USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY05 Final Performance Report (approx. May 05 – April 06)
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Cover Page

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Fiscal Year:	**2005**
FY05 ARS Agreement ID:	**58-3640-2-139**
Agreement Title:	**Use of Gene Expression Analysis to Study Pathogenicity in Gibberela zeae.**
FY05 ARS Award Amount:	**$ 38,005**

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
EDM	Yeast Two-Hybrid Libraries for Studying Protein-Protein Interactions in Gibberella zeae.	$ 38,005
	Total Award Amount	**$ 38,005**

7/14/2006

Principal Investigator Date

* BIO – Biotechnology
CBC – Chemical & Biological Control
EDM – Epidemiology & Disease Management
FSTU – Food Safety, Toxicology, & Utilization
GIE – Germplasm Introduction & Enhancement
VDUN – Variety Development & Uniform Nurseries

Project 1: *Yeast Two-Hybrid Libraries for Studying Protein-Protein Interactions in Gibberella zeae.*

1. What major problem or issue is being resolved and how are you resolving it?

Understanding molecular mechanisms regulating pathogenesis and toxin production of *Fusarium graminearum* is critical for developing new disease control methods for head blight or scab. Several genes, including *TBL1, ZIF1, MGV1, LIP1 and FMK1*, that are involved in fungal-plant interactions have been characterized in *F. graminearum* recently. However, it is not clear how these genes interact with other components of signaling complexes and relaying various plant signals. The yeast two-hybrid approach has been widely used in studying direct interaction between human, plant, and fungal genes. In this study we constructed a yeast two-hybrid library with the HybridZap2.1 system using RNA samples isolated from nitrogen-starved cultures. This library consists of about 500, 000 original cDNA clones. Several *TBL1*-interacting clones, including a putative transcription factor homologous to Atf-2, have been identified by screening the yeast two-hybrid library. Verification and functional characterization of these genes are under the way. We also have conducted microarray analysis with the *tbl1* mutant. In comparison with the wild-type, over 200 genes had more than five-fold changes in expression levels in the *tbl1* mutant.

In addition, we have generated gene replacement mutants of predicted genes FG00060, FG00061, FG00062, and FG10551. These genes are unique to *F. graminearum* and are expressed during plant infection. Structurally, they are similar to the KP4 killer toxin encoded by a mycovirus in *Ustilago maydis*. Deletion of FG00060, FG00061, and FG00062 in *F. graminearum* has no obvious effects on growth or infection in laboratory conditions. However, mutants deleted of FG10551 were defective in inhibiting the growth of a *U. maydis* killer-sensitive strain, indicating that FG10551 may be important for niche competition in the nature. We also used the split-marker approach to delete a 7 kb cluster (FG11025-11026) and a 8 kb cluster (FG02313-FG02315) of genes that are unique to *F. graminearum*. These *F. graminearum* specific genes may be important for wheat head infection.

2. List the most important accomplishment and its impact (how is it being used?). Complete all three sections (repeat sections for each major accomplishment):

<u>Accomplishment:</u>

A yeast-two hybrid cDNA library was constructed with the HybridZap2.1 system. Several putative *TBL1*-interacting genes have been isolated. Four putative toxin-like genes unique to *F. graminearum* were functionally characterized. These genes may be important for *F. graminearum* to infect wheat plants or to compete with other microorganisms in nature. The FG10551 deletion mutants were defective in inhibiting the *Ustilago maydis* b3 strain. In addition, we have deleted two clusters of genes that have no homologs in GenBank. Further characterization of these mutants is under the way and will be useful to understand the function of genes unique to *F. graminearum*.

Impact:

This is the first yeast two-hybrid library available for *F. graminearum.* This yeast two-hybrid library will be distributed to the Fusarium community. It will be useful to identify proteins that directly interact with known important pathogenicity factors or genes regulating mycotoxin production.

We have functionally characterized four putative KP4-like toxin genes in *F. graminearum*. Further characterization of FG10551 is under the way to understand its role in plant infection and competition against other microbes. Transgenic plants expressing the KP4 toxin have been reported to be resistant to several fungal pathogens.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?:

Currently, there is no yeast two-hybrid library available for *F. graminearum.* The availability of this library will be helpful to further characterize molecular mechanisms of pathogenesis and toxin production in *F. graminearum.* Further characterization of FG10551, a KP4-like toxin gene, may lead to the development of a new anti-fungal gene suitable for generating disease-resistant transgenic plants.

Include below a list of the publications, presentations, peer-reviewed articles, and non-peer reviewed articles written about your work that resulted from all of the projects included in the grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

Peer-reviewed articles:

1. Ramamoorthy, V., Zhao, X., Snyder, A. K., Xu, J. –R., and Shah, D. M. 2006. Two Mitogen-Activated Protein Kinase Signaling Cascades Regulate Sensitivity to Antifungal Plant Defensins in *Fusarium graminearum*. Submitted to Plant Cell.
2. Xu, J. -R., Peng, Y., Dickman, M. B., and Sharon, A. 2006.The dawn of fungal pathogen genomics. *Annual Reviews of Phytopathology* 44: 337-366 (invited review).
3. Goswami, R. S., Xu, J. –R., Trail, F., Hilburn, K., and Kistler, H. C. 2006. Genomic analysis of host-pathogen interaction between *Fusarium graminearum* and wheat during early stages of disease development. *Microbiology* 6: 1877-1890.
4. Güldener, U., Seong, K., Boddu, J., Cho, S., Trail, F., Xu, J. –R., Adam, G., Mewes, H., Muehlbauer, G. J., and Kistler, H. C. 2006. Development of a *Fusarium graminearum* Affymetrix GeneChip for profiling fungal gene expression in vitro and *in planta*. *Fungal Genetics and Biology* 43: 316-325.
5. Seong, K., Li, L., Hou, Z., Kistler, H. C., and Xu, J. –R. 2006. Cryptic promoter activity of the *HMR1* coding region in the wheat scab fungus *Fusarium graminearum. Fungal Genetics and Biology* 43: 34-41.
6. Seong, K., Hou, Z., Kistler, H. C., and Xu, J. –R. 2005. Random Insertional Mutagenesis Identifies Genes Associated with Virulence in the Wheat Scab Fungus *Fusarium graminearum. Phytopathology* 95 (7): 744-750.

Now peer-reviewed articles:

Anderson, J. M., Cambron, S. E., Crane, C., Goodwin, S. B., Johnson, A., Nemacheck, J. A., Scofield, S., Schemerhorn, B., Shukle, R. H., Williams, C. E., Ohm, H. W., Kong, L., Sharma, H. C., Shen, X., Uphaus, J., Buechley, G., Huber, D., Shaner, G., Xu, J. R., and Stuart, J. 2005. Annual Wheat Newsletter. Volume 51: 178-184.

Seong, K., J. R., and Kistler, H. C. 2005. Functional genomic studies of pathogenicity in *Fusarium graminearum*. p. 168, The 2005 National Fusarium Head Blight Forum, Milwaukee, WI., (Dec. 11-13, 2005).

Xu, J. R., Trail, F., and Kistler, H. C. 2005. Gene expression analysis of conidium germination in *Fusarium graminearum*. p171, The 2005 National Fusarium Head Blight Forum, Milwaukee, WI., (Dec. 11-13, 2005).

Invited presentations:

Functional genomic studies of pathogenicity in *Fusarium graminearum*. Invited presentation at the 2005 National Fusarium Head Blight Forum. Milwaukee, WI. Dec. 11-13, 2005.

Genomic studies of plant pathogens *Fusarium graminearum* and *Magnaporthe grisea*. Invited presentation at Fujian Agricultural and Forestry University. Fuzhou, P. R. China. August, 2005.