PATHOGEN GENETICS AND GENOMICS

THE IDENTIFICATION OF A GENE IN *FUSARIUM GRAMINEARUM* THAT CONTRIBUTES TO BUTENOLIDE SYNTHESIS.

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ABSTRACT

The development of expressed sequence tag (EST) databases, directed transformation and a sequenced genome have facilitated the functional analyses of *Fusarium graminearum* genes. Extensive analysis of 10,397 ESTs, derived from thirteen cDNA libraries of *F. graminearum* grown under diverse conditions, identified a novel cluster of eight genes (gene loci *fg08077 – fg08084*) located within a 17 kb region of genomic sequence contig 1.324. The expression of these genes, as detected by Northern analysis and qPCR, is concomitantly up-regulated under growth conditions that promote mycotoxin production. Gene disruption experiments followed by metabolite analysis of the transformants indicated that one of the genes, *fg08079*, is directly involved in butenolide synthesis, a secondary metabolite derived from glutamic acid. The mycotoxin butenolide is produced by several *Fusarium* species and has been suggested, but not proven, to be associated with tall fescue toxicoses in grazing cattle. To confirm that this gene is involved in butenolide biosynthesis, the complete, intact gene was added back to the disruption mutants. The add-back transformants were once again able to synthesize butenolide. As expression of these genes can be detected very early in wheat and barley infection, butenolide may play a role in plant infection. However, greenhouse testing for FHB (Fusarium Head Blight) using disruption mutants of *fg08079*, showed that this gene did not contribute significantly to virulence in wheat heads. We will continue to exploit genomic and proteomic tools to identify genes that are involved in FHB disease.

HAPLOTYPE NETWORKS FROM *FUSARIUM GRAMINEARUM* REVEAL PATTERNS OF EVOLUTION.

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ABSTRACT

DNA sequences of two nuclear genes (*MAT1-1-3*, and *Tri101*) were examined from over 500 isolates of *Fusarium graminearum* collected from South American and Korean wheat, maize and sorghum. Haplotype networks were developed for each gene that illustrate the relationships between the DNA sequences of isolates in this study based on the minimum number of base pair changes that separate the isolates. Some lineage diagnostic single nucleotide polymorphisms (SNPs) are not conserved in this strain set. The lack of dichotomous branching suggests that the lineages did not evolve in a stepwise fashion. The haplotype networks cannot be resolved without cycles, which is consistent with recent gene flow between the lineages.

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EMERGENT POPULATIONS OF *FUSARIUM GRAMINEARUM SENSU STRICTO* IN THE UPPER MIDWESTERN U.S. DISPLAY GRADIENT OF FREQUENCY AND A HIGH MYCOTOXIN POTENTIAL.

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ABSTRACT

Based on preliminary analyses of 4,957 fungal cultures gathered from wheat in Minnesota (MN), North Dakota (ND) and South Dakota (SD) in 2003 and 2004, we previously reported that *Fusarium graminearum sensu stricto* in the Upper Midwest consists of genetically divergent populations (Proceedings of the 2005 National Fusarium Head Blight Forum, page 158). Besides the common Midwestern 15ADON population, chemotyping using PCR and VNTR analysis revealed the presence of two additional and genetically divergent populations (emergent populations) that have increased dramatically in frequency in ND and northwestern MN over a short time period. Plotting the frequency of populations according to sample location clearly reveals a gradient of decreasing frequencies of the emergent populations toward southern regions of MN and ND and with highest frequency being observed at locations close to the Canadian border (Manitoba). This distribution implies that introduction and spread of these emergent populations probably has a North to South direction. Potential spread further south is currently being examined by a collection from 2006 consisting of approximately 1,200 wheat samples from 36 locations in ND, MN and SD that concentrated on the southern range of distribution of these emergent populations. Knowledge of the distribution and frequency of these emergent populations is important as greenhouse experiments conducted in spring 2006 that included a total of 60 strains (with three repetitions) from the three known populations clearly indicated that the susceptible cultivar Norm accumulated substantially more deoxynivalenol when inoculated with strains from the two emergent populations than when inoculated with strains from the common and widespread MW 15 ADON population. Whether higher mycotoxin levels also accumulate under field conditions to at least partially explain the increase in frequency of these emergent populations in some regions needs to be determined further.

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REAL-TIME QUANTITATIVE EXPRESSION STUDIES OF THE ZEARALENONE BIOSYNTHETIC GENE CLUSTER IN *FUSARIUM GRAMINEARUM*.

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ABSTRACT

Zearalenones are estrogenic mycotoxins produced by several *Fusarium* species and can cause reproductive problems in animals. We have previously described the use of *Fusarium pseudograminearum* to identify the polyketide synthase gene *PKS4* as having a major role in zearalenone (ZON) production (Lysøe et al. 2006. Appl. Environm. Microbiol. 72: 3924-3932). An *Agrobacterium tumefaciens*-mediated transformation protocol was used to replace the *PKS4* gene with a *hygB* resistance gene in a high zearalenone-producing *F. graminearum* strain. PCR and Southern analysis of transformants identified isolates with single insertional replacements of *PKS4*, and HPLC analyses were used to confirm the lack of the ability of this mutant to produce ZON. Barley root infection studies showed no alteration in the pathogenicity of the wild type and the *PKS4* mutant. Also others have proved the involvement of *PKS4* as well as the neighboring gene *PKS13* in the synthesis of ZON (Gaffoor et al 2005. Eukaryot. Cell 4: 1926-1933; Kim et al. 2005. Mol. Microbiol. 58: 1102-1113). Expression experiments of the genes located in the cluster where *PKS4* and *PKS13* are positioned, however, have to date been limited. This study focuses on the real-time expression of seven genes in the cluster in ZON-producing and ZON-deficient mutant strains, under inducing conditions on inoculated sterile rice and during wheat infection. The two polyketide synthase genes *PKS4* and *PKS13* and the alcohol oxidase *FG12056* showed similar gene expression pattern as the putative transcriptional regulator *FG02398*, while the non-ribosomal peptide synthase *FG02394*, the K^+ channel ß subunit *FG12015* and the protein kinase *FG02399* displayed a somewhat different expression pattern. The expression of *PKS4, PKS13, FG12056* and *FG02398* genes were quite consistent in relation to each other between the two experiments, while the others varied. *PKS13* had slightly higher gene expression than *PKS4* in both experiments, possibly suggesting individual enzymatic activity as opposed to an enzyme complex. *FG02398* and *FG12015* showed higher gene expression in wheat than on parboiled rice, suggesting a role during wheat infection. Based on these expression data, the knowledge that ZON is able to bind to the estrogen receptor, and literature studies, we suggest a potential role of ZON in *Fusarium*.

TWO MITOGEN-ACTIVATED PROTEIN KINASE SIGNALING CASCADES REGULATE SENSITIVITY TO ANTIFUNGAL PLANT DEFENSINS IN *FUSARIUM GRAMINEARUM*.

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ABSTRACT

Two cysteine-rich antifungal defensins, MsDef1 and MtDef4, from *Medicago* spp., share 41% amino acid sequence identity and inhibit the growth of the fungal pathogen *Fusarium graminearum* at micromolar concentrations. However, the molecular mechanisms by which these defensins inhibit the growth of this fungus remain largely unknown. In order to determine the fungal signaling cascades that are modulated by these defensins, we have screened 4,800 insertional mutants of *F. graminearum* and isolated several mutants that selectively exhibit hypersensitivity to MsDef1, but not to MtDef4. The molecular characterization of two of these mutants, designated *enhanced sensitivity to defensin (esd)*, has revealed that the Mgv1 and Gpmk1 MAP kinase signaling cascades play a major role in regulating sensitivity of *F. graminearum* to MsDef1, but not to MtDef4. The Hog1 MAP kinase pathway, which is responsible for adaptation of this fungus to hyperosmotic stress, does not participate in the fungal response to these defensins. Significantly, the *esd* mutants also exhibit hypersensitivity to other defensins used in this study except MtDef4 and are highly compromised in their pathogenesis on wheat heads and their ability to cause infection in wounded tomato fruits. The studies reported here for the first time implicate two MAP kinase signaling cascades in a plant defensin-mediated alteration of fungal growth. Based on our findings, we propose that specific MAP kinase signaling cascades are essential for protection of a fungal pathogen from the antimicrobial proteins of its host plant.

SPATIAL PATTERNS OF TRICHOTHECENE GENOTYPES OF *GIBBERELLA ZEAE* IN WHEAT FIELDS.

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ABSTRACT

Grain infected with *Gibberella zeae* often contains the trichothecene mycotoxins deoxynivalenol (DON) and nivalenol (NIV), threatening the health of humans and domesticated animals. Isolates of *G. zeae* that produce DON may also produce two acetylated derivatives, 3-ADON and 15-ADON. These derivatives may vary in toxicity, and NIV is considered to be ten times more toxic to animals than DON. Little is known about the spatial distribution of trichothecene genotypes of *G. zeae* (3-ADON, 15-ADON, and NIV) in wheat fields. We collected GPS-referenced FHB samples from individual wheat fields in Virginia, New York, and North Carolina. Singleplex and multiplex PCR assays were used to evaluate trichothecene genotypes of *G. zeae* in these geographically-referenced populations. Spatial patterns of trichothecene genotypes were visualized by contour plots of genotype counts over entire fields. Knowledge of the spatial patterns of trichothecene genotypes of *G. zeae* in wheat fields may aid in developing and/or excluding strategies for disease management. Little or no testing for NIV is currently performed in the eastern United States. Should the NIV genotype be present in wheat fields in the eastern United States, it would be essential to implement appropriate assays for detecting NIV contamination in these regions.

TRICHOTHECENE GENOTYPES IN ATMOSPHERIC POPULATIONS OF *GIBBERELLA ZEAE*.

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ABSTRACT

Gibberella zeae (*Fusarium graminearum* sensu stricto) is the principal causal agent of Fusarium head blight (FHB) of wheat and barley in the USA. Grain infected with *G. zeae* often contains the trichothecene mycotoxins deoxynivalenol (DON) and nivalenol (NIV), threatening the health of humans and livestock. Isolates of *G. zeae* that produce DON may also produce two acetylated derivates, 3-ADON and 15-ADON. These derivates may vary in toxicity, and NIV is considered to be ten times more toxic to animals than DON. We used singleplex and multiplex PCR assays to evaluate trichothecene genotypes (3-ADON, 15-ADON, and NIV) in atmospheric populations of *G. zeae* collected over three years in New York, USA. Results indicated that the majority of the isolates were of the 15-ADON genotype. Knowledge of the distribution and spread of trichothecene genotypes of *G. zeae* in the atmosphere may be used to infer sources of inoculum for regional epidemics of FHB, and may aid in the development of strategies for disease management. Immigrant strains of *G. zeae* with altered toxin profiles, if transported long distances through the atmosphere, have the potential to spread rapidly across North America and displace native strains.

GENE EXPRESSION ANALYSIS OF CONIDIUM AND ASCOSPORE DEVELOPMENT IN *FUSARIUM GRAMINEARUM*.

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ABSTRACT

To understand the infection cycle of the head blight pathogen *F. graminearum*, gene expression profiles were monitored during both ageing and germination for conidia and ascospores. Ascospores and conidia were treated in a similar manner, either aged under desiccating conditions or suspended in liquid germination medium. RNA was extracted from spores and cultures and used to query the 18K feature *F. graminearum* Affymetrix GeneChip. Overall, a slightly greater number of probe sets corresponding to genes were detected in ascospores (9,207) than in conidia (8,815; detection p value <0.001). However, the large majority of probe sets (8,068) were shared between conidia and ascospores. While a similar number of genes were detected at most stages of development, the biggest difference among spore types was upon desiccation where the number of probe sets detected in ascospores (6,801) was more than twice the number detected in conidia (2,916). These results indicate that ascospores remain more metabolically active than conidia upon ageing. Peroxisomal proteins and genes involved in lipid beta-oxidation are strongly up-regulated both in fresh conidia and in ascospores. After suspending conidia or ascospores in liquid germination medium, numerous genes involved in transcription, RNA splicing, protein synthesis, and amino acid and nucleotide metabolism were highly induced. Up-regulation of proteasome components and secretory proteins were observed as spores established polarized growth after 8h of incubation. Comparing gene expression in spores with expression in hyphae under a variety of environment regimes indicates that a total of 328 probe sets were specific for ascospores and another 150 were specific for conidia. Spore-specific gene expression may be used to develop hypotheses concerning spore maturation, dormancy and initiation of germination that ultimately may serve as the underlying basis for novel disease control strategies.

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A NOVEL G-BETA LIKE PROTEIN IS ESSENTIAL FOR PATHOGENESIS IN THE WHEAT SCAB FUNGUS *FUSARIUM GRAMINEARUM*.

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ABSTRACT

Fusarium head blight (FHB) or scab caused by *Fusarium graminearum* is an important disease of wheat and barley. In addition to yield losses, infected grains are reduced in grain quality and contaminated with mycotoxins. In our previous study, we identified several mutants with reduced virulence by random insertional mutagenesis. In one of these mutants, the transforming vector was inserted in a predicted gene named *TBL1* (for transducin beta-like gene 1). *TBL1* is homologous to the mouse *TBLR1*, which encodes a putative nuclear receptor corepressor. The Tbl1 protein contains three WD40 repeats and an N-terminal LisH domain, which is involved in protein-protein interactions. We generated the *tbl1* deletion mutant by the gene replacement approach. The •*tbl1* mutant was non-pathogenic and significantly reduced in conidiation. It was defective in colonizing flowering wheat heads and more sensitive to a plant defensin MsDef1. Conidium germination was delayed in the "*tbl1* mutant. The "*tbl1* mutant accumulated a red pigment that may be related to the upregulation of the aurofusarin synthesis cluster based on our microarray analysis. Interestingly, *TBL1* is the only LisH domain-containing gene in the *F. graminearum* genome. To determine its function, we generated a *TBL1*$^{\text{"LisH}}$ allele and transformed it into the "*tbl1* mutant. Phenotype analysis of the resulting transformants expressing *TBL1*$^{\text{"LisH}}$ suggests that LisH is essential for the *TBL1* function and plant infection. We also generated a *TBL1*-GFP fusion construct and examined its expression and localization. Our results indicate that *TBL1* plays a critical role in conidium germination, response to plant defense compounds, and colonization of wheat tissues.

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