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Functional reconstitution of the Trypacidin Gene Cluster in Aspergillus fumigatus by Advanced Gene Editing

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624F Mycosarcoma, a resurrected generic name for corn smut (Ustilago maydis). *D. Begerow*[1], A. McTaggart[2], R. Shivas[3] 1) Geobotany, Ruhr-Universität Bochum, Bochum, DE; 2) Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; 3) Plant Pathology Herbarium, Biosecurity Queensland, Department of Agriculture and Fisheries, Queensland, Australia.

Ustilago is a polyphyletic genus of smut fungi found mainly on Poaceae. The development of a taxonomy that reflects phylogeny requires subdivision of *Ustilago* into smaller monophyletic genera. Several separate systematic analyses have determined that *Macalpinomyces mackinlayi*, *M. tubiformis*, *Tolyposporella pachycarpa*, *Ustilago bouriquetii* and *U. maydis*, occupy a unique phylogenetic position within the Ustilaginaceae. A previously introduced monotypic generic name typified by *U. maydis*, *Mycosarcoma*, is available to accommodate these species, which resolves one component of polyphyly for *Ustilago* s. lat. in Ustilaginaceae. An emended description of *Mycosarcoma* is provided to reflect the morphological synapomorphies of this monophyletic group. A specimen of *Ustilago* maydis that has had its genome sequenced is designated as a neotype for this species. Taxonomic stability will further be provided by a forthcoming proposal to conserve the name *Uredo maydis* over *Lycoperdon zeae*, which has priority by date, in order to preserve the well-known epithet *maydis*.

625W Population structure and drivers of genetic diversity in *Candida glabrata*. *E. Shor*[1], K. Healey[1], C. Jimenez-Ortigosa[1], S. Lockhart[2], V. Loparev[2], D. Perlin[1] 1) Public Health Research Institute, Rutgers University, Newark, NJ; 2) Centers for Disease Control, Atlanta, GA.

The prevalence of *Candida glabrata* infections has been rising for several decades, and it now accounts for approximately 25% of all Candida bloodstream infections in the U.S. and can in some settings predominate as the principal bloodstream fungal pathogen. Furthermore, *C. glabrata* exhibits elevated intrinsic resistance to triazoles and readily acquires resistance to echinocandins. It also becoming increasingly apparent that *C. glabrata* has a highly genetically diverse population structure. However, how this diversity arises and whether it contributes to the virulence and drug resistance of *C. glabrata* is unknown. A multi-locus sequence typing (MLST) scheme has thus far identified over 80 different *C. glabrata* sequence types (STs); however, our preliminary analysis indicates that this number is likely much higher. We also found that different STs carry different alleles of DNA repair gene *MSH2*, some of which cause elevated rates of drug resistance *in vitro*, indicating that different STs have different propensity towards mutability and acquiring drug resistant gene variants. *C. glabrata* also displays a high degree of variability at the level of chromosomal architecture. For instance, it has been reported that clinical *C. glabrata* isolates show great diversity in terms of chromosomal number and structure, displaying a high number of chromosomal rearrangements relative to the reference laboratory strain. This observation has led to the hypothesis that emergence of new chromosomes is a virulence mechanism in *C. glabrata* and may underlie emergence of drug resistance. However, we find that strains of the same ST have similar chromosomal patterns, suggesting that specific chromosomal configurations may pre-exist emergence of virulence and may be a feature of commensal *C. glabrata* populations. To identify new determinants of virulence and/or drug resistance in *C. glabrata*, we are performing optical mapping and long read genome sequencing (PacBio) of several STs that are prevalent in the U.S. We are also investigating the importance of the DNA replication checkpoint in facilitating chromosomal rearrangements in *C. glabrata*.

626T Comparative genomics of hybrids reveal potential host specificity genes in *Microbotryum*. Britta Büker[1], Michael E. Hood[2], Andreas Brachmann[3], Sven Rahmann[4], *Dominik Begerow*[1] 1) Geobotanik, Ruhr-Universität Bochum, Bochum, DE; 2) Department of Biology, Amherst College, Amherst, MA, USA ; 3) Genetics, Faculty of Biology, Ludwig-Maximilians-University Munich, Planegg-Martinsried, Germany; 4) Genominformatik, Institut für Humangenetik, Medizinische Fakultät, Universität Duisburg-Essen, Essen, Germany.

The evolution of obligate plant pathogens like smut fungi is often characterized by lineage tracking, resulting in host specificity and one-to-one relationships. Therefore, adaptation and specialization to the host plant seems to be crucial and should involve genes or regulatory pathways governing host specificity. To identify genes relevant for host specificity of *Microbotryum* species, we produced artificial hybrids between the two host-specific species *M. lychnidis-dioicae* and *M. silenes-acaulis* and applied strong experimental selection on different host plants to identify genes necessary for successful infections.

Genome comparison of the two species revealed that most gene families are shared and the majority of genes are conserved, indicating very similar biological features of both species, including host adaptation and infection processes. Lower nucleotide identity of genes encoding for secreted proteins might indicate their importance for host specific interaction, as it is known from other plant pathogens. Moreover, we identified 211 candidate genes that occur in each hybrid and backcross genome that were posed under host-driven selection and might therefore play a crucial role in host specialization. The analysis of hybrid genomes also demonstrates the effect of genetic homogeneity on the fitness of hybrid individuals including the occurrence of species-specific mating type chromosomes.

In conclusion, the combination of comparative genomics with experimental selection and hybridization is a promising way to identify potential host specificity factors. Our data suggest that only a limited set of genes is required for successful infection, but also demonstrate the strong influence of intra-genomic conflicts on the viability of hybrids.

Synthetic Biology

627F Functional reconstitution of the Trypacidin Gene Cluster in *Aspergillus fumigatus* by Advanced Gene Editing. *J. Weber*[1,2], V. Valiante[3], C.S. Nødvig[4], D.J. Mattern[1,2], R.A. Slotkowski[1,2], U.H. Mortensen[4], A.A. Brakhage[1,2] 1) Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (HKI) –, Jena, Germany; 2) Institute of Microbiology, Friedrich Schiller University Jena, Germany; 3) Leibniz Research Group – Biobricks of Microbial Natural Product Syntheses, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany; 4) Eukaryotic Molecular Cell Biology, Section for Eukaryotic Biotechnology, Department of Systems Biology, Technical University of Denmark, Søltofts Plads, Kongens Lyngby, Denmark.

The human pathogenic fungus *Aspergillus fumigatus* is known to produce various spore-borne natural products. One of these is the polyketide trypacidin which has been shown to be involved in the interactions with alveolar macrophages as well as with the amoeba *Dictyostelium discoideum.* Even though recent studies could elucidate the corresponding gene cluster in *A. fumigatus*, it still remained elusive why several isolates do not produce trypacidin. We addressed this question employing a CRISPR/Cas9-based gene editing strategy. Thus, we could link a single nucleotide insertion in the polyketide synthase of the trypacidin biosynthetic pathway and also

reconstitute its production in a nonproducing strain. In addition, we developed a split-marker approach for the selection of edited strains, since the selectable marker could not be directly linked to the target site. The here established tool could be useful in next generation fungal genetics *e.g.* for the investigation of single nucleotide polymorphism, or amino acid substitutions.

628W Identification of gene targets for improved heterologous enzyme production in *Aspergillus niger*. *M.C. Reilly*[1], J. Kim[1], J. Lynn[1], J.M. Gladden[1,2], J.K. Magnuson[1,3], S.E. Baker[1,3] 1) Joint BioEnergy Institute, Emeryville, CA; 2) Sandia National Laboratory, Livermore, CA; 3) Pacific Northwest National Laboratory, Richland, WA.

Efficient and economical deconstruction of biomass is critical for the success of lignocellulosic biorefineries. The use of ionic liquids (ILs) in the pretreatment of biomass leads to increased biomass saccharification efficiency at lower cellulose loadings. However, some ILs can inhibit the activity of commercial cellulases and must be removed from the biomass prior to the application of the lignocellulosic enzyme cocktail, a costly additional step in the conversion process. To overcome this issue, cellulolytic enzymes that can maintain function in the presence of ILs have been identified. Many of these enzymes originate from bacterial species that are difficult to cultivate in the laboratory. One possible solution are filamentous fungi, which have been widely utilized for enzyme production in industry. Here, a forward genetics approach was utilized to increase heterologous enzyme production in *Aspergillus niger*: a strain engineered to secrete an IL-tolerant bacterial beta-glucosidase (BG) was mutagenized and the resulting progeny screened for increased BG activity. Subsequent full-genome sequencing revealed several loci associated with the hyper-production of the heterologous enzyme. Deletion of one of these, a putative sugar transporter, results in a doubling of heterologous BG enzyme activity detected in culture supernatants. Future studies will further characterize the role of this sugar transporter in heterologous enzyme production.

629T An *in-silico* reconstructed gene regulation network for *Aspergillus niger* for the prediction of protein functions. *Sascha Jung*, Norman Paege, Paul Schäpe, Vera Meyer Institute of Biotechnology, Department Applied and Molecular Microbiology, Berlin University of Technology, Gustav-Meyer-Allee 25, 13355 Berlin, Germany.

The genome of *A. niger* belongs to the best annotated genomes among *Aspergillus* species; however, only 2% of its ~14,000 genes are functionally verified and 50% of the predicted open reading frames encode for hypothetical proteins. Hence, the genetic basis for almost all cellular processes in *A. niger* and its physiological peculiarities is unknown. On the other hand, hundreds of post-genomic data including transcriptomic and proteomic data are available for *A. niger* for more than 150 different growth conditions. This holistic dataset can be scrutinized and used to predict gene functions and gene interactions. For a proof-of-concept, we have focused on the *anafp* gene encoding the antifungal protein AnAFP known to selectively inhibit the growth of filamentous fungi. As more than 50 AnAFP orthologs have been identified in many different genera of the *Ascomycota* tree of life, we wished to understand which regulatory systems control expression of the *anafp* gene.

Our analysis uncovered that *anafp* displays a highly coordinated temporal and spatial transcriptional profile which is concomitant with key nutritional and developmental processes. Its expression profile coincides with early starvation response and parallels with genes involved in nutrient mobilization and autophagy. Using fluorescence- and luciferase reporter strains we could demonstrate that the *anafp* promoter is indeed under control of CreA and FlbA as predicted by the *in silico* data. A co-expression network analysis further predicted that *anafp* expression is embedded in several cellular processes including allorecognition, osmotic and oxidative stress survival, development, secondary metabolism and autophagy, and predicted StuA and VelC as additional regulators (1). We currently prove these predictions by respective wet-lab experiments. First results match the expected knock out phenotype in *A. niger*.

(1) Paege N, Jung S, et al. A Transcriptome Meta-Analysis Proposes Novel Biological Roles for the Antifungal Protein AnAFP in *Aspergillus niger*. PlosOne 2016; DOI:10.1371/journal.pone.0165755.s009

630F Genome wide consequences of the deletion of the Aspergilli non-homologous end joining (NHEJ) DNA repair mechanism. I. Álvarez-Escribano[1], C. Sasse[2], J. Woo Bok[3], A. Lipzen[4], W. Schackwitz[4], J. Marin[4], K. Barry[4], I. Grigoriev[4], A.T. Marcos[1], N.P. Keller[3], G.H. Braus[2], *D. Canovas*[1] 1) Department of Genetics, Faculty of Biology, University of Seville, Spain; 2) Department of Molecular Microbiology and Genetics and Göttingen Center for Molecular Biosciences (GZMB), Georg-August-University, Göttingen, Germany; 3) Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, Wisconsin, USA; Department of Bacteriology, University of Wisconsin-Madison, Madison, Wisconsin, USA; 4) DOE Joint Genome Institute, Walnut Creek, California, USA.

The relevance of *Aspergillus* for human daily life is immeasurable: not only because of its industrial applications but also because of its clinical implications. Most *Aspergillus* laboratories world-wide use mutants in the non-homologous end joining (NHEJ) pathway (*KU70* or *KU80*) to do genetic modifications in the strains under study due to a higher frequency of homologous integrations after transformation of DNA constructs. *KU70* is involved in a DNA repair mechanism highly conserved in eukaryotes, and genetic manipulations are much faster in NHEJ mutant (*?KU70*) strains in many fungal species.

Three *Aspergillus* species were selected for this study to allow for comparative genetics and genomics: *A. flavus, A. fumigatus* and *A. nidulans*. Wild-type and NHEJ mutants were grown on solid media for 60 growth passages using asexual spores (ca. 3,000 mitosis). In the case of *A. nidulans* ten passages with sexual cleistothecia were also independently performed. Genome sequences were obtained and analyzed. In both *A. flavus* and *A. fumigatus*, the *KU70* mutants accumulated on average more mutations than the wild type strains (8.37 +/- 1.80 vs 6.12 +/- 1.76 in *A. flavus*, and 5.75 +/- 2.33 vs 4.12 +/- 1.76 in *A. fumigatus*). The higher number of non-synonymous mutations in *A. fumigatus* and of mutations in the intergenic regions in *A. flavus* in the *KU70* mutants was statistically significant. None of the sexual passages of the *A. nidulans KU70* strain could be completed due to the lack of cleistothecia formation, while the majority of the wild type passages reached ten passages. Collectively here we provide an assessment of the effects of the NHEJ pathway in the genomic stability in Aspergilli.

631W CRISPR/Cas9 systems can be used to manipulate gene function in the thermally dimorphic fungus *Histoplasma capsulatum*. *Saori Amaike Campen*, Sinem Beyhan J Craig Venter Institute, La Jolla, CA.

The thermally dimorphic fungus, *Histoplasma capsulatum* is a causal agent of the severe pulmonary and systemic human disease histoplasmosis. *H. capsulatum* can cause disease in both immunocompromised and immunocompetent individuals and is endemic in the