New Insights into the Evolution of *Fusarium* Pathogenesis in Wheat

Kemal Kazan
Where are we?

CSIRO Plant Industry, Brisbane

Queensland Bioscience Precinct – BRISBANE

Research Station – GATTON
Wheat - Fusarium Research in CSIRO

Four inter-connected strands to deliver industry and science outputs
A tale of two diseases
wet finish = head blight - dry finish = crown root

F. pseudograminearum and F. graminearum can cause both
crown rot and head blight
Australian Wheat Belt & Fusarium

Northern Region
- Summer rain

Southern and Western Regions
- Winter rain

Crown rot found in all regions.
Mainly F. pseudograminearum
Fusarium species causing crown rot and head blight in Australia

Fusarium species from infected plant parts

- Others
- F. avenaceum
- F. crookwellense
- F. graminearum
- F. pseudograminearum

Sukumar Chakraborty, CSIRO
2010 wheat season had a very wet finish

Obanor et al. (2012) Plant Pathology
Wheat crops were sampled from 25 sites in QLD and 19 in NSW at soft dough or early maturity stage.

FHB was widespread on bread wheat and durum.

Both *Fusarium graminearum* and *F. pseudograminearum* caused FHB epidemic.

*Obanor et al. (2012). Plant Pathology*
FHB epidemic 2010

DON concentration (mg/kg)  Proportion of spikelets

F. pseudograminearum  F. graminearum

Obanor et al. (2012). Plant Pathology
What happened to the big wet after December 2010?
Major Questions

• Why is *F. pseudograminearum* predominant in crown rot?

• Why is *F. pseudograminearum* so broadly adapted?

• What cereal defences are overcome by *F. pseudograminearum* and how?
## F. pseudograminearum genome

<table>
<thead>
<tr>
<th>Species</th>
<th>Size</th>
<th>Technology</th>
<th>Coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. pseudograminearum</td>
<td>37Mbp</td>
<td>Illumina</td>
<td>179x</td>
</tr>
<tr>
<td>F. graminearum</td>
<td>36Mbp</td>
<td>Sanger</td>
<td>10x</td>
</tr>
<tr>
<td>F. oxysporum f. sp. lycopersici</td>
<td>60Mbp</td>
<td>Sanger</td>
<td>6x</td>
</tr>
<tr>
<td>F. oxysporum 5176</td>
<td>55Mbp</td>
<td>454</td>
<td>8x</td>
</tr>
<tr>
<td>F. verticillioides</td>
<td>41Mbp</td>
<td>Sanger</td>
<td>8x</td>
</tr>
</tbody>
</table>

1 Cuomo et al 2007; 2 Ma et al 2010; 3 Thatcher et al 2012; Gardiner et al 2012
Comparison of *F. pseudograminearum* & *F. graminearum* genomes

- **Fp specific-sequences (6%)**
- **Gaps**
- **Fg SNPs**
Comparative genomics pipeline

- *Fp* predicted gene set (12,448)
- Reciprocal BLASTp against 16 cereal pathogens & 11 non-pathogens
- 156 *Fp* genes no match
- 239 Genes exclusively in other cereal pathogen(s)
- Functional and phylogeny analysis
Examples of *F. pseudograminearum* genes exclusively shared by cereal pathogens with some acquired from bacteria

<table>
<thead>
<tr>
<th>Grass pathogens</th>
<th>Other fungi &amp; bacteria</th>
</tr>
</thead>
</table>

### Fp genes

| *F. pseudograminearum* | *F. oxysporum* | *F. verticillioides* | *F. solani* | *T. virens* | *C. higginsianum* | *C. graminicola* | *N. crassa* | *M. poae* | *M. oryzae* | *G. graminis* | *B. cinerea* | *C. sativus* | *C. graminicola* | *A. nidulans* | *C. heterost. C5* | *C. heterost. C4* | *P. teres* | *P. tritici-rep.* | *P. nodorum* | *P. fijiensis* | *P. chrysosp.* | *U. maydis* | *S. cerevisiae* | *P. triticina* | *P. graminis* | *Bacteria* |
|------------------------|---------------|---------------------|-------------|-------------|------------------|------------------|-------------|----------|-----------|--------------|-------------|-------------|----------------|---------------|----------------|----------------|------------|----------------|----------------|-------------|-------------|-------------|-------------|----------------|-------------|----------------|----------------|
| FPSE_00725             | 1099          | 724                 |             |             |                  |                  |             | 63       | 32        | 33           |             |             |                |               |                |                |             |                |                |             |                |             |             |                |             |               |                |
| FPSE_07536             | 1885          | 1673                |             |             |                  |                  |             | 32       | 33        | 1264        |             |             |                |               |                |                |             |                |                |             |                |             |             |                |             |               |                |
| FPSE_07775             | 624           | 549                 |             |             |                  |                  |             |          | 32       | 360          |             |             |                |               |                |                |             |                |                |             |                |             |             |                |             |               |                |
| FPSE_08088             | 215           | 724                 |             |             |                  |                  |             |          | 32       | 591          |             |             |                |               |                |                |             |                |                |             |                |             |             |                |             |               |                |
| FPSE_08088             | 622           | 336                 |             |             |                  |                  |             | 29       | 32        | 41           | 43          | 43           |                |               |                |                |             |                |                |             |                |                |             |                |             |             |                |             |               |                |
| FPSE_11907             | 1452          | 724                 |             |             |                  |                  | 601         | 660      | 634      | 635          | 409         | 631         | 613               |               |                |                |             |                |                |             |                |                |             |                |             |             |                |             |               |                |
| FPSE_02381             | 492           | 299                 |             |             |                  |                  |             |          | 1240     | 1013         |             |             |                |               |                |                |             |                |                |             |                |             |             |                |             |               |                |
| FpAH1                  | 365           | 254                 |             |             |                  |                  | 113         | 133      | 98       | 437          |             |             |                |               |                |                |             |                |                |             |                |             |             |                |             |               |                |
| FPSE_05718             | 357           | 507                 |             |             |                  |                  |             |          | 502      | 437          |             |             |                |               |                |                |             |                |                |             |                |             |             |                |             |               |                |
| FPSE_05719             | 365           | 254                 |             |             |                  |                  |             |          | 113      | 133          | 98           |             |                |               |                |                |             |                |                |             |                |             |             |                |             |               |                |
| FPSE_06956             | 357           | 507                 |             |             |                  |                  |             |          | 502      | 437          |             |             |                |               |                |                |             |                |                |             |                |             |             |                |             |               |                |
| FpDLH1                 | 1240          | 1013                |             |             |                  |                  |             |          | 861      | 55           |             |             |                |               |                |                |             |                |                |             |                |             |             |                |             |               |                |
| FPSE_08937             | 274           | 541                 |             |             |                  |                  |             |          | 113      | 133          | 98           |             |                |               |                |                |             |                |                |             |                |             |             |                |             |               |                |
| FPSE_10646             | 365           | 254                 |             |             |                  |                  |             |          | 113      | 133          | 98           |             |                |               |                |                |             |                |                |             |                |             |             |                |             |               |                |

Numbers indicate BLAST bit score

Gardiner et al., 2012, PLOS PATHOGENS
Where did FpAH1 come from?

Genetic exchange between Stagonospora and Fp?

or

Independent acquisition from bacteria?

SNP analysis of AH1 genes in *F. pseudograminearum* & *P. nodorum* indicates an independent & ‘ancient’ origin in each species

*Fp* haplotypes

*Phaeosphaeria* haplotypes

Gardiner et al., 2012, PLOS PATHOGENS
FpAH1 is expressed during crown infection

FpAH1 promoter-GFP fusion and Real-time RT-PCR
FpAH1 is important for full virulence on wheat

Gardiner et al., 2012, PLOS PATHOGENS
FpAH1 is required for full virulence on barley

FpAH1 knockouts have reduced virulence (i.e. increased plant survival)

FpAH1 complementation restores virulence in knock outs

Gardiner et al., 2012, PLOS PATHOGENS
What is the mode of action of FpAH1?

Amidohydrolases are a diverse superfamily of enzymes which catalyse the hydrolysis of amide bonds in a large number of different substrates.

Amide-containing cereal defence molecules

Hydroxamic acids (DIMBOA)

Gramine (indole alkaloid)

Hordatine
Evolution of pathogenicity in cereal pathogens: possible roles for horizontal gene transfer

- Stagonospora nodorum
  - ToxA gene
- Pyrenophora tritici-repentis
- Colletotrichum graminicola
- Fusarium verticillioides
- Fusarium pseudograminearum
  - Amidohydrolase
  - Dienelactone hydrolase + Amidase

Plant endophytic bacteria
# Genomes now under comparative analysis

<table>
<thead>
<tr>
<th>Species</th>
<th>Isolate</th>
<th>Interest</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F. pseudograminearum</em></td>
<td>CS3220</td>
<td>Moderate pathogenicity</td>
</tr>
<tr>
<td></td>
<td>CS3427</td>
<td>High pathogenicity</td>
</tr>
<tr>
<td></td>
<td>CS3487</td>
<td>Promiscuous mating isolate</td>
</tr>
<tr>
<td></td>
<td>CS5834</td>
<td>WA isolate</td>
</tr>
<tr>
<td><em>F. acuminatum</em></td>
<td>CS5907</td>
<td>CR pathogen occasionally isolated in field</td>
</tr>
<tr>
<td><em>F. equiseti</em></td>
<td>CS3069</td>
<td>CR pathogen occasionally isolated in field</td>
</tr>
<tr>
<td><em>F. culmorum</em></td>
<td>CS7071</td>
<td>Globally important species for FCR and FHB</td>
</tr>
</tbody>
</table>
Thank you

CSIRO Plant Industry
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