Historical Diversity and Molecular Diagnosis of Bacterial Pathogens on Tomato in Pennsylvania

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Bureau of Plant Industry
Division of Plant Health
PDA historical collection of bacterial strains from tomato.

266 strains from more than 5,000 clinical tomato samples.
Bacterial speck
*(P. syringae pv. tomato)*
38 strains (1987 – 2015)

• Positive by specific PCR
  (Bereswill et al., 1994; Zaccardelli, 2005)

“Pith necrosis”
*(Pseudomonas spp.)*
Bacterial canker on tomato in PA

*Clavibacter michiganensis*
subs. *michiganensis*

Positive by specific real-time PCR (Luo et al., 2008)
Bacterial Spot Xanthomonads (BSX) on tomato in PA

*X. perforans* (100%, XV938)
1994-2012, 90 strains

*X. gardneri* (99-100%, XCGA2)
1995-2015, 32 strains

*X. vesicatoria* (100%, XV1111)
1996, 1 strain

*X. euvesicatoria* (100%, XV155)
not found on tomato but present on pepper (53 strains, 1987-2015)

Based on sequencing of 16S rRNA and BOX PCR.
Bacterial Spot Xanthomonads (BSX) on pepper and tomato in PA.

X. euvesicatoria  X. vesicatoria  X. perforans  X. gardneri
Box PCR (rep-PCR) with PA BSX strains

(Versalovic et al., 1991).

Representatives of PA BSX strains: lanes 2 -10 - *X. gardneri*; lanes 11-17, 19, 21, 22 - *X. perforans*; lanes 20 and 23 *X. euvesicatoria*; lane 24 - *X. vesicatoria*; non-BSX strains: lanes 25-27 *X. vitians*; lane 28 and 29 – *X. campestris* pv. *campestris*
Specificity of conventional PCR primers to PA BSX strains.

| Primers (target)
<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>X. gardneri</td>
</tr>
<tr>
<td>X. perforans</td>
</tr>
<tr>
<td>X. euvesicatoria</td>
</tr>
<tr>
<td>X. vesicatoria</td>
</tr>
<tr>
<td>Non-BSX</td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>RST65/RST69 (HrpB gene, <em>vesicatoria</em> group)</td>
</tr>
<tr>
<td>1/28(^a)</td>
</tr>
<tr>
<td>BSX1/2 (KK1750 sequence, <em>vesicatoria</em> group)</td>
</tr>
<tr>
<td>18/23</td>
</tr>
<tr>
<td>XCVF/XCVR (<em>Hrs</em> protein, <em>X. euvesicatoria</em>)</td>
</tr>
<tr>
<td>0/28</td>
</tr>
<tr>
<td>Xeu2.4/Xeu2.5 (Rep PCR fragment, <em>X. euvesicatoria</em>)</td>
</tr>
<tr>
<td>0/28</td>
</tr>
<tr>
<td>Bs-XeF/ Bs-XeR (AFLP product, <em>X. euvesicatoria</em>)</td>
</tr>
<tr>
<td>0/28</td>
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<tr>
<td>Bs-XvF/ Bs-XvR (AFLP product, <em>X. vesicatoria</em>)</td>
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<tr>
<td>0/28</td>
</tr>
<tr>
<td>Bs-XgF/ Bs-XgR (AFLP product, <em>X. gardneri</em>)</td>
</tr>
<tr>
<td>27/28</td>
</tr>
<tr>
<td>Bs-XpF/ Bs-XpR (AFLP product, <em>X. perforans</em>)</td>
</tr>
<tr>
<td>0(2)/28</td>
</tr>
</tbody>
</table>

\(^a\) 133 PDA BSX strains isolated from tomato and pepper were tested with published PCR primer pairs specific for BSX (Obradovic et al., 2004; Cuppels et al., 2006; Park et al., 2009; Moretti et al., 2009; Koenraadt et al., 2009). Results are based on three replications. Number of positive samples/Total tested samples.
Specific Multiplex real-time PCR for detection of *X. gardneri* from plant samples.

Two primer and probe sets:

1) Specific for *X. gardneri*
   Primers: Xg263F/Xg438R
   Probe: Xg360P
   Target: *arvBs1* effector gene

2) Specific for plant DNA (internal control)
   Primers: COX-F/COX-R
   Probe: COX-P
   Target: COX gene (Weller et al., 2000)
Multiplex real-time PCR for detection of *X. gardneri* from plant samples. Total DNA extracted with Qiagen kit.

Simplex real-time PCR for detection of *X. gardneri* from bacterial cultures without DNA extraction.

Cepheid real-time PCR machine
Time: >1 h
Detection of *X. gardneri* in tomato tissue samples.

<table>
<thead>
<tr>
<th>Bacterial CFU</th>
<th>Tomato/ <em>X. gardneri</em></th>
<th>Tomato/ <em>X. perforans</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Xg, Ct&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Cox, Ct&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>16.95&lt;sup&gt;b&lt;/sup&gt; ± 0.43</td>
<td>20.3 ± 1.30</td>
</tr>
<tr>
<td>10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>20.21 ± 0.87</td>
<td>20.47 ± 0.08</td>
</tr>
<tr>
<td>10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>23.38 ± 1.12</td>
<td>21.53 ± 0.23</td>
</tr>
<tr>
<td>10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>27.45 ± 1.23</td>
<td>21.71 ± 1.08</td>
</tr>
<tr>
<td>10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>34.21 ± 1.87</td>
<td>21.01 ± 1.70</td>
</tr>
<tr>
<td>10&lt;sup&gt;2&lt;/sup&gt;</td>
<td>&gt;40</td>
<td>22.65 ± 0.46</td>
</tr>
<tr>
<td>10&lt;sup&gt;1&lt;/sup&gt;</td>
<td>&gt;40</td>
<td>23.12 ± 0.58</td>
</tr>
<tr>
<td>10&lt;sup&gt;0&lt;/sup&gt;</td>
<td>&gt;40</td>
<td>21.80 ± 0.07</td>
</tr>
<tr>
<td>Control&lt;sup&gt;d&lt;/sup&gt;</td>
<td>&gt;40</td>
<td>22.47 ± 0.29</td>
</tr>
</tbody>
</table>

100 mg healthy tissues of tomato were mixed with 0.1 ml bacterial suspension of *X. gardneri* strain Rf-1. DNAs from plant/bacterial mixtures were extracted using Qiagen kit. <sup>b</sup> The Ct values <sup>c</sup> No fluorescence was detected after 40 cycles of PCR amplification. <sup>d</sup> Healthy tomato tissues.
Effectiveness of real-time PCR for detection of *X. gardneri* from PDA clinical samples

<table>
<thead>
<tr>
<th>PDA Clinical #</th>
<th>Host</th>
<th>Plant parts</th>
<th>Purified DNA&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Culture</th>
<th>Identification&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013-99-99-0054</td>
<td>Tomato</td>
<td>Leaves</td>
<td><strong>19.23</strong> 20.39</td>
<td>Yes</td>
<td><em>X. gardneri</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stems</td>
<td><strong>25.94</strong> 20.84</td>
<td>Yes</td>
<td><em>X. gardneri</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fruits</td>
<td><strong>18.35</strong> 21.5</td>
<td>Yes</td>
<td><em>X. gardneri</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flowers</td>
<td><strong>21.48</strong> 19.62</td>
<td>Yes</td>
<td><em>X. gardneri</em></td>
</tr>
<tr>
<td>2011-02-35-0028</td>
<td>Tomato</td>
<td>Leaves</td>
<td><strong>26.18</strong> 22.68</td>
<td>Yes</td>
<td><em>X. gardneri</em></td>
</tr>
<tr>
<td>2011-99-99-0063</td>
<td>Tomato</td>
<td>Leaves</td>
<td><strong>21.26</strong> 20.77</td>
<td>Yes</td>
<td><em>X. gardneri</em></td>
</tr>
<tr>
<td>2012-99-99-0094</td>
<td>Tomato</td>
<td>Leaves</td>
<td><strong>24.73</strong> 27.52</td>
<td>Yes</td>
<td><em>X. gardneri</em></td>
</tr>
<tr>
<td>2012-07-41-0035</td>
<td>Tomato</td>
<td>Leaves</td>
<td><strong>17.06</strong> 20.93</td>
<td>Yes</td>
<td><em>X. gardneri</em></td>
</tr>
<tr>
<td>2015-99-99-0107</td>
<td>Tomato</td>
<td>Fruits</td>
<td><strong>23.56</strong> 22.04</td>
<td>Yes</td>
<td><em>X. gardneri</em></td>
</tr>
</tbody>
</table>

<sup>a</sup> Samples expressing Bacterial Spot symptoms (PDA: Harrisburg, PA). DNA from plant tissues was extracted and purified using Qiagen kit.

<sup>b</sup> Identification based on culture characteristics and conventional and/or BOX PCR.
### Effectiveness of real-time PCR for detection of *X. gardneri* from PDA clinical samples

<table>
<thead>
<tr>
<th>PDA Clinical</th>
<th>Host</th>
<th>Plant parts</th>
<th>Purified DNA&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Culture</th>
<th>Identification&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010-99-99-0113</td>
<td>Tomato</td>
<td>Leaves</td>
<td>&gt;40.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21.56</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stem</td>
<td>&gt;40.00</td>
<td>22.58</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fruits</td>
<td>&gt;40.00</td>
<td>20.99</td>
<td>Yes</td>
</tr>
<tr>
<td>2012-99-99-0108</td>
<td>Tomato</td>
<td>Leaves</td>
<td>&gt;40.00</td>
<td>21.83</td>
<td>Yes</td>
</tr>
<tr>
<td>2013-07-31-0067</td>
<td>Pepper</td>
<td>Leaves</td>
<td>&gt;40.00</td>
<td>21.18</td>
<td>Yes</td>
</tr>
<tr>
<td>2012-03-13-0029</td>
<td>Pepper</td>
<td>Leaves</td>
<td>&gt;40.00</td>
<td>21.44</td>
<td>Yes</td>
</tr>
<tr>
<td>2012-99-99-0089</td>
<td>Tomato</td>
<td>Leaves</td>
<td>&gt;40.00</td>
<td>19.87</td>
<td>Yes</td>
</tr>
<tr>
<td>2012-99-99-0109</td>
<td>Tomato</td>
<td>Leaves</td>
<td>&gt;40.00</td>
<td>20.72</td>
<td>Yes</td>
</tr>
</tbody>
</table>

<sup>a</sup> Samples expressing Bacterial Spot symptoms (PDA: Harrisburg, PA). DNA from plant tissues was extracted and purified using Qiagen kit.

<sup>b</sup> Identification based on culture characteristics and BOX PCR.

<sup>c</sup> No fluorescence was detected after 40 cycles of PCR amplification.
Multiplex real-time PCR for detection of *X. gardneri*. Conclusions:

- Xg263F/Xg438R real-time PCR is fast and specific for *X. gardneri* detection.

- Sensitivity of detection of *X. gardneri*
  - 30 fg of target DNA from extracted DNA;
  - 4 CFU from bacterial culture without DNA extraction;
  - $10^3$ CFU per 100 mg of plant samples.
PDA exotic disease survey on Solanaceous (Farm Bill 2015)

Bacterial wilt
*(Ralstonia solanacearum)*

Phytoplasma diseases

Photo Source: Phil Hamm, Oregon State University
Exotic Phytoplasmas
(not present in US):

• Australian Grapevine Yellows (Ca. Phytoplasma australiense)
• Bois noir/Stolbur Phytoplasma (Ca. Phytoplasma solani)

General Phytoplasmas:

• Tomato Big Bud Disease
  (found in CA, AR, NY) Granett, 1974; Shaw et al., 1993; Dale et al., 1975
• “Brote Grande” on pepper (found in NM, AR) Randall et al., 2009
• American Potato Purple Top Wilt
  (found in TX, NE) Lee et al., 2006;
• Potato Aster Yellows, Potato Purple Top Wilt
  (found in CA, AK, OR, WA, ID) Lee et al., 2004; Lee et al., 2006
Phytoplasma symptoms on Tomato:

- Witches' brooms
- Enlarged buds form like a cyst
- Leaves become yellow-green or purplish and roll along their margins.
- Flower distortion.
- Fruit development is arrested following infection.

Persley D. & Cooke T. AU

www.apsnet.org
Phytoplasma

- Very small bacteria.
- 37 species described. All of them plant pathogens.
- Cause diseases in hundreds of plants.
- Reside inside plant phloem.
- Do not have cell walls.
- Symptoms can be unevenly distributed on the plants.
- Insect-transmitted (mainly by Leafhoppers) or seed born.
- Cannot be cultured in a lab.
- Identification can be done only at USDA approved Lab!
PDA Phytoplasma 2015 survey on Solanaceous

Diagnostics:

- Visual symptoms
- Molecular:
  - PDA Lab – USDA approved for Phytoplasma detection
  - Modified Phytoplasma-USDA approved specific real-time PCR
  - COX primers/probe as internal control (Weller et al., 2000)

Our Results:

>100 symptomatic samples

- Tomato – 13 PA counties
- Potato – 6 PA counties
- Pepper – 4 PA counties

No Phytoplasma positive found.
Bacterial wilt survey  
(*Ralstonia solanacearum*)

- Affects potato, tomato, pepper, and eggplant.
- Symptoms: wilting, leaf chlorosis (yellowing), necrosis (browning) of vascular tissue, stunting, vascular rings, and rotting of tubers.
- *R. solanacearum* bv2 is on USDA PPQ Select Agent list!
PDA Ralstonia solanacearum survey (2015)

Diagnostics:

- Visual symptoms
- ImmunoStrips (Agdia, Inc.) - species level
  USDA work instruction

Our Results:

35 symptomatic samples

- Tomato – 7 PA counties
- Potato – 4 PA counties

- No *Ralstonia* positive found.
Contributors:

- **Ekaterina (Katya) Nikolaeva**, PhD
- Seong Hwan Kim, PhD
- Ruth Welliver, PhD
- Tracey Olson
- Tucker Piergallini
- Seogchan Kang, PhD

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