The cereal pathogen Fusarium pseudograminearum produces a mimic of cytokinin plant hormones

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The cereal pathogen *Fusarium pseudograminearum* produces a mimic of cytokinin plant hormones. *D.M. Gardiner*[1], J.L. Sørensen[2], A.H. Benfield[1], R.D. Wollenberg[2], K Westphal[2], R Wimmer[2], K.F. Nielsen[3], J. Carere[1], L. Covarelli[4], G. Beccari[4], J. Powell[1], T. Yamashino[5], H. Kogler[6], T.E. Sondergaard[1] 1) Agriculture and Food, CSIRO, St Lucia, Queensland, AU; 2) Department of Chemistry and Bioscience, Aalborg University, DK-9000 Aalborg, Denmark; 3) Department of Biotechnology and Biomedicine, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark; 4) Department of Agricultural, Food and Environmental Sciences, University of Perugia, IT-06121 Perugia, Italy; 5) Laboratory of Molecular Microbiology, School of Agriculture, Nagoya University, Furocho, Chikusa-ku, Nagoya, 464-8601 Japan; 6) Karlsruhe Institute of Technology, D-76131 Karlsruhe, Germany.

The necrotrophic pathogen *Fusarium pseudograminearum* infects a broad range of agronomically important crops including barley and wheat. During a survey of secondary metabolites produced by this fungus, a novel class of cytokinins, which are plant hormones, was identified as being produced by the pathogen during plant infection. Cytokinins are generally thought of as having growth promoting and anti-senescence activity and the production of a cytokinin mimic by a necrotrophic pathogen challenges the view that these pathogens invade by a simple barrage of lytic enzymes and toxins. Through genome mining, a gene cluster in the *F. pseudograminearum* genome for the production of these compounds was identified and the biosynthetic pathway established using gene knockouts. The *F. pseudograminearum* cytokinins can activate cytokinin signalling demonstrating their genuine hormone mimicry. *In planta* analysis of the transcriptional response to one of the *F. pseudograminearum* cytokinins suggests extensive reprogramming of the host environment by these molecules, possibly through cross talk with defence signalling pathways.

Conserved *veA*-dependent genetic elements, *rtfA* and *mtfA*, regulate secondary metabolism, morphogenesis and virulence in *Aspergillus* spp. *A.M. Calvo* Dept Biological Sci, Northern Illinois Univ, Dekalb, IL.

In the model fungus *Aspergillus nidulans* the global regulatory gene *veA* is necessary for the biosynthesis of several secondary metabolites, including the mycotoxin sterigmatocystin (ST). In order to identify additional *veA*-dependent genetic elements involved in regulating ST production, we performed a mutagenesis on a deletion *veA* strain to obtain revertant mutants (RM) that regained the capability to produce toxin. Genetic analysis and molecular characterization of two of the revertant mutants, RM3 and RM7, revealed that point mutations occurred at the coding region of the *rtfA* and *mtfA* genes respectively. *rtfA* encodes a RNA-pol II transcription elongation factor-like protein, similar to *Saccharomyces cerevisiae* Rtf1, while *mtfA* encodes a novel putative C2H2 zinc finger domain transcription factor. Both genes are conserved in *Aspergillus* spp and in other fungal genera. Further research revealed that in a *veA* wild-type background, *rtfA* controls the production of several secondary metabolites, or natural products, in *A. nidulans*, as well as in the agriculturally and medically important *A. flavus* and *A. fumigatus. mtfA* also governs the biosynthesis of natural products in *Aspergillus* spp by regulating the expression of secondary metabolite gene clusters, as demonstrated in *A. nidulans* and *A. fumigatus* functional genomics studies. Additionally, both regulators strongly influence other biological processes, including morphological development and virulence.

Mushroom polyketide synthase produces polyenes for chemical defense. *P. Brandt*[1], M. García-Altares[2], M. Nett[3], C. Hertweck[2], D. Hoffmeister[1] 1) Friedrich-Schiller-Universität, Department Pharmaceutical Microbiology at the Hans-Knöll-Institute, Winzerlaer Straße 2, 07745 Jena (Germany); 2) Leibniz Institute for Natural Product Research and Infection Biology, Department Biomolecular Chemistry, Beutenbergstraße 11a, 07745 Jena (Germany); 3) Technische Universität Dortmund, Department Biochemical and Chemical Engineering, Technical Biology, Emil-Figge-Straße 66, 44227 Dortmund (Germany).

Basidiomycetes have evolved a diverse repertoire of bioactive chemical defense compounds. After wounding of its mycelium, the taxonomically undetermined white-rotting basidiomycete BY1 produces yellow pigments *de novo*, which massively inhibit the pupation of insect larvae. These natural products were identified as the polyunsaturated fatty-acid like polyenes, 18-methyl-19-oxoicosaoctaenoic acid and 20-methyl-21-oxodocosanonaenoic acid. [1]

The objective of this study was to understand the genetic and biochemical basis of this basidiomycete defense compounds. We succeeded in identifying both alleles of a candidate gene, *PPS1*, in the genome of BY1, coding for a six-domain reducing polyketide synthase (HR-PKS). Quantitative real-time PCR showed a 9.5-fold upregulation of *PPS1* expression 48 hours past injury of the BY1 mycelium. To verify that PPS1 has polyene synthase activity, the polyene biosynthesis was heterologously reconstituted in *Aspergillus niger*. To that end, *PPS1* was placed under the control of the *terA* promoter and *trpC* terminator using plasmid SM-Xpress. [2] Combining liquid chromatography, mass spectrometry, and NMR, the structures of the PPS1 products were elucidated, and proved identical to the polyenes initially isolated from BY1. MALDI-MS imaging indicated polyene accumulation in the wounded mycelial area. Our work represents the first characterized basidiomycete HR-PKS and sets the stage for a more profound understanding of basidiomycete chemical ecology.

[1] D. Schwenk, M. Nett, H.-M. Dahse, U. Horn, R. A. Blanchette, D. Hoffmeister, *J Nat Prod* **2014**, *77*, 2658-2663.
[2] M. Gressler, P. Hortschansky, E. Geib, M. Brock, *Front Microbiol* **2015**, *6*, 184.

Copper-responsive isocyanide biosynthetic cluster in *Aspergillus fumigatus*. *F. Lim*[1], J. Baccile[2], T. Won[2], P. Wiemann[1], A. Lind[3], A. Rokas[3], F. Schroeder[2], N. Keller[1] 1) University of Wisconsin-Madison, Madison, WI, U.S.A; 2) Cornell University, Ithaca, NY, U.S.A; 3) Vanderbilt University, Nashville, TN, U.S.A.

Isocyanide-containing natural products are of immense interest in the biological and chemical milieu owing to its broad range of bioactivity, which is attributed by the highly reactive isocyano- functional groups tethered to structurally diverse carbon scaffolds. In contrast to the fast-growing list of naturally-occurring isocyanides, knowledge on the biosynthetic machineries that give rise to such unique chemistry is still in its infancy with only four characterized biosynthetic gene clusters in bacteria, and unprecedented in eukaryotes. A hallmark function for these isocyanide natural products is their capability for metal coordination, which is shown to impart the bioactivites for many of these naturally-occurring isocyanides and from an ecological perspective, crucial to the pathogenesis of the entomopathogenic bacterium, *Xenorhabdus nematophila* (by disabling the innate immune defense via inhibition of the cuproenzyme, phenoloxidase). Here we report on the identification of four isonitrile synthases (INS) in the genome of the human opportunistic pathogen, *Aspergillus fumigatus* and the discovery of a novel copper-responsive INS-NRPS-like hybrid enzyme (CrmA) involved in the synthesis of xanthocillin analogues, the first reported biosynthetic pathway dedicated to this family of naturally-occurring isocyanides. This work also elucidates the regulatory circuitry that bridges cellular metal homeostasis and fungal development.