USDA-ARS / USWBSI
FY03 Final Performance Report (approx. May 03 – April 04)
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Cover Page

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Year:	**FY2003 (approx. May 03 – April 04)**
FY03 ARS SCA ID:	**58-3640-2-139**
FY03 ARS Agreement Title:	**Use of gene expression analysis to study pathogenicity in Gibberella zeae.**
FY03 ARS Award Amount:	**$ 72,637**

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
EDM	REMI mutagenesis in Fusarium graminearum for identifying genes important for fungal development and pathogenesis.	$ 29,757
EDM	Genomics of Gibberella zeae, the head scab fungus.	$ 42,900
	Total Amount Recommended	**$ 72,637**

7/14/2004

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: ***REMI mutagenesis in Fusarium graminearum for identifying genes important for fungal development and pathogenesis.***

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

Fusarium head blight (scab) is a disease of wheat and barley that can cause reduction both in crop yield and grain quality. Molecular study of *F. graminearum* is critical because of the lack of effective fungicides and highly resistant plant varieties. Unfortunately, there are few molecular studies on fungal development and pathogenicity in this important pathogen.

In this study, we generated over 7, 500 REMI (restriction-enzyme mediated integration) transformants. All these REMI transformants were screened using the corn-silk and flowering wheat head infection assays. To date, 12 mutants defective in plant infection have been isolated. Genes disrupted in seven of these pathogenicity mutants have been identified. Mutants M7, M8, M30, and M75 are reduced in colonizing flowering wheat heads. In M8, the cystathionine β-lyase (*CBL1*) gene required for methionine synthase is disrupted. Mutants deleted of the *CBL1* or the methionine synthase (*MSY1*) gene displayed phenotypes similar to M8, indicating that methionine synthesis is critical for fungal pathogenicity in *F. graminearum*. In M7 and M75, a bZIP transcription factor and a transducin-like protein are disrupted, respectively. None of them have significant orthologs known to be important pathogenicity factors in other fungal pathogens. In M30, the transforming DNA was integrated between two *F. graminearum* genes (predicted ORFs) without known homologs in GenBank. Mutant 222 shows no ability to induce plant cell death and cause necrotic symptoms on the plant. The HMG-CoA reductase gene (*HCR1*), a key enzyme involved in isoprenoid biosynthesis, is disrupted in this mutant. Mutants 25C3 and M28 are significantly reduced in wheat head infection. Preliminary analyses indicated that a putative hydrolase gene and an iron/sulfur protein maturation factor are disrupted in mutant 25C3 and M28, respectively. Further characterization of these genes are under way.

2. What were the most significant accomplishments?

We have isolated 12 REMI mutants that are defective in plant infection. Genes disrupted in seven of these pathogenicity mutants have been identified. Five of these genes are novel pathogenicity or virulence factors that play important roles in colonizing wheat heads.

Project 2: *Genomics of Gibberella zeae, the head scab fungus.*

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

F. graminearum is an important pathogen suitable for studying many different aspects of fungal pathogenicity. Our studies aimed at functional analyses of candidate pathogenicity genes identified in EST and genome sequences. In the past year, we have generated gene replacement mutants of 12 *F. graminearum* genes, including two G-protein coupled receptor genes *FgGPR11* (FG05006.1) and *FgSTE3* (FG07270.1) that are homologs of *GPR1* and *STE3* in the budding yeast, a methionine synthase gene (*MSY1*), a MAP kinase kinase gene *FgSTE7* (FG09903.1), and a MAP kinase kinase kinase gene *FgSTE11* (FG05484.1). Preliminary data indicated that *FgSTE7* and *FgSTE11* are essential for pathogenicity in *F. graminearum* and likely functional above the *GMK1* gene for regulating plant infection process. The *msy1* mutant is significantly reduced in virulence, suggesting that methionine synthesis is important for pathogenesis in *F. graminearum.*

In order to verify the role of choline in the wheat scab disease, we generated knockout mutants of one putative choline transporter gene that displayed the highest homology with the yeast *HNM1* gene. We also isolated mutants deleted of two genes (*NLS1* and *NLS2*) that have both signal peptide and nuclear localization signal and may function as novel virulence factors in *F. graminearum*. Corn-silk and wheat head infection assays indicated that the *nls1, nls2,* and *hnm1* deletion mutants have no obvious defect in plant infection. We have also generated gene replacement mutants of two *HetS* homologs and two polyketide synthase genes (*PKS1* and *PKS2*) highly homologous to the *F. verticillioides FUM1* gene. While the *pks2* deletion mutant is normal, the *pks1* deletion mutant is reduced in virulence. However, it appears that the defect of the *pks1* mutant in plant infection is dependent of culture conditions. In *Podospora anserina*, *HetS* regulates self-recognition and vegetative incompatibility, which may play important role in population structures in the nature. Preliminary data indicated that the *HetS2 and HetS2* genes are dispensable for fungal pathogenicity in corn silk assays. These two mutants will be further examined for their defects in vegetative incompatibility and virulence on flowering wheat heads.

2. What were the most significant accomplishments?

In this study, we have examined the function of 12 candidate genes in *F. graminearum* and identified at least three more genes that play important roles in plant infection processes and fungal development. In addition, we applied the split-marker approach for generating knockout mutants and found it works efficiently in *F. graminearum*.

With the preliminary data generated in this project and available genome sequence, we have received funding from USDA to generate the whole-genome microarray of *F. graminearum.* This microarray will be very useful for large-scale functional analysis.

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 you grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

Seong, K., Tracy, M., Hou, Z., Kistler, H.C. and Xu, J.R. 2003. REMI mutagenesis in the wheat scab fungus *Fusarium graminearum*. Abstract presented at 2003 US National Fusarium Head Blight Forum. Dec. 13-15, 2003. St. Paul. MN.

Trail, F., Xu, J. -R., San Miguel, P., Halgren, R. G., and Kistler, H. C. 2003. Analysis of Expressed Sequence Tags from *Gibberella zeae* (anamorph *Fusarium graminearum*). *Fungal Genetics and Biology* 38: 187-197.

Goswami, R. S., Trail, F**.,** Xu, J. -R., and Kistler, H. C. 2003. Fungal genes expressed during plant disease development in the *Fusarium graminearum*/wheat interaction. Abstract 292 presented at the 22nd Asilomar Fungal Genetics Conference, Pacific Grove, CA. March 18-23, 2003.

The whole genome sequence of the wheat and barley scab fungus, *Fusarium graminearum*. H. Corby Kistler, Bruce Birren, Sarah Calvo, James Galagan, Liane R. Gale, Li-Jun Ma, Frances Trail, and Jin-Rong Xu. The XII Plant and Genome Conference, San Diego, CA. Jan. 10-14, 2004.

Genomics of the wheat scab fungus *Fusarium graminearum.* H. Corby Kistler, Bruce Birren, James Galagan, Frances Trail, and Jin-Rong Xu. Abstract presented at The 15th International Plant Protection Congress (ICPP) held from May 11-16, 2004, Beijing, P. R. China.