Reduced intracellular immune response of the potato psyllid, *Bactericera cockerelli*, to ‘*Candidatus Liberibacter solanacearum*’ at the gut interface

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A story of cell death...

--Edited by a bacterium

From: http://sc.chinaz.com/tupian/121217371100.htm
Introduction and background

Interaction of bacteria---vector---plants

Solanaceous plants

“Zebra chip”

Disease

Candidatus Liberibacter solanacearum

“Lso”

Bactericera cockerelli

“Potato psyllid”

Liefting et al. (2008)
Hansen et al. (2008)
Munyaneza et al. (2006)
Introduction and background

Lso distribution and haplotypes

Glynn et al. (2012)
Lin et al. (2012)
Nelson et al. (2011)
Swisher Grimm and Garczynski, (2018)
Circulative transmission of LsoA and LsoB

The ability of the bacteria to infect the gut depends on the insect immune responses as well as the bacterial strategies deployed to disrupt the host immunity.

Vyas et al. (2015)
Reduced innate immune system in psyllids

- **Reduced** innate immune system;
- Lacking a number of **anti-microbial peptides**;
- Lacking **Imd** pathway – Gram-negative bacteria.

Nachappa et al. (2012)
Arp et al. (2016)
Apoptosis was induced in the gut of ACP by CLas

Uninfected

Infected

Blue -- cell nuclei

Asian citrus psyllid adult

Ghanim et al. (2016)
Question

If Lso could induce apoptosis in the gut of potato psyllid?
Objectives

- **Objective 1:** Investigate gene expression of apoptosis genes in psyllid gut
  - Identify apoptosis related genes from transcriptome dataset
  - qPCR for evaluating gene expression upon early Lso infection

- **Objective 2:** Verify apoptosis process at microscopic and DNA level
  - Nuclear architecture assay
  - Actin cytoskeleton assay
  - DNA degradation assay
Materials & Methods

Lso-free (200 ♀) → transfer → early infection (2, 3, 5, 7 days) → infected

I am also a control

Three replicates

Gene expression
Nuclear architecture Actin cytoskeleton
DNA degradation

The instrument images are from Google
Results of Objective 1

Identify the genes involved in apoptosis

<table>
<thead>
<tr>
<th>Immune responses</th>
<th>Gene name</th>
<th>Code</th>
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</thead>
<tbody>
<tr>
<td>Apoptosis</td>
<td>Inhibitor of apoptosis isoform X1</td>
<td>IAPX1</td>
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<tr>
<td></td>
<td>Baculoviral IAP repeat-containing protein 2</td>
<td>IAPP2</td>
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<tr>
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<td>Apoptosis inhibitor 5-like</td>
<td>IAP5</td>
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<td></td>
<td>TP53-regulated inhibitor of apoptosis 1</td>
<td>IAP1</td>
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<td>Apoptosis-inducing factor 3-like</td>
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<td>Caspase-1 isoform X1</td>
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<tr>
<td></td>
<td>Caspase-3-like</td>
<td>Caspase3</td>
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</tbody>
</table>

Transcriptome
Nachappa et al. (2012)

- **Inhibitor of apoptosis** (IAP): 6
- **Apoptosis-inducing factor** (AIF): 2
- **Caspase** (apoptosis): 3
Results of Objective 1

Apoptotic response to LsoA in the gut

Three inhibitors of apoptosis were up-regulated at day3 or day5. Other genes were not induced.

(N--Non infected; A-2d,3d,5d,7d--Infected by LsoA for 2,3,5,7 days)
Results of Objective 1

Apoptotic response to LsoB in the gut

Two inhibitors were up-regulated, while one inducer of apoptosis was up-regulated at day2.

(N--No infected; B-2d,3d,5d,7d--Infected by LsoB for 2,3,5,7 days)
Results of Objective 2

Nuclei, cytoskeleton and DNA integrity of gut cell

- Uniform and round shape cell nuclei as well as organized structure of actin filaments;

- No DNA ladder was detected for both Lso-free and Lso-infected DNA samples.

Blue -- cell nuclei; Green -- actin filaments, 20 replicates

N: No infected; A, B: LsoA, B colony; A, B-5d: Infected by LsoA, B for 5 days.
Conclusions

Objective 1: Investigate gene expression of apoptosis genes in psyllid gut

Conclusion 1: Both LsoA and LsoB induced several apoptosis related genes especially inhibitors in psyllid gut

Objective 2: Verify apoptosis process at microscopic and DNA level

Conclusion 2: Both LsoA and LsoB did not induce apoptosis phenotype in psyllid gut
Summary

Potato psyllid mounts a **reduced** intracellular immune response to Lso at the gut interface.

Lso-exposed activation level of immune pathways did not reach or exceed the threshold to induce the intracellular immune reaction.

Lso induced the inhibitors of cell death to repress the host immunity to get more acquisition.
Significance of our research

Understanding the adaptive significance of the immune response has the potential to create new approaches for disrupting the transmission of bacteria in insect and controlling ZC.
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