January 27th 2017

Zürich Mycology Symposium 2017

Swiss Federal Institute of Technology (ETH Hönnggerberg), Room HIL E9, Stefano-Franscini-Platz 5, 8093 Zürich

08:30 - 09:00  **Arrival and Registration** (fee CHF 10.-)

**Morning session I: Animal parasites and pathogens** *(chaired by Markus Künzler)*

09:00 - 09:05  **Opening remarks**

09:05 - 09:25  Christina Braunsdorf (Group Leibundgut), VetSuisse, University of Zürich
*Candida albicans* strain specific differences in host-pathogen interaction in oral candidosis

09:25 - 09:45  Dominique Sanglard, Institute of Microbiology, CHUV Lausanne
Understanding multidrug resistance in *Candida lusitaniae*

09:45 - 10:05  Deborah Kaiser (Group Vogelsang), Agroscope ART, Zürich
Biological control of pollen beetles with the entomopathogenic fungus *Beauveria bassiana* – the tricky path to an efficient formulation

10:05 - 10:25  Johanna Mayerhofer (Group Widmer), Agroscope ART, Zürich
Non-target effects of *Metarhizium brunneum* on soil microorganisms in biocontrol of Agriotes spp. in potato production

10:25 - 10:55  **Coffee break**

**Morning session II: Saprophytes** *(chaired by Markus Künzler)*

10:55 - 11:15  Niels van der Velden (Group Künzler/Aebi), Institute of Microbiology, ETH Zürich
Identification of a novel class of fungal RiPPs featuring backbone N-methylation

11:15 - 11:35  Claude Herzog (Group Brunner), Eidg. Forschungsanstalt WSL, Birmensdorf
Fungal succession on decomposing pine roots

11:35 - 12:25  **Microsynth-Lecture**
Stefanie Pöggeler, Georg-August Universität Göttingen
Sexual development in *Sordaria macrospora*: signaling and nutrient recycling during fruiting-body formation
12:30 - 14:00  **Lunch break** *(at mensa 'Fusion Meal', 1st floor across the street)*

**Afternoon session I: Plant pathogens and symbionts** *(chaired by Pilar Junier)*

14:00 - 14:20  Krishnan Parvathy (Group McDonald), Institute of Integrative Biology, ETH Zürich
Variable levels of melanization in *Zymoseptoria tritici* population is mediated by differential expression of a transcription factor

14:20 - 14:40  Clémence Plissonneau (Group McDonald), Institute of Integrative Biology, ETH Zürich
The evolution of orphan regions in genomes of the fungal pathogen *Zymoseptoria tritici*

14:40 - 15:00  Gioele Fiori (Group Egli/Peter), Eidg. Forschungsanstalt WSL, Birmensdorf
Analysis of the genetic diversity and mating type distribution of the Burgundy truffle - Using a fine-scale partition plan in a productive Swiss *Tuber aestivum* orchard

15:00 - 15:20  Maira de Freitas Pereira (Group Egli/Peter), Eidg. Forschungsanstalt WSL, Birmensdorf
The role of Small Secreted Proteins (SSPs) in the interaction of the ectomycorrhizal fungus *Cenococcum geophilum* with its host plants

15:20 - 15:50  **Coffee break**

**Afternoon session II: Interaction with bacteria** *(chaired by Pilar Junier)*

15:50 - 16:10  Laure Weisskopf, University of Applied Sciences and Arts Western Switzerland, Nyon
The potential of bacterial volatiles for plant protection against diseases

16:10 - 16:30  Andrea Lohberger (Group Junier), Institute of Microbiology, University of Neuchâtel
Organic nitrogen exploitation as a benefit stabilizing mutualistic interaction of soil fungi and bacteria

16:30 - 16:50  Lucrezia Comensoli (Group Junier/Joseph), Institute of Microbiology, University of Neuchâtel
The role of fungi in the geochemical cycle of iron

16:50 - 16:55  **Closing remarks**

Organisation: Markus Künzler and Ilka Riedel
Abstracts - Zürich Mycology Symposium, January 27th 2017, ETH Hönggerberg

Salome Leibundgut-Landmann, VetSuisse, University of Zürich

_Candida albicans_ strain specific differences in host-pathogen interaction in oral candidosis

The opportunistic fungal pathogen _Candida albicans_ can cause a variety of infections ranging from superficial mucosal infections to life threatening systemic infections with huge economical impact. _C. albicans_ is found as a commensal in healthy individuals, where fungal overgrowth is restricted by epithelial barrier functions and by the immune system. In particular, neutrophil granulocytes and IL17-dependent responses are relevant for fungal control both in murine and human hosts. If the immune system is compromised or epithelial barrier functions are breached, _C. albicans_ can disseminate in the host leading to Candida sepsicaemia with mortality rates up to 50%. There is an increasing prevalence of fungal infections due to increasing numbers of patients with immunosuppression and at the same time fungal isolates with resistance to the available antifungal drugs are on the rise. Our aim is to better understand the interaction between _C. albicans_ and its host to be able to identify patients at risk and develop novel strategies for antifungal therapies. In particular, we are interested to understand the impact of the natural diversity of _C. albicans_ on the outcome of the interaction with the host. For this we use a murine infection model and cell-based in vitro assays. Here we would like to discuss current advances in investigating the differences among _C. albicans_ isolates that use distinct infection strategies, resulting in different levels of immune recognition and persistence in the host.

Dominique Sanglard, Institute of Microbiology, CHUV Lausanne

Understanding multidrug resistance in _Candida lusitaniae_

Antifungal resistance is an inevitable phenomenon when fungal pathogens get exposed to antifungals. Multidrug resistance (MDR) has emerged in hospitals due to the use of several agents administered in combination or sequentially to the same individual. We investigated MDR in Candida lusitaniae during therapy with azoles and candins within a 3 months time lapse and resolved MDR mechanisms.

Isolates were obtained during therapy with azoles (targeting sterols) and candins (targeting cell wall). Four different resistance profiles from 5 different isolates (P1-P5) were identified against 2 to 3 different drug classes. To investigate MDR, analysis of specific drug resistance genes or comparisons of genomes obtained by PacBio sequencing were used.

Azoite resistance in P3 and P4 was associated with upregulation of a multidrug transporter (MFS7) and coincided with resistance to 5-flucytosine (FK5). Candida resistance in P3 and P5 was related to upregulation of P2 and P5. Thus, P2 to P5 accumulated different drug resistance mutations as compared to P1 that emerged during the short time lapse therapy. MDR emergence the variety of the resistance profiles is intriguing. By analogy with a recent study of MDR in Candida glabrata which highlighted the role of defects of the DNA mismatch repair machinery in MDR, we are currently investigating this possibility in _C. lusitaniae_.

Deborah Kaiser (Group Vogelsang), Agroscope ART, Zürich

Biological control of pollen beetles with the entomopathogenic fungus _Beauveria bassiana_ – the tricky path to an efficient formulation

Pollen beetles, _Meligethes_ spp., cause substantial yield loss in oilseed rape throughout Europe. Increasing insecticide resistance and lack of sustainable control options urge the development of novel treatment methods. In previous studies with the entomopathogenic fungus _Beauveria bassiana_, high pollen beetle mortality in laboratory experiments but no significant increase in yield was seen. To improve efficiency, we explored synergistic interactions between _B. bassiana_ spores and vegetable oils or stone dusts that have previously been shown to affect adult pollen beetles. The combined application of fungus spores and vegetable oil achieved higher and faster pollen beetle mortality in laboratory experiments. A field experiment indicated reduction in pollen beetle number, higher amount of pods and increased yield. However, field samples showed resistance in P1, but decreased resistance after three days, which is probably due to the susceptibility of spores to ultraviolet irradiation. Consequently, we examined natural compounds to protect the fungus’ spores from ultraviolet irradiation when applied in the field. Promising substances are tested as UV-protectant additives to spore formulations. Mortality and mycosis of collected pollen beetles treated with different combinations of _B. bassiana_ spores and vegetable oils was assessed in bioassays and field experiments. Survival of fungus spores in field applications was assessed by plating processed plant material to agar plates and counting grown colony forming units. Effect of UV protectants was tested on survival of fungus spores applied to agar plates or leaf discs and exposed to artificial UV irradiance. The combined application of _B. bassiana_ spores and vegetable oil caused a mortality of up to 80% in pollen beetles in laboratory experiments. Fungus spores isolated from collected plant material showed a decrease in viability of 90% and 100% three and seven days after field application, respectively.

Formulation of _B. bassiana_ spores with natural UV protectants increased the number of surviving spores up to a factor of two, relative to untreated spores. Screenings of further potential UV protectants in various concentrations are currently ongoing on lab scale.

Johanna Mayerhofer (Group Widmer), Agroscope ART, Zürich

Non-target effects of _Metarhizium brunneum_ on soil microorganisms in biocontrol of _Agriotes_ spp. in potato production

Biological control using entomopathogenic fungi includes application of large amounts of fungal propagules to the soil, often resulting in densities of up to 1014 propagules per ha. Such mass applications potentially affect soil microorganisms (fungi and prokaryota) and the ecosystem functions they fulfill. A biocontrol experiment was performed to control _A. obsecus_ larvae with the fungal entomopathogen _Metarhizium brunneum_ in potato production in a pot and a field experiment. Four different formulations of _M. brunneum_, two co-formulations of _M. brunneum_ and garlic extract, garlic extract alone as well as an insecticide and one formulation control were tested. Non-target effects of _M. brunneum_ on soil fungi and prokaryota were assessed using illumina high throughput sequencing of ribosomal RNA (fungi: ITS2, prokaryota 16S V3-V4). _M. brunneum_ densities and soil microbial communities were monitored over a period of four months. _M. brunneum_ formulated as fungus colonized barely kernels was the most efficient treatment in controlling _Agriotes obsecus_ larvae in the pot experiment with...
77% reduction of potato tuber damage compared to untreated control pots. None of the treatments resulted in efficient Agrilotes control in the field experiment. Fungal treatments had small effects on fungal communities and garlic treatments affected prokaryotic communities in the pot experiment. Effects detected were in the range of the effects caused by formulations alone or observed in the controls over time. In the field experiment neither treatment-nor time-effects but spatial differences in fungal and prokaryotic communities were detected. Results obtained in this study suggest that the application of M. brunneum does not pose a risk for soil microorganisms.

Niels van der Velden (Group Künzler/Aebi), Institute of Microbiology, ETH Zürich

**Identification of a novel class of fungal RiPPs featuring backbone N-methylation**

N-Methylated backbone amides alter the physicochemical properties of peptides and are critical for the activity and stability of blockbuster drugs like the immunosuppressant cyclosporin A. In nature, backbone N-methylations have not been observed in proteins, but only in peptides not synthesized by the ribosome. Here, we show that omphalotins, cyclic backbone N-methylated peptides from the fungus *Omphalotus olearius*, are ribosomally synthesized and post-translationally modified peptides (RiPPs). Expression of a single gene, ophA, in *Escherichia coli* revealed auto-o-N-methylation activity of the encoded protein. Remarkably, the sequence destined to be the nematotoxic natural products is encoded in the C-terminus of OphA, and we demonstrate that OphA catalyzes iterative α-N-methylation of this sequence. Thus, OphA is the first observed case of backbone N-methylations as post-translational modifications (PTMs) and the first RiPP precursor found to be capable of catalysis or self-modification. The omphalotins represent, therefore, a new RiPP family, one of the few so far identified in fungi. We also report that OphA can methylate engineered peptide sequences exchanged for the core omphalotin sequence, providing a straightforward and convenient biotechnological strategy to produce custom peptides with backbone N-methylation.

Claude Herzog (Group Brunner), Eidg. Forschungsanstalt WSL, Birmensdorf

**Fungal succession on decomposing pine roots**

Forest carbon sink potential will be strongly influenced by the changing climate. Expected longer drought periods increase root mortality on a short time scale and affect root production and decomposition on a longer scale. Primary production is one part of a very complex system in which environmental factors and microbial community composition are key for the decomposition of roots. Root litter which is not or not entirely degraded becomes soil organic matter. In comparison to foliar litter, which is known to decompose fast and nearly completely, root litter contains more recalcitrant components (e.g. lignin) and is partly protected by soil from decomposing.

In our study we focused on the fungal succession on decaying roots during two. We hypothesized, ubiquitous fungi at an early stage and specialized fungal degrader of recalcitrant litter components at a later stage.

An irrigation system was installed in a drought prone pine forest to counteract summer drought events. Litterbags were buried with two classes of roots (fine, coarse) and gradually sampled over two years. Decomposition was measured as mass remaining, and the fungal community was assessed by Illumina® amplicon sequencing of ITS II.

The effect of irrigation on decomposition rate was minor for fine and coarse roots. Therefore, more frequent summer drought events rather do not limit microbial decomposition rate. Nonetheless, a clear successional shift in the fungal community during decomposition was detected. Moreover, fungal communities differed significantly between control and irrigation. This leads to the conclusion that fungal community shifts but the function (e.g. decomposition rate) remains. This phenomenon is known as functional redundancy. Decreasing water availability influenced the fungal community but was redundant for root decomposition rate. Root derived carbon in soils remains difficult to quantify, but maybe the root derived inputs prove to be more stable than expected.

**Microsynth-Lecture:**

Stefanie Pöggeler, Georg-August Universität Göttingen

**Sexual development in *Sordaria macrospora* signaling and nutrient recycling during fruiting-body formation**

The homothallic filamentous ascomycete *Sordaria macrospora* is an ideal model organism to study multicellular fruiting-body development. The natural habitat of *S. macrospora* is the dung of herbivores. The fungus lacks asexual reproduction and is strictly dependent on the sexual cycle for the production of ascospores. The availability of a library of mutants with a genetic block at different stages of fruiting-body formation is an excellent source for the isolation of developmental genes. A number of *S. macrospora* developmental mutants exhibit defects in the conserved eukaryotic striatin-interacting phosphatase and kinase (STRI/PAK) protein complex [1], indicating that crosstalk between kinases and phosphatases is crucial for the coordinated regulation of sexual differentiation processes.

In addition, it turned out that supply and homeostasis of nutrients are important issues for sexual development. We therefore analyzed the role of autophagy during fruiting-body formation. Autophagy is a degradation process in which eukaryotic cells digest their own cell constituents [2]. To analyze whether nutrient recycling by autophagy is required for the formation of fruiting bodies, we have characterized conserved components of the autophagic machinery and could show that autophagy is an essential and constitutively active process to sustain high energy levels for multicellular development [3-5].

Krishnan Parvathy (Group McDonald), Institute of Integrative Biology, ETH Zürich

Variable levels of melanization in Zymoseptoria tritici population is mediated by differential expression of a transcription factor

In fungal pathogens melanin play a major role in tolerating harmful radiation, virulence, and resistance to fungicide. Even though the biosynthetic pathway of melanin is highly conserved across different fungal species, we observed a high variability in the degree of melanization among the different isolates of the wheat pathogen Zymoseptoria tritici. A mapping population derived from a cross between two Swiss isolates 3D1 (lighter) and 3D7 (darker) was used to understand the genetic basis of the differences in melanin accumulation. Of the 6 candidate genes identified on chromosome 11, two belong to the melanin biosynthetic gene cluster, including a transcription factor, CMR1 (Colletotrichum Melanin Regulation), that regulates the expression of the genes in the melanin biosynthetic gene cluster in other fungal species. Disruption of CMR1 homologue in Z. tritici leads to loss of melanin deposition, confirming its role in melanin biosynthesis. Although, we did not observe any sequence polymorphism in the coding sequence of CMR1 that will explain the differences in melanin accumulation between 3D1 and 3D7. CMR1 expression levels were significantly lower in the lighter isolate (3D1) compared to the darker one. Interestingly, a transposable element 1.8 kb upstream of the start codon of CMR1 was identified only in 3D1 and not in 3D7. Preliminary data indicates that CMR1 expression in 3D1 is repressed by the transposable element. Thus, gene expression level polymorphism of a major melanin regulator, leads to differences in melanin accumulation in Z. tritici and is linked to the presence/absence of a TE that regulates its expression.

Clémence Plissonneau (Group McDonald), Institute of Integrative Biology, ETH Zürich

The evolution of orphan regions in genomes of the fungal pathogen Zymoseptoria tritici

Fungal plant pathogens rapidly evolve virulence on resistant hosts through mutations and loss or gains of genes. The rapidly evolving genes often encode proteins modulating the immune responses by the host. However, the mechanisms creating adaptive genetic variation and the levels of genomic diversity in populations are poorly understood. We aimed to quantify structural variations and the mechanisms underlying their creation in the highly polymorphic fungal wheat pathogen Zymoseptoria tritici. The genetic basis of virulence in Z. tritici is complex and populations harbor significant genetic variation for virulence. We used high-coverage PacBio sequencing, genetic maps and transcriptomics data to generate four fully assembled and annotated genomes of Z. tritici sampled from a Swiss population. Comparative genomic analyses against the complete reference genome IPO323 identified large inversions and the complete gain or loss of transposable element clusters, explaining the chromosomal length polymorphisms found in this species. All genomes harbored long tracts of sequences exclusive to them. In each isolate, we identified from 296 to 340 genes which were absent from the reference genome IPO323, including new candidate effector-coding genes. These newly identified genes tended to be organized in clusters and showed evidence for mutational decay. Our study showed that pathogen populations harbor extensive structural polymorphism that may drive virulence evolution and highlights the "pangenomics" approach to effector research on plant pathogens fungal plant pathogens.

Gioele Fiori (Group Egli/Peter), Eidg. Forschungsanstalt WSL, Birmensdorf

Analysis of the genetic diversity and mating type distribution of the Burgundy truffle - Using a fine-scale partition plan in a productive Swiss Tuber aestivum orchard

The domestication of ectomycorrhizal (ECM) truffles has increased in importance worldwide as an economical alternative to traditional agriculture. Particularly the Burgundy truffle (Tuber aestivum) seems to be most suitable for cultivation due to its wide ecological range, being less selective to soil, host tree, and climate than other Tuber species. Several aspects of the sexual reproduction of truffles have been elucidated only recently. However, the life cycle and dispersal of these edible mushrooms are still poorly understood. This work aims to improve knowledge about the genetic factors involved in the sexual reproduction of T. aestivum and to implement it in an agricultural management context. 445 ECM root tips were investigated from 27 host plants within an eight-year-old T. aestivum orchard located in Eastern Switzerland. Additional 42 ECM root tips were collected from three native plants neighboring the orchard. Moreover, all sampled fruit bodies of T. aestivum were analyzed to identify parental gametes. T. aestivum mating type (MAT) markers were used to examine the distribution of the two idiomorphs, master regulators MAT1-1-1 and MAT1-2-1. Genotypes of host plants and of T. aestivum on ECM root tips and fruit bodies were analyzed using specific SSR markers to identify the diversity, genetic isolation, potential vegetative incompatibility and maternal and paternal elements.

The first results show that almost 80% of the 27 host plants are colonized by T. aestivum, the majority of which is colonized by both MATs. However, the EMCs show a skew mating type distribution in favor of MAT1-1-1. Concerning the spatial distribution, an aggregation of the mating types was observed. Further results of the genetic diversity will concretize whether a real mating type aggregation exists and will elucidate the population genetic structure of T. aestivum as well as the inter- and intra-species relationships of ECM fungi in this truffle orchard.

Maira de Freitas Pereira (Group Egli/Peter), Eidg. Forschungsanstalt WSL, Birmensdorf

The role of Small Secreted Proteins (SSPs) in the interaction of the ectomycorrhizal fungus Cenococcum geophilum with its host plants

In nature, soil-born fungi associate with roots of trees to form ectomycorrhizas (ECM). In exchange for carbohydrates, ECM fungi improve mineral supply of host trees, protect them against stresses such as drought and directly contribute to the exclusion of competitive microbes. To facilitate nutrient exchanges, root morphology is strikingly altered and a symbiotic organ, the ectomycorrhiza, is formed. How beneficial organisms such as mycorrhizal fungi manage to colonize plant tissues while avoiding plant defense systems remains elusive. Recent studies highlight that mycorrhizal fungi, as pathogenic ones, use effectors in the form of Small Secreted Proteins (SSPs) as molecular keys to promote symbiosis. Functionally characterized, however, is only one of such a mycorrhiza-induced SSP (MISsP7) from the first sequenced ECM basidiomycete Laccaria bicolor.

Our aim is to elucidate the role of effector-like SSPs in the mycorrhiza formation and functioning of the ecologically very important species Cenococcum geophilum by taking advantage of available genomic and transcriptomic resources. The specific objectives are to (i) conduct an in-depth genome-wide survey of SSPs in C. geophilum and mining the available gene expression data to identify candidate effectors (ii) use 15 re-sequenced C. geophilum strains and closely related dothideomycetous genomes to study intra- and interspecific diversity in the sequences of the candidate effectors to elucidate their evolution and conservation and (iii) functionally characterize a few selected candidate effectors through a) gene
Iron is essential to complete our knowledge of the biogeochemical processes involved in iron cycling. Biomass for microbial growth was measured by quantifying siderophore production in liquid cultures of Pseudomonas putida. Of the iron, with 10 mM of iron citrate or iron chloride. Moreover, in terms of iron uptake, Alternaria sp. was capable of accumulating 80% of the iron present.

This study aimed at generating basic knowledge concerning the interaction between filamentous fungi and iron through the evaluation of the most important metabolic activities that could involve this metal. Spectrophotometric analyses and scanning electron microscopy were conducted in order to evaluate reduction, uptake and biogenic mineral production from soluble iron. The role of fungi in the geochemical cycle of iron is the second most abundant element on Earth; nevertheless, only a small part of it is bioavailable. Hence in order to access this element, microbes have developed different strategies that have significant influence on iron geochemical cycling. Although most studies have focused on bacteria, microbial diversity is much larger than that and fungi are often overlooked. Except for pathogenic fungi and yeast, the understanding of the interaction between filamentous fungi and iron is incomplete. This study aimed at generating basic knowledge concerning the interaction between filamentous fungi and iron through the evaluation of the most important metabolic activities that could involve this metal. Spectrophotometric analyses and scanning electron microscopy were conducted in order to evaluate reduction, uptake and biogenic mineral production from soluble iron phases in filamentous fungi. A screening of 12 strains allowed to observe the abilities of Beauveria bassiana to chelate iron as aggregates with extracellular polymeric substances, as well as to form biogenic minerals when inoculated in cultures amended with 10 mM of iron citrate or iron chloride. Moreover, in terms of iron uptake, Alternaria sp. was capable of accumulating 80% of the iron supplied as iron chloride in the medium after only 4 days of incubation. Further, the bioavailability of this iron-rich biomass for microbial growth was measured by quantifying siderophore production in liquid cultures of Pseudomonas putida. Iron is an important element for microbial growth, and a better understanding of the interactions between filamentous fungi and iron is essential to complete our knowledge of the biogeochemical processes involved in iron cycling.