Focused Meeting 2016: The Dynamic Fungus
Poster Abstract Book

P15	Alessandra Gasperini, Cranfield University, UK	Comparison of ecology of *Aspergillus flavus* strains isolated form GM and non-GM maize	17
P16	Azlina Mohd Danial, Cranfield University, UK	Screening of lactic acid bacteria and a Streptomyces strain (AS1) for efficacy against fungal pathogen	18
P17	Dale Lall, London Metropolitan University, UK	An investigation into the antimicrobial effects of *Azadirachta Indica* plant extracts against selected microorganisms.	19
P18	Alaa Baazeem, Cranfield University, UK	What impact will climate change scenarios have on aflatoxin contamination of pistachio nuts?	20
P19	David S. Foster, University of Nottingham, UK	Non-genotypic heterogeneity as an adaptation of fungi to environmental stress.	21
P20	Daniel Sayon, University of Exeter, UK	Targeting Synthetic Lethal Genetic Interactions of *PDR1+* as a Paradigm for Treating *Candida glabrata* infections.	22
P21	Xia Yan University of Exeter, UK	Transcriptional regulation of effector in rice blast fungus Magnaporthe oryzae	23
P22	Xia Yan University of Exeter, UK	Characterization of *Magnaporthe oryzae* effectors and their interacting partners in rice	24
P23	Dafna Ben-Yaacov, Tel-Aviv University, Israel	Identification and characterization of Hemofungin, a novel antifungal compound which inhibits the final step of heme biosynthesis	25
P24	David Mwongera Thuranira, University of Exeter, UK	Determining the population structure and avirulence gene repoitoire of the rice blast fungus *Magnaporthe oryzae* in Kenya by comparative genome analysis	26
P25	Vincent Were, University of Exeter, UK	Identification and Characterisation of effector proteins in the rice blast fungus *Magnaporthe oryzae*	27
P26	Afra Khiralla, Université de Lorraine, France	Future perspectives on the endophyte *Curvularia papendorfii:* a source of cytotoxic and antibacterial agents	28

P1

A novel thermotolerant β-glucosidase from *Aspergillus nidulans* has activity across a broad pH profile and a likely bacterial origin

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This study reports the purification and characterization of a recombinant β-glucosidase expressed from an *AOX1* promoter in *Pichia pastoris* carrying an *Aspergillus nidulans* (*A. nidulans*) cloned gene. β-glucosidase was optimally active at 50 ^{o}C and pH 5.5, though it had a broad pH range of pH 3.0 – 10.0 and a broad temperature range of 10 – 80 ^{o}C. β-glucosidase showed very high affinity to para-Nitrophenyl β-D-glucopyranoside (pNPG). Evidence for a bacterial origin of this gene (AN1804) was provided by the absence of introns, absence of some fungal specific amino acid insertions in its encoded protein sequence, automatic annotation as "periplasmic" and unusual positions in phylogenetic trees showing similarities to bacterial proteins.

Keywords: β-glucosidase, *A. nidulans*, horizontal gene transfer, para-Nitrophenyl β-D-glucopyranoside

P2

Diverse growth strategies and drug adaptations in clinical strains of the fungal human pathogen *Candida glabrata*

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Fungal human pathogens cause as many deaths annually as tuberculosis or malaria but their adaptation dynamics and drug susceptibilities are poorly characterised within diverse host environments. Species of the *Candida* genus are highly prevalent in bloodstream infections; *C. glabrata* is responsible for 50% clinical mortality and multi-drug resistance, with strain variation existing between host sites and individuals. Therefore understanding the influence of environmental conditions, strain genetics and virulence on drug susceptibility is crucial for managing the evolution of resistance. We grew five strains of *C. glabrata* in minimal medium at various glucose concentrations, measuring optical density and determining growth rates, carrying capacities and yields (efficiency of biomass production). We then compared the antifungal dose response and evolution of resistance over 14 days of serial transfers, for two clinical strains with contrasting growth kinetics, isolated from different host environments. We identified rate-yield trade-offs between different glucose concentrations across all five strains, with growth rates highest at intermediate glucose. For the two most contrasting strains, drug adaptations and fitness costs varied both between and within replicate populations, and virulence differed between the strains in a wax moth larva infection model. These findings could improve characterisation and personalised treatment of fungal infections.

P3

Does Differential Regulation of Hsp90 Cochaperones Contribute to Virulence in *Candida albicans*?

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Treatment options for systemic candidaemia, caused by the leading fungal pathogen *Candida albicans*, are limited by drug target paucity and emergence of antifungal drug resistances. Fungal virulence, morphogenesis, and drug resistance are regulated by the essential and highly conserved molecular chaperone heat shock protein 90 (Hsp90). While Hsp90 itself may be an unsuitable drug target, its less conserved cochaperones, which regulate Hsp90 ATP-dependent chaperoning of up to 10% of the proteome, may provide suitable venues for drug development if they regulate *C. albicans* virulence. When measuring protein levels of Hsp90 and five of its cochaperones, we noticed that cochaperones appear to be co-regulated during filamentous growth. Cdc37, Sba1, and Sti1, which block Hsp90 ATPase activity, have increased expression, while Cpr6 and Aha1, which support Hsp90 ATPase activity, have reduced levels relative to yeast planktonic growth. This suggests that Hsp90 cochaperone co-regulatory expression patterns may be linked to *C. albicans* virulence-associated hyphal morphology, though further studies are needed in other virulence-associated conditions. Our findings contribute to understanding the virulence potential and regulatory expression of Hsp90 cochaperones and thus will yield insights into fungal virulence strategies to further the development of Hsp90 cochaperones as future drug targets.

P4

Molecular Identification of the Fusarium Species Complex

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Background: Fusarium species are common plant pathogens implicated in superficial and systemic infections in humans with poor prognosis depending on the patient's immune state and the causative Fusarium species. Accurate identification of Fusarium species is required for efficacious treatment of patients. Fusarium isolates implicated in human disease share many morphological similarities making it difficult to identify the individual species. In accordance, different set of variables are needed to classify the members of the Fusarium species complex that infect humans.

Aim: Identify the distinct morphological phenotypes of the species complex and correlate the molecular identification via taxonomic strategy.

Methodology: 130 isolates were sub cultured for 5 days on Potato Dextrose Agar. DNA was extracted from mycelium using a glass bead DNA extraction method followed by PCR amplification of the Pan universal fungal marker using ITS-1 and ITS-4 primers. The amplicons were sequenced using Sanger sequence method. The molecular identification was performed by using Blast algorithm against UNITE database. Multiple *alignment analysis* was *performed using* Clustalw and tree constructed with PhyLM algorithm.

Results: Based on taxonomic analysis, the molecularly identified isolates belong to three species complexes, F. oxysporum species complex (FOSC) being the most abundant (65), F. solani species complex (FSSC) (42), F. proliferatum species complex (23). F. equsitei and F. dimerum included in different species complex, while they were genetically related to FOSC and FFSC, respectively.

Conclusion: Molecular methods were more reliable than Conventional for identification of clinical Fusarium isolates and should be used for resolving the multi-member Fusarium species complex.

P5

Effects of predicted climate change environmental fluctuations on growth, gene expression, and aflatoxin production by *Aspergillus flavus* both *in vitro* and *in maize*

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There is a significant interest in the impact that climate change factors may have on mycotoxigenic fungi. We have examined on conducive media and maize grain the impact that three way interactions between water availability, temperature and elevated CO_2 have on: (i) growth, (ii) the relative expression of all genes in the aflatoxin gene cluster using both RT-qPCR and RNAseq, and (iii) the phenotypic aflatoxin B_1 production by *Aspergillus flavus*. On conducive media, interactions between water activity (a_w; 0.97, 0.95, 0.92), temperature (34, 37^oC) and CO_2 exposure (350, 650, 1000 ppm) were considered and the growth, AFB_1 production and expression of biosynthetic genes (*aflD*, *aflR*) studied. For maize grains, interactions between a_w (0.99, 0.91), temperature (30, 37^oC) and CO_2 exposure (350, 650, 1000 ppm) were included. The results showed that for growth there was relatively little effect. In contrast, the three-way interacting conditions had a profound effect on aflatoxin B_1 production both in media and maize grains. Under slightly elevated CO_2 conditions there was a stimulation of aflatoxin B_1 production. In stored maize grain RNA-seq analysis revealed differential expression of several genes in the aflatoxin gene cluster in relation with these interacting factors. Aflatoxin B_1 production increased under elevated CO_2 conditions at both temperatures and a_w tested. This is the first study to examine these three-way interacting climatic factors on growth and mycotoxin production by a strain of *A.flavus*. This provides data which is necessary to help predict the real impacts of climate change on mycotoxigenic fungi.

P6

Modelling the relationship betwqeen environmental factors, growth, secondary metabolite gene clusters and mycotoxin production

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There has been interest in understandoing the relationship between interacting environmental factors and the growth of folamentous fungi and their ability to produce secondary metabolites. Particular interest has been in the production of mycotoxins by food spoilage fungi. We have utilised a systems approach to model the effect of different water activities and temperatures on growth, gene clusters involved in mycotoxin proiduction and phenotypic toxin production by Fusarium sppecies and Aspergillus flavus. By using a mixed growth model associated production model and linking this with a linear combination of key genes in the biosynthtic pathways for the production of the toxins it was possible to predict the optimum and boundary conditions for growth and toxin production. By examining the relative expression of key genes it was also possible to identify, using ternary diagrams the impact of interacting environmental factors on both growth and toxin production (deoxynivalenol, fumonisins and aflatoxin B1).

P7

Deciphering the role of BAR domain proteins during plant penetration by *Magnaporthe oryzae*

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Rice blast disease caused by *Magnaporthe oryzae* starts when a spore lands on the surface of a rice leaf where it develops a specialised infection cell called an appressorium. This cell switches from isotropic expansion to polarised growth at its base by a septin-mediated process that re-models the F-actin cytoskeleton to generate a penetration peg that ruptures the leaf cuticle. We have observed that, concomitant with these morphological changes, an asymmetric re-distribution of phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) occurs at the plasma membrane, indicating that differences in membrane composition may be involved in appressorium morphogenesis and host invasion. Membrane-deforming BAR domain family proteins generally bind to the negatively charged inner surface of the plasma membrane through $PI(4,5)P_2$ and phosphatidylserine. In *M. oryzae*, seventeen BAR domain proteins have been identified. We are currently characterising homologs of Rvs167, Rvs161, KAR3, Cdc24, Snx4, Gvp36, Vps17, Vps5, Snx41, Rgd1, Rgd2, Bzz1, Hof1, Pil1, Lsp1 and SIP3 to determine their function and their relative contribution to appressorium morphogenesis and function. Progress in understanding the function of BAR domain proteins in septin-mediated appressorium penetration will be presented.

P8

Bambusicola loculata sp. nov. (Bambusicolaceae), a new ascomycete species from bamboo

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A new ascomycete species, Bambusicola loculata, inhabiting decaying bamboo, is introduced based on morpho-molecular studies. Bambusicola loculata is characterized by immersed, dark, stromatic and loculate ascostromata, bitunicate, cylindrical-clavate asci and 1-septate, hyaline, narrowly fusiform ascospores, surrounded by an inconspicuous mucilaginous sheath. Maximum likelihood and Bayesian analyses of combined LSU, SSU, RPB2 and TEF1 gene sequence data as well as morphological characters show that our new taxon belongs to Bambusicola, Bambusicolaceae. The new species is compared with other morphologically and phylogenetically similar species.

P9

Is the *Candida albicans* Hsp90 co-chaperone complex essential for virulence?

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Genes regulating microbial virulence are often located close to the telomere, such as the *TLO* genes in *Candida albicans* (Anderson et al., 2012) and the *VAR* genes in *Plasmodium falciparum* (Su et al., 1995). *C. albicans* Hsp90 co-chaperone genes are near the telomeres. When considering that homologs in the benign baker's yeast are spread evenly across the chromosome, could Hsp90 co-chaperones play a key role in virulence of *C. albicans*?
C. albicans is a leading fungal pathogen of humans, killing ~700 people in the UK annually (Brown et al., 2012). Life-threatening systemic infections are difficult to treat due to the limited number of antifungal drug targets and the increasing prevalence of antifungal resistance. Hsp90 co-chaperones may recommend themselves as a solution to this problem if essential for virulence, but not for commensal growth.
In order to ascertain whether co-chaperones could be exploited as novel drug targets their role in *C. albicans* virulence must first be assessed. Homozygous deletion mutants were generated and examined for growth and virulence using assays such as iron sequestration, survival of oxidative stress, biofilm formation, and killing of an invertebrate host model. Data thus far shows Ssa1 and Sba1 play a role in survival of oxidative stress, Ssa2 and Sba1 are necessary for biofilm formation, and Sti1 plays a role in the heat shock response.

P10

Elucidating the role of the AP-2 endocytic adaptor complex in *Candida albicans* virulence.

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Fungi such as Candida species are a major cause of hospital-acquired infections especially in elderly and immuno-compromised patients, and invasive candidiasis is associated with a high mortality rate. Central to Candida albicans virulence is its ability to colonise different body niches, and in each distinct environment it must remodel its cell surface to ensure appropriate levels of transporters and cell wall synthesis enzymes. Endocytosis is known to be a critical pathway in surface remodelling allowing cells to internalise proteins that are no longer needed at the plasma membrane. There is now robust evidence implicating the endocytic pathway in the mechanism of Candida albicans bud to hyphal transition, in the maintenance of polarised growth in hyphae and in virulence. The aim of this study is to investigate the role of the AP-2 endocytic adaptor complex in endocytosis within C.albicans. Homozygous deletions were generated in an essential subunit of the AP-2 complex. The deletion did not affect rates of cell growth but using fluorescence and electron microscopy, defects were observed in polarization and in cell wall organization. Current studies aim to determine how the AP-2 complex is activated to interact with specific cargoes and internalize them in response to changing environmental conditions.

P11

Fungal and Oomycete Biodiversity in the Falkland Islands and South Georgia

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Soil microbial communities are extremely complex and heterogeneous entities which are not fully understood at all levels, from soil pore structural microclimates to global diversity and dispersion. This is despite their crucial involvement in biogeochemical cycles, which affects agricultural and natural ecosystems. A major component of the community are fungi, which have critical roles in breaking down soil organic matter. A subgroup can also form symbiotic relationships with plants, known as mycorrhizae, which can increase plant productivity. The Falkland Islands and South Georgia represent isolated natural ecosystems with introduced herbivores such as sheep and reindeer. To investigate fungal diversity in these circumstances, soil samples from multiple sites in the Falklands have been taken to carry out high throughput sequencing on barcode regions. The subsequent data will allow fungal community analyses to be carried out. An investigation into the temporal impact will also be established with future sampling in 2017.

P12

Two independent S-phase checkpoints regulate appressorium-mediated plant infection by the rice blast fungus *Magnaporthe oryzae*

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Rice blast disease is caused by the fungus *Magnaporthe oryzae*, which enters the host by means of a specialised infection structure called an appressorium. Appressorium development requires extreme changes of polarity. First, apical growth of the germ tube gives way to isotropic expansion to generate the dome shaped appressorium. Once the appressorium is fully formed a switch from isotropic to apical growth at the base of the appressorium occurs, which translates turgor pressure into vertical mechanical force and requires a septin-dependent re-orientation of F-actin cytoskeleton to lead into the formation of a penetration peg and break the host cuticle. Here, we show that the changes in polarity associated with appressorium development are tightly linked to cell cycle control. In this study we show that the S-phase checkpoint controlling the switch from apical to isotropic growth, leading to initiation of an appressorium, is mediated through the conventional DNA damage response (DDR). We also present an integrated model to show that the second S-phase checkpoint controlling appressorium-mediated plant penetration and cytoskeletal re-polarisation at the base of the appressorium that lead into the formation of a penetration peg and infection, are regulated by an novel S-phase checkpoint that is linked to turgor control.

P13

Using inherent variabilty for increased high value aromatic production in yeast

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Industrial fermentations are characterised by expensive equipment and control mechanisms required to maintain sterility, limiting their use to expensive pharmaceuticals or ethanol production whereby the product prevents invasion. Alternative sources of fossil fuel derived chemicals such as 2-phenylethanol, are required to prevent further climate change. However, the low cost of these molecules from present sources prevents their replacement using micro-organisms despite the discovery of many of these compounds in relevant organisms. Metschnikowia pulcherrima is a ubiquitous budding yeast with highly robust growth phenotypes and with the ability to outcompete a variety of micro-organisms. M. pulcherrima is able to produce 2PE, a common flavour and fragrance molecule currently derived from fossil fuels despite many potential natural sources. By analysing growth and 2PE production in a set of local and type strains in a variety of conditions, it is clear that 2PE production is a highly variable phenotype, with this variation arising separately from genetic lineage. By combining this phenotypic variation with genome sequencing, as well as adaptive laboratory evolution the molecular mechanisms of this variability will be utilised to produce novel strains capable of maintaining axenical growth and producing industrially relevant titres.

P14

Tracking tracheomycosis: the distinct host affinities seen in populations of the coffee wilt pathogen, *Gibberella xylarioides*, suggests evolution in action.

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Tracking tracheomycosis: distinct host affinities of coffee wilt pathogen (*Gibberella xylarioides*) populations and evolution in action

Coffee wilt disease (CWD; also known as tracheomycosis) has been recognised for more than 80 years and affects all coffee species in Africa including the commercially important *Coffea arabica* and *C. canephora* (robusta coffee). A recent multidisciplinary study employed a variety of molecular tests to identify and characterise fungi isolated from symptomatic coffee plants. The investigation confirmed that all isolates belonged to the same fungal species, *Gibberella xylarioides,* but revealed two host-specific (and geographically separate) populations i.e. those infecting arabica or robusta coffee. Further, when the two populations were compared against 'historic' strains obtained from CWD-affected plants in previous serious outbreaks, they were shown to be distinct from most of the 'historic' strains, whilst still conforming to *G. xylarioides sensu lato*. These findings may have important implications for coffee breeders and for national authorities providing management advice to farmers. It also provides an insight into potential speciation events which will require the use of genomic analyses to elucidate them fully. Finally, comparisons are made with another plant-pathogenic fungus (*Ganoderma*) to highlight some further complexities of tackling fungal phytopathogens.

P15

Comparison of ecology of *Aspergillus flavus* strains isolated form GM and non-GM maize

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Maize is an important commodity worldwide and can be infected by fungi during the growing season, harvest and storage. While adoption of GM crops continues to increase on a global scale, few studies have compared the influence of GM maize with either herbicide-tolerance or herbicide-tolerance+insect-resistance on colonisation by *Aspergillus flavus* and aflatoxin contamination, with non-GM maize cultivars. Thus, the objective of the present work was to study the ecology of *A. flavus* on GM- and non-GM based maize matrices to examine the impact on growth and aflatoxin B_1 (AFB_1) production. Strains of *A. flavus* (n=4) isolated from GM/non-GM maize were select and grown on milled maize media from 3 GM and 3 non-GM related maize cultivars where the water availability (water activity, a_w) was modified with glycerol to 0.90, 0.95 and 0.99 and incubated at 25 and 30°C. The relative growth rates and AFB_1 production were compared. Using GM- and non-GM maize as nutritional substrate showed that both toxigenic and non-toxigenic strains colonised both types of maize-based matrices. There was similar growth of strains on GM and non-GM nutritional media with optimum at 0.99 a_w and 30^{o}C. More AFB_1 was produced by a toxigenic strain on non-GM than on GM substrates. Studies are in progress to examine the interactions between toxigenic *A. flavus* strains and other fungal colonists to identify potential antagonists for controlling AFB_1 production and to examine whether climate change parameters may have differential impacts on colonization and toxin contamination of GM and non-GM maize cultivars.

P16

Screening of lactic acid bacteria and a Streptomyces strain (AS1) for efficacy against fungal pathogen

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Lactic acid bacteria (LAB) have been used for food preservation for many years and are considered safe as bio-preservatives because of their production of lactic acid, acetic acid and bacteriocins. Actinomycete strains particularly strains of Streptomyces are known to produce metabolites with antimicrobial properties. The objective of this study was to screen 66 lactic acid bacteria (LAB) isolated from Malaysian fermented foods and a Streptomyces strain (AS1) isolated from peanuts for efficacy against Aspergillus, Fusarium, Penicillium, Trichophyton and C. albicans. In agar well diffusion assays five LABs showed antifungal activity against F. verticillioides. Using the agar overlay method only four LAB strains (L. plantarum strain MCC 2156, L. plantarum strain HT-W104-B1, P. acidilactici 1498 and P. pentosaceus 1426) showed efficacy against F. verticillioides and strong activities against T. rubrum. The Streptomyces AS1 strain had a broad spectrum of antifungal activity and reduced growth of all the fungi examined with inhibition between 40-90% in dual culture assays. P. verrucosum was particularly sensitive to metabolites produced by the AS1 strain. Studies are in progress to examine the relative concentrations of the metabolites required to inhibit germination, growth and mycotoxin production (ochratoxin A, fumonisin B_1 and gliotoxin)

P17

An investigation into the antimicrobial effects of *Azadirachta Indica* plant extracts against selected microorganisms.

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The objective was to investigate the antimicrobial capabilities of *Azadirachta Indica* (Neem) plant extracts, specifically the leaves with a dry herb to menstruum ratio of 1:15 and the seeds of the plant containing a cold press concentration of 2000ppm. This was done via the method of disc diffusion assays against five micro organisms including two Gram positive organisms (*Bacillus subtilis*& *Staphylococcus epidermidis*) and two Gram negative organisms (*Escherichia coli* & *Pseudomonas fluorescens*) as well as a yeast strain (*Saccharomyces cerevisiae*) the controls used were Levofloxacin and Nystatin respectively, results indicate no antimicrobial properties from the Neem leaf extract while the Neem seed oil did show promise of good anti fungal activity but further investigations are needed, such as the minimum inhibition concentration (MIC) test as well as compound isolation and analysis into the specific mode of HSP90 interaction the compounds have. Other eukaryotic models have shown inhibition of HSP90 from the use of *Azadirachta Indica* isolates, with current anti-fungal treatments proving ineffective and multi drug resistance on the increase this is a key issue for research.

P18

What impact will climate change scenarios have on aflatoxin contamination of pistachio nuts?

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Pistachio nuts can be contaminated by Aspergillus flavus under warm and humid conditions*. This can result in contaminate with aflatoxins, classified as a class 1a carcinogen.* There have been no studies on the impact that interacting climate change (CC) factors on pistachio nuts. Thus the objectives of this study were to examine the effect of CC interacting factors of temperature x water activity x CO_2 (350 and 1000 ppm) on (a) growth of *A.flavus*, (b) on relative genes expression of the *aflD* and *aflR* genes, and (c) on AFB_1 production. These studies showed that the effect of interacting CC factors on growth of *A. flavus* colonisation was not significant. However, AFB_1 production was stimulated. The expression was higher at 1000 ppm CO_2 than with existing atmospheric CO_2 levels at 37°C for both strains in some cases. AFB_1 production was higher at 35°C at the two CO_2 levels for both strains. At the same temperature, AFB_1 production was significantly increased at 1000 ppm CO_2 and 0.98 a_w. At 37°C, AFB_1 production was either decreased in strain AB3 or similar as in strain AB10 when exposed to 1000 ppm CO_2. This suggests that CC factors may have a differential effect depending on the interacting conditions of temperature (35 or 37°C) as in some cases AFB_1 production was stimulated while in others remained the same. Studies are in progress to examine whether acclimatisation to elevated CO_2 of *A.flavus* strains affects AFB_1 production under interacting CC conditions and compare it with non-acclimatised culture.

P19

Non-genotypic heterogeneity as an adaptation of fungi to environmental stress.

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Non-genotypic heterogeneity (NGH) describes the phenotypic variation that is evident between individual cells of an isogenic population. The extent of NGH in a given phenotype may be determined by epigenetic or genetic factors, e.g. specific gene-promoter sequences producing nosier gene expression. A number of phenotypes displaying NGH have been identified as fitness-determining, therefore it may have a bearing on survival under fluctuating-stress conditions. Recent evidence from our laboratory suggests that environmental stress selects for increased levels of NGH. Wild yeast populations isolated from polluted sites were found to show elevated NGH in resistance to pollutants, in comparison to populations from nearby control sites. However, the influence of environmental stability on selection for NGH is not understood, nor the long-term stability of this trait. Yeast and filamentous fungal isolates from long-term stressed sites are being studied to provide insight. Dose-response gradients, amongst other measures of cell-cell variation, are being used to provide further evidence for NGH selection under stress. Additional experiments are to understand the main causal factors of NGH selection, by investigating isolates from plots subjected to different temporal-regimes of stressor addition, and to ascertain the relationship between evolution of NGH versus mean resistance (IC50).

P20

Targeting Synthetic Lethal Genetic Interactions of *PDR1+* as a Paradigm for Treating *Candida glabrata* infections.

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Candida glabrata has recently emerged as the second most common cause of invasive candidiasis, and now accounts for ~25% of candidosis cases. *Candida glabrata* infections often exhibit multidrug resistance associated with single point mutations in the *PDR1* gene. This gene encodes a zinc-finger transcription factor for which more than 50 gain-of-function mutations have been shown to drive drug resistant phenotypes. In this study, we propose a paradigm for anti-fungal drug discovery which consists in performing Synthetic Genetic Array (SGA) analysis of *PDR1+* mutant alleles to identify synthetic lethal interaction partners that can be used as novel drug targets. These experiments will provide an inventory of gene deletions that are synthetically lethal with *PDR1+* mutations. *Saccharomyces cerevisiae* gene deletions that consistently interact with *PDR1+* mutations are assessed by scoring for growth defects and these interactions are then confirmed in *C. glabrata* by mutating or inhibiting the orthologous interactor. It is expected that this project will establish the characterisation of synthetic interactions as a paradigm for drug discovery in the context of antimicrobial resistant pathogens.

P21

Characterization of *Magnaporthe oryzae* effectors and their interacting partners in rice

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Plants detect pathogen-associated molecular patterns to trigger PAMP-triggered immunity (PTI) as a first line of defense. Pathogens that overcome PTI and successfully invade host cells do so by delivering effectors that interfere with PTI, leading to effector-triggered susceptibility (ETS). A number of rice blast pathogen *Magnaporthe oryzae* effectors have been characterized in our study by a combination of RNA-seq, targeted gene deletion and live-cell imaging. Apoplastic effectors outlined invasive hyphae while cytoplasmic effectors accumulated at the biotrophic interfacial complex (BIC). Protein-protein interactions studies have been used to identify *M. oryzae* effector targets in rice in this study. Both apoplastic effectors and cytoplasmic effectors have been screened against rice cDNA yeast two hybrid libraries and co-immunoprecipitation experiments carried out. A range of rice proteins were found to interact with *M. oryzae* effectors using yeast two hybrid assay and also confirmed by Bimolecular Fluorescence Complementation (BiFC) assay. Effector structural characteristics were found to be important for proper interaction. Interestingly, many of the effectors target the same rice proteins, which means the rice plant and rice blast pathogen interaction may involve suppression of key defense signaling hubs.

P22

Transcriptional regulation of effector in rice blast fungus Magnaporthe oryzae

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To cause rice blast disease, the filamentous fungus, *Magnaporthe oryzae*, secretes effectors to modulate metabolism of its host and suppress plant immunity. Effector genes are only expressed *in planta* once the pathogen has invaded its host, and show only low basal expression levels in other developmental stages. In *M. oryzae* a large number of effectors have been identified and some have been partially characterized by means of identifying their interacting partners in host plants. To date, however, very little is known regarding how plant-specific expression of fungal effectors is regulated. To investigate the mechanisms which govern transcriptional regulation of effectors, we established a mutant screen to identify putative regulators of effector gene expression. We have screened for mutants showing constitutive expression of GFP-labelled effectors in conidia and during mycelial growth in order to identify potential transcriptional regulators and signaling components. We are now carrying out genome sequencing and bulked segregant analysis to identify novel effector regulators and will report on progress toward their characterization.

P23

Identification and characterization of Hemofungin, a novel antifungal compound which inhibits the final step of heme biosynthesis

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The incidence of fungal infections such as invasive pulmonary aspergillosis (IPA) caused by *Aspergillus fumigatus* (*A. fumigatus*), has risen dramatically throughout recent years, especially among immunocompromised patients. Despite this rise in invasive infections, there is only a limited number of antifungal drugs active against fungal pathogens and even with treatment, the mortality rate remains high. Therefore, there is an urgent need to develop novel effective antifungal drugs to treat fungal infections.

The aim of this study was to evaluate CW-208/hemofungin as a novel antifungal compound. This molecule was previously identified in our laboratory by a library screening of synthetic drug-like compounds and shown to cause swelling and lysis of growing fungal cells. Hemofungin inhibited growth of pathogenic isolates at micromolar concentrations, while only weakly affecting the growth of mammalian cell lines. Genetic and biochemical analyses in *A. nidulans* indicated that hemofungin primarily inhibits ferrochelatase, the last enzyme in the heme biosynthetic pathway that converts protoporphyrin IX to hemin, suggesting that its effect on the cell-wall is indirect. Hemofungin significantly reduced mortality rates of larvae infected with *A. fumigatus*, in a dose-dependent manner without signs of toxicity. Additional findings strengthened our hypothesis that ferrochelatase is the target of hemofungin. For instance, addition of hemin canceled inhibition by hemofungin in *A.fumigatus* in a dose-dependent manner and enzymes in the hemin biosynthetic pathway were strongly up-regulated.

In summary our approach in finding novel antifungals has yielded a promising novel compound, hemofungin. Further investigation in additional animal models of fungal infection is strongly warranted.

P24

Determining the population structure and avirulence gene repoitoire of the rice blast fungus *Magnaporthe oryzae* in Kenya by comparative genome analysis

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A total of 270 isolates of rice blast fungus *Magnaporthe oryzae,* were collected from rice growing regions in Kenya. Genotyping of the isolates was undertaken using the internal transcribed spacer regions (ITS 1 and ITS 2) from the ribosomal RNA gene cluster. This clustered the isolates into two groups supported by bootstrap analysis with clade support of 83%. Isolates from Mwea region in Central Kenya clustered separately from isolates from Ahero in Western Kenya and Kwale regions in Coastal Kenya. Isolates from Ahero clustered together with isolates from Kwale region. Genome analysis of a wider collection of isolates from sub-Saharan Africa (SSA) was undertaken using high throughput next generation sequencing on the Illumina Hiseq 2500 system. Single nucleotide polymorphism (SNP) indicated that East African isolates clustered separately from West African isolates with clades support of 100%. Pathotype analysis of Kenyan isolates was undertaken using a set of differential varieties for rice blast. Rice blast resistance genes *Pi*9 and *Piz*5, conferred resistance to all the isolates tested. Other resistance genes that conferred resistance to majority of isolates tested are *Pib*, *Pia* and *Piz*. These resistance genes are suitable candidates for introgressing into the commercially grown varieties in SSA We are currently introgressing resistance gene *Pi*9 into Basmati 370 and Basmati 217 which are the widely grown rice varieties in East Africa. Our research also aims to identify rice blast avirulence genes. We are using comparative genome analysis approach and virulence pattern on rice blast differential varieties to identify rice blast avirulence genes from the SSA isolates. We are undertaking gene deletion and complementation for one gene candidate for putative *AvrPiz5* in a Kenyan strain KE002. The presence/absence of the gene candidate in the genomes of 7 Kenyan isolates correlates with the virulence pattern on rice differential line harbouring resistance gene P*iz5*. The gene candidate is also upregulated in the infection of the rice blast susceptible variety, Mokoto, by KE002. We will employ a similar approach to identify other avirulence genes.

P25

Identification and Characterisation of effector proteins in the rice blast fungus *Magnaporthe oryzae*

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To cause disease in plants, microbial pathogens secrete effector proteins that are able to suppress basal plant immunity mechanisms and help facilitate proliferation of the pathogen in plant tissue. The rice blast fungus, Magnaporthe oryzae, causes the most serious disease of cultivated rice. We are characterising effector proteins from M. oryzae and using this knowledge in an applied project, funded by the Bill and Melinda Gates foundation and BBSRC, to develop durable rice blast disease control in Sub-Saharan Africa. To identify effectors in M. oryzae we have first used RNA-seq analysis of rice blast infections and identified secreted protein-encoding genes that are differentially regulated during plant infection. This has enabled identification of more than 50 putative effectors, which can then be classified into cytoplasmic effectors that localize to the Biotrophic Interfacial Complex (BIC) during secretion and apoplastic effectors that accumulate between the fungal cell wall and plant plasma membrane. We have then identified putative interacting partners of a sub-set of effectors and generate null mutants to test their role in virulence. In order to understand the relationship between the effector repertoire of M. oryzae and their potential recognition as avirulence determinants in blast populations in Sub-Saharan Africa (SSA), we have sequenced the genomes of 29 rice blast isolates in which we have classified virulence using rice monogenic lines differing in 24 major rice blast resistance genes. In this way, we have identified a number of putative Avr genes that encode secreted effectors. We will report on the identification and characterization of these effectors and how their identification could lead to a durable rice blast disease control breeding strategy for Sub-Saharan Africa.

P26

Future perspectives on the endophyte *Curvularia papendorfii:* a source of cytotoxic and antibacterial agents

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Latest researches on endophytic fungi, the hidden fungi which colonizing inside the healthy tissues of the host plant, proved that they are a new source of novel natural products. However, the probability of isolation of a powerful bioactive agents from endophytic fungi associated with medicinal plants, is higher than other plants. This study investigated, for the first time, the fungal endophytes associated with *Vernonia amygdalina* a tropical medicinal plant from Sudan. *V. amygdalina* has several uses in Sudan folk medicine. Bioassay-guided fractionation of the ethyl acetate extract of one selected fungus was performed, using antibacterial assay against methicillin-resistant *Staphylococcus aureus* in order to isolate pure compounds. Three endophytic fungi were isolated from *V. amygdalina*. The fungal strains were identified by sequencing of internal transcribed spacer (ITS) regions of rDNA as: *Cladosporium cladosporioides*, *Curvularia papendorfii* and *Hansfordia sinuosa*. Endophytic fungus, *C. papendorfii* was selected for further investigations. Bioassay-guided fractionation led to isolation of ten pure compounds. Some fractions and one pure compound were showed a potent cytotoxicity with IC_{50} values ranged from 5.3 to 29.78µg/mL. In addition, the structure of one pure compound was elucidated by NMR, MS and IR spectral data. The pure compound, 3,7,11,15-tetrahydroxy-18-hydroxymethyl-14,16,20,22,24-pentamethyl-hexacosa-4E,8E,12E,16,18-pentaenoic acid, is a new acid which revealed antibacterial activity against *S. aureus* with MIC value 62.5 µg/mL. Herein, the endophye *Curvularia papendorfii* isolated from *Vernonia amygdalina* a medicinal plant from Sudan, produced some active compounds which may serve as potential candidates for anti-cancer and anti-bacterial drugs development in the future.