Pseudomonas syringae pv. actinidifoliorum and strains of Pseudomonas syringae with low virulence found in kiwifruit orchards in Spain

F. Morán¹, A. Abelleira², E. Landeras³, and M. M. López¹

¹Instituto Valenciano de Investigaciones Agrarias (IVIA), Spain
² Diputación de Pontevedra, Estación Fitopatolóxica de Areeiro, Spain
³Laboratorio de Sanidad Vegetal (LSV) de Asturias, Spain
**Pseudomonas syringae pv. actinidiae populations**

- Population or biovar 1 (Psa 1): strains detected in Japan.

- Population or biovar 2 (Psa 2): strains detected in Korea.

- Population or biovar 3 (Psa 3): (Psa-V, virulent strains), very aggressive strains detected in Italy, France, New Zealand, Portugal, Spain and other countries.

- Population or biovar 4 (Psa 4): (Psa-LV, less virulent strains), detected in New Zealand, Australia, France and recently in the North of Spain. Named *P. syringae pv. actinidifoliorum* (Psaf) (Cunty *et al.* 2014)
EPPO Protocol
Flow chart for detection and identification of Psa

Plant sample (symptomless)
Pathogen extraction

**RAPID Screening tests**
PCR according to Rees-George *et al.* (2010) or Gallelli *et al.* (2011) and isolation

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**P. syringae pv. actinidiae not detected**

**PCR test positive and colonies with typical morphology**

**Identification tests on pure cultures**
Use at least two tests based on different biological principles

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Positive  
*Psa detected*  
Confirm pathogenicity

Negative

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Negative

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Yes

No
PCR for rapid screening of samples

- Isolation.

- Conventional PCR (Rees-George et al. 2010) → Duplex PCR (Gallelli & Loreti, 2011)

The first *P. syringae* Spanish strains showed pathogenic, phenotypic and molecular characteristics identical to *P. syringae* pv. *actinidiae* V (Psa 3) (Abelleira et al. 2011, 2013)
Pathogenicity test with Spanish strains in comparison with strains from New Zealand
Detection of Ps-LV from leaves, flower sepals and asymptomatic canes

- Inoculations with Spanish strains showed the Psa-LV causes tiny spots in leaves but do not cause symptoms in kiwi canes, just a callus at the inoculation wound.

- They also cause symptoms in pepper fruits but not on Prunus cerasus.
Test in branches with strains LV

4439  4440  4441  4442-1
Dendrogram from 16SrRNA gene sequences for classification of Spanish strains
Psa V (Psa3) and Psa LV (Psa4 = Psaf)

Similarities and differences

-Similar symptomatology in leaves, differences in virulence

-Colony morphology: Psa 1, 2, 3 are creamy-white and non fluorescent at 72h in KB. Strains with LV show variable fluorescence.

- Some biochemical tests and API 50 CH show different utilisation of compounds for both types.

-Similar results in Rees-George et al (2010) PCR, but different in duplex PCR (Gallelli and Loretti 2011), multiplex PCR and BOX-PCR.
**Effector gene amplification of different Psa isolates**

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The LV Spanish strains show pathogenic, phenotypic and molecular characteristics of *P. syringae* pv. *actinidifoliorum*

Abelleira et al. 2015. Journal of Applied Microbiology (in press)
Test in leaves with Psaf look-alike strains of LV

Strain NZ10627 (Psa –V)

Strain 4515-2
Test in leaves with strains LV

Virulence in leaves similar to Psaf
Dendrogram from 16SrRNA gene sequences for classification of Spanish strains

- Spanish strains inside the group *P. syringae*. 
New Psaf look-alike Spanish strains

*Pseudomonas* considered as Psaf look-alike, isolated from asymptomatic kiwi plants (leaves and flowers), are being investigated:

- **Test LOPAT:** + - - - + and ----+ (Psaf =+----+)
- Hypersensitivity reaction in tobacco positive
- **Profile API 50 CH ≠** to Psaf and Psa
- Negative for phaseolotoxin and coronatine (equal to Psaf)
- Pathogenic on Hayward leaves but do not produce canker
- PCR Gallelli and Loreti (2011) positive for both targets
- **16S rRNA** reveals proximity to *P. syringae* pv. *tomato*
- Different by biochemical and molecular tests of Psa4.
- PCR Balestra et al. (2013) no geographical origin assigned.
Test in branches
MLSA
New Psaf look-alike Spanish strains

- Maximun-likelihood tree constructed with the concatenated (2230pb) partial sequences of four housekeeping genes gapA, gltA (cts), gyrB and rpoD of 88 strains

- Strains close to Psaf but not inside lineages described

- Strains separated significantly (Value bootstraps > 75%) of Psa and Psaf
## Comparison Psa, Psaf and psaf look-alike

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<th>Psaf</th>
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<td>Profile ≠ to Psaf and Psa</td>
<td>Typical profile Psa</td>
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The new LV Spanish strains show pathogenic, phenotypic and molecular characteristics close but not identical to *P. syringae* pv. *actinidifoliorum*.
Preliminary conclusions

Spanish isolates of *P. syringae* from kiwi plants are grouped in three groups:

- *Pseudomonas syringae* pv. *actinidia*
- *Pseudomonas syringae* pv. *actinidifoliorum*
- *Pseudomonas syringae* pv. *actinidifoliorum* look-alike

- Psaf look–alike show low virulence in *Actinidia deliciosa*
- Virulence and pathogenicity of Psaf and Psaf look–alike are similar.
- Analysis MLSA suggest a difference between Psaf and Psaf look–alike.
- For a more accurate taxonomic classification of Psaf look–alike strains:
  - Presence of effector genes
  - Host range test
  - Genome sequencing
Thank you for your attention