Genome Sequencing and Comparative Transcriptomics of Race1 and Race4 of Banana pathogen: \textit{Fusarium. oxysporum f.sp. cubense}

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Outline

I. Introduction
II. Generation of Genome
III. Gene evolution
IV. Transcriptome analysis
V. Virulence associated genes

\textit{Fusarium oxysporum f.sp. cubense} is a fungal plant pathogen that causes Panama disease of banana (Musa spp.), also known as fusarium wilt of banana.

Four races of this pathogen have been described which attack different banana cultivars:
- Race 1 (Foc1) attacks cultivars in the Musa (AAA group) 'Gros Michel' and caused the 20th century epidemic. It also attacks Musa (AAB group) 'Pome' and its subgroups, Musa (AAB group) 'Silk' and Musa (ABB group) 'Pisang Awak'.
- Race 2 (Foc2) attacks Musa (ABB group) 'Bluggoe' and its close relatives.
- Race 4 (Foc4) attacks Musa (AAA group) 'Dwarf Cavendish' as well as the hosts of races 1 and 2.
### Sequencing Data

<table>
<thead>
<tr>
<th>Insert Size (bp)</th>
<th>500</th>
<th>1000</th>
<th>10000</th>
</tr>
</thead>
<tbody>
<tr>
<td>100_100</td>
<td>2.04</td>
<td>2.75</td>
<td>4.79</td>
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<tr>
<td>44_44</td>
<td>45.36</td>
<td>61.18</td>
<td>106.54</td>
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<tr>
<td>Raw Total</td>
<td>1.76</td>
<td>1.97</td>
<td>3.76</td>
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<tr>
<td>After Filter</td>
<td>39.83</td>
<td>43.83</td>
<td>83.48</td>
</tr>
<tr>
<td><strong>Total Data (G)</strong></td>
<td><strong>1.91</strong></td>
<td><strong>2.16</strong></td>
<td><strong>0.73</strong></td>
</tr>
<tr>
<td><strong>Sequence coverage (X)</strong></td>
<td><strong>42.44</strong></td>
<td><strong>45.36</strong></td>
<td><strong>25.71</strong></td>
</tr>
</tbody>
</table>

### Statistics of assembly

<table>
<thead>
<tr>
<th>Contig</th>
<th>Foc1</th>
<th>Foc4</th>
</tr>
</thead>
<tbody>
<tr>
<td>N90</td>
<td>6.063</td>
<td>2.109</td>
</tr>
<tr>
<td>N80</td>
<td>10.179</td>
<td>1.517</td>
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<tr>
<td>N70</td>
<td>14.753</td>
<td>1.131</td>
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<td>N60</td>
<td>18.773</td>
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<tr>
<td>N50</td>
<td>23.057</td>
<td>1.997</td>
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<tr>
<td><strong>Total size (bp)</strong></td>
<td><strong>47,081,305</strong></td>
<td><strong>47,838,312</strong></td>
</tr>
</tbody>
</table>

### Gene prediction

Methods: A combination of homologous gene searching, de novo gene prediction and transcription evidence.

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### Gene families and phylogenetic tree

The most recent common ancestor (MCRA) has 13,974 gene families
Gene families expanded in Foc/Fol branch

- **Gene families**: Major facilitator superfamily (MFS) transporters, ATP-binding cassette (ABC) transporters, Oxidation-reduction activity, Glycoside hydrolase, Tetra-tricopeptide repeat
- **Function**: MFS transporters are typically involved in the transport of a wide range of substrates and may function as nutrient sensors.
- **Function**: The ABC transporters are usually implicated in defending the pathogen from host-produced secondary metabolites.
- **Function**: Adaptation to the huge amounts of reactive oxygen species (ROS) released by the host plant during early stage of infection.
- **Function**: It is crucial for fungi to penetrate plant cell wall and establish successful infection.
- **Function**: Have been reported involved in intracellular processes, regulate morphological transition and virulence in Candida albicans.

Differences in genome structure between Foc and Fol

- The size of window is 100 bp.
- (a) Fol chromosomes.
- (b) The average depth of Foc1 reads mapped on Fol chromosomes.
- (c) The average depth of Foc4 reads mapped on Fol chromosomes.
- (d) The SNP and InDel number of Foc1.
- (e) The SNP and InDel number of Foc4.
- (f) Gene density of Fol.
- (g) Repetitive sequence density of Fol.

Gain and lost gene in Foc1 and Foc4

Taking Fol as out group, comparison of gene content between Foc1, Foc4 and Fol allows us to identify genes gained and lost during evolution.

<table>
<thead>
<tr>
<th>Gene cluster</th>
<th>Foc1</th>
<th>Total</th>
<th>Duplication</th>
<th>Horizontal gene transfer</th>
<th>Retroposition</th>
<th>De novo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foc1</td>
<td>177</td>
<td></td>
<td>111</td>
<td>8</td>
<td>8</td>
<td>58</td>
</tr>
<tr>
<td>Foc4</td>
<td>131</td>
<td></td>
<td>130</td>
<td>11</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

| Lost         |      |       |             |                          |               |         |
| Foc1         | 186  |       |             |                          |               |         |
| Foc4         | 211  |       |             |                          |               |         |

Fast evolution

- Foc1/Foc4: 183
- ortholog: Foc1/Foc4: 15140

Functional enrichment of gain genes in Foc4

- **Gene cluster**: Major facilitator superfamily (MFS) and ATP-binding cassette (ABC) transporters, Cytochrome P450 enzymes, Transcription factors
- **Function**: Defending the pathogen from host-produced secondary metabolites, as well as sensing nutrient source.
- **Function**: Participate in the production of diverse metabolites, and play critical roles in organism’s adaptation to specific ecological and/or nutritional niches by modifying potentially harmful environmental chemicals.
- **Function**: Some transcription factors are specifically required for pathogenicity, such as Fow2 in F. oxysporum f. sp. melonis and Sge1 in Fol.

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We used RNA-Seq to compare transcriptional responses of Foc1 and Foc4 at 0 hour and 48 hour after they infect the optically clear Musa (AAA group) Cavendish cv. Brazil, respectively.
Genes up-regulated in Foc1 and Foc4 after inoculation to banana. Compare to Foc1, Foc4 have more genes up-regulated implicated in extracellular region, virion, virion part, structural molecule, biological adhesion, death, developmental process and reproduction.

Genes down-regulated in Foc1 and Foc4 after inoculation to banana. Compare to Foc4, Foc1 have more genes down-regulated implicated in extracellular region, membrane-enclosed lumen, cell killing, developmental process and reproduction.

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PHI database


There are more PHI annotated gene in Foc4 compared to Foc1, including genes GRABC1, CBL1, ZIP1, TBL1 and MGG_02731 which have been tested by experiments that mutant will lead to reduce in virulence, as well as MGG_00883, PEX6 and MGG_02731 which have been empirically tested that gene disruption or deletion may lead to loss of pathogenicity.

SIX genes

Recent research with Fol has elucidated the role of SIX-genes (secreted in xylem, SIX1-SIX8) in pathogenicity in the Fol-tomato pathosystem.

<table>
<thead>
<tr>
<th>SIX genes</th>
<th>Foc4 gene ID</th>
<th>Foc1 gene ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIX1</td>
<td>FOC4_g10000240</td>
<td>FOC1_g10001632</td>
</tr>
<tr>
<td>SIX2</td>
<td>FOC4_g10000575</td>
<td>none</td>
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<tr>
<td>SIX3</td>
<td>FOC4_g10000324</td>
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<tr>
<td>SIX4</td>
<td>FOC4_g10000731</td>
<td>none</td>
</tr>
<tr>
<td>SIX5</td>
<td>FOC4_g10000351</td>
<td>FOC1_g10000211</td>
</tr>
<tr>
<td>SIX6</td>
<td>FOC4_g10000520</td>
<td>none</td>
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</tbody>
</table>

Similarity tree of SIX proteins. The unrooted tree describes the similarity relationships among the SIX proteins of the three Foc1 (green), Foc4 (red), and Fol (black).
Secondary Metabolite Clusters

Secondary Metabolites (SMs), including mycotoxins, antibiotics and pharmaceuticals, provide protection against various environmental stresses and during antagonistic interactions with other soil inhabitants or a eukaryotic host.

We employed the web-based software SMURF (SECONDARY METABOLITE UNIQUE REGIONS FINDER, www.jcvi.org/smurf) to systematically predict clustered SM genes based on their genomic context and domain content.

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