Advancement in Biocontrol Research: A Cellular Level Approach to Study Trophic Interactions

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Outline

- Rapid screening of biocontrol agents
- Microaspiration technology to study interactions at the cellular level
- RNA extraction method for cell specific transcriptome
- Suppression of potential nematode parasitism genes due to biocontrol agents
Interactions

➢ Nematophagous activity

➢ Targeting specific developmental stages of nematode life cycles
Potato plantlets

Microscopy Rhizosphere Chambers (Micro-ROCs)

Biocontrol fungi

G. pallida juveniles stained with PKH26 (1 week after fungal inoculation)

Microscopic observation after 4 days

Imaging techniques
Potato root colonization by *C. globosum*

*G. pallida* in potato root
## Effect of biocontrol fungi on infection by *G. pallida*

<table>
<thead>
<tr>
<th>Treatments</th>
<th><em>G. pallida</em> juveniles (J2s)/root system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>38.6 ± 9.6 a</td>
</tr>
<tr>
<td><em>F. oxysporum</em></td>
<td>24.0 ± 20.6 bd</td>
</tr>
<tr>
<td><em>M. bolleyi</em></td>
<td>23.2 ± 8.2 b</td>
</tr>
<tr>
<td><em>P. lilacinum</em></td>
<td>22.8 ± 12.1 b</td>
</tr>
<tr>
<td><em>F. solani</em></td>
<td>18.2 ± 12.8 ed</td>
</tr>
<tr>
<td><em>P. cucumerina</em></td>
<td>15.0 ± 7.8 ebd</td>
</tr>
<tr>
<td><em>F. tricinctum</em></td>
<td>13.2 ± 5.8 ced</td>
</tr>
<tr>
<td><em>C. globosum</em></td>
<td>7.4 ± 4.1 e</td>
</tr>
</tbody>
</table>

Values with the same letter are not significantly different at $P \leq 0.05$

### Effect of PKH26 or acid fuchsin

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Number of infective juveniles (J2s) in potato roots</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acid fuchsin</td>
</tr>
<tr>
<td>Non-inoculated control</td>
<td>30.6 ± 9.3 a</td>
</tr>
<tr>
<td>C. globosum</td>
<td>8.4 ± 4.8 b</td>
</tr>
</tbody>
</table>

Values with the same letter are not significantly different at $P \leq 0.05$

Molecular Methods

- Systemic and local responses
- Competition for nutrients and niches
- Biotic/abiotic factors
Changes in gene expression at site of infection may be diluted by the relative abundance of uninfected cells.
Process of microaspiration and RNA isolation

- Allows isolation of RNA, DNA, proteins and metabolites from a heterogeneous cell population
- "Touch-free" approach: Contents from the cells of interest can be aspirated
Quality assessment of RNA isolated from microaspirated samples

28S/18S: 1.3
RQN: 7.8

Genes amplified from microaspirated cell specific cDNA
Pathogen defense against biological control

- Understanding pathogen self-defense mechanisms against biocontrol agents
- Developing approaches to improving the durability of biocontrol agents
- Implications for the deployment of transgenes

Nematode inoculation and RNA isolation

- Single roots of *S. tuberosum* and *S. sisymbriifolium* were inoculated with sterile *G. pallida*.
- Extraction of infected nematodes from the plant roots
- RNA isolation and transcriptome analysis

Differential expression of nematode defence genes

- Clp-1
- CTSS
- Exn-1
- Bath-38
Concluding Remarks

➢ Tools are required to study trophic interactions at the molecular level.

➢ Recent developments in microscopic and “Omics based” techniques are promising to study these interactions at the cellular level.
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Thank you