Potential of *Trichoderma asperellum* for biocontrol of Fusarium wilt in banana

Nancy Chaves, Charles Staver, Miguel Dita

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Introduction
Fusarium wilt

- *Fusarium oxysporum* f. sp. *cubense* (F.o.c) is responsible for Fusarium wilt in banana.
- This fungi survives in soil for many years.
- There are four races of F.o.c.
- Race 1 affects Gros Michel banana in Costa Rica.
- Control by chemical methods is generally ineffective.
Production area and total of Gros Michel farmers in the country

<table>
<thead>
<tr>
<th>Zone</th>
<th># farmers</th>
<th>% participation</th>
<th>Average area</th>
<th>Total area estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Talamanca</td>
<td>1.589*</td>
<td>81,7%</td>
<td>2,4 ha</td>
<td>3.813,6 ha</td>
</tr>
<tr>
<td>Limón</td>
<td>44</td>
<td>2,3%</td>
<td>1,1 ha</td>
<td>48,4 ha</td>
</tr>
<tr>
<td>Siquirres/Matina</td>
<td>80</td>
<td>4,1%</td>
<td>2,0 ha</td>
<td>160,0 ha</td>
</tr>
<tr>
<td>Pérez Zeledón</td>
<td>30</td>
<td>1,5%</td>
<td>1,0 ha</td>
<td>30,0 ha</td>
</tr>
<tr>
<td>Turrialba</td>
<td>153</td>
<td>7,9%</td>
<td>2,0 ha</td>
<td>306,0 ha</td>
</tr>
<tr>
<td>Otras zonas</td>
<td>50</td>
<td>2,6%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td><strong>1.946</strong></td>
<td><strong>100%</strong></td>
<td><strong>4.358 ha</strong></td>
<td></td>
</tr>
</tbody>
</table>

(Escobedo 2010)
Panama disease in Costa Rica

• Association with coffee, cocoa, casava and trees in traditional and organic production systems.

• In Talamanca, 40% of farms growing GM with cocoa are infected.

• In Turrialba, GM in more than 45% of farms. 90% of farms are infected.

• Plant propagation material (suckers) is the main way for dissemination of Foc.

- Local consumption.
- Small-scale farmers (food security).
- Poor management of banana plantation.

Functional endophytes

- Disease prevention is mostly base on the tissue culture (TC) banana plants.
- TC-plants are more susceptible than conventional planting material.
- Inoculating TC-plants with endophytes would increase the plant fitness.
- Several species have been reported as successful biological control agents.

(Nowak 1998, Smith et al. 2008)
Isolate and characterize endophytic microorganisms that can reduce incidence and/or severity of Fusarium wilt of banana.
Endophytes isolation
Samples collection

Map: Bioversity Internacional/D. Brown

Corms

Roots
Endophytes isolation

- NaOCl (2%) and sterilized water (3 times)

Bacteria isolation

Fungi isolation

Surface sterilization protocol (Pocasangre et al. 2000)
Results

**Isolation**

**122 Endophytic bacteria:**

- 27% Central cylinder of the corm
- 33% Cortex of the corm
- 39% Roots

**72 Endophytic fungi:**

- 31% Cortex of the corm
- 33% Roots
- 36% Central cylinder of the corm
In vitro evaluation
Dual culture technique

Fungi

Bacteria
Results

*In vitro test*

**Endophytic bacteria:**
(A) Clear zone of inhibition.
(B) No inhibition.
(C) Fusarium control plate.

**Endophytic fungi:**
(D) Strong inhibition.
(E) No inhibition.
(F) Fusarium control plate.

Antagonism of endophytes to *Fusarium oxysporum* f. sp. *cubense* after 7 days of dual growth on culture media
## Results

<table>
<thead>
<tr>
<th>Tratamiento</th>
<th>CRP (mm)</th>
<th>MICMO</th>
<th>PICR</th>
</tr>
</thead>
<tbody>
<tr>
<td>End 2</td>
<td>7,00</td>
<td>3 ab</td>
<td>78 a</td>
</tr>
<tr>
<td>E6</td>
<td>7,00</td>
<td>4 a</td>
<td>78 a</td>
</tr>
<tr>
<td>GM15</td>
<td>7,33</td>
<td>4 a</td>
<td>77 a</td>
</tr>
<tr>
<td>GM39</td>
<td>7,67</td>
<td>1 bc</td>
<td>76 a</td>
</tr>
<tr>
<td>End1</td>
<td>7,67</td>
<td>1 bc</td>
<td>76 a</td>
</tr>
<tr>
<td>Cart36</td>
<td>10,00</td>
<td>4 a</td>
<td>69 a</td>
</tr>
<tr>
<td>P58</td>
<td>15,33</td>
<td>4 a</td>
<td>52 b</td>
</tr>
<tr>
<td>F7B3</td>
<td>15,67</td>
<td>4 a</td>
<td>51 b</td>
</tr>
<tr>
<td>F7B13</td>
<td>16,00</td>
<td>4 a</td>
<td>50 b</td>
</tr>
<tr>
<td>F7B5</td>
<td>16,00</td>
<td>4 a</td>
<td>50 b</td>
</tr>
<tr>
<td>P52</td>
<td>25,33</td>
<td>4 a</td>
<td>21 c</td>
</tr>
<tr>
<td>B21</td>
<td>31,00</td>
<td>4 a</td>
<td>3 d</td>
</tr>
<tr>
<td>Testigo Foc</td>
<td>32,00</td>
<td>4 a</td>
<td>0 d</td>
</tr>
</tbody>
</table>

Letras distintas indican diferencias significativas (p = 0,05)
In vivo evaluation
In vivo evaluation

Gros Michel
TC-banana plants

Endophytes suspension
$1 \times 10^6$ cfu, 5 min
In vivo evaluation

Planting

Infested corn grains
In vivo evaluation

Weekly evaluation symptoms and growth

After 12 weeks

Final evaluation of roots and corms
In vivo evaluation

Scale for evaluation of symptoms of Fusarium wilt in banana under greenhouse conditions
Endophytes microorganism vs. plant performance
Inoculated with Foc

- Bacillus spp P52 +F
- Bacillus spp P58+F
- T. asperellum C36+F
- T. asperellum End2+F
- Bacillus spp B13+F
- Control Foc
- Bacillus spp B3+F
- T. asperellum E6+F
- T. asperellum GM 15+F

Y-axis:
- Height increase (cm)
- Diameter increase (mm)
Endophytes microorganisms vs. F. o. f. sp. cubense in greenhouse

![Bar chart showing % Yellowing reduction and % Rhizome discoloration reduction for different microorganisms.](image_url)
### Results

Effect of endophytic isolates on the external and internal symptoms expression of Fusarium wilt in Gros Michel banana plantlets under greenhouse conditions after 12 weeks of inoculation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Yellowing % Reduction</th>
<th>Wilt % Reduction</th>
<th>Corm discoloration % Reduction</th>
<th>Number of infected plantlets</th>
<th>Number of dead plantlets</th>
<th>Foliar weight</th>
<th>Root weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusarium</td>
<td>3.10 a</td>
<td>0</td>
<td>2.80 ab</td>
<td>0</td>
<td>3.8 a</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>B13+F</td>
<td>3.10 a</td>
<td>0</td>
<td>3.10 a</td>
<td>0</td>
<td>3.6 a</td>
<td>5.26</td>
<td>10</td>
</tr>
<tr>
<td>P58+F</td>
<td>3.40 a</td>
<td>0</td>
<td>3.20 a</td>
<td>0</td>
<td>3.3 ab</td>
<td>13.15</td>
<td>10</td>
</tr>
<tr>
<td>C36+F</td>
<td>3.10 a</td>
<td>0</td>
<td>2.70 a</td>
<td>3.57</td>
<td>3 abc</td>
<td>21.05</td>
<td>10</td>
</tr>
<tr>
<td>B3+F</td>
<td>3.00 ab</td>
<td>3.22</td>
<td>2.70 ab</td>
<td>3.57</td>
<td>3.1 abc</td>
<td>18.42</td>
<td>10</td>
</tr>
<tr>
<td>P52+F</td>
<td>2.90 ab</td>
<td>6.45</td>
<td>2.40 abc</td>
<td>14.28</td>
<td>2.9 abcd</td>
<td>23.68</td>
<td>10</td>
</tr>
<tr>
<td>End2+F</td>
<td>2.20 bc</td>
<td>29.03</td>
<td>1.70 cd</td>
<td>39.28</td>
<td>2.1 cd</td>
<td>44.73</td>
<td>10</td>
</tr>
<tr>
<td>E6+F</td>
<td>2.20 bc</td>
<td>29.03</td>
<td>1.30 bcd</td>
<td>53.57</td>
<td>2.3 bcd</td>
<td>39.47</td>
<td>9</td>
</tr>
<tr>
<td>GM15+F</td>
<td>2.00 c</td>
<td>35.48</td>
<td>1.50 d</td>
<td>46.42</td>
<td>1.9 d</td>
<td>50.00</td>
<td>9</td>
</tr>
</tbody>
</table>

Average of 10 repetitions. Values in a column followed by the same letter are not statistically different ($P < 0.05$).
Isolates identification

Free living fungi, common in soil and root ecosystems. Opportunistic, avirulent plant symbionts. Some strains establish robust and long-lasting colonization of root. They produce a variety of compounds that induce resistance responses. Root colonization by *Trichoderma* spp. protects the plant from pathogens and enhances root growth and development, crop productivity, resistance to abiotic stresses and the uptake and use of nutrients (Harman *et al.* 2004)

University of Costa Rica
PCR gen 18S

University of Maryland
PCR gen FE1α

Bioinformatics
Embrapa

4 isolates 99-100%
*Trichoderma asperellum*
General conclusions

• Endophytes may play diverse roles in Fusarium wilt development.
• Understanding of antagonistic mechanisms displayed by endophytes is needed.
• Suitable application technologies have to be developed.
• Microorganisms with more than one beneficial effect are of great interest.
Thank you

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