Copper tolerance in *Pseudomonas syringae* pv. *tomato* from tomatoes in Queensland, Australia

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These slides will be made available on the APPS website.
Study Overview

- *Pseudomonas syringae pv. tomato* = Bacterial Speck Disease
- Control reliant on Copper based products
Study Overview

- Intensive copper use in crops
- Risk of copper tolerance development
- Environmental impacts
- Copper tolerance in *P. syringae pv. tomato* reported globally, 2 Australian studies
Study Overview

- Sampled commercial tomato crops 2015-2017
- 101 plant samples
- 31 *P. syringae pv. tomato* isolates
Copper-tolerance in *Pseudomonas syringae* pv. *tomato* and *Xanthomonas* spp. and the control of diseases associated with these pathogens in tomato and pepper. A systematic literature review

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in vitro copper tolerance screening

- Methodology testing: Effect of media type and pH on in vitro results (Paper)
- All isolates copper tolerant – MIC 0.8-1.5 mM on CYEG (Casitone yeast extract glycerol agar)
- Confirmed in vivo
- Herbarium isolates from 1970-80, also copper tolerant
PCR to identify cop genes

- Plasmid encoded cop genes linked to copper tolerance
- Normal metabolism requires low levels of copper ions – chromosomally encoded
- Initial focus on copA

Fig. 1. The cop operon of *Pseudomonas syringae* and probable locations and functions of its protein products. $P_{cop}$ is the copper-inducible promoter that drives $copA-D$, while $P_{copR}$ is the constitutive promoter that drives $copRS$. Figure extracted from Cooksey 1994
<table>
<thead>
<tr>
<th>Isolate ID/s</th>
<th>P. syringae pv.</th>
<th>Source</th>
<th>MIC (mM)</th>
<th>Copper status</th>
<th>Primers producing a fragment</th>
<th>No. Cu binding motifs (Cha &amp; Cooksey 1991)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*M19930</td>
<td>tomato</td>
<td>USA</td>
<td>N/A</td>
<td>Tolerant</td>
<td>N/A</td>
<td>4</td>
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<tr>
<td>*JQ418536</td>
<td>syringae</td>
<td>Spain 2016 USA 2005</td>
<td>N/A</td>
<td>Tolerant</td>
<td>N/A</td>
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<tr>
<td>*KY362372</td>
<td>tomato</td>
<td>UK 1960</td>
<td>0.5</td>
<td>Sensitive</td>
<td>None</td>
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<tr>
<td>*CP000075</td>
<td>strain DC3000</td>
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<tr>
<td>*AE016853</td>
<td>tomato</td>
<td>This Study</td>
<td>1.5</td>
<td>Tolerant</td>
<td>PCCop, KGCop1&amp;2</td>
<td>4</td>
</tr>
<tr>
<td>63.1</td>
<td>tomato</td>
<td>This Study</td>
<td>1.5</td>
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<td>KGCop1</td>
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<td>16 study isolates</td>
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<td>KGCop1</td>
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<tr>
<td>BRIP38744</td>
<td>tomato</td>
<td>Aust. 1970-80</td>
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<td>Tolerant</td>
<td>PCCop</td>
<td>?</td>
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</table>

Primer details

PCCop = Almost entire copA
KGCop2 = 1\textsuperscript{st} half of copA
KBCop1 = 2\textsuperscript{nd} half of copA
63.1 Cu Tolerant (This Study) – 4 motifs

1.1 Cu Tolerant (This Study) – 3 motifs

94.1 Cu Tolerant (This Study) – 3 motifs

- 63.1 100% identity* to M19930 reference (nt and aa)
- Rest of study isolates ~65% nt identity* & ~70% aa identity*

*273 aa & 842 nt region
Conclusions

- *P. syringae* pv. *tomato* from QLD, NSW & VIC are tolerant to copper
- Study isolates carry the *copA* gene
  - Sequence differences in the copper binding region
- Genome sequences
Acknowledgments

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  - Roger Shivas and Yu Pei Tan: The Plant Pathology Herbarium of Biosecurity Queensland Department of Agriculture and Fisheries
  - Eric Newberry & Matthews Paret: University of Florida USA
References


