forest microbial communities

Spatial genetic structure of *Tuber melanosporum* in a productive orchard determined by mating type genes and microsatellites analysis

Herminia De la Varga[1], Claude Murat[1], Mélanie Lagoguet[1], Emila Akroume[1], François Le Tacon[1], Christophe Robin[2], Fabien Halkett[1], Francis Martin[1]

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Truffles are ascomycetes fungi belonging to the genus *Tuber* and forming ectomycorrhizal associations with trees and shrubs. The black truffle of Périgord (*Tuber melanosporum* Vittad.) is one of the most popular species because of its organoleptic qualities. The sequencing of its genome allowed the characterization of highly polymorphic microsatellite markers and the identification of mating type (it is a heterothallic species), which enables us to better understand the biology and evolution of this truffle.
A truffle plantation in Rollainville was selected to better understand 1) the genetic structure within a productive truffle orchard; 2) the dynamics of genotypes and 3) the distribution of genotypes of the two mating types. To carry out these analyses fruiting bodies, ectomycorrhizas and soil were sampled and mapped during 4 growing seasons around 7 productive trees. In order to investigate the genetic exchange in this truffle orchard both maternal and paternal genotypes were mapped by gleba and spores genotyping. Genotyping was performed by combining 10 microsatellite markers and the locus that determines the mating type.
We observed a great diversity with up to 13 genotypes in 50 m^2. Only few genotypes could be found in several seasons. A clear and stable division for the 4 seasons was observed with distinct zones with multiple genotypes of the same mating type. This result suggests a non-random distribution of the maternal genotypes for the colonization of the root system as a function of mating type. Moreover, in soil samples, it was possible to identify the two mating types in most of the samples. In conclusion, truffle orchards are dynamic ecosystems with a renewal of individuals suggesting that *T. melanosporum* invest preferentially in sexual reproduction for spreading.

Functional characterization of bacterial communities associated with black Perigord truffle during its life cycle

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The black truffle *Tuber melanosporum* is a hypogeous ectomycorrhizal ascomycete fungus. In contrast to many other mushrooms which quickly form above ground fruiting bodies, truffles slowly develop belowground in a six month process. During this time, truffle fruiting bodies are colonized by very specific bacterial communities that differ from those colonizing mycorrhizae. Thus, it is a good model for studying processes involved in bacterial communities selection in ascocarps or mycorhizae. Functional characterization of bacterial strains isolated from the ascocarp, mycorrhizae and the surrounding bulk soil at two stages of development illustrates a taxonomic and functional structuration. The truffle compartments appeared enriched in bacterial strains with high enzymatic potential compared to those of the surrounding soil. Although the functional tests used here did not allow us to conclude about the role of bacterial communities. Nevertheless, the results suggest a potential role in the maturation of the truffle.

"Bacteria are speaking!" Intercellular communication systems

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Quorum sensing (QS) is a widespread communication system used by bacteria for the regulation of functions involved in relation to their environment or host, such as the production of biofilms, motility patterns, luminiscence or virulence factors (Nealson et al., 1970).
This communication system involves the accumulation of signal molecules in the extracellular medium until a critical value is reached and the expression of target genes is triggered (Jayaraman and Wood, 2008). In response to this, some bacteria have developed different strategies to interrupt the QS pathway. One of them is known as quorum quenching (QQ) and it involves the enzymatic degradation of the signalling molecules. This suggests that disrupting the QS could be a valuable approach to develop new therapeutic strategies aimed at pathogens that rely upon QS for the regulation of their pathogenicity (Uroz et al., 2009).
Up to date, many QS systems have been described. For example, the production of exoenzymes in the plant pathogen *Pectobacterium carotovorum* is QS-regulated, as well as the production of siderophores in *Burkholderia cepacia* or the formation of biofilms in *Pseudomonas aeruginosa.* However, few studies are related to the QS and QQ systems in saline environments such as sea water or hypersaline soils (Llamas et al., 2005).
The aims of this work are the following . First, the study of the QS systems in fish pathogens and the isolation of new QQ bacteria capable of disrupting those communication mechanisms. Second, the screening for the QS and QQ genes in salt concentrated soil.

Nealson KH, Platt T, Hastings JW. (1970). Cellular control of the synthesis and activity of the bacterial luminescence system. J Bacteriol, 104, pp 313–322.
Jayaraman A and Wood TK. (2008). Bacterial quorum sensing: signals, circuits and implications for biofilms and disease. Annu Rev Biomed Eng, 10, pp 145-167.
Uroz S, Dessayux Y, Oger P. (2009). Quorum sensing and quorum quenching: the Yin and Yang of bacterial communication. Chem Bio Chem, 10, pp 205-216.
Llamas I, Quesada E, Martínez-Cánovas MJ, Gronquist M, Eberhard A, González JE. (2005). Quorum sensing in halophilic bacteria: detection of *N*-acyl-homoserine lactones in the exopolysaccharide-producing species of *Halomonas*. Extremophiles, 9, pp 333-341.

MOS long-term monitoring network: elaboration and characterization of initial state of MOS network by NIRS/MIRS

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MOS network is an 18 sites network for long-term monitoring about French forest ecosystems. It focuses on impacts of logging residues removal on forest soil fertility and biodiversity. Indeed, current energy policies are characterized by combined willing to reduce fossil energy consumption and to develop renewable energies market, particularly woodfuel sector. In such environment, wood harvesting is expected to increase for next years.
This study is focusing on two aspects: the interactions between carbon and nitrogen cycling in forest soils and impacts of this forest management on soil microbial communities. Four management treatments will be studied on each site with 3 repetitions by treatments: current managing, remnant removal, remnants and leave litter removal, remnants removal and ashes fertilization. Three tree species will be studied: oak, beech and douglas fir.
Eleven sites in deciduous forests have been set up during last year, with an important prospecting work for initial characterization. In particular, choice of treatments positions on each site has been determined by random drawing, based on soil mid/near infra-red spectroscopy analysis. MIRS/NIRS data provide a precise and global mapping of soil heterogeneity at site scale. It permits to develop an efficient method to get quickly a global view of site plot. At the same time, microbial diversity analysis for initial description have been started this year (Cf abstract Master 2 P. Royer).

Impact of massive forest remnant removal on fungal richness and community structure

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Since the "Grenelle" debate, the public policy aims to reduce fossil energy consumption and to develop renewable energies (20% of the energy through renewable resources before 2020). Thus, forest managers have to increase the export of wood, brushwood, and wood residues. However the impact of these biomass exportations on soil fertility and soil biodiversity are poorly known.
The subject of my research Master takes place in this context. To measure those effects, a long-term monitoring network of eleven experimental sites has been setup in France (MOS network). My task is to describe the functional and taxonomical diversity of fungi communities of those soils before wood export. Indeed, the biogeographical patterns of fungal assemblages have been little explored and, consequently, the factors driving the diversity and the composition of these communities are poorly understood. The first step of the projet was to determine whether the diversity and composition of soil fungal assemblages vary with soil geographic gradients and plant host (beech versus oak). High-throughput sequencing of the ITS-1 region was used to explore fungal assemblages in 88 composite soil samples from the MOS network. Moreover, the functional diversity of these soil microbial communities was investigated with enzymatic tests developed for hydrolytic and oxidative functions involved in the degradation of ligno-cellulose, chitin and P-containing organic compounds. In this propose, I established an experimental protocol for the study of soil fungal functions from different horizons, before and after the bud break of dominant species. This approach could determine whether the ecological niches and environmental parameters affect microbial functions.

Key words: Environmental genomics, functional diversity, fungi, organic matter.

Theme 5: Mechanisms and evolution of poplar-poplar rust interactions

Evolution of the determinants of gene-for-gene interactions: the case of poplar rust *Melampsora larici-populina* during a major resistance breakdown

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Host-pathogen interactions imply a molecular dialogue between partners, likely governed by the gene-for-gene model. Among the molecules involved, effectors produced by the pathogen allow it to interfere with the defense responses of the plant, leading to the successful infection and disease [1]. Effectors discovered so far show common characteristics, covered by the term of SSP (Small Secreted Protein).
In order to monitor the molecular consequences of an evolutionary change in gene-for-gene interaction, we propose to identify loci subject to selection during a drastic breakdown event. Our model for the study is *Melampsora larici-populina* agent of poplar rust, primary disease of the European poplar. Its genome has been sequenced, assembled and a systematic search for SSP was performed [2].
A population genetics analysis based on 600 isolates *Melampsora larici-populina* allowed the detection three distinct genetic groups. Building on that result, a first comparative genomics study of 14 re-sequenced genomes revealed SSP potentially under selection, but also several weaknesses of this "SSP search based" approach. In the following we aimed at combining this approach with a more throughout population genomics study, based on the resequencing of a hundred of isolates. Ongoing genome scan analyzes have already pointed to new genomic regions of interest, some of which encode SSP representing effector candidates for functional analysis.

[1] Dangl, JL. Horvath, DM., Staskawicz, BJ (2013). “Pivoting the plant immune system from dissection to deployement.” Science **341**:746-751.
[2] Duplessis, S., C. A. Cuomo, et al. (2011). "Obligate biotrophy features unraveled by the genomic analysis of rust fungi." Proc Natl Acad Sci U S A **108**(22): 9166-71.

Identification of candidate effectors of *Melampsora larici-populina*

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Rust fungi are serious pathogens of many plants. One group of rust fungi is the Melampsoraceae family. And although the life cycle of this fungal group it is known very well, the molecular mechanisms of plant-fungi interaction still need to be studied. The first step of our work is to identify Melampsora effectors which initiate plant infection. To perform this task 25 Melampsora candidates were screened by B. Pêtre at the beginning of the project. We recloned 9 of them to E. coli expression system and tried to purify these molecules. We were able to produce 7 proteins from 9 in heterological system, but we concentrate our work on 2 of them, because expression of these proteins was the highest and they were easiest to purify. Thus we managed to obtain sufficient amounts of 2 effectors candidates for future study.

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