Regulation of mycotoxin production and kinome analysis in *Fusarium graminearum*

Chenfang Wang

Northwest Agricultural & Forestry University
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Jin-Rong Xu, Purdue University
Purdue – NWAFU Joint Research Center
Nationwide Outbreak of Wheat Scab in China - 2012
DON

陕西 杨凌: 1891.5 ppm

陕西 华县: 772.7 ppm

(FDA: 1ppm human consumption, 5 ppm animal feeds)

Organizing a wheat scab initiative/working group
Unique features of *Fusarium graminearum*

- Ascospores – primary inoculum
- Sexual reproduction critical in its infection cycle
- No microconidia
- ‘Tissue-specific’ for flowering heads
  wheat, barley, Brachypodium, and others
Unique genomic features of *F. graminearum*

- Very few repetitive sequences (0.03%, 15x less than Sc)
- No active transposable elements
- Rare recent duplications
- Localized polymorphism and pathogen specialization

Many *TRI* genes were up-regulated during plant infection.

**TRI Gene Functions**

- **TRI3**: C-3 Acetylation
- **TRI4**: Trichodiene Oxygenation
- **TRI5**: Trichodiene Synthesis
- **TRI6**: Transcription factor
- **TRI7**: C-4 Acetylation
- **TRI8**: C-3 Deacetylation
- **TRI9**: ‘Unknown’
- **TRI10**: Transcription factor
- **TRI101**: Trichotheecene 3-0-acetyltransferase
- **TRI11**: C-15 Hydroxylation
- **TRI12**: Transporter
- **TRI13**: C-4 Hydroxylation
- **TRI14**: Transcription factor
- **TRI16**: Transporter
**Tri6**: Cys2His2 zinc-finger domain

**Tri10**: Fungal specific Zn(2)-Cys(6) binuclear cluster domain

- Both Tri6 and Tri10 are important for *TRI5* expression
- Tri6 appears to be more important than Tri10

*TRI5 Expression*

(Seong et al., 2010)

*tri6 tri10* double mutants
WT tri6 tri10

TRI genes down-regulated in the tri6 and tri10 mutants
Microarray analysis
(Seong et al., 2010)

1/37 genes positively regulated by Tri10
87/208 genes positively regulated by Tri6

Tri10: quite specific for trichothecene biosynthesis
Tri6: other genes related to plant infection
Evolution: co-regulation of isoprenoid and trichothecene biosynthesis genes in *F. graminearum*

(Seong et al., 2010)
Tri6-Tri10 interaction may be conditional -
Environmental or host factors affect DON production may converge on Tri6/Tri10

pH: acidic pH induced TRI genes

Nitrogen sources

NH$_4$ inhibits DON synthesis
Polyamines stimulates (Gardiner et al., 2010)

ROS + other stresses

Plant factors (flowering heads)

(DON not produced in rice scab)
A. TRI6/10 expression
   Binding to TRI6/10 promoter
B. Tri6/10 activities
   Interacting with Tri6/10

‘Tri6/Tri10 model’

PacC

pH N ROS/Stresses Plant factors

TRI6/10

TRI genes
A type factors: binding to TRI6 promoter

Conserved PacC-binding sites:
GCCAAG (-512) TRI6 promoter

- yeast one-hybrid assays
- searching for conserved promoter elements
B type factors: Tri6–interacting proteins

- Screening 2 yeast two-hybrid libraries

- Affinity purification -MS analysis
  TRI6-GFP
  TRI6-3xFLAG
  TRI6-S-tag
  (may need to be over-expressed)
Three MAPK pathways (Gpmk1, Mgv1, FgHog1)

The cAMP-PKA pathway

Putative PKA & MAPK phosphorylation sites in Tri6 MAPK docking site

Why Tri6 is ‘global’ regulator?
Project 1. Functional analyses of predicted protein kinase genes (Kinome)

Project 2. Genes unique to *F. graminearum* (‘Uniquitome’)

Targeted gene deletion – highly efficient (80%) in *F. graminearum*
## Comparative Analysis - Protein Kinase Genes

<table>
<thead>
<tr>
<th>Kinase group</th>
<th>Yeast</th>
<th>FOXG</th>
<th>FVEG</th>
<th>FGSG</th>
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<td>AGC</td>
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<td>10</td>
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<td>CAMK</td>
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<tr>
<td>PIKK</td>
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<td>5</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>RIO</td>
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<td>1</td>
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<tr>
<td>Other</td>
<td>18</td>
<td>39</td>
<td>28</td>
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<tr>
<td>Putative</td>
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<td></td>
<td>8</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>127</strong></td>
<td><strong>130</strong></td>
<td><strong>109</strong></td>
<td><strong>116</strong></td>
</tr>
</tbody>
</table>

20 genes - essential

Mutants identified for 96 PK gene
At least two mutants for each gene
>30 of them - complementation assays
(subcellular localization)

Specifically localized to the septal pore
17 phenotypes scored
- growth rate
- colony morphology (pigmentation)
- hyphal growth and branching
- conidiation
- conidium morphology & germination
- sexual reproduction
- infection assays with flowering wheat heads
- DON production
- stress responses: NaCl, H₂O₂, SDS, CR

Searchable database
http://www.fgkinome.nwsuaf.edu.cn

Wang et al., PLoS Pathogens. 7(12): e1002460
42 PK genes are important for plant infection
Networks of PK-PK interactions predicted with the interlog approach.

Three MAP kinase cascade
PK genes essential in *S. cerevisiae* or *S. pombe* but not in *F. graminearum*

<table>
<thead>
<tr>
<th><em>F. graminearum</em> genes</th>
<th><em>S. cerevisiae</em> orthologs</th>
<th><em>S. pombe</em> orthologs</th>
<th>Number of mutants</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGSG_02399 ^a ^b</td>
<td><em>IPL1</em></td>
<td>arkl</td>
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<td>FGSG_00433</td>
<td><em>RAD53</em></td>
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<td>FGSG_04947</td>
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<td>cdc7</td>
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<td>FGSG_08468 ^b ^c</td>
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<td>cdc2</td>
<td>12</td>
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<tr>
<td>FGSG_03132 ^b ^c</td>
<td><strong>CDC28</strong></td>
<td>cdc2</td>
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<td>FGSG_01188</td>
<td><em>CBK1</em></td>
<td>orb6 ^d</td>
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<td><em>KIC1</em></td>
<td>nak1 ^d</td>
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<td>SPCC70.05C</td>
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<tr>
<td>FGSG_04053</td>
<td>- ^c</td>
<td>prp4</td>
<td>7</td>
</tr>
</tbody>
</table>

Key regulator of cell cycle
Two Cdc28/Cdc2 genes

FGSG_03132 mutant: Reduced 30% in conidiation

FGSG-08468 mutant: similar to Fg04947 (CDK kinase)
Defective in pathogenesis (DI <1.5)
Blocked in ascosporogenesis

Double mutants – not viable
Cell cycle and fungal pathogenesis

Some CDKs: infection-specific functions
Hyphal growth: *in vitro* vs. *in planta*
Project 2. Genes unique to *F. graminearum*

- Unique genes with signal peptide (secreted)
- Unique genes with conserved domains

Six of them have distinct functions
**SSP1 mutant**: reduced growth rate

+ colony morphology defects

A *F. graminearum* unique gene important for hyphal growth
SSP2 is required for normal sexual reproduction

PH-1

Mutant

Empty perithecia (60%)

aborted

abnormal
Two *F. graminearum* specific genes important for plant infection

- PH-1
- ssp3
- sdp1

*In planta expression*
Acknowledgements

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