Werkgroep Fusarium

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Chromatin-mediated silencing in Fusarium graminearum

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We use fungi as model organisms to study chromatin-mediated gene silencing. We found that in many filamentous fungi centromeric chromatin is heterochromatic (i.e. largely transcriptionally silent) and marked by histone H3 trimethylation at lysine 9 (H3K9me3). Some fungi also have H3K27me3 enriched at centromeres, though most of this mark is subtelomeric. In fusaria, as in the genus *Neurospora* [1], H3K27me3 is found in non-syntenic, non-conserved regions [2]. In *Fusarium graminearum*, H3K27me3 controls more than 20% of all genes. Deletion of the gene encoding the catalytic subunit for the Polycomb Repressive Complex 2, the lysine methyltransferase KMT6 resulted in expression of much of the «cryptic genome», regions that are usually transcriptionally silent when fungi are grown under lab and not environmentally conducive conditions (e.g.*in planta* or in competition with other microbes). Our work has implications on the study of secondary metabolite gene clusters and pathogenicity of fungi on animals and plants.

References:

[1] Jamieson *et al.* 2013: DOI: 10.1073/pnas.1303750110
[2] Connolly, Smith and Freitag 2013: DOI: 10.1371/journal.pgen.1003916

Transfer and stability of Lineage Specific chromosomes in F. oxysporum

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The *F. oxysporum* genome consists of a number of core chromosomes which are present in all strains and required for normal functioning of the fungus as well as a number of Lineage Specific (LS) chromosomes. These LS chromosomes often encode genes involved in pathogenicity. Transfer of these chromosomes to a non-pathogenic strain leads to gain of pathogenicity while loss of these chromosomes presumably leads to loss of pathogenicity. We have devised and applied methods to assess which chromosomes are amenable to transfer or loss.

Several hundred random insertions of a marker in a pathogenic strain were tested for transfer to a non- pathogenic strain. Four of these showed consistent transfer of the marker and the LS chromosome 14, indicating only this chromosome is amenable to transfer. The 5 smallest chromosomes were then tested independently with the same result: only transfer of chromosome 14 was observed.

To test the stability of LS and core chromosomes these were marked with a fluorescent marker and then screened for loss of the marker using flow cytometry. Non-fluorescent spores were collected and individually cultured and analyzed. When comparing a core chromosome with an LS chromosome, loss of the marker was much more frequent when expressed from the LS chromosome. Loss of fluorescence expressed from the core chromosome could often be explained by partial or complete deletion of the marker itself. Loss of fluorescence expressed from an LS chromosome could often be linked to complete loss of the chromosome or to very large deletions spanning several 100Kb.

Fusarium oxysporum mitochondria in the Next Generation Sequencing era

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In recent years more and more WGS (whole genome sequencing) projects are becoming publicly available. Despite this fact, the number of published mitochondrial genomes is lagging behind. There are only six mitochondrial genomes ready for *Fusarium* spp., but there are more than twenty WGS projects available.
Our group has developed a program, GRAbB (Genomic Region Assembly by Baiting), which can selectively assemble regions of the genome from next generation sequencing reads. Using this program and the publicly available WGS reads we have assembled and annotated twenty-seven mitochondrial genomes of *Fusarium oxysporum* strains. We also re-sequenced the first *F. oxysporum* strain (F11) that had its mitochondrion sequenced and a *F. proliferatum* strain to be used as an outgroup. Besides the mitochondrial genomes we also extracted seven nuclear marker sequences that have been used for phylogenetic study of the FOSC.
Previous studies have identified a highly variable region in the mitogenome of *Fusarium spp*, which is found between MT-RNR2 and MT-ND2 genes. This variable region encodes a large (~6kb) ORF. In our dataset we found that within the FOSC there are two more variants of this region. All three variants contain the same tRNA genes, except for one of the variants, which contains an additional tRNA gene. Only one of the variants contains the typical large ORF, which was described in other *Fusarium spp*. The variants are not clade specific and the trees inferred from the variable regions are similar to the trees inferred using an eight-marker dataset. These findings make it likely that there is mitochondrial recombination going on within the species.

Relocation and co-regulated gene expression patterns in Fusarium graminearum

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Genome comparisons between closely related species often show non-conserved regions across chromosomes. Some of them are located in specific regions of chromosomes and some are even confined to one or more entire chromosomes. The origin and biological relevance of these non-conserved regions are still largely unknown. The genome of *Fusarium graminearum* genome was studied to elucidate the significance of non-conserved regions. In the genome of *F. graminearum* harbours thirteen non-conserved regions dispersed over all of the four chromosomes. Using RNA-Seq data from the mycelium of *F. graminearum*, we found weakly expressed regions on all of the four chromosomes that exactly matched with non-conserved regions. Comparison of gene expression between two different developmental stages (conidia and mycelium) showed that the expression of genes in conserved regions is stable, while gene expression in non-conserved regions is much more influenced by the developmental stage. In addition, genes involved in the production of secondary metabolites and secreted proteins are enriched in non-conserved regions, suggesting that these regions could also be important for adaptations to new environments, including adaptation to new hosts. Finally, we found evidence that non-conserved regions are generated by sequestration of genes from multiple locations. Gene relocations may lead to clustering of genes with similar expression patterns or similar biological functions, which was clearly exemplified by the *PKS2* gene cluster. Our results showed that chromosomes can be functionally divided into conserved and non-conserved regions, and both could have specific and distinct roles in genome evolution and regulation of gene expression.

Reference:

Relocation of genes generates non-conserved chromosomal segments in *Fusarium graminearum* that show distinct and co-regulated gene expression patterns. C Zhao, C Waalwijk, PJGM de Wit, D Tang, T van der Lee. BMC genomics 15 (1), 191

Combination antifungal activity on conidia and hyphae of Fusarium species

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Objectives
Data on antifungal susceptibility of hyphae, elements that better represent the fungal form in tissues, are scarce. We aimed to study the in vitro combined activity of antifungals against conidia and hyphae of Fusarium species, using a pharmacodynamic-based methodology. Methods Sixteen clinical strains were tested in total: 5 *F. solani* species complex (SC), 8 *Gibberella fujikuroi* SC (5 *F. verticillioides*, 3 *F. proliferatum*), 2 *F. oxysporum* SC and one *Fusarium* spp. Hyphae were formed after a 12h incubation of conidia at 37°C. Dual combinations of Amphotericin B (AmB), anidulafungin (AND), posaconazole (POS) and voriconazole (VOR) were tested against the conidia or hyphae in a chequerboard assay based on the EUCAST methodology. The MICs were determined with the XTT tetrazolium salt method and synergy was estimated by calculation of the fractional inhibitory concentration (FIC) indices. *Aspergillus fumigatus* ATCC 204305 and *Candida krusei* ATCC 6258 were used as quality control strains.

Results
The MICs of the antifungals alone did not differ significantly between conidia and hyphae. The FICs against conidia or hyphae were comparable regardless of the drug combination used. AmB/AND was the combination showing synergy more frequently against conidia and the combination AmB/POS against hyphae. Synergy was not species related. Antagonism was in no case observed.

Conclusions
Antifungal drug activities were equally represented by conidia or hyphae in the above setting. The study is ongoing with more strains and combinations and is supported by an investigator-initiated research grant from Pfizer.

Genomic and proteomic based species detection of clinically relevant Fusarium fujikuroi species complex

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Fusarium is a hyaline hyphomycete fungus commonly found in the environment, isolated from soil, plants and water systems. Some Fusarium species cause a broad spectrum of opportunistic infections in humans, it is reported to cause invasive and disseminated infections that occur predominantly in severely immunocompromised patients. Risk factors for the development of invasive fusariosis are neutropenia, hematologic malignancies, hematopoitic cell transplantation, and patient's deficit with cellular immunity. Superficial infections, such as keratitis or endophtalmitis and onychomycosis, are frequently manifested in immunocompetent persons and may be associated with trauma.
The *Fusarium fujikuroi* species complex (FFSC) contains at least 13 species that have been found in human infections, causing the whole range of infections. FFSC is involved in the deep and disseminated infections causing fusariosis next to members of the *Fusarium solani* and *Fusarium oxysporum* species complexes.
We collected more than 120 clinical and environmental samples of all the known human pathogenic species and some non-pathogenic species within the FFSC including their type strains. Based on 15 nuclear sequences we developed an MLST for all these strains, grouping them in well-supported clades and confirming their identifications. Finally we validated this MLST with other new set of samples.
Conventional mycological identification has some disadvantages: it is frequently slow, reliability is sometimes low, and an extensive experience is required. Well-characterised strains can be used to build up a database with reference spectra that will then be used for the identification of etiological agents. Our sets of strains were used to construct such the first database ever, which now allows distinguishing between even the closely related species. The correlation in *Fusarium* identification between genomic and MALDI-TOF MS was extremely high (95.2% to the species level and 100% to the genus level).

Effector gene expression in Fusarium oxysporum is regulated by the transcription factor FTF1

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In the tomato pathogen *Fusarium oxysporum* f. sp. *lycopersici*, most known effector genes reside on an accessory chromosome that can be exchanged between strains through horizontal transfer. Expression of these effector genes is massively upregulated upon infection, but the mechanism by which this is regulated is unknown. In addition to effector genes, the accessory chromosome also encodes 10 predicted transcription factors. Among these are three homologs of *FTF1* (Fusarium transcription factor 1, Reyes-Dominguez *et al.*, 2012) and one copy of *EBR2* (Enhanced Branching 2, Jonkers *et al.*, 2013). Of all transcription factor genes on the accessory chromosome, except one, there is a homolog in the core genome. To test whether the transcription factors on the accessory chromosome affect effector gene expression, overexpression transformants of eight of the transcription factor genes on the accessory chromosome were generated. For one of the *FTF1* homologs, overexpression greatly enhanced expression of the effector gene *SIX1*. Using oligo-DNA arrays, DNA binding sites could be reliably inferred for four of the transcription factors on the accessory chromosome and three of the homologs on the core genome. The binding site for core and accessory homologs is in all cases highly similar or identical. Remarkably, the DNA binding site for *FTF1* (accessory) and *FTF2* (core) corresponds to a motif found earlier to be enriched in effector (*SIX*) gene promoters (Schmidt *et al.*, 2013). And indeed, transformants overexpressing *FTF2* also have a highly induced expression of *SIX1*.

References:

Reyes-Dominguez *et al.*, FGB, 2012
Jonkers *et al.*, Environ. Microbiol. 2013
Schmidt *et al.*, BMC Genomics, 2013

Fusarium poae: proposed pathway for a novel trichothecene chemotype

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Fusarium poae is a cosmopolitan fungus occurring on the ears of many important cereal crops such as barley, oats and wheat. While the species is not as aggressive as the most important “Fusarium Head Blight” pathogen, *F. graminearum*, it does the capacity of producing more toxic trichothecenes, mycotoxins that through deposition in the grains end up in the feed and food chain. We found that *F. poae* isolates produce an exceptional blend of mycotoxins, a combination between type A and type B trichothecenes, which differ at their functional group placement. The trichothecene chemotype of *Fusarium* species depends on whichever alleles of certain key *Tri* genes are present. With a combination of phylogeny, LC-MS/MS targeted analysis, *in silico* protein modeling and domain swapping, we are able to trace back the unique chemotype to two important “molecular switches” in the biosynthetic pathway, ie. *Tri1* and *Tri13*. These genes have respectively a *F. graminearum*-like function (type B producer) and *F. sporotrichioides*-like function (type A producer). The implications of this in the broader framework of the trichothecene gene cluster evolution are discussed.

Evolution of races within f.sp. lycopersici of Fusarium oxysporum

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Race 1 isolates of *Fusarium oxysporum* f.sp. *lycopersici* (Fol) are characterized by the presence of *AVR1* in their genome. The product of this gene, Avr1, triggers resistance in tomato cultivars carrying resistance gene *I*. In Fol race 2 and race 3 isolates, *AVR1* is absent and hence they are virulent on tomato cultivars carrying *I*. In this study, we analyze an approximately 100 kb genomic fragment containing the *AVR1* locus of race 1 isolate Fol004, and compare it to the sequenced genome of Fol race 2 isolate 4287 (Fol4287). A genomic fragment of 31 kb containing *AVR1* was found to be missing in Fol4287. Further analysis suggests that race 2 evolved from race 1 by deletion of this 31 kb fragment due to a recombination event between two transposable elements bordering the fragment. A worldwide collection of 71 Fol isolates represents races 1, 2 and 3, all known VCGs and five different geographical origins was subjected to PCR analysis of the *AVR1* locus, including the two bordering transposable elements, avirulence genotypes. Based on phylogenetic analysis using *EF1*-α, five evolutionary lineages for Fol were identified that correlate well with VCGs. More importantly, we show that Fol races evolved in a stepwise manner within each VCG by the loss of function of avirulence genes in a number of alternative ways.

Interaction between the fungal pathogen Fusarium graminearum and the aphid Sitobion avenae in wheat ears

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We investigate how the fungal pathogen *Fusarium graminearum* (*Fg*), known to cause Fusarium Head Blight disease and producer of deoxynivalenol (DON), and the grain aphid *Sitobion avenae* influence each other feeding both on the nutrients of grain ears. Experiments elucidated that pre-exposure of wheat ears to grain aphids five days prior to inoculation had a positive influence on the subsequent ear colonization by *Fg*, leading to more symptomatic spikelets and a higher fungal biomass in the ears six days after spray inoculation. Investigation of the plant responses showed an upregulation of defense genes due to *Fg* infection, but the defense was higher when the ears had been previously infested with aphids. Conversely, the influence of the fungus and DON on grain aphids showed that the aphids, although they do not specifically prefer *Fg*- or DON-contaminated ears, were able to survive on common field concentrations of DON without a loss of survival and reproduction. Interestingly, we also noticed that the grain aphid can tolerate DON much better than the pea aphid *Acyrthosiphon pisum* which has a host specificity for vegetables. In conclusion, these results indicate that grain aphids *S. avenae* can favor a subsequent *Fg* infection in wheat and that they are able to thrive well on common field concentrations of DON. The high sensitivity of pea aphids to DON compared to grain aphids might point to an adaptation of *S. avenae* to cope with DON in wheat ears.

Lineages in Nectriaceae: Generic status of Fusarium

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The ascomycete family *Nectriaceae* (*Hypocreales*) includes numerous important plant and human pathogens, several of which are used extensively in industrial and commercial applications as biodegraders and biocontrol agents. Members of the family are unified by phenotypic characters such as uniloculate ascomata that are yellow, orange-red to purple, not immersed in a well-developed stroma and with phialidic asexual morphs. Presently, the generic concepts in *Nectriaceae* are still poorly defined, since sequence data are only now becoming available for many of these genera. To address this issue we performed a multi-gene phylogenetic analysis using partial sequences of the *acl1*, *act*, *cmdA*, *hisH3*, ITS, LSU, *rpb1*, *rpb2*, *tef1* and *tub1* gene regions for available type and authentic strains representing known genera in *Nectriaceae*, including several genera for which no sequence data were previously available. Supported by morphological observations, the data resolved more than 40 genera in the *Nectriaceae*. We re-evaluated the generic status of several genera, including the genus *Fusarium*, which were shown to represent several genera previously introduced for these fungi. Additionally, two new genera are introduced for fungi previously treated as *Fusarium*.