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Instituto Nacional de Investigación
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Plant Health at the Age of Metagenomics, 26th
of September 2019



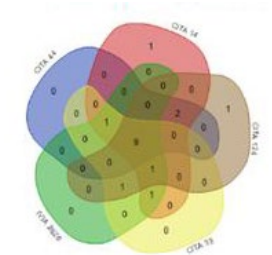
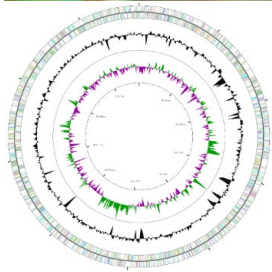
Genomics as a tool for the diagnosis of bacterial diseases: the case of *Xanthomonas arboricola* pv. *pruni*, the causal agent of bacterial spot of stone fruits

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Summary

- Brief introduction on some diseases caused by plant pathogenic bacteria:
...*Xanthomonas citri* subsp. *citri* (*Xcc*) and *Xanthomonas arboricola* pv. *pruni* (*Xap*)
- Some cases of misidentification of strains and consequences:
Xcc and *Xap*
- *Xanthomonas arboricola* pv. *pruni*: genomics
 - Discovering factors involved in virulence
 - Precise bacterial identification and disease diagnosis
 - Strategies to control the disease
- Other model where genomic analysis is mandatory:
 - *Candidatus Liberibacter*

The genus *Xanthomonas*

Plant pathogenic bacteria of high economic impact

- Diverse pathogenicity

High host specificity

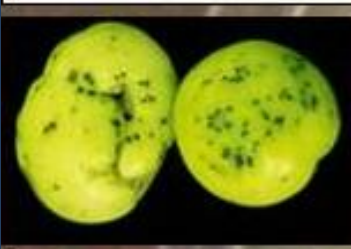
- More than 100 species/pathovars

Different infection, colonization processes and disease diversity

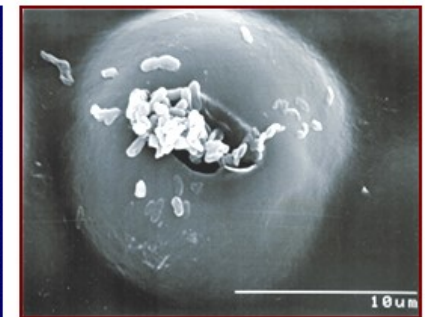
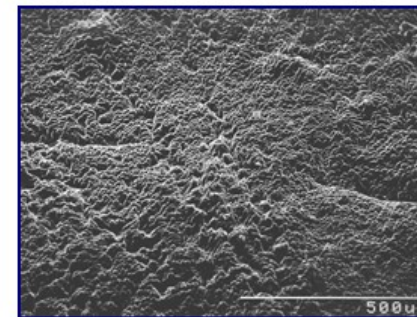
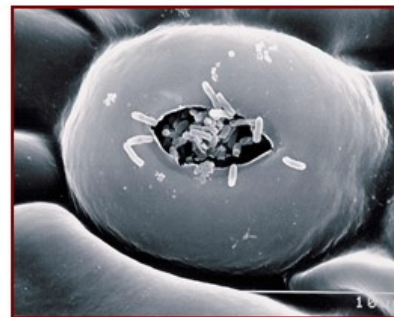
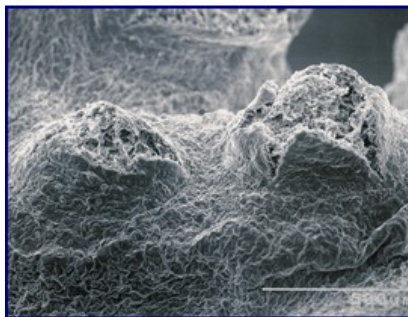
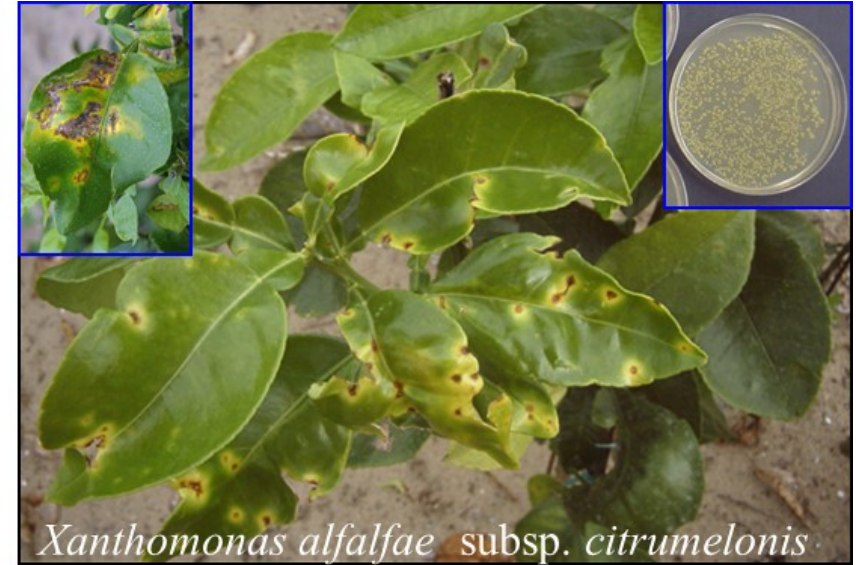
Diseases and bacterial species:

- ***Xanthomonas citri* subsp. *citri***
 - Citrus bacterial canker
- *Xanthomonas oryzae*
 - Bacterial blight
- *Xanthomonas vesicatoria*
 - Bacterial spot of tomato and pepper
- ***Xanthomonas arboricola* pv. *pruni***
 - Bacterial spot of stone fruits
- *Xanthomonas fragariae*
 - Bacterial angular leaf spot of strawberry
- *Xanthomonas campestris*
 - Black rot of cole crops rot of cole crops)

Yes, but....there are also non pathogenic xanthomonads in plants



Citrus Bacterial Spot (CBS) and Citrus Bacterial Canker (CBC)



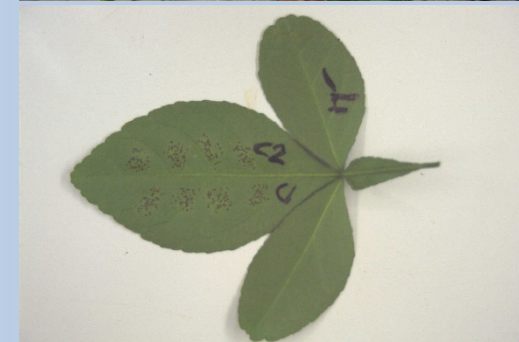
Citrus Bacterial Canker (CBC)

Citrus Bacterial Spot (CBS)

Similar to *Xap*...

Before starting with *X. arboricola* pv. *pruni* in Spain....*X. citri* in USA

- In 1984 a novel foliar disease, now known as citrus bacterial spot (CBS), caused by *Xanthomonas campestris* pv. *citrumelo* appeared first
- The disease had characteristic flat leaf lesions with necrotic centers and water-soaked margins
- Initially in 1984, the new nursery disease was thought by scientists at the Florida Department of Agriculture and Consumer Services the USDA to be a new form of citrus canker
- This diagnosis triggered the implementation of the Citrus Canker Disease Action Plan developed in 1982 by USDA-APHIS. The purpose of the plan was to ensure regulators to be prepared to eradicate citrus canker. Eradication was to be accomplished by:
 - 1) burning plants in a nursery where an infected plant was found,
 - 2) destroying all trees with canker symptoms within orchards and defoliating surrounding trees,
 - 3) using fruit from diseased or exposed trees in orchards for processing only



- Over 20 million trees in more than 100 nursery and orchard locations were destroyed at a cost of \$94 million. During this process, nurseries were subjected to lengthy quarantines. These measures were costly and inconvenient to growers, and markets for fruit were lost. There was a great deal of controversy over the quarantines and restrictions in the regulatory, industrial, and scientific arenas alike
- In September 1990, all regulations of the “Florida nursery strain of citrus canker” were removed based on scientific evidences and experience wich indicate that this CBC bacteria did not cause a disease dangerous to citrus or other plants or fruit



Graham, J.H.; Gottwald, T.R. (1991) Research perspectives on eradication of citrus bacterial diseases in Florida. *Plant Disease* **75**, 1193-1200.

All this was necessary?...



Bacterial taxonomy or bacterial identification are not just academic subjects...

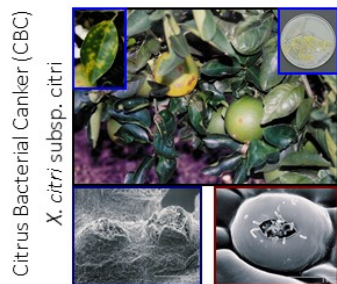
Same genus, different specie....



Felis concolor
Florida Panther



Felis catus
Domestic cat



If you know the enemy and know yourself, you need not fear the result of a hundred battles...

Sun Tzu (孫子; pinyin: Sūnzǐ) 孫武 c. 544-496 BC)



Knowledge of genome content means better knowledge of the microorganism

Genomics and Next Generation Sequencing

Precise identification of disease causal agents

Understand the genomic mechanisms that underlie diseases

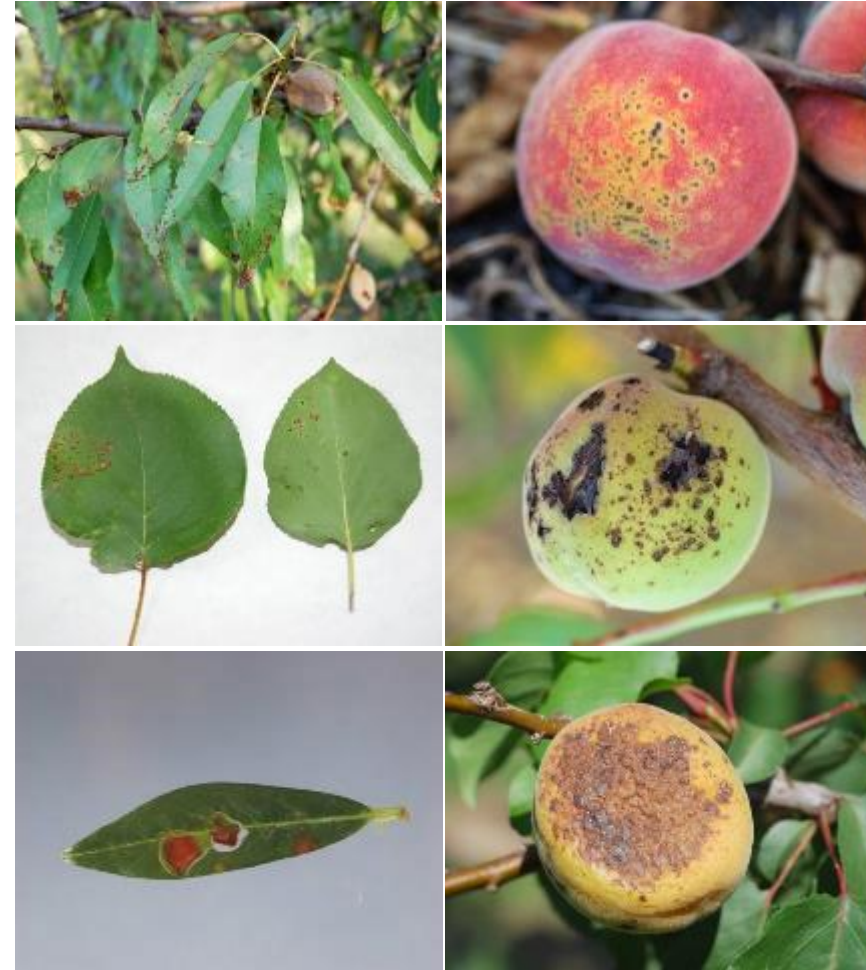
Microbiome as a factor affecting plant health

And the same thing from *X. arboricola* pv. *pruni*... genomics may clarify here too

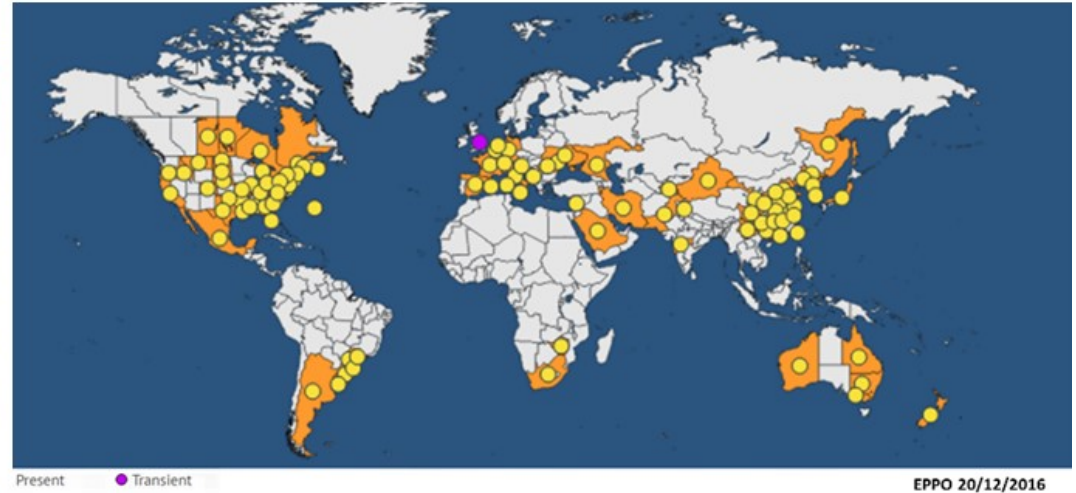
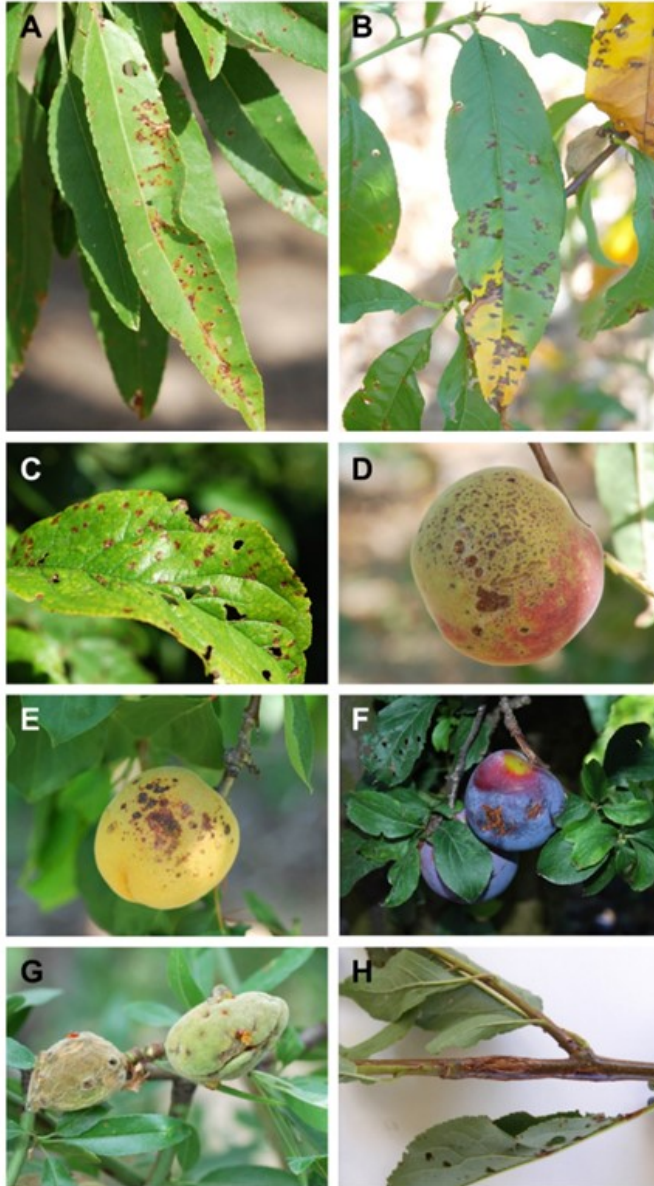
Bacterial spot of stone fruits and important disease in an important crop...

BS of stone fruits symptoms

- Bacterial disease presents in EU although quarantine
- Lesions on fruits, leaves and branches
- Symptomatic fruit not commercialized
- Premature fruit dropping
- Economic losses:
 - Quarantine disease
 - Limited commercialization
 - Control treatments



X. arboricola pv. *pruni* symptoms and worldwide situation



1, Badajoz (2002); 2, Valencia (2004); 3, Alicante (2006); 4, Zaragoza (2008); 5, Huesca (2008); 6, Lérida (2009); 7, Navarra (2009); 8, Mallorca (2010).

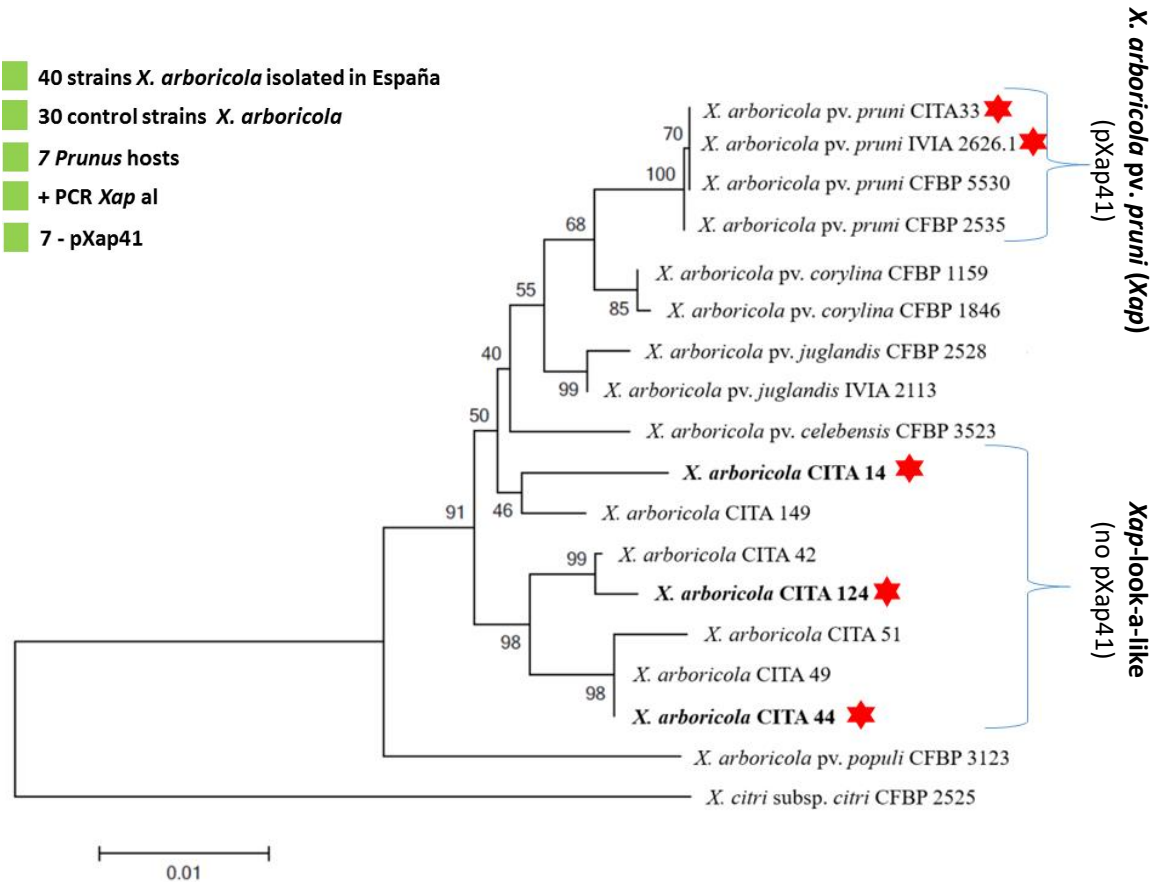
Firts identification in Spain: specificity of PCR reactions?

The beginning of the disease in Spain

Study in Spain....many *Xanthomonas* isolated from Prunus spp. were PCR+ for ABC primers (transporter gene)

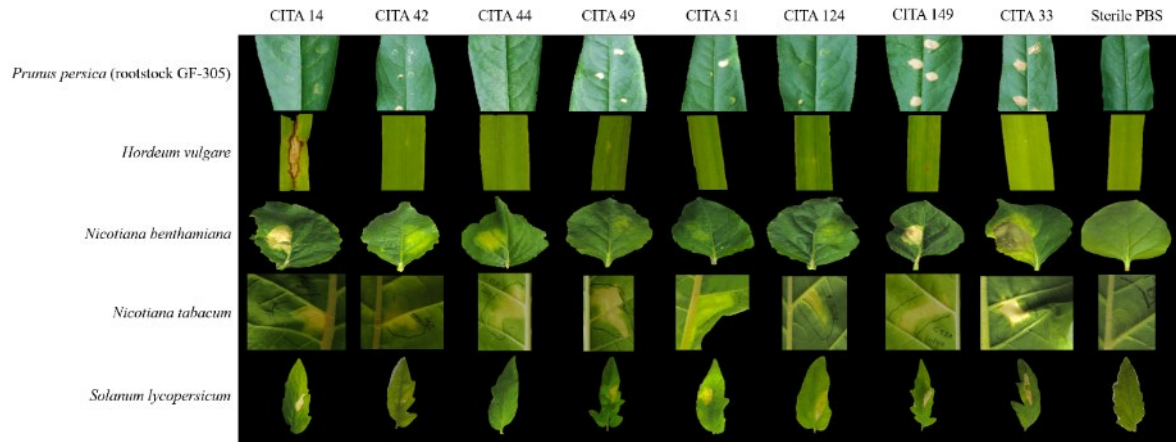
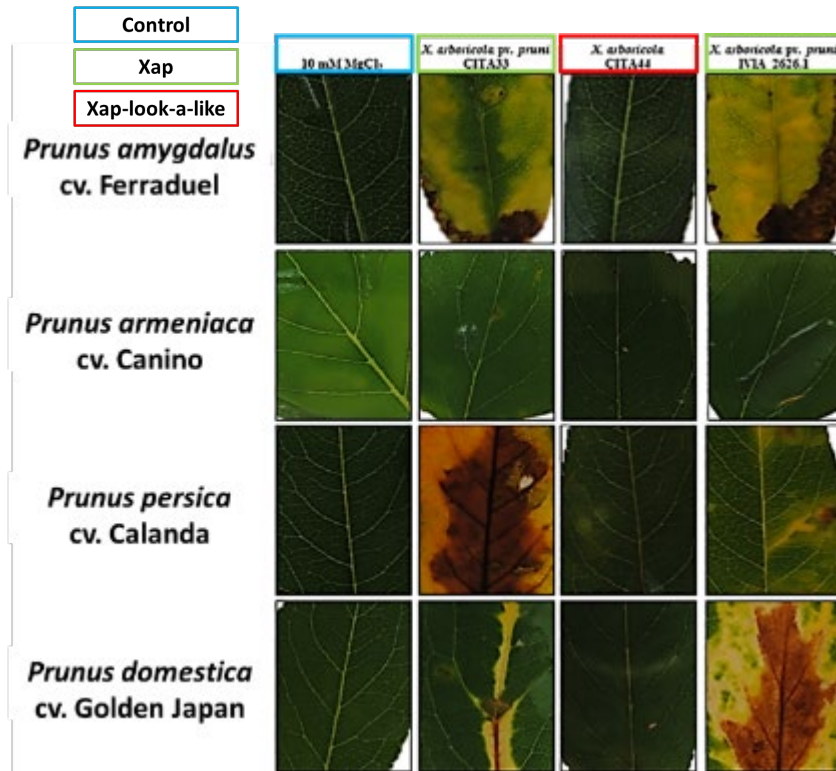
Taxa	Strain	Result ABC	Result xopE3	Blind identification
Agrobacterium tumefaciens	Agrob-6	-	-	unknown
Agrobacterium tumefaciens	Agrob-9	-	-	unknown
Agrobacterium tumefaciens	IVIA 1245-80	-	-	unknown
Agrobacterium tumefaciens	IVIA 2304-10	-	-	unknown
Pantoea	IVIA 2261-1	-	-	unknown
Pseudomonas syringae pv. syringae	Psy-13	-	-	unknown
Pseudomonas syringae pv. syringae	Psy-13	-	-	unknown
Pseudomonas syringae pv. syringae	IVIA 3514-2	-	-	unknown
X. arboricola	CITA 44	+	-	unknown
X. arboricola	CFBP 3566	-	-	unknown
X. arboricola	IVIA 4302 col 5	-	-	unknown
X. arboricola	IVIA 2835-1	-	-	unknown
X. arboricola	IVIA 4185	+	-	unknown
X. arboricola	CITA 42	+	-	unknown
X. arboricola	CITA 149	+	-	unknown
X. arboricola	IVIA 4302_eol7	-	-	unknown
X. arboricola pv. corylina	CFBP 1846	+	-	unknown
X. arboricola pv. corylina	IVIA 3978	-	-	unknown
X. arboricola pv. corylina	RIPF-X08	-	-	unknown
X. arboricola pv. fragariae	CFBP 6771	-	-	unknown
X. arboricola pv. juglandis	IVIA 2113	-	-	unknown
X. arboricola pv. juglandis	Xaj-2	-	-	unknown
X. arboricola pv. juglandis	Xaj-3	-	-	unknown
X. arboricola pv. juglandis	Xaj-4	-	-	unknown
X. arboricola pv. juglandis	Xaj-5	-	-	unknown
X. arboricola pv. populi	CFBP 3123	-	-	unknown
X. arboricola pv. pruni	CITA 9	+	+	xap
X. arboricola pv. pruni	CITA 11	+	+	xap
X. arboricola pv. pruni	CITA 46	+	+	xap
X. arboricola pv. pruni	CITA 70	+	+	xap
X. arboricola pv. pruni	100343	+	+	xap
X. arboricola pv. pruni	100400	+	+	xap
X. arboricola pv. pruni	CITA 154	+	+	xap
X. arboricola pv. pruni	IVIA 4490.1	+	+	xap
X. arboricola pv. pruni	IVIA 4491.1	+	+	xap
X. arboricola pv. pruni	IVIA 4493	+	+	xap
X. arboricola pv. pruni	IVIA 4492.2	+	+	xap
X. arboricola pv. pruni	IVIA 4494.1	+	+	xap
X. arboricola pv. pruni	IVIA 2626-3	+	+	xap
X. arboricola pv. pruni	IVIA 2626-6	+	+	xap
X. arboricola pv. pruni	IVIA 2626-7	+	+	xap
X. arboricola pv. pruni	IVIA 2647-1.3	+	+	xap
X. arboricola pv. pruni	IVIA 2647-13	+	+	xap
X. arboricola pv. pruni	IVIA 2647-3.2	+	+	xap
X. arboricola pv. pruni	IVIA 2649-3	+	+	xap
X. arboricola pv. pruni	IVIA 2649-7	+	+	xap
X. arboricola pv. pruni	IVIA 2649-10	+	+	xap
X. arboricola pv. pruni	IVIA 2758-2	+	+	xap
X. arboricola pv. pruni	IVIA 2758-3	+	+	xap
X. arboricola pv. pruni	IVIA 2826.3	+	+	xap
X. arboricola pv. pruni	IVIA 2826-4	+	+	xap
X. arboricola pv. pruni	IVIA 2826-5	+	+	xap
X. arboricola pv. pruni	IVIA 2826-6	+	+	xap
X. arboricola pv. pruni	IVIA 2826-9	+	+	xap
X. arboricola pv. pruni	IVIA 2826-10	+	+	xap
X. arboricola pv. pruni	IVIA 2826-11	+	+	xap
X. arboricola pv. pruni	IVIA 2832-5	+	+	xap
X. arboricola pv. pruni	IVIA 2832-17	+	+	xap
X. arboricola pv. pruni	IVIA 2832-19	+	+	xap
X. arboricola pv. pruni	IVIA 2832-21	+	+	xap
X. arboricola pv. pruni	IVIA 2832-24	+	+	xap
X. arboricola pv. pruni	IVIA 2832-26	+	+	xap
X. arboricola pv. pruni	IVIA 2832-30	+	+	xap
X. arboricola pv. pruni	IVIA 3177-1-6	+	+	xap
X. arboricola pv. pruni	IVIA 3177-3-4	+	+	xap
X. arboricola pv. pruni	IVIA 3177-3-8	+	+	xap
X. arboricola pv. pruni	IVIA 3181-3-1	+	+	xap
X. arboricola pv. pruni	IVIA 3181-3-3	+	+	xap
X. arboricola pv. pruni	CITA 33	+	+	xap
X. arboricola pv. pruni	CITA 99	+	+	xap
X. arboricola pv. pruni	CFBP 3894	+	+	xap
X. arboricola pv. pruni	CFBP 5530	+	+	xap
X. arboricola pv. pruni	CFBP 5724	+	+	xap
X. axonopodis	Xp-2	-	-	unknown
X. axonopodis pv. phaseoli	NCPPB 381	-	-	unknown
X. axonopodis pv. phaseoli	IVIA 1518	-	-	unknown
X. campestris	Xca-1	-	-	unknown
X. campestris	Xca-2	-	-	unknown
X. campestris	IVIA 2734-1	-	-	unknown
X. campestris	IVIA 1609	-	-	unknown
X. citri subsp. citri	IVIA 2889-1	-	-	unknown
X. citri subsp. citri	IVIA 3026-1	-	-	unknown
X. citri subsp. citri	str. 306	-	-	unknown
X. hortorum	IVIA 1575.1	-	-	unknown
X. vesicatoria	IVIA 3619-1	-	-	unknown
Curatobacterium sp.	EP-2.2	-	-	unknown
Pantoea sp.	EP-14.1	-	-	unknown
Microbacterium sp.	EP-16.1	-	-	unknown
Sphingomonas sp.	EP-16.2	-	-	unknown
Pseudoaerobacter sp.	EP-16.4	-	-	unknown
Terrabacter sp.	EP-16.6	-	-	unknown
Pseudomonas sp.	EP-17.1	-	-	unknown
Curatobacterium sp.	EP-18.1	-	-	unknown
Rhodococcus sp.	21/14-7.B3	-	-	unknown
Pseudomonas sp.	21/14-12.B8	-	-	unknown

- 40 strains *X. arboricola* isolated in España
- 30 control strains *X. arboricola*
- 7 Prunus hosts
- + PCR *Xap a1*
- 7 - pXap41



A few strains were misidentified...

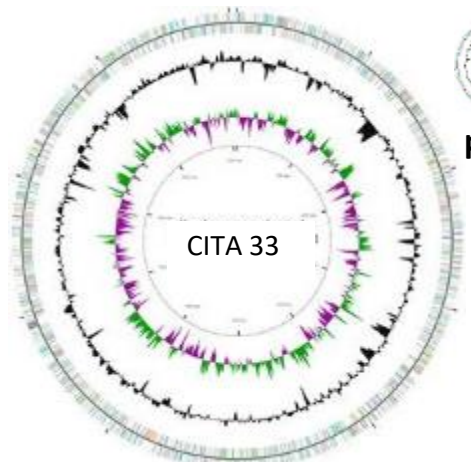
Firts identification in Spain: all strain were really *Xap*?



- Main differences:**
- Pathogenicity/virulence
 - Motility
 - Chemotaxis
 - Carbon source compounds

Genomes of *X. arboricola* strains isolated from *Prunus* species in Spain

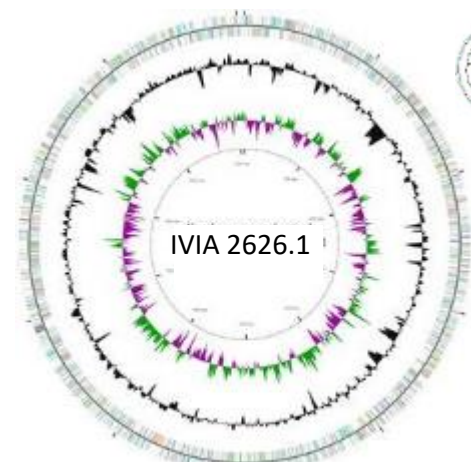
Plant Health at the Age of Metagenomics, Paris 2019



pXap41

X. arboricola pv. *pruni*

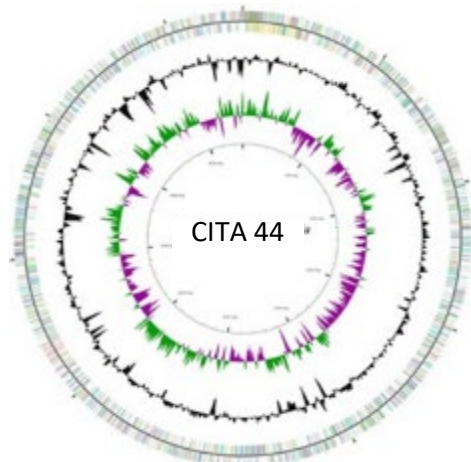
Garita-Cambronero et al. 2014 Genome Announcements



pXap41

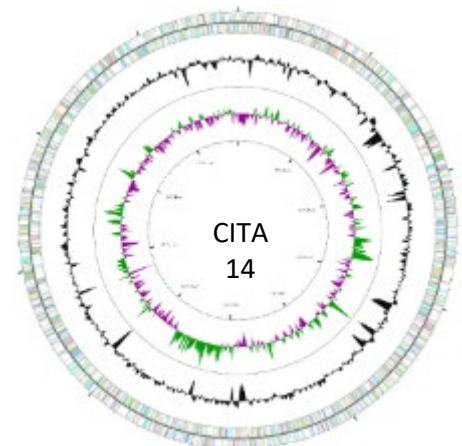
X. arboricola pv. *pruni*

Garita-Cambronero et al. 2016 Standards in Genomic Sciences



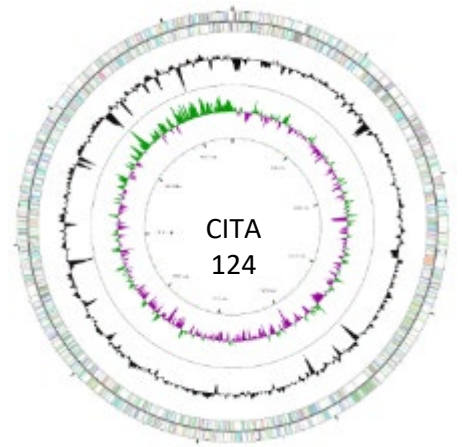
X. arboricola

Garita-Cambronero et al. 2016 Standards in Genomic Sciences



X. arboricola

Garita-Cambronero et al. 2016 Genome Announcements



X. arboricola

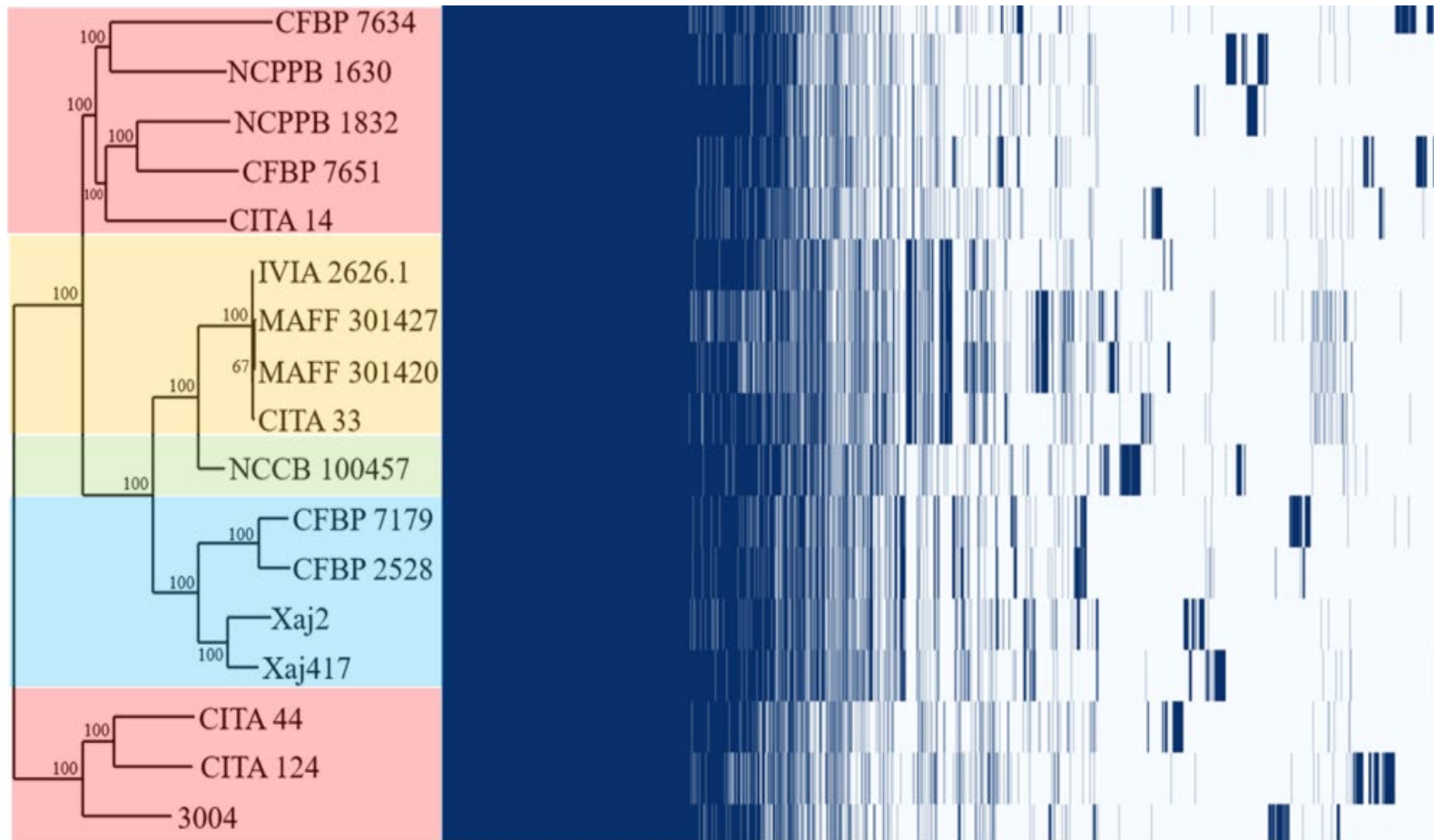
Garita-Cambronero et al. 2016 Genome Announcements

Clusters of orthologous groups (COG)

- A:00G
- B:00G
- J:00G
- K:00G
- L:00G
- M:00G
- O:00G
- P:00G
- Q:00G
- R:00G
- S:00G
- T:00G
- U:00G
- V:00G
- W:00G
- X:00G
- Y:00G
- Z:00G
- C:00G
- G:00G
- F:00G
- H:00G
- I:00G
- D:00G
- E:00G
- S:00G
- Unk/unknown:00G
- GC content
- GC skew
- GC skew

Xa strains different from Xap...plasmid content difference

X. arboricola isolated from *Prunus* species within *X. arboricola* species



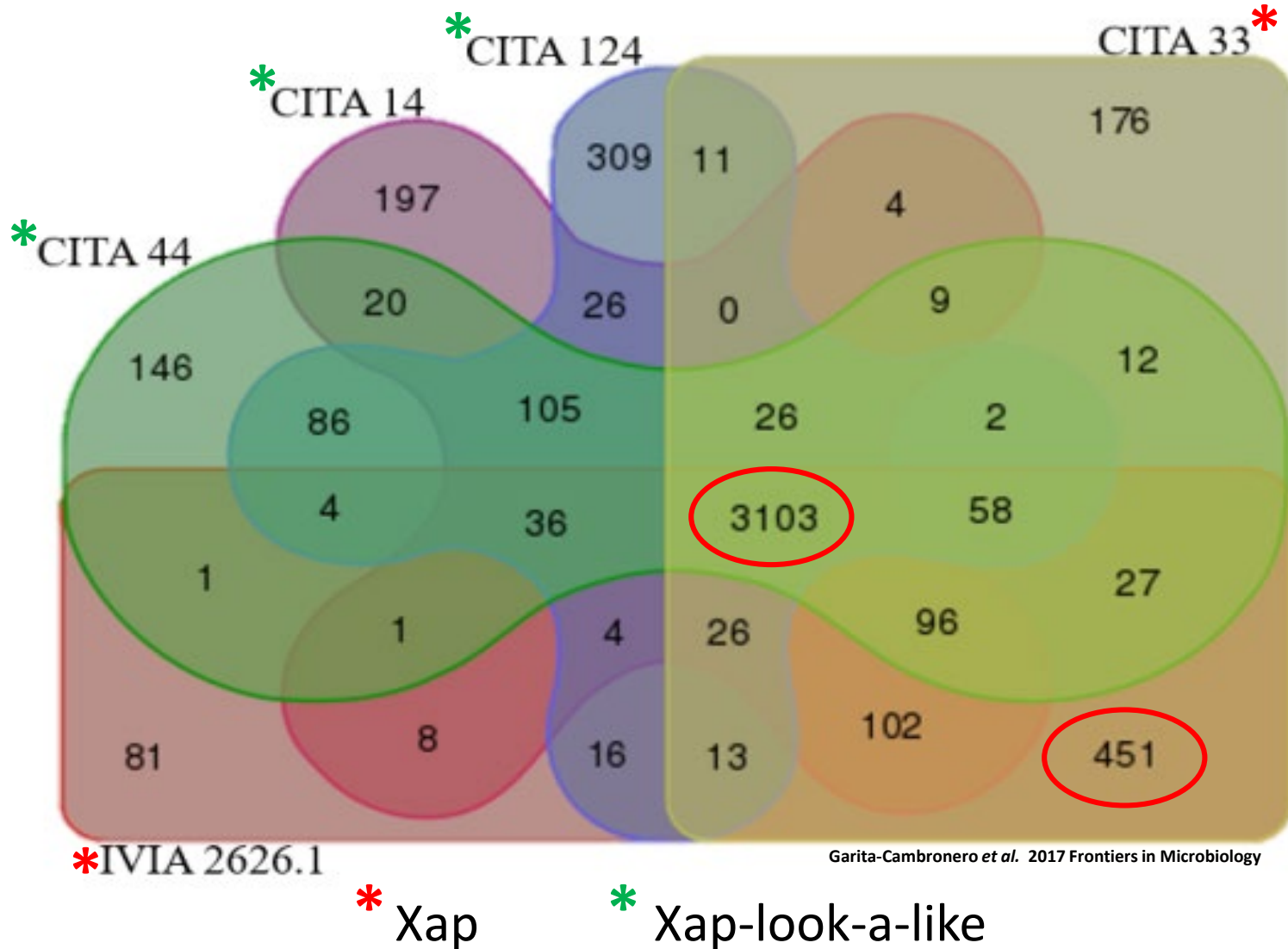
Garita-Cambronero *et al.* 2017 *Frontiers in Microbiology*

- *X. arboricola* + *X. arboricola* pv. *celebensis*
- *X. arboricola* pv. *juglandis*
- *X. arboricola* pv. *pruni*
- *X. arboricola* pv. *corylina*

Phylogenetic analysis of 17 strains of *Xanthomonas arboricola* based on the core genome sequence (2,714 potential groups of orthologous genes) and representation of the distribution of the potential orthologous cluster genes of the pangenome (7,074) within the analysed genome sequences.

***Xa* strains different from *Xap*...core genome content difference**

Characteristics of some *X. arboricola* isolated from *Prunus* species



Venn diagram showing the groups of orthologous genes shared by five genome sequences of pathogenic (CITA 33 and IVIA 2626.1) and non-pathogenic (CITA 14, CITA 44 and CITA 124) strains of *X. arboricola* isolated from *Prunus* spp.

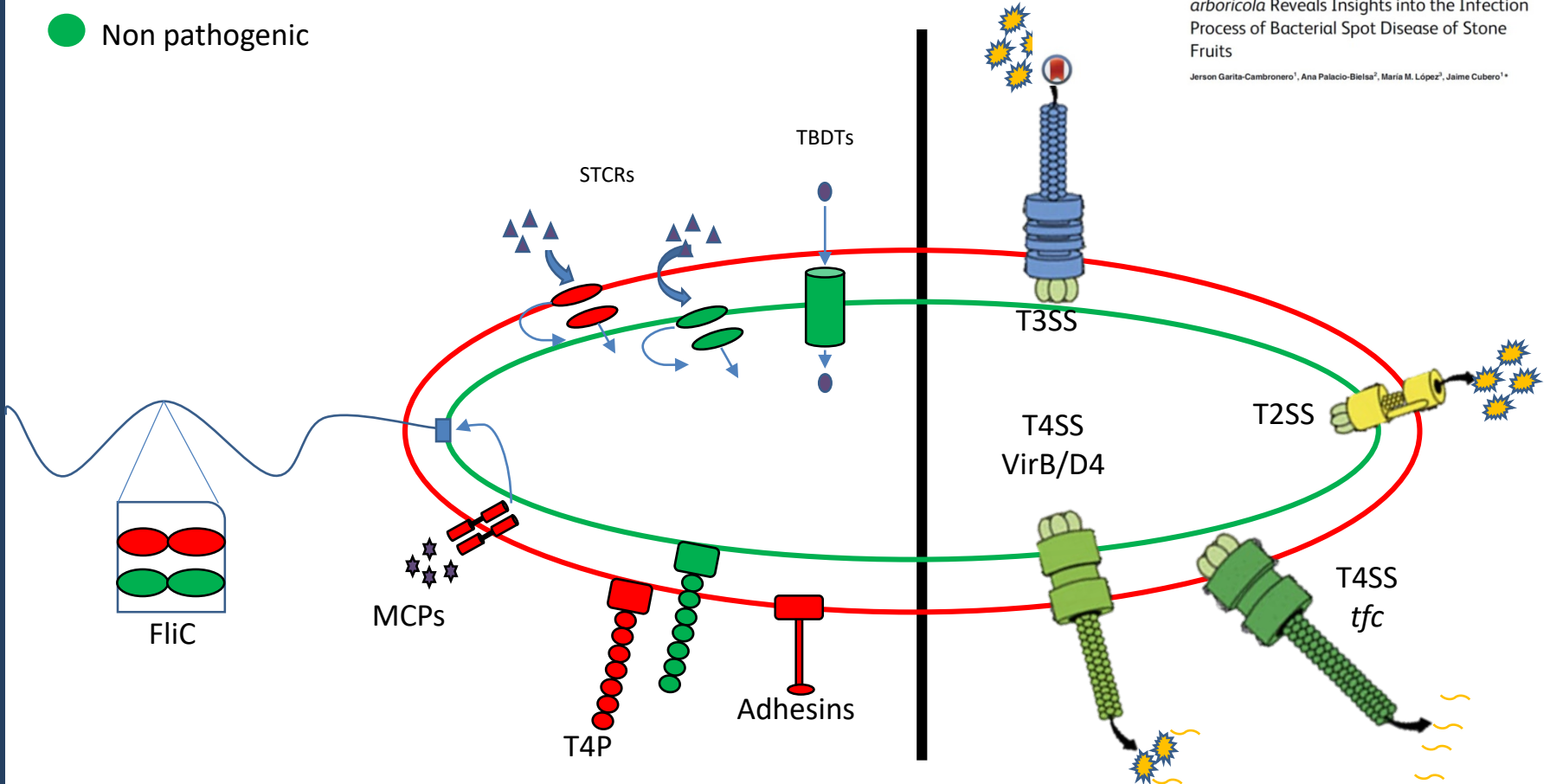
Xa strains different from Xap...gene content difference

X. arboricola pv. *pruni* vs. *X. arboricola* pv. *pruni* look-a-like: infection factors

RESEARCH ARTICLE
 Comparative Genomic and Phenotypic Characterization of Pathogenic and Non-Pathogenic Strains of *Xanthomonas arboricola* Reveals Insights into the Infection Process of Bacterial Spot Disease of Stone Fruits

Jerson Garita-Cambronero¹, Ana Palacio-Bielsa², María M. López², Jaime Cubero^{1*}

- Pathogenic
- Non pathogenic



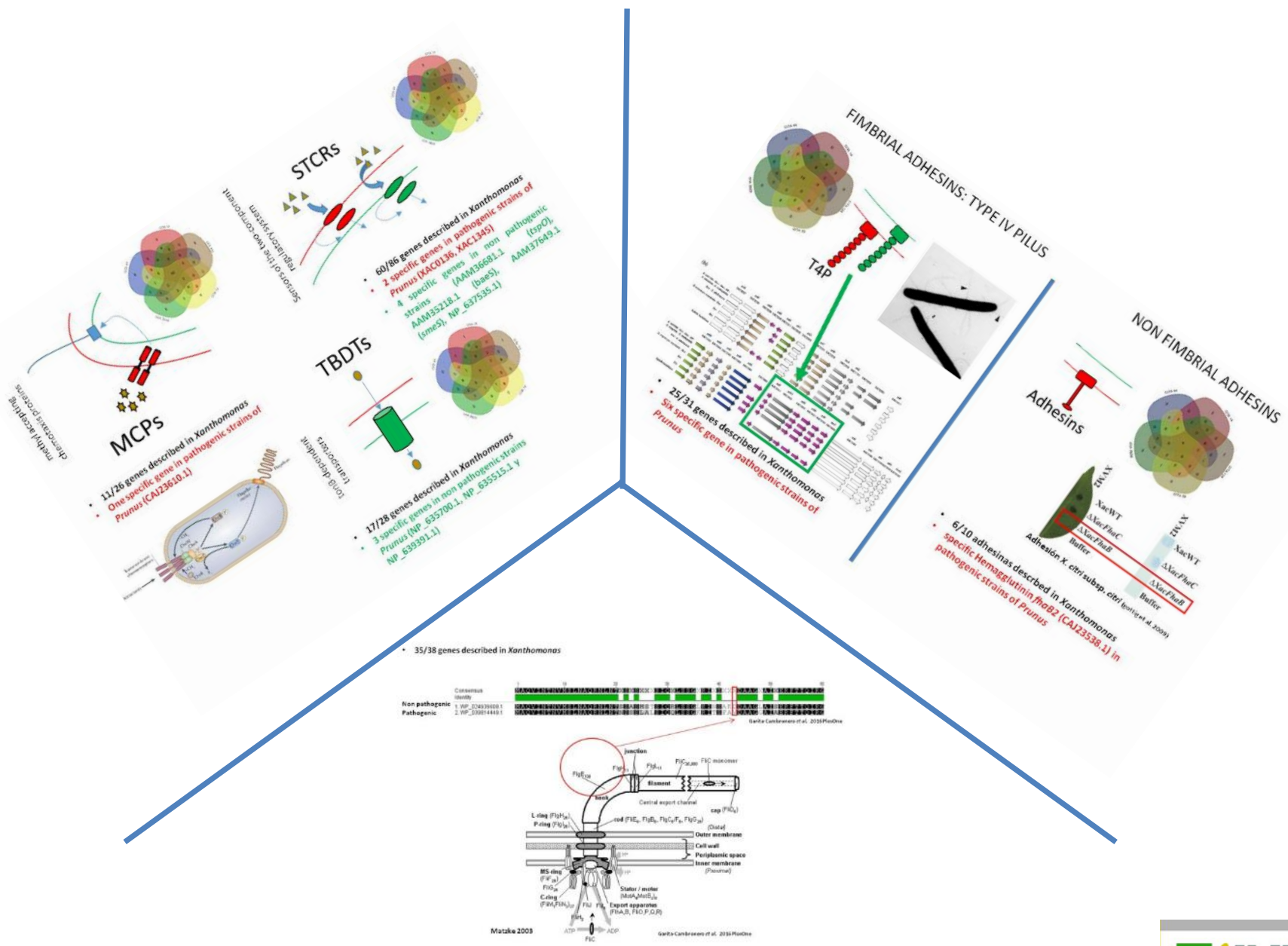
Initial steps

Advanced steps

Xa strains different from *Xap*...virulence gene content difference

X. arboricola pv. pruni vs. X. arboricola pv. pruni look-a-like: environmental sensors

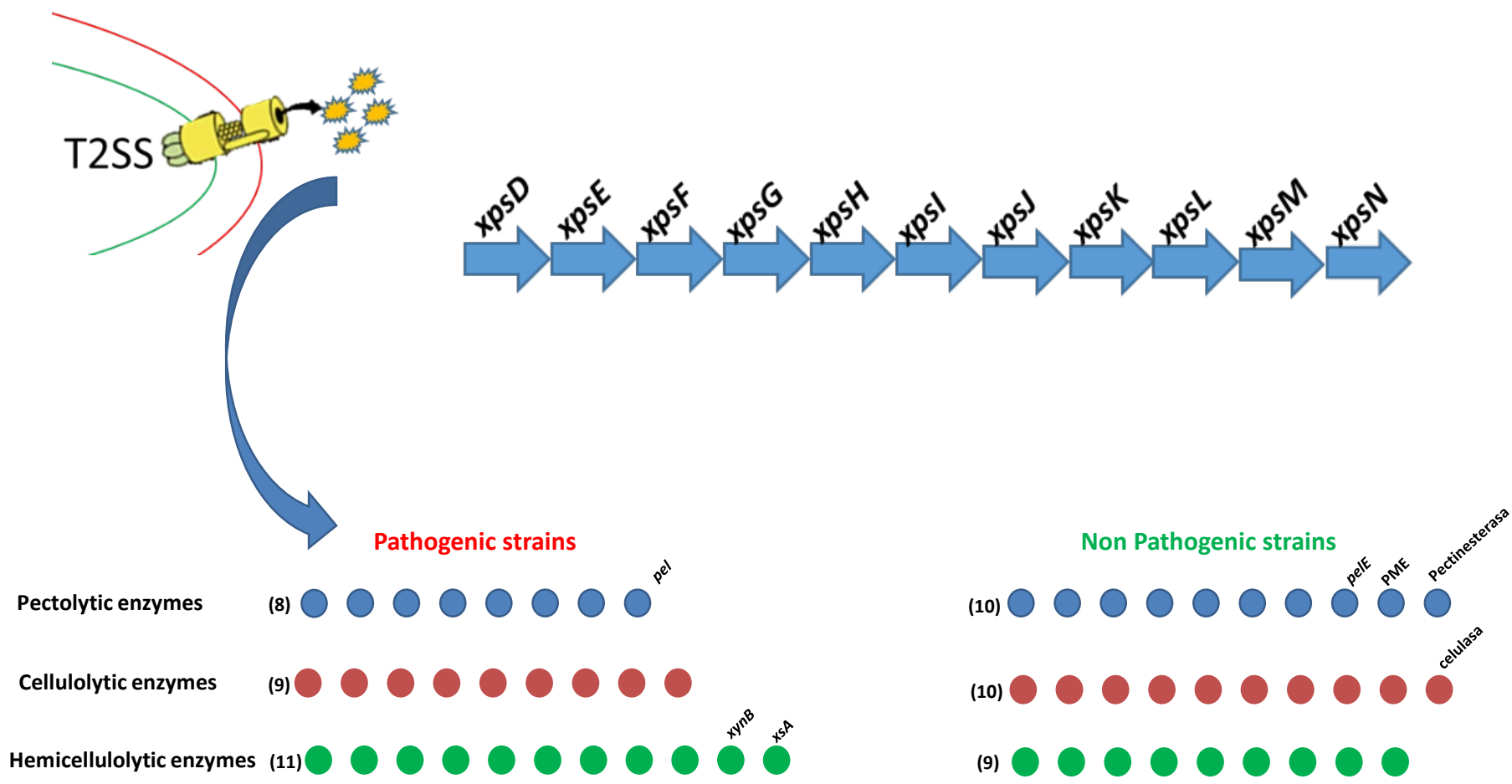
Plant Health at the Age of Metagenomics, Paris 2019



Aspartic acid by Valine in pathogenic strains in aa 43

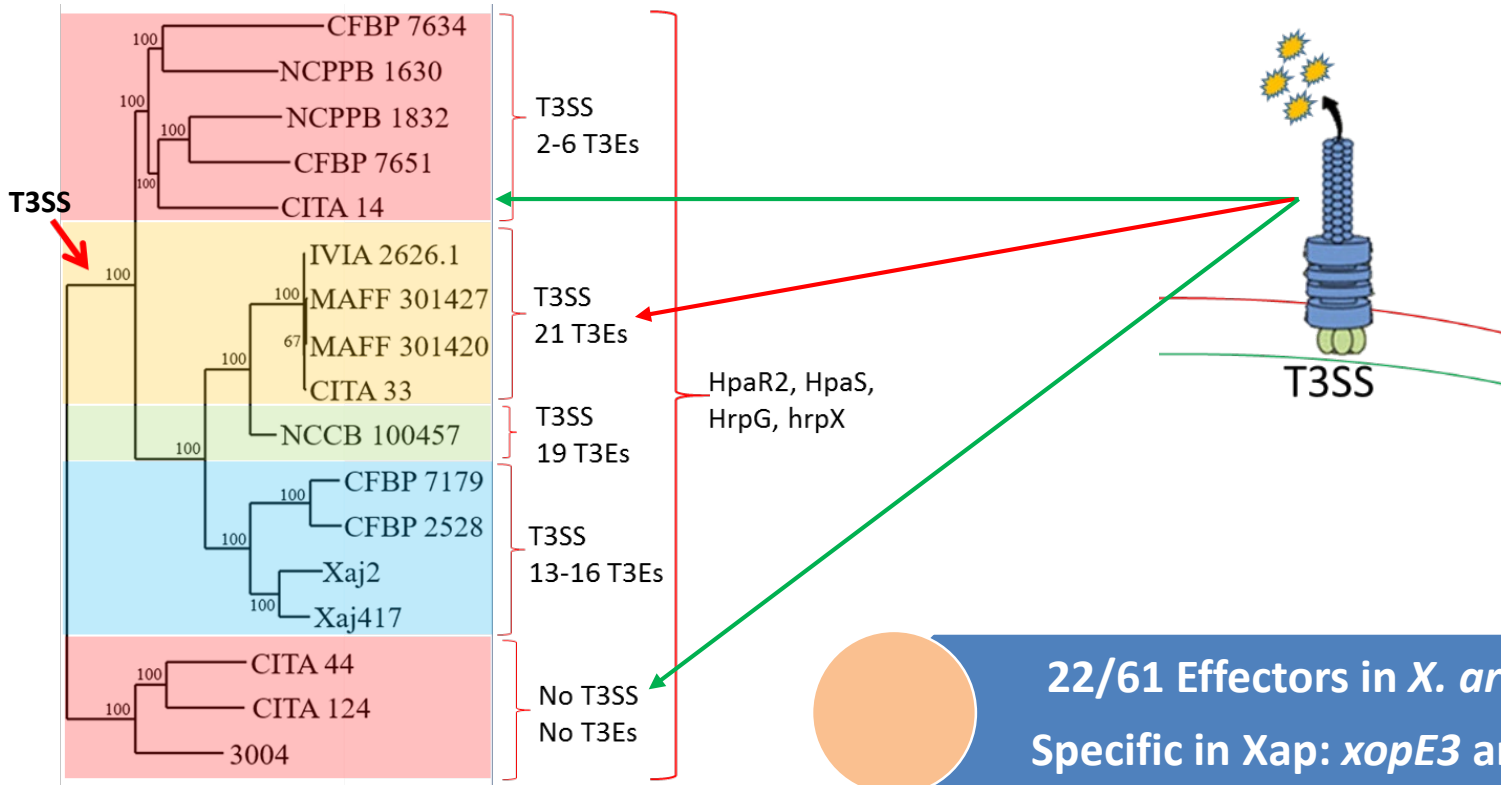
Xa strains different from Xap...sensor gene content difference

X. arboricola pv. *pruni* vs. *X. arboricola* pv. *pruni* look-a-like: T2SS



Xa strains different from *Xap*...cellulolytic gene content difference

X. arboricola pv. *pruni* vs. *X. arboricola* pv. *pruni* look-a-like: T3SS



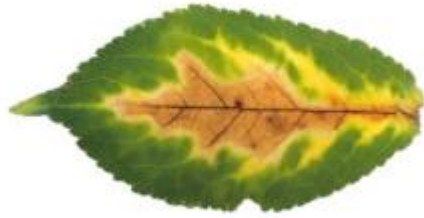
- *X. arboricola* + *X. arboricola* pv. *celebensis*
- *X. arboricola* pv. *pruni*
- *X. arboricola* pv. *juglandis*
- *X. arboricola* pv. *corylina*

Garita-Cambronero et al. 2017 Frontier in Microbiology

22/61 Effectors in *X. arboricola*.
Specific in Xap: *xopE3* and *xopAQ*

xopB, *xopE2*, *xopL*, *xopN*, *xopV*, *xopX*, *xopZ* => interfere PAMP-triggered immunity (PTI) mediated by Flg22

Xa strains different from Xap...T3SS and T3E content difference



CITA 33 and IVIA 2626.1



1 MCP (CAJ23610.1) y 2 STCRs (XAC1036, XAC1345)

FliC polymorfism

6 genes related to minor pillins

2 no fimbrilar adhesins (*fhaB2*, XAC0444)

1 pectolityc enzyme (*pel*)

2 hemycellulolityc ezymes (*xynB* & *xsA*)

1 T3SS

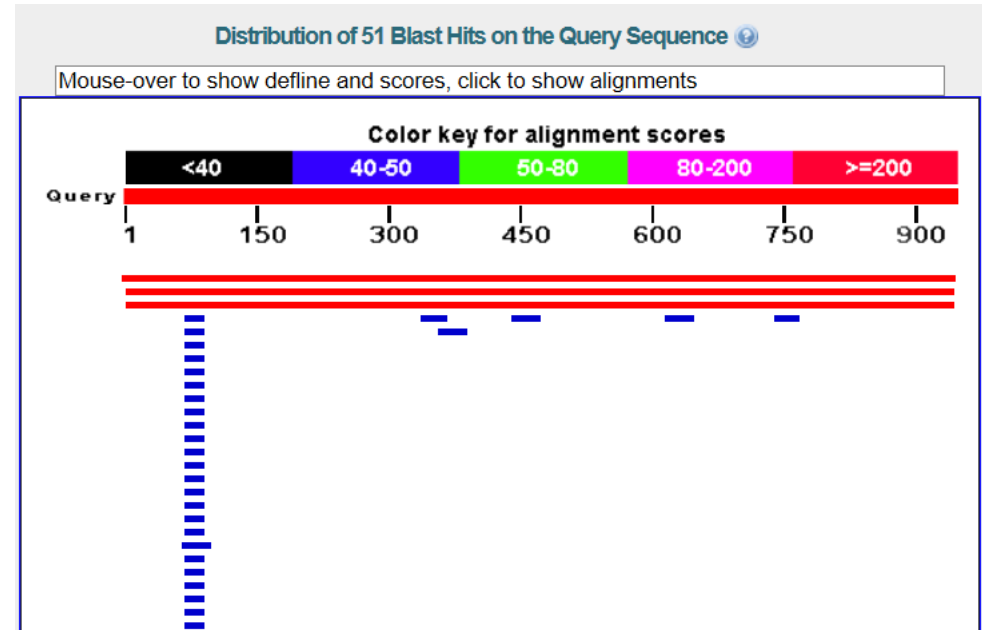
21 T3Es

Plasmid pXap41

Coming back to misidentification of Xap...

ABC transporter (*ftsx*): used in PCR conventional and Real Time (Pagani, 2004, Palacio-Bielsa et al. 2011)

ABC transporter in other *Xanthomonas* sp.!!!



Sequences producing significant alignments:

Select: [All](#) [None](#) Selected:0

[Alignments](#) [Download](#) [GenBank](#) [Graphics](#) [Distance tree of results](#)

	Description	Max score	Total score	Query cover	E value	Ident
<input type="checkbox"/>	Xanthomonas arboricola pv. pruni strain CFBP 5530 ABC transporter ATP-binding protein (ftsX) pseudogene, partial sequence >gb HQ8964	1700	1700	100%	0.0	100%
<input type="checkbox"/>	Xanthomonas albilineans GPE PC73 complete genome	753	753	99%	0.0	78%
<input type="checkbox"/>	Xanthomonas oryzae pv. oryzicola BLS256, complete genome	697	697	99%	0.0	77%

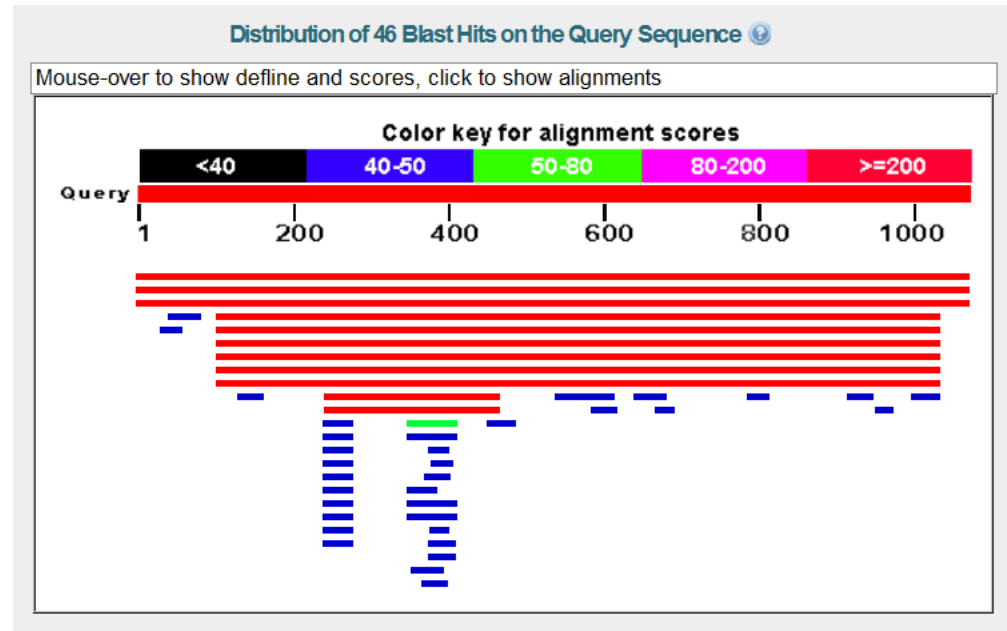
ABC transporters are a large superfamily of proteins found in all living organisms including bacteria, archaea, and eukarya. They are highly conserved across species...

Specificity of PCR reactions

XopE3: TS3 efector specific *X. arboricola* pv. *pruni* (Hajri et al. 2011)

Efector present in other *Xanthomonas* sp.!!!

Involved in virulence...



Sequences producing significant alignments:

Select: [All](#) [None](#) Selected: 0

	Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/>	Xanthomonas arboricola pv. <i>pruni</i> str. CFBP 5530 plasmid pXap41	1932	1932	100%	0.0	100%	FR875157.1
<input type="checkbox"/>	Xanthomonas citri subsp. <i>citri</i> Aw12879, complete genome	1882	1882	100%	0.0	99%	CP003778.1
<input type="checkbox"/>	Xanthomonas axonopodis pv. <i>citri</i> str. 306, complete genome	1882	1882	100%	0.0	99%	AE008923.1
<input type="checkbox"/>	Xanthomonas arboricola pv. <i>pruni</i> strain CFBP5724 type III effector protein (<i>xopE3</i>) gene, partial cds	1678	1678	86%	0.0	100%	JF826916.1
<input type="checkbox"/>	Xanthomonas arboricola pv. <i>pruni</i> strain CFBP7100 type III effector protein (<i>xopE3</i>) gene, partial cds	1678	1678	86%	0.0	100%	JF826915.1
<input type="checkbox"/>	Xanthomonas arboricola pv. <i>pruni</i> strain CFBP5722 type III effector protein (<i>xopE3</i>) gene, partial cds	1678	1678	86%	0.0	100%	JF826914.1
<input type="checkbox"/>	Xanthomonas arboricola pv. <i>pruni</i> strain CFBP5575 type III effector protein (<i>xopE3</i>) gene, partial cds	1678	1678	86%	0.0	100%	JF826913.1
<input type="checkbox"/>	Xanthomonas arboricola pv. <i>pruni</i> strain CFBP3894 type III effector protein (<i>xopE3</i>) gene, partial cds	1678	1678	86%	0.0	100%	JF826912.1
<input type="checkbox"/>	Xanthomonas arboricola pv. <i>pruni</i> strain CFBP5530 type III effector protein (<i>xopE3</i>) gene, partial cds	1678	1678	86%	0.0	100%	JF826911.1
<input type="checkbox"/>	Xanthomonas campestris pv. <i>campestris</i> strain 528t <i>avrXacE2</i> gene, partial cds	354	354	21%	8e-94	95%	FJ815242.1
<input type="checkbox"/>	Xanthomonas campestris pv. <i>campestris</i> strain B-30 <i>avrXacE2</i> gene, partial cds	338	338	21%	6e-89	93%	FJ815241.1

Specificity of PCR reactions

X. arboricola from *Prunus* spp

MLSA analysis

Gene analysis

Xap

Xap-look-a-like



Initial steps of infection

Late steps of infection

NGS

Phylogenetic analysis
(ML-“core-genome”)

Similarity analysis
(Pangenome)

Sensors

Adhesion

Chemotaxis

Xanthano

Quorum-sensing

T2SS

T3SS

T4SS

PCR protocol development

X. arboricola pv. Pruni (Xap) vs. X. arboricola pv. pruni look-a-like

TABLE 2 | Components of the type three secretion system, repertoire of the type three effectors and other secreted proteins, presence of the plasmid pXap41 and pathogenicity of X. arboricola strains isolated from Prunus spp.

	Gene/Strains	CITA 14	CITA 42	CITA 44	CITA 49	CITA 51	CITA 124	CITA 149	CFBP 5530 ^P	CITA 33 ^P
Components of the type III secretion system	<i>hrcC</i>	■							■	■
	<i>hrcJ</i>	■							■	■
	<i>hrcN</i>	■						■	■	■
	<i>hrcR</i>	■						■	■	■
	<i>hrcS</i>	■						■	■	■
	<i>hrcT</i>	■						■	■	■
	<i>hrcU</i>	■						■	■	■
	<i>hrcV</i>	■						■	■	■
	<i>hrpB1</i>	■						■	■	■
	<i>hrpD5</i>	■						■	■	■
	<i>hrpF</i>	■						■	■	■
	Type III effectors and other type III secreted proteins	<i>avrBs2</i>	■							■
<i>avrXccA2</i>		■							■	■
<i>hpaA</i>		■						■	■	■
<i>hrpW</i>		■						■	■	■
<i>xopA</i>		■						■	■	■
<i>xopAF</i>		■							■	■
<i>xopAH</i>		■							■	■
<i>xopAI</i>		■							■	■
<i>XopAQ</i>		■							■	■
<i>xopE2</i>		■							■	■
<i>xopE3</i>		■							■	■
<i>xopF1</i>		■							■	■
<i>xopG</i>		■							■	■
<i>xopK</i>		■							■	■
<i>xopL</i>		■							■	■
<i>xopN</i>		■							■	■
<i>xopQ</i>		■							■	■
<i>xopR</i>		■							■	■
<i>xopV</i>	■							■	■	
<i>xopX</i>	■							■	■	
<i>xopZ</i>	■							■	■	
pXap41	<i>repA1</i>	■							■	■
	<i>repA2</i>	■							■	■
	<i>mobC</i>	■							■	■
Pathogenicity	<i>Hordeum vulgare</i>	N, C	NS	NS	NS	NS	NS	NS	ND	NS
	<i>Nicotiana benthamiana</i>	N, C	C	C	MC	C	MC	N, C	ND	N, C
	<i>N. tabacum</i>	N	C	C	C	C	C	C	ND	N
	<i>Solanum lycopersicum</i>	N	MN, C	NS, MC	N, C	N, C	N, C	N, C	ND	N, C
	<i>Prunus persica</i> (GF-305)	MN	N	NS	N	N	MN	N	ND	N, C
	CFU/ml 21 dpi	0–10 ⁵	10 ¹ –10 ⁵	10 ² –10 ⁴	10 ² –10 ⁴	10 ³ –10 ⁵	10 ³ –10 ⁵	10 ³ –10 ⁵	ND	10 ⁶ –10 ¹⁰
	Real-time PCR*	+	+	+	+	+	+	+	ND	+

Positive/negative PCR amplification are represented in gray or white, respectively. P, Prunus pathogenic strains of X. arboricola pv. pruni (Xap), the remaining tested strains were considered as atypical Xap-look-a-like strains; N, necrosis; C, chlorosis; NS, not visible symptoms; MC, mild chlorosis; MN, mild necrosis; ND, no data.

*According to the protocol described by Palacio-Bielsa et al. (2011, 2015).

CITA, Centro de Investigación y Tecnología Agroalimentaria de Aragón, Zaragoza, Spain; CFBP, Collection Française de Bactéries Phytopathogènes, Angers, France.

Specific factors involved in virulence in Xap

X. arboricola pv. *pruni* (Xap) vs. *X. arboricola* pv. *pruni* look-a-like

group_1372, group_4503, group_4329, group_3971, group_471, relE2_1, group_4064, group_2647, group_4486, group_4371, group_3988, hin_1, prkC, hopW1-1, group_4346, group_3474, group_3958, group_4259, pel_2, group_4324, group_4297, group_4350, viaA_2, group_4532, group_3984, group_3966, group_4005, wapA_1, group_4207, group_1003, mltB_1, group_4385, virB6_1, group_4290, group_1710, group_4301, group_4344, group_4009, group_4298, group_4528, group_3946, group_2204, group_3975, gnl_3, group_4107, group_3967, group_1335, group_4361, group_3952, group_1708, group_4098, group_187, group_506, group_4233, group_4507, virB6_2, group_3981, basR, group_4498, group_2644, group_4212, mobA_5, vraR_2, group_4119, group_4420, group_1835, group_4304, group_4248, yejK, group_3970, group_4054, group_4294, group_4441, group_3962, group_2965, group_4186, wapA_2, group_6572, group_4210, group_4489, group_3959, group_1109, menB, group_4097, group_3945, group_3976, group_4076, group_2648, group_390, group_4012, group_4208, group_4370, group_4164, group_3965, group_2290, apxB_1, group_4384, group_3990, group_4302, group_4515, group_4236, group_4502, group_1844, group_1289, group_4343, group_4357, group_3985, group_4330, group_3989, group_4325, group_562, group_1893, group_4496, group_4120, group_4214, group_4362, group_4326, group_3949, group_4506, group_3995, group_3961, group_2427, group_3982, group_4499, group_4353, group_1793, group_4529, group_4008, icd_1, group_4106, group_4387, group_4443, group_4421, group_4439, group_4526, group_4293, **group_3954**, group_4247, group_4310, group_4279, group_4418, group_4055, group_2183, traD, group_274, group_4211, group_1469, group_3311, group_2645, group_4001, group_4358, group_3986, group_4530, group_3964, group_4383, group_3991, group_4209, group_4243, group_4292, group_3978, group_4056, group_3951, group_3263, group_3996, group_3977, virB5, group_6482, nicB_1, group_4235, cat, group_4206, ydiO, group_4355, group_4188, group_4373, group_3983, group_604, group_3972, group_4146, paiB_2, group_2795, group_2789, ompR_1, group_4075, group_4354, group_3955, group_4438, group_4007, group_3960, group_4327, group_4351, group_395, pcrA, group_1327, group_4442, group_4296, group_4261, group_151, group_4493, group_4105, group_2426, group_2988, group_4077, group_4112, group_2236, parA, group_3969, group_4213, group_4314, group_4066, group_4169, group_4488, group_4527, group_4185, group_4501, group_4096, group_3948, group_4367, group_4121, group_4118, group_4202, group_4238, group_2243, group_4010, group_4241, group_3963, group_3992, group_3979, group_4250, group_4437, group_1907, group_4300, group_2923, group_4491, group_3980, ccp, group_4229, cvaA, mobA_4, tagG, group_4500, group_1178, group_4487, group_4184, group_4216, group_3053, group_4365, group_4205, group_4002, group_4347, group_4063, nicB_2, group_4177, group_3987, group_4356, vapC, group_4108, group_3157, ykvP, group_1706, group_4323, group_3974, group_2260, group_2055, group_4100, group_4531, group_277, group_4352, group_4372, group_4101, group_3494, group_2989, group_4295, group_3968, group_4492, group_655, group_4303, group_4099, intA_1, group_3973, hin_2, group_4234, group_2735, group_4417, group_4299, group_4201, group_4189, group_4034, group_2635, group_3993, group_2990, group_4043, group_2233, group_2646

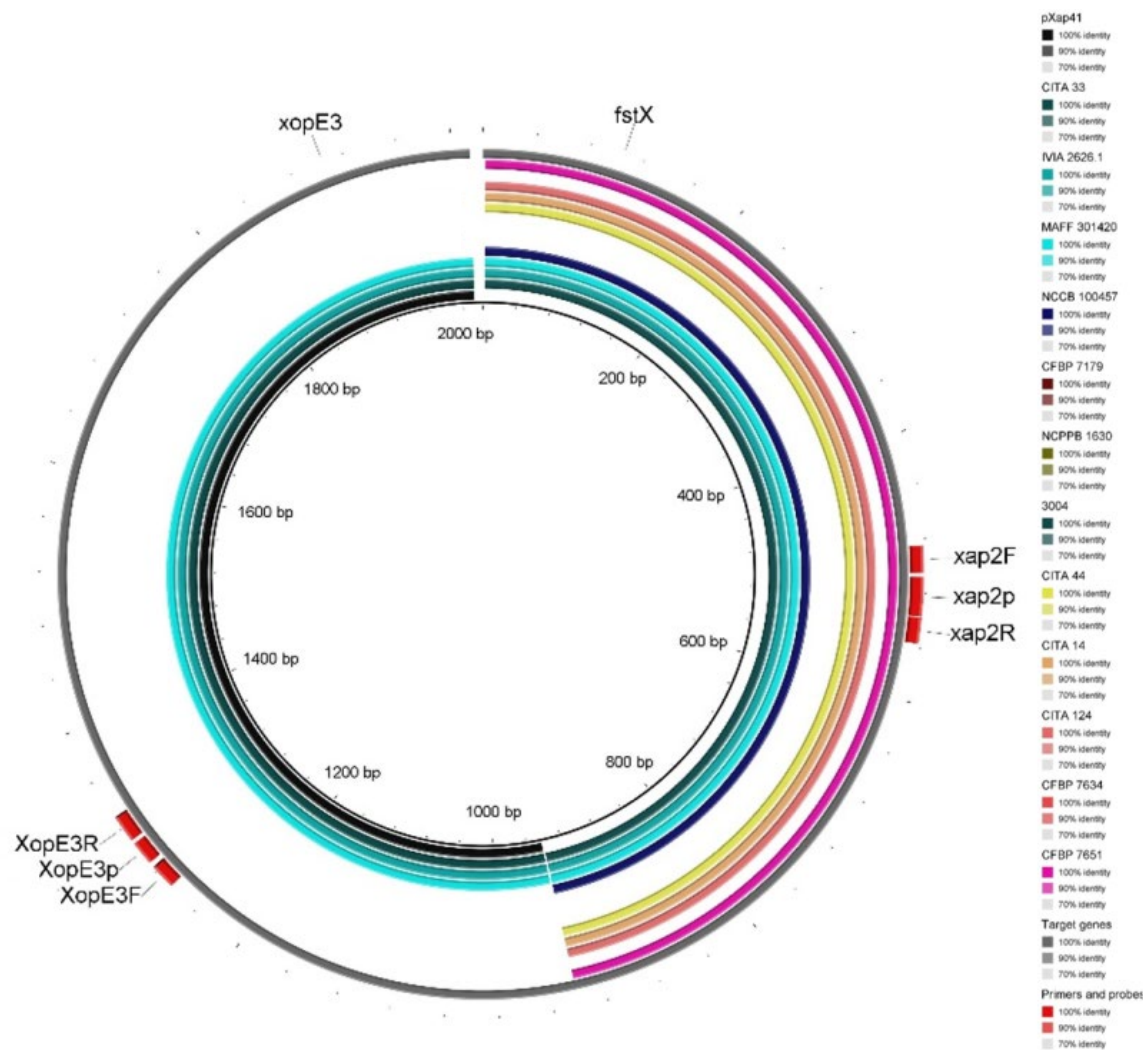
- ***xopE3***:

5'GTAAATGGGCTGTTGAGCGATTGCCCCAAGCGTCGGCGTCGTGGTGGGTCGCTCTCCGATCCTGATATCTCCCTCGCCGGGCTACTGCTGAGCAAAGCCGTCATCGGGGATCCGGT
CGAGCCCCAGGACATCCCTCGGCTGCACAAGGCCGAACAACCGGTGCAGAAGACACGGGCGCGCTTCCCATACGGACGCGGCAACGTCGCCACCGACATTGCCGTCAAGCGATCAC
GCATCCAGTCAACATGCGCAGGCCGCACATGATGTGTTTGTGATCTGGTGCAGCGGCGCGCGCCGCGCTCAATGCTCAGCAATCCGACGCTGGGGCATTGCGGTCTGATCTGAATT
CGTCCAGGGCGGTCCTGGCCGGATGCTGCCGTAGCAACCATCGACATGTACAGAAGCTTCAGCCAGAAGAAAGCGTCCACTATGTCCAGCACAAACCATCAGGGATCAGT
GGGCTGAGTCGCGCGCTGCCGACGGACATCAACAAGCCATCGTCTAGACCCCTTGGGCCAAGGACCGGCGGTGTTTGCCTCCGACAGCAGATTTCCGGCAAAATGCTCAACACACC
CAAGAACGCTTGGCCTTGAATGCCAAGGACGGAGATGATATTGCCGCCAAGACCGGCGAGTGCAGATATCTTGGAAAACTGCCCTCCCTGACTGAAACACACTCAAGAG
ACTGAAAGCCAGGATTCATTGCGCGCTGAGGAAGTCTGGCAACCACAACCTGTGGTAAGCGATCGTTCCTAGAAAGGTTCCGCAAAGTCTGGCTACATTGACCAACTTTC
AGAGCTTGGCTTGTGCGGGCAGAGCAGAGCAAGGCTTTGAAGAAGATGTCCATCAAATCGGCATGCGCACTGGGTTTCGGCAAGAAGCAGCAAGCGCAGCTGCCAAGG
CATT-3'

- **Primer XopE3F: 5`-TCA GCG ATC ACG CAT CCA-3`**
- **Primer XopE3R: 5`-CGC ACC AGA TCG ACA AAC AC-3`**
- **Probe XopE3p: 5`-/56-FAM/ CAT GCG CAG GCC GCA CAT G/36-TAMSp/-3`**

RT PCR approach...

X. arboricola pv. *pruni* (Xap) vs. *X. arboricola* pv. *pruni* look-a-like: specificity *xopE3*, *fstx*



***In silico* scheme of *xopE3* and *fstX* genes and hybridization areas for primers for real time PCR methods developed for Xap in *Xanthomonas arboricola*. (Blastn, E-value de 0.001).**

Garita-Cambrero *et al.* 2017 *Frontiers in Microbiology*

Primers and probe for *XopE3* looks Xap specific

X. arboricola pv. *pruni* (Xap) vs. *X. arboricola* pv. *pruni* look-a-like: specificity *xopE3*, *fstx*

Taxa	Strain	Result ABC	Result xopE3	Blind identification
Agrobacterium tumefaciens	Agrob-6	-	-	unknown
Agrobacterium tumefaciens	Agrob-9	-	-	unknown
Agrobacterium tumefaciens	IVIA 1245-80	-	-	unknown
Agrobacterium tumefaciens	IVIA 2304-10	-	-	unknown
Pantoea	IVIA 2261-1	-	-	unknown
Pseudomonas syringae pv. syringae	Psy-13	-	-	unknown
Pseudomonas syringae pv. syringae	Psy-13	-	-	unknown
Pseudomonas syringae pv. syringae	IVIA 3514-2	-	-	unknown
<i>X. arboricola</i>	CITA 44	+	+	unknown
<i>X. arboricola</i>	CFBP 3566	-	-	unknown
<i>X. arboricola</i>	IVIA 4302 col 5	-	-	unknown
<i>X. arboricola</i>	IVIA 2835-1	-	-	unknown
<i>X. arboricola</i>	IVIA 4185	+	+	unknown
<i>X. arboricola</i>	CITA 42	+	+	unknown
<i>X. arboricola</i>	CITA 149	+	+	unknown
<i>X. arboricola</i>	IVIA 4302 col7	-	-	unknown
<i>X. arboricola</i> pv. <i>corylina</i>	CFBP 1846	+	+	unknown
<i>X. arboricola</i> pv. <i>corylina</i>	IVIA 3978	-	-	unknown
<i>X. arboricola</i> pv. <i>corylina</i>	RIPF-X08	-	-	unknown
<i>X. arboricola</i> pv. <i>fragariae</i>	CFBP 6771	-	-	unknown
<i>X. arboricola</i> pv. <i>juglandis</i>	IVIA 2113	-	-	unknown
<i>X. arboricola</i> pv. <i>juglandis</i>	Xaj-2	-	-	unknown
<i>X. arboricola</i> pv. <i>juglandis</i>	Xaj-3	-	-	unknown
<i>X. arboricola</i> pv. <i>juglandis</i>	Xaj-4	-	-	unknown
<i>X. arboricola</i> pv. <i>juglandis</i>	Xaj-5	-	-	unknown
<i>X. arboricola</i> pv. <i>populi</i>	CFBP 3123	-	-	unknown
<i>X. arboricola</i> pv. <i>pruni</i>	CITA 9	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	CITA 11	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	CITA 46	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	CITA 70	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	100343	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	100400	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	CITA 154	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 4490.1	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 4491.1	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 4493	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 4492.2	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 4494.1	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 2626-5	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 2626-6	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 2626-7	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 2647-1.3	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 2647-13	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 2647-3.2	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 2649-3	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 2649-7	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 2649-10	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 2758-2	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 2758-3	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 2826.3	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 2826-4	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 2826-5	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 2826-6	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 2826-9	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 2826-10	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 2826-11	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 2832-5	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 2832-17	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 2832-19	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 2832-21	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 2832-24	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 2832-26	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 2832-30	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 3177-1-6	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 3177-3-4	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 3177-3-8	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 3181-3-1	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	IVIA 3181-3-3	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	CITA 33	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	CITA 99	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	CFBP 3894	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	CFBP 5530	+	+	xap
<i>X. arboricola</i> pv. <i>pruni</i>	CFBP 5724	+	+	xap
<i>X. axonopodis</i>	Xp-2	+	+	unknown
<i>X. axonopodis</i> pv. <i>phaseoli</i>	NCPPB 381	-	-	unknown
<i>X. axonopodis</i> pv. <i>phaseoli</i>	IVIA 1518	+	+	unknown
<i>X. campestris</i>	Xca-1	-	-	unknown
<i>X. campestris</i>	Xca-2	-	-	unknown
<i>X. campestris</i>	IVIA 2734-1	-	-	unknown
<i>X. campestris</i>	IVIA 1609	-	-	unknown
<i>X. citri</i> subsp. <i>citri</i>	IVIA 2889-1	+	+	unknown
<i>X. citri</i> subsp. <i>citri</i>	IVIA 3026-1	+	+	unknown
<i>X. citri</i> subsp. <i>citri</i>	str. 306	+	+	unknown
<i>X. hortorum</i>	IVIA 1575.1	+	+	unknown
<i>X. vesicatoria</i>	IVIA 3619-1	+	+	unknown
<i>Curatobacterium</i> sp.	EP-2.2	-	-	unknown
<i>Pantoea</i> sp.	EP-14.1	-	-	unknown
<i>Microbacterium</i> sp.	EP-16.1	-	-	unknown
<i>Sphingomonas</i> sp.	EP-16.2	-	-	unknown
<i>Pseudoclavibacter</i> sp.	EP-16.4	-	-	unknown
<i>Ferribacter</i> sp.	EP-16.6	-	-	unknown
<i>Pseudomonas</i> sp.	EP-17.1	-	-	unknown
<i>Curatobacterium</i> sp.	EP-18.1	-	-	unknown
<i>Rhodococcus</i> sp.	21/14-7.B3	-	-	unknown
<i>Pseudomonas</i> sp.	21/14-12.B8	-	-	unknown

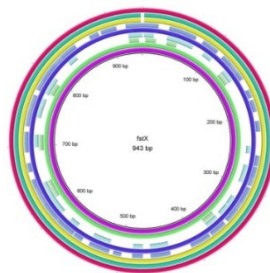
Primers ABC (*ftsx*) or *xopE3* are not 100% specific on RT-PCR: **problem?**

		<i>XopE3</i>	
		+	-
<i>ftsx</i>	-		
	+	<i>Xap</i> 54/54*	<i>Xap</i> -look-a-like 7/7

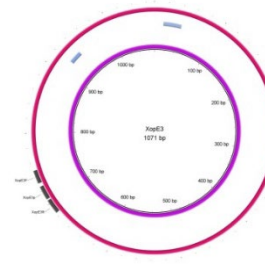
*Non desire PCR + in from *Xanthomonas citri* subsp. *citri*

		<i>XopE3</i>	
		+	-
ABC	+	<i>Xap</i>	<i>Xap</i> -a-like
	-	<i>X. spp</i>	No X

ftsx ABC



xopE3



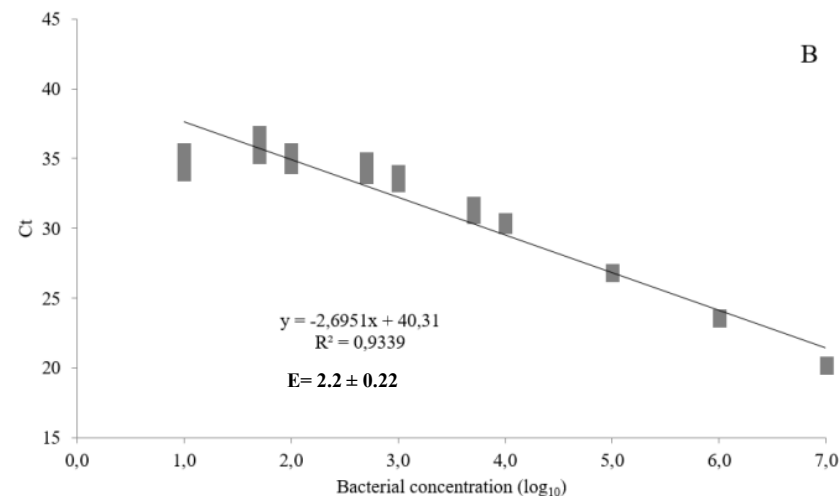
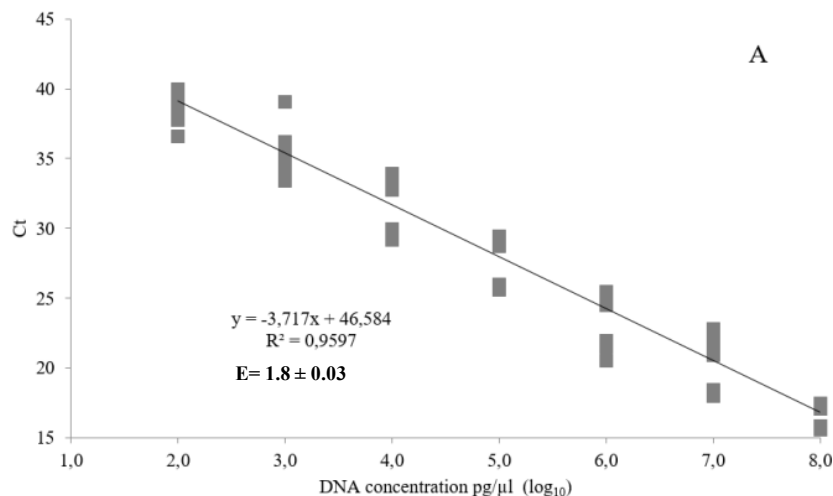
A combination is the best solution

Pan-Genomic Analysis Permits Differentiation of Virulent and Non-virulent Strains of *Xanthomonas arboricola* That Cohabit *Prunus* spp. and Elucidate Bacterial Virulence Factors

Jerson Garita-Cambronero¹, Ana Palacio-Bielsa², María M. López³ and Jaime Cubero^{1*}

Protocol provided for bacterial spot of stone fruit diagnosis

Sensitivity

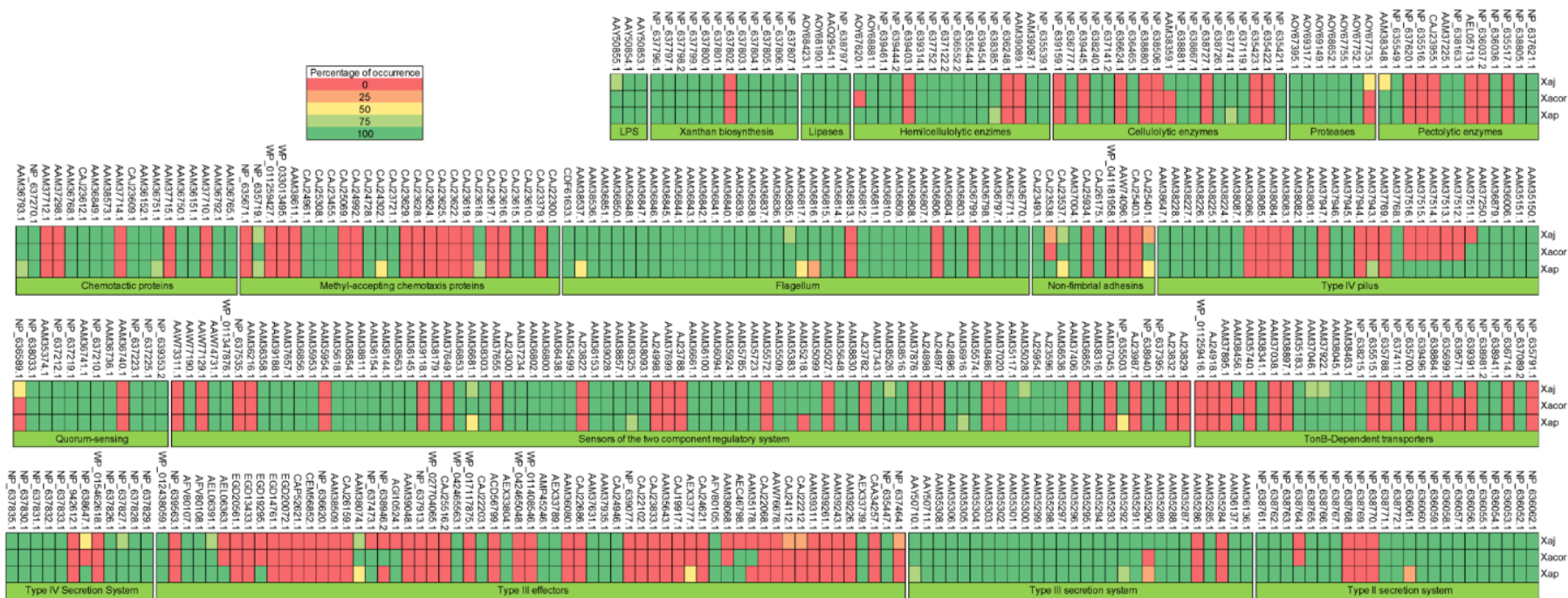


Calibration curves for PCR/*xopE3* for *Xanthomonas arboricola* pv. *Pruni*. DNA (A) cells (B)

X. arboricola pv. *pruni* (Xap) vs. *X. arboricola* pv. *juglandis* (Xaj) and *corylina* (Xacor)

We have many other options of genes involved in virulence in *Xanthomonas arboricola* sp.

Summary of virulence factors in most pathogenic pv. of the species



Alternative for new PCR approaches

Pathogen profile
Xanthomonas arboricola* pv. *pruni, causal agent of bacterial spot of stone fruits and almond: its genomic and phenotypic characteristics in the *X. arboricola* species context

JERSON GARITA-CAMBRONERO^{1,2}, ANA PALACIO-BIELSA³ AND JAIME CUBERO^{1,3*}



Candidatus Liberibacter: HLB and Zebra chip

HLB and Zebra chip: very important diseases in relevant crops worldwide

Candidatus *Liberibacter*
asiaticus (CLas), africanus (CaLaf), americanus (CLam)



Candidatus *Liberibacter solanacearum* (CLso)
Different haplotypes: A, B, C, D, E...



Quarantinable diseases...precise identification needed!!!! (nonculturable)

Candidatus Liberibacter species: diagnosis

PCR diagnosis HLB/Calsol/Zebra chip

reference	gene
Li et al.	16S
Morgan et al.	<i>hyvI/hyvII</i>
Bertolini et al.	16S
Hocquellet et al.	<i>rplA (rib operon)</i>

Primers based on ribosomal sequences or general functions

Again....is it a good approach to use genes not involved in virulence?

Non pathogenic Liberibacter

False positive?

Herein...a positive QPCR result was generated using HLB primers

Full genomic comparison validated by functional analysis will give new tools for Liberibacter diseases diagnosis....

microbial biotechnology

Open Access

Novel '*Candidatus Liberibacter*' species identified in the Australian eggplant psyllid, *Acizzia solanicola*

Jacqueline Morris,^{1,2,3} Jason Shiller,^{1,3,4}
Rachel Mann,^{1,3} Grant Smith,^{1,5,6} Alan Yen^{1,2,3} and
Brendan Rodoni^{1,2,3,*}



'*Candidatus Liberibacter europaeus*' sp. nov. that is associated with and transmitted by the psyllid *Cacopsylla pyri* apparently behaves as an endophyte rather than a pathogen

Noura Raddadi,¹ Elena Gonella,¹ Caterina Casarà,¹
Alan Pizzinat,¹ Rosemarie Tedeschi,¹ Elena Crotti,¹
Mauro Mandrici,¹ Piero Attilio Bianco,¹
Daniela Daffonchio^{2*} and Alberto Alma^{2*}

MPMI Vol. 27, No. 2, 2014, pp. 169-176 <http://dx.doi.org/10.1094/MPMI-09-13-0290-R>

The Complete Genome Sequence of '*Candidatus Liberibacter americanus*', Associated with Citrus Huanglongbing

Nelson A. Wulff,¹ Shujian Zhang,² João C. Setubal,² Nalvo F. Almeida,² Elaine C. Martins,¹ Ricardo Harakava,³ Dibyendu Kumar,³ Luiz Thiberio Rangel,² Xavier Foissac,² Joseph M. Bové,² and Dean W. Gabriel²

Phytoparasitica (2012) 40:213-221
DOI 10.1007/s12600-012-0225-5

Incidence of '*Candidatus Liberibacter europaeus*' and phytoplasmas in *Cacopsylla* species (Hemiptera: Psyllidae) and their host/shelter plants

Caterina Camerota · Noura Raddadi · Alan Pizzinat · Elena Gonella · Elena Crotti · Rosemarie Tedeschi · Netta Mozes-Daube · Ibolya Ember · Zoltan Acs · Maria Kolber · Einat Zchori-Fein · Daniele Daffonchio · Alberto Alma

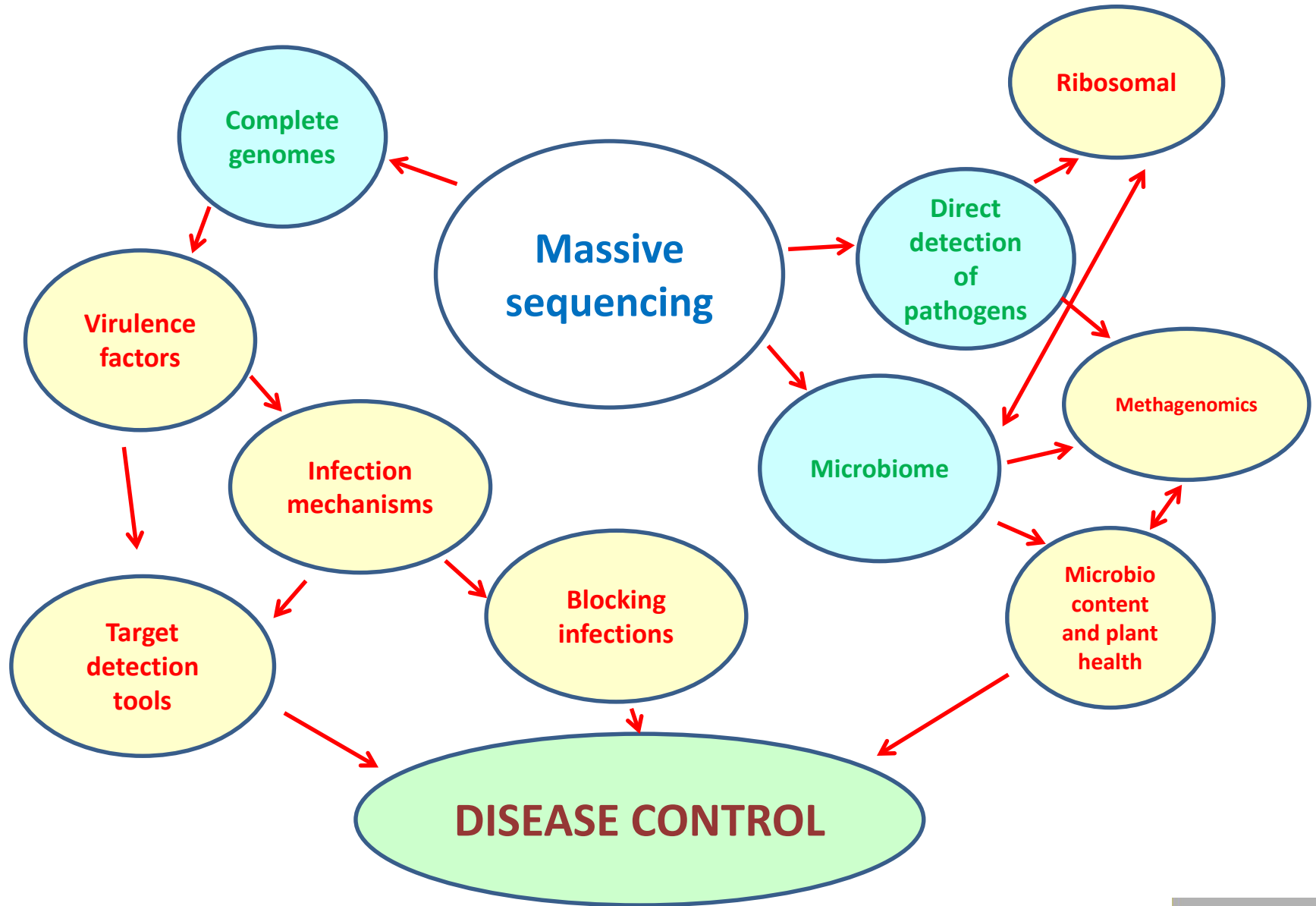


RESEARCH ARTICLE

Genomic sequence of '*Candidatus Liberibacter solanacearum*' haplotype C and its comparison with haplotype A and B genomes

Jinhui Wang^{1*}, Minna Haapalainen¹, Thomas Schott², Sarah M. Thompson^{3,4}, Grant R. Smith^{3,4,5}, Anne I. Nissinen⁶, Minna Pirhonen¹

Summary of applications of massive sequencing in plant pathogenic bacteria



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Valencia:

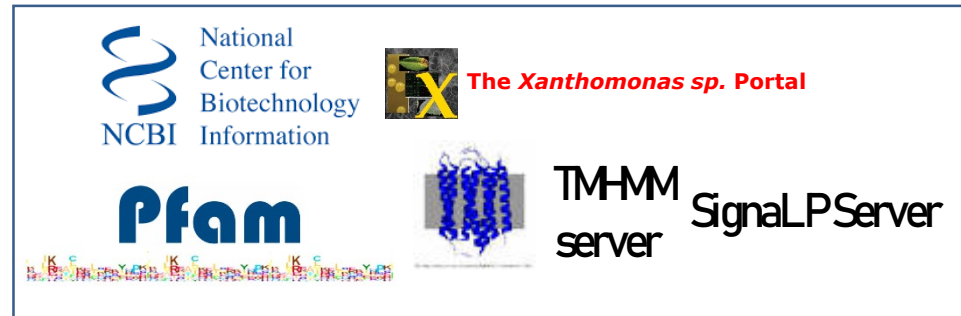
- María Milagros López
- Pablo López



Facilities and resources



Essential databases



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