Exploration of microbial communities associated to Fruitlet Core Rot (FCR) disease in 'Queen' pineapple from Reunion Island

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Ananas comosus

- ‘Queen’ Variety
- 1st fruit production of Reunion
- Economic losses due to various diseases including FCR disease
Black Spot disease

• First described in 1896
• originates in the « field »
• Difficult to anticipate
  – Black spots inside the fruit
  – No visible symptoms from the outside

Jean-Christophe Meile, unpublished

Avalone et al., 2003
Fruitlet Core Rot

• 3 main Phytopathogenic Fungal species
  ◦ *Penicillium funiculosum (Talaromyces funiculosa)*
  ◦ *Fusarium moniliforme*
  ◦ *Fusarium ananatum*

• Fruitlet Browning

<table>
<thead>
<tr>
<th></th>
<th>Healthy Fruitlets</th>
<th>Infected Fruitlets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Browning</td>
<td>0.33 ± 0.04***</td>
<td>0.58 ± 0.12***</td>
</tr>
<tr>
<td>Polyphenol oxydase</td>
<td>0.34 ± 0.35***</td>
<td>3.37 ± 2.26***</td>
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<tr>
<td>Laccase</td>
<td>0.17 ± 0.18***</td>
<td>1.35 ± 0.46***</td>
</tr>
<tr>
<td>Saccharose</td>
<td>4.15 ± 0.89***</td>
<td>1.61 ± 0.76***</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.91 ± 0.19&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>1.38 ± 0.41&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.98 ± 0.17&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>1.18 ± 0.28&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Adapted from Avallone, et al, 2003 Current Microbiology
Hypotheses

- FCR occurrence linked to various factors:
  - Pedoclimatic conditions
  - Fruit Biochemical composition
  - Agricultural practices
  - Microbial Communities?

Are there specific microbial communities associated to FCR disease?
Methodology

Study of microbial communities associated to:

- Healthy and diseased fruitlets
- In various climatic contexts
- In various production contexts (agricultural practices)

Comparative study of microbial communities to search for microbial markers of FCR disease
Fruit Sampling

- 20 producers
- 6 fruits/plot
- Parameters:
  - Climate
  - Agricultural practices
  - Fruit Biochemical Composition
- Total of 126 samples

0 -300 m of altitude
300 -600 m of altitude
600-800 m of altitude

Julie De Stefano, non publiée
Fruitlet Sampling

Healthy and diseased fruitlets

Adaptée de : http://www.snv.jussieu.fr
Microbiological analyses of fruitlets

Healthy / Diseased fruitlet samples

Classical Microbiology

Molecular Microbiology (culture-independent)

Culturing and isolation
- Fresh and frozen samples

Total DNA extraction
- Frozen samples

Identification (DNA extraction, PCR amplification and sequencing (Sänger))

Microbial ecology analysis (PCR-DGGE)
- (bacterial 16S and fungal ITS regions)
Bacterial Communities (PCR amplification)

- PCR amplification:
  - 16S rDNA V3 region
  - DNA primers 338F and 518R

- 200 bp amplicons

- Bacterial DNA amplified in most samples (120/126)
Bacterial Communities Profiling

8 different bacterial profiles

One major DNA band / bacterial species
Bacterial groups

- Principal Component Analysis (PCA)
  - F1+ F2 represent 85 % of the total variance
  - 4 bacterial groups
  - 2 major groups
    - Green : 45 samples (on 107)
    - Yellow : 34 samples
Bacterial groups on the map I

Legend:
- **H**: Healthy Fruitlet or Floral Cavity
- **D**: Diseased Fruitlet or Floral Cavity

Major Bacterial groups
- **Yellow**

Minor bacterial group
- **Red**

Floral receptacle
- **Blue**

Fruitlet or Floral Cavity
- **Green**

North: 0 - 300 m of altitude

West

East

South
Bacterial groups on the map II

Legend:
- **H**: Healthy Fruitlet or Floral Cavity
- **D**: Diseased Fruitlet or Floral Cavity
- **Floral receptacle**
- **Fruitlet**
- **Floral Cavity**

- **Major Bacterial groups**
- **Minor bacterial group**

- 300 - 600 m of altitude

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Bacterial groups on the map III

Legend:
- **H**: Healthy Fruitlet or Floral Cavity
- **D**: Diseased Fruitlet or Floral Cavity

Map showing bacterial groups with color-coding:
- Yellow: Major Bacterial groups
- Green: Minor bacterial group

Map details:
- 600-800 m of altitude
- North
- East
- West
- South

Map area marked:
- Floral receptacle
- Fruitlet
- Floral Cavity

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Bacterial Communities : Conclusions

• Most bacterial groups are present at all altitudes and type of fruitlet (Healthy and Diseased)

• Blue bacterial group
  – Low altitude
  – North and East sides of the Island

• Yellow bacterial group not found in St Louis samples (Soil management ? Climate ?)
Fungal Communities (PCR amplification)

• PCR amplification:
  – Genomic region ITS1-5,8S-ITS2
  – DNA primers ITS1F and ITS4

  ◦ 500-700 bp amplicons
  ◦ Fungal DNA amplified for 120 samples on 126

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Fungal Communities Profiling

• 9 different fungal profiles
Fungal Communities Profiling

- 9 different fungal profiles

Two Major DNA bands / Fungal species

35 samples

51 samples (on 97)
Fungal Groups

• PCA
  – F1 + F2 represent 87% of the total variance
  – 5 fungal groups
  – Yellow & Orange major groups
    • Yellow : 32 samples on 97, Mostly Diseased fruitlets
    • Orange : 35 samples
      Healthy and Diseased fruitlets
Map of fungal communities

Legend:
- Healthy Fruitlet or Floral Cavity (H)
- Diseased Fruitlet or Floral Cavity (D)
- Major Fungal groups
- Minor Fungal groups

0-300 m of altitude

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Map of fungal communities II

Legend:
- H: Healthy Fruitlet or Floral Cavity
- D: Diseased Fruitlet or Floral Cavity
- Major Fungal groups
- Minor Fungal groups
- Floral receptacle
- Fruitlet
- Floral Cavity

300 - 600 m of altitude
Map of fungal communities III

Legend:

- **H**: Healthy Fruitlet or Floral Cavity
- **D**: Diseased Fruitlet or Floral Cavity
- **Fruitlet**
- **Floral Cavity**

Major Fungal groups

Minor Fungal groups

- **Floral receptacle**

600-800 m of altitude
Fungal Communities : Conclusions

• Red Fungal group :
  – Detected in diseased fruitlets at low altitude (<300m)

• Green fungal group
  – Only detected at medium altitude (300-600m)

• Blue Fungal group
  – Detected in diseased fruitlets at high altitude (600-800m)
Conclusions

• Microbial (bacterial and fungal) DNA detected in almost all (>95%) sampled fruitlets

• Potential microbial markers of FCR disease:

  – At low altitude (<300m)
    • Yellow fungal & bacterial groups (North)
    • Red fungal group (East)
    • Green bacterial group (South)

  – At medium altitude (300-600m)
    • Green & Blue fungal & red bacterial groups (East)
    • Orange Fungal group (South-West)
    • Blue Fungal group (South)

  – At high Altitude (600-800m)
    • Blue Fungal group
Conclusions - Perspectives

• Potential microbial markers of FCR vary according to:
  – Altitude
  – Region of production (climate, practices)

• Perspectives
  • Identification of microbial DNA sequences composing the different microbial groups/profiles (current)
  • Get complementary data with Next Generation Sequencing approaches
  • Functional approaches (RNA) to identify which microbial species are metabolically actives (RT-PCR-DGGE, RNAseq)
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Thank you for your attention!
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