



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

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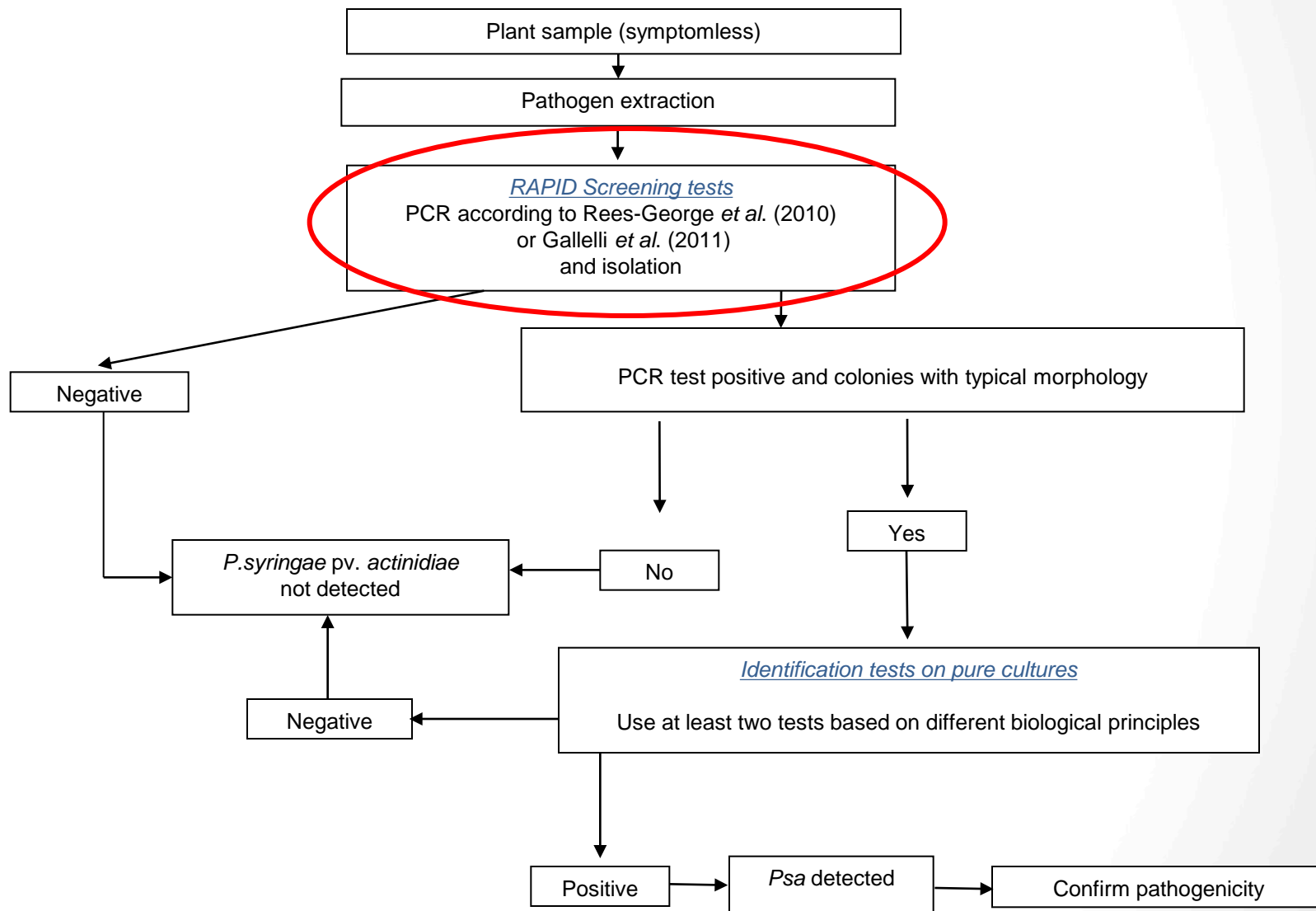
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## *Pseudomonas syringae* pv. *actinidiae* populations

- 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)

# EPP0 Protocol

## Flow chart for detection and identification of Psa

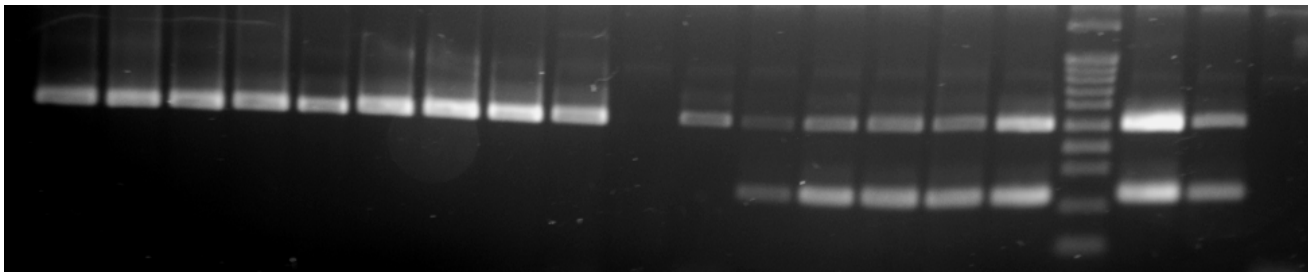


# PCR for rapid screening of samples

-Isolation.

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

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 M 17 18 19



Psa-LV

Psa-V

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



Psa-V



Psa-LV

- Inoculations with Spanish strains showed the Psa-LV causes tiny spots in leaves but do not 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



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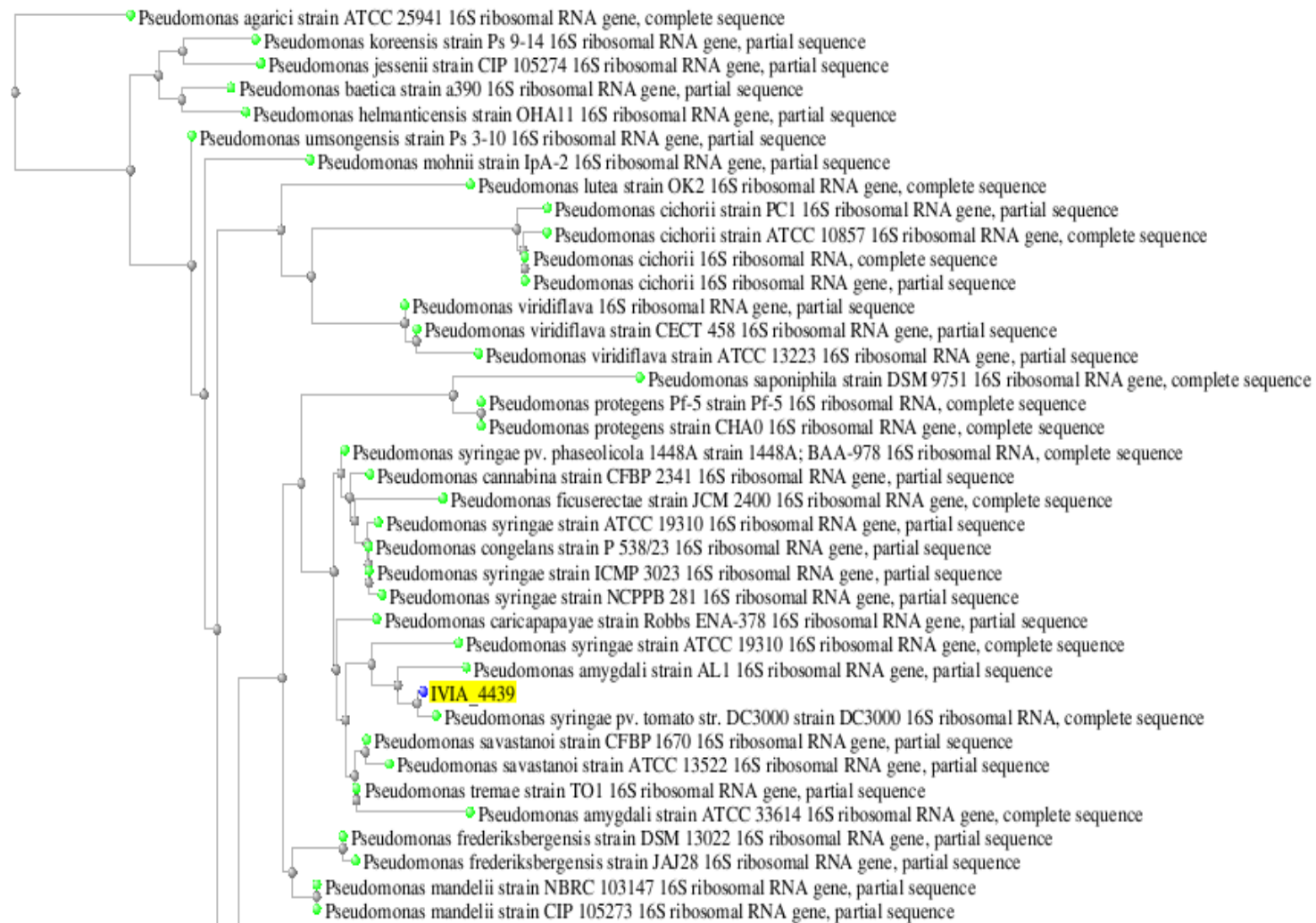


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# Dendrogram from 16SrRNA gene sequences for classification of Spanish strains





# 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 Loretta 2011), multiplex PCR and BOX-PCR.**

# Effector gene amplification of different Psa isolates

Country of origin	Japan	Italy (1992 outbreak)	Korea 1994	Korea 2011	China	Italy (2008-9 outbreak)	New Zealand	Chile	Spain Pontevedra	New Zealand	Australia	Spain A Coruña
MLSA group	Psa1	Psa1	Psa2	Psa2	Psa3	Psa3	Psa3	Psa3	Psa3	Psa4	Psa4	Psa4
Gene Name												
<i>avrPto1</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>avrD1</i>	+	+	+	+	+	+	+	+	+	-	-	-
<i>avrE1</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>hopA1</i>	-	-	-	+	+	+	+	+	-	+	+	+
<i>hopC1</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>hopF2</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>hopG1</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>hrpK1</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>hopAF1</i>	-	-	-	-	-	-	-	-	-	+	+	+
<i>hopAN1</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>hopH1</i>	+	+	-	+	-	+	+	-	+	-	-	-
<i>phaseolotoxine</i>	+	+	-	-	-	-	-	-	-	-	-	-
<i>coronatine</i>	-	-	+	+	-	-	-	-	-	-	-	-

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

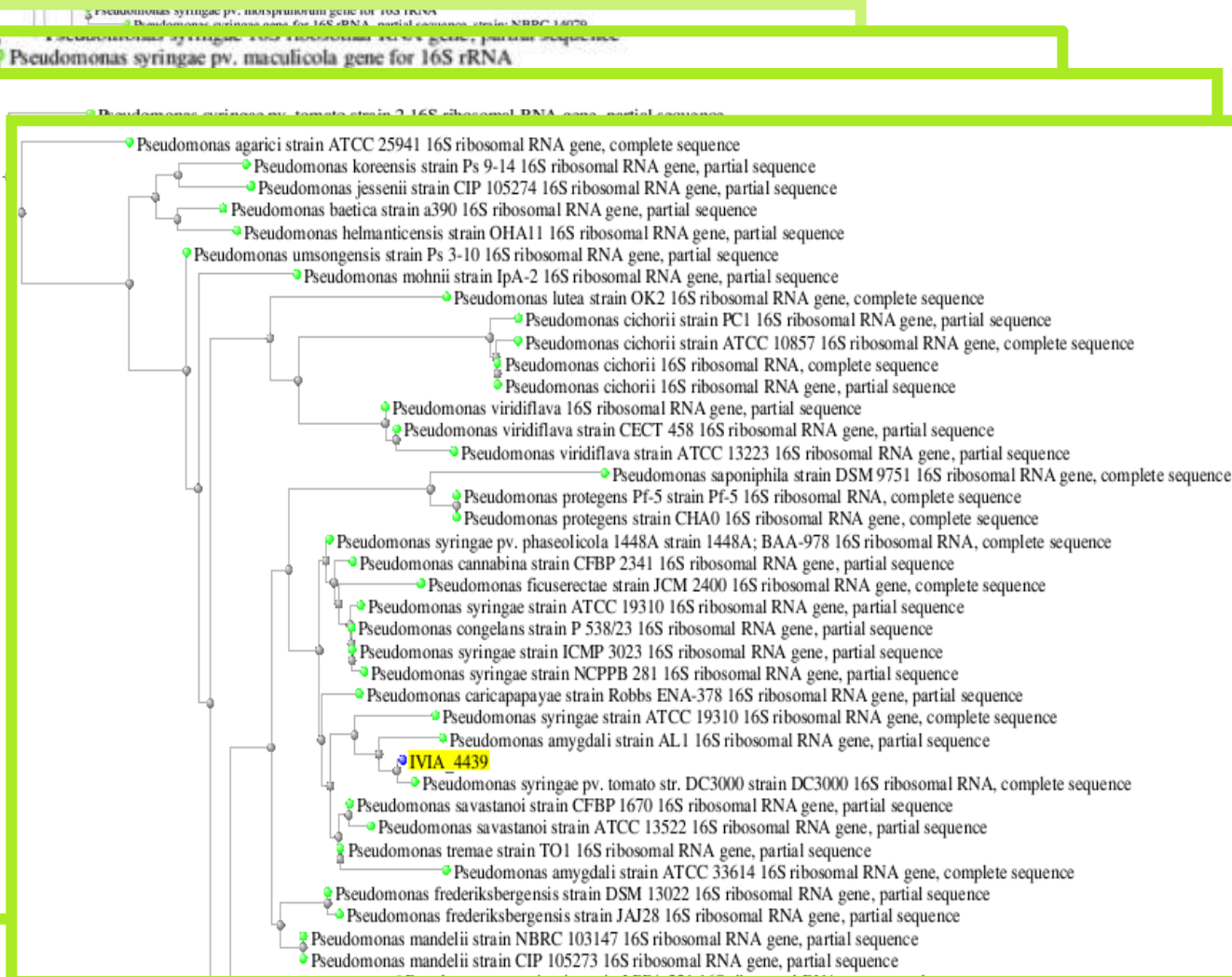
## Test in leaves with strains LV



Virulence in leaves  
similar to Psaf



# Dendrogram from 16SrRNA gene sequences for classification of Spanish strains



- Spanish strains inside the group *P. syringae*.

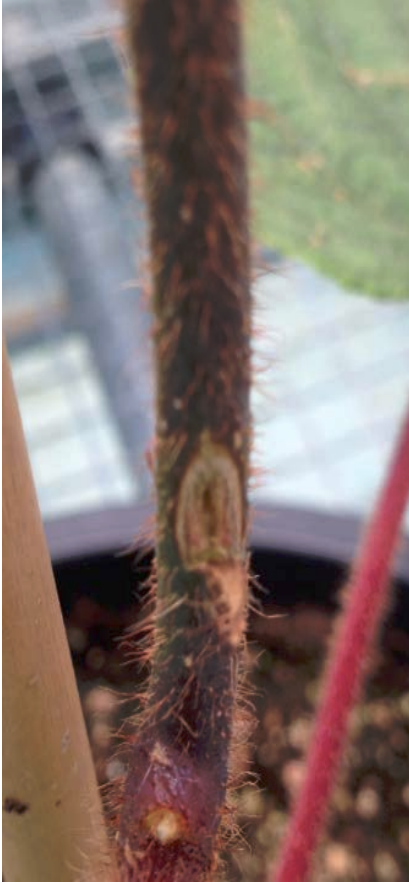


# New Psaf look-alike Spanish strains

*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 Rees-George et al ( 2010 ) positive.
- 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



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4446-2

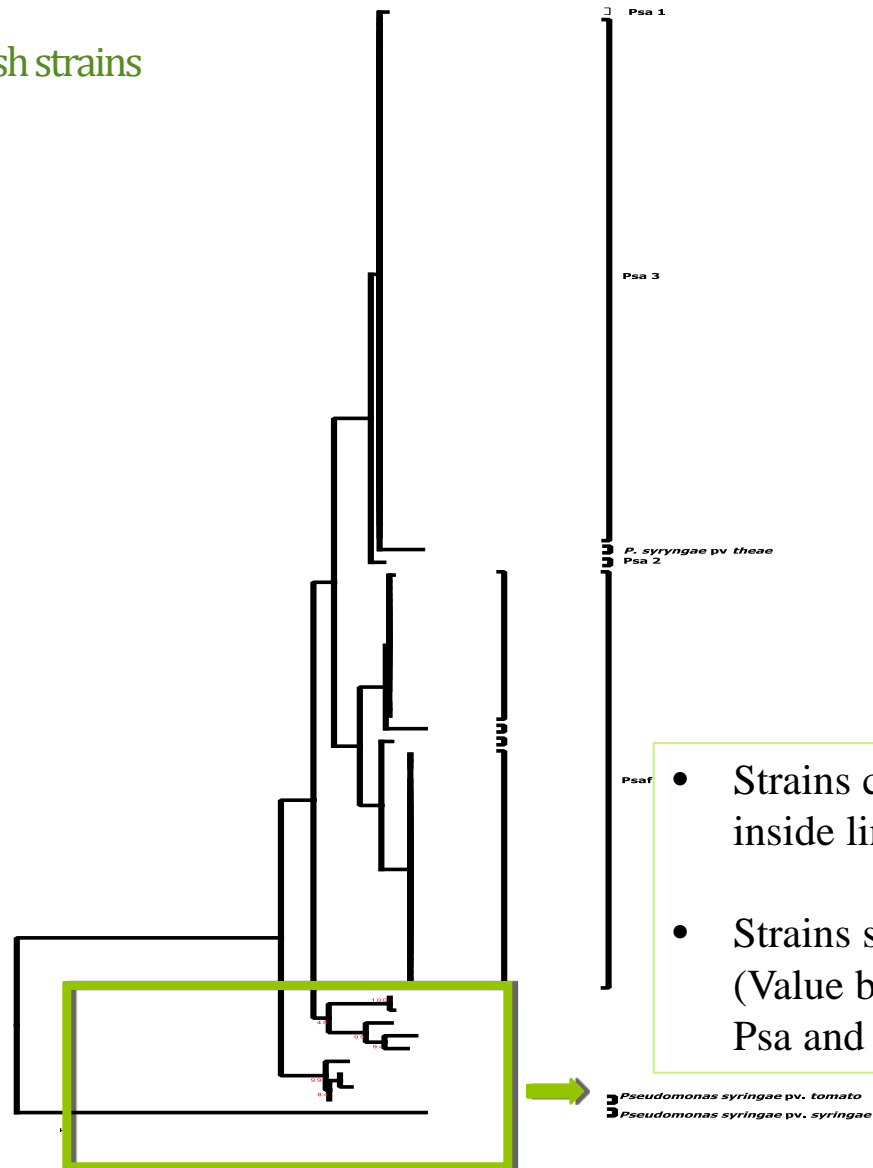


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# MLSA

## New Psaf look-alike Spanish strains

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



# Comparison Psa, Psaf and psaf look-alike

	Psaf	Psaf look-alike	Psa3
Virulence/Pathogenicity	+	+	+++
Test LOPAT	+---+	+---+ and ----+	+---+
Test INA	-	-	-
MLSA group	Psaf	≠ Psaf and Psa	Psa3
Phaseolotoxin	-	-	-
Coronatine	-	-	-
Production of canker	-	-	+
Test GATT	+ + +/- -	+ + +/- -	- - - -
API 50 CH	Typical profile Psa	Profile ≠ to Psaf and Psa	Typical profile Psa

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. *actinidiae*

*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

