

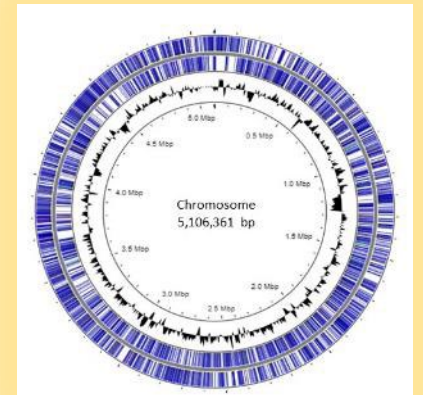
Mass sequencing: a useful tool for the control of plant diseases



Jaime Cubero

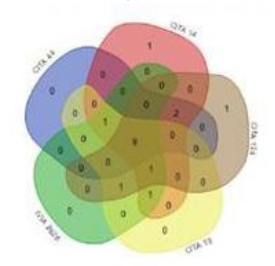
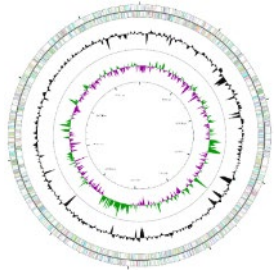
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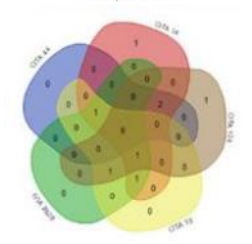
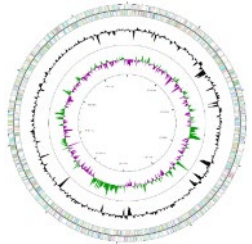
Why NGS and genomics in a plant pathology/bacteriology lab?

Summary



- Summary of Applications of High-Throughput Sequencing in Plant Pathology Laboratories
- Brief introduction on some diseases caused by plant pathogenic bacteria: ...*Xanthomonas citri* pv. *citri* (*Xcc*) and *Xanthomonas arboricola* pv. *pruni* (*Xap*)
- Some cases of misidentification of strains and consequences: Citrus bacteria canker (CBC), Bacterial spot of stone fruits and almond
- *Xanthomonas arboricola* pv. *pruni*: genomics
 - Precise bacterial identification and disease diagnosis
 - Discovering factors involved in virulence
- Other non-xanthomonads model where genomic analysis is mandatory:
 - *Candidatus Liberibacter*

Why NGS and genomics in our plant pathology/bacteriology group?



- **Genetic Diversity and Strain-Specific Traits:**

Genomic studies by NGS reveal the diversity within the pathogens, allowing to identify different strains and understand their unique genetic features. This information is essential for tracking the spread of specific strains and designing targeted interventions.

- **Identification of Virulence Factors:**

Genomic analyses help pinpoint genes associated with virulence, the factors that enable pathogens to cause disease in plants. By identifying these virulence factors, develop strategies to disrupt or neutralize them, ultimately enhancing plant resistance to pathogens infections.

- **Evolutionary Insights:**

Comparative genomics enables to trace the evolutionary history of pathogen species. Understanding how these pathogens evolve over time provides valuable information for predicting future trends in their pathogenicity and developing long-term strategies for disease management.

- **Genomic Surveillance and Diagnostic Tools:**

The genomic data obtained from different pathogens serve as a foundation for creating surveillance tools. Diagnostic assays based on specific genomic markers allow for rapid and accurate identification of pathogen infections, aiding in early detection and containment efforts.

- **Precision Agriculture and Disease Management:**

Genomic insights facilitate the development of precision agriculture techniques, tailoring interventions based on the specific characteristics of pathogens present in a given region. This targeted approach can optimize the use of resources and minimize the environmental impact of disease management strategies.

- **Genomic Editing for Crop Improvement:**

The knowledge gained through genomics opens avenues for genetic manipulation to enhance crop resistance to pathogens infections. CRISPR-based technologies and other genomic editing tools precisely modify plant genomes, introducing resistance genes or altering susceptibility factors.

Why NGS and microbiome analysis in our plant pathology/bacteriology group?

- **Microbiota Analysis:**

NGS allows for a comprehensive analysis of the plant microbiota, identifying a vast array of microorganisms, including bacteria, fungi, viruses, and other microbes, which may not be detectable through traditional methods.

- **High Resolution:**

The high resolution of NGS enables the detection of low-abundance species and the differentiation between closely related microbial taxa, providing a detailed understanding of the microbial community structure.

- **Rapid and Efficient :**

NGS is a rapid and efficient method for microbiota analysis, offering faster turn around times compared to conventional culture-based methods and allowing for the simultaneous processing of multiple samples.

- **Culture-Independent:**

As a culture-independent technique, NGS can identify and characterize microorganisms that are difficult or impossible to culture in the laboratory, giving a more accurate representation of the plant microbiota.

- **Functional Insights:**

NGS can be used to study the functional potential of the microbiota through metagenomics and transcriptomics, providing insights into the roles and interactions of different microorganisms within the plant environment.

- **Disease Diagnosis:**

By identifying pathogenic microbes and their relative abundances, NGS can aid in the diagnosis of plant diseases and the identification of potential biocontrol agents.

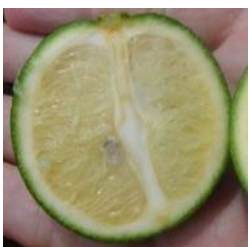
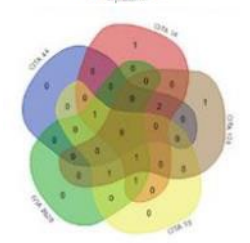
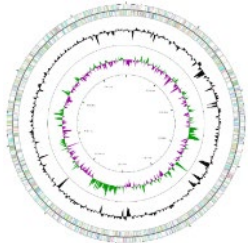
- **Ecological Studies:**

NGS facilitates ecological studies by enabling the examination of microbial diversity, dynamics, and interactions in different plant-associated environments and under various conditions.

- **Longitudinal Studies:**

NGS is suitable for longitudinal studies, allowing researchers to monitor changes in the microbiota over time and under different environmental or treatment conditions

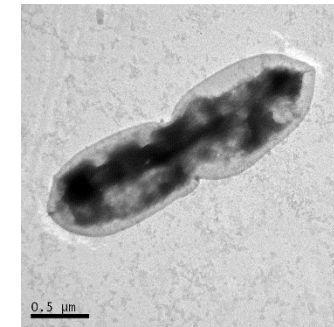
These advantages make NGS a powerful tool for advancing the understanding and management of plant health and disease but...



Xanthomonas genus: plant pathogenic bacteria...



- 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



Yes, but...there are also non pathogenic xanthomonad strains in plants ... or less “virulent/harmful”

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

An old history...



Xanthomonas citri subsp. *citri*

Xanthomonas alfalfae subsp. *citrumelonis*

Citrus Bacterial Canker (CBC)

Citrus Bacterial Spot (CBS)

- 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.
- Over 20 million trees in more than 100 nursery and orchard locations were destroyed at a cost of \$94 million.
- In September 1990, all regulations of the “Florida nursery strain of citrus canker” were removed based on scientific evidences.

Same genus, different specie....



Felis concolor
Florida Panther



Felis catus
Domestic cat

Precise identification!

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

Graham, J.H.; Gottwald, T.R. (1991)

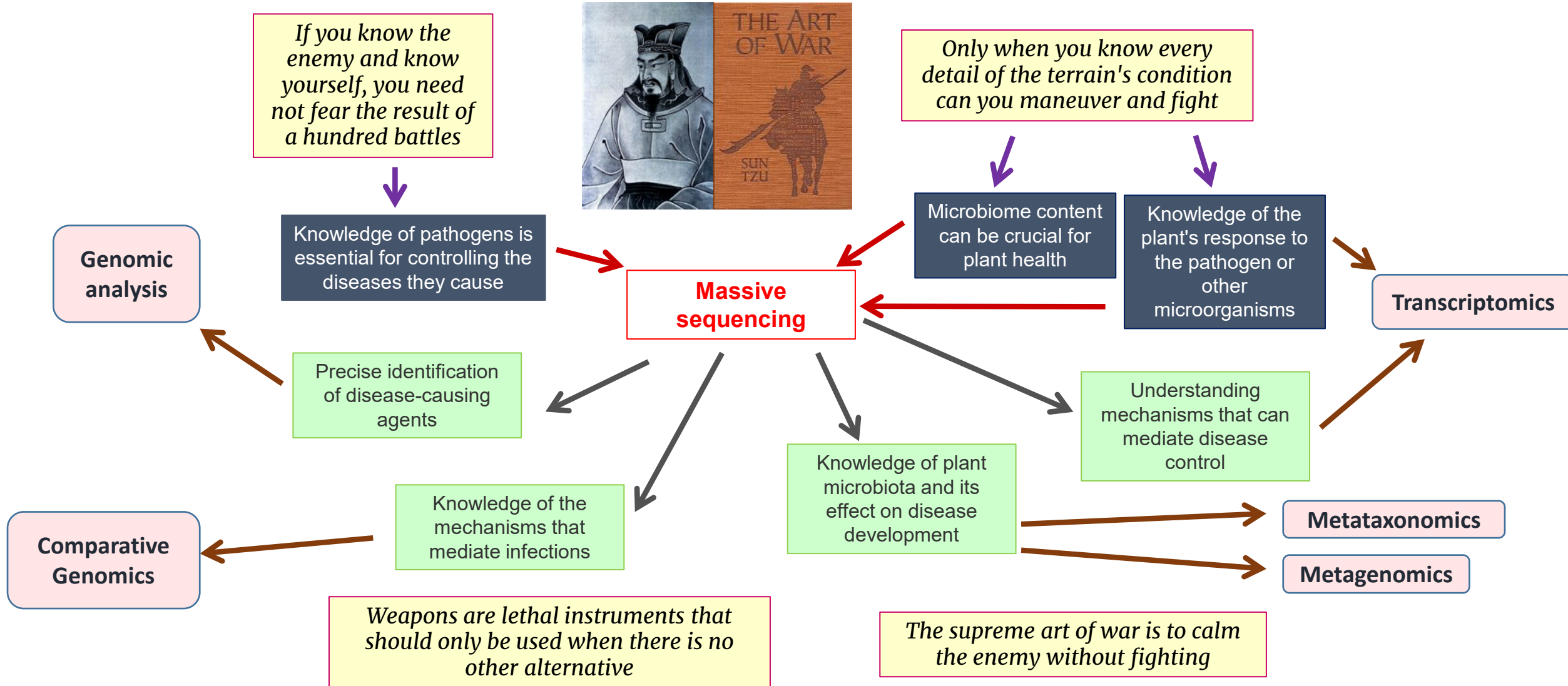


was all this really necessary?...

Disease control: a war against pathogens

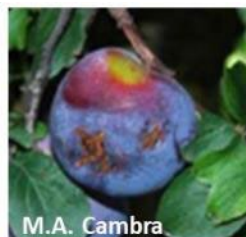
The art of the war
Sun Tzu (孫子; pinyin: Sūnzǐ) 孫武 c. 544-496 BC).

...strategies that can lead to success in any competitive endeavor

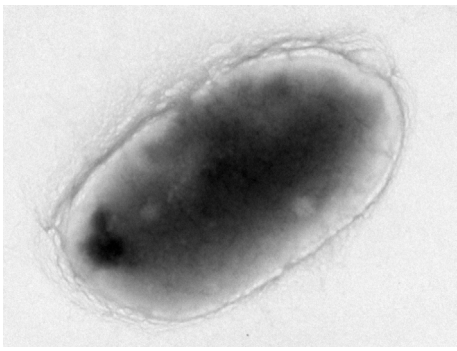
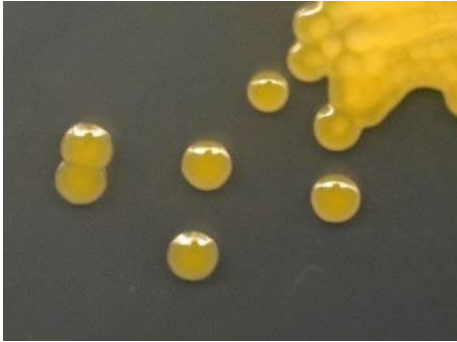




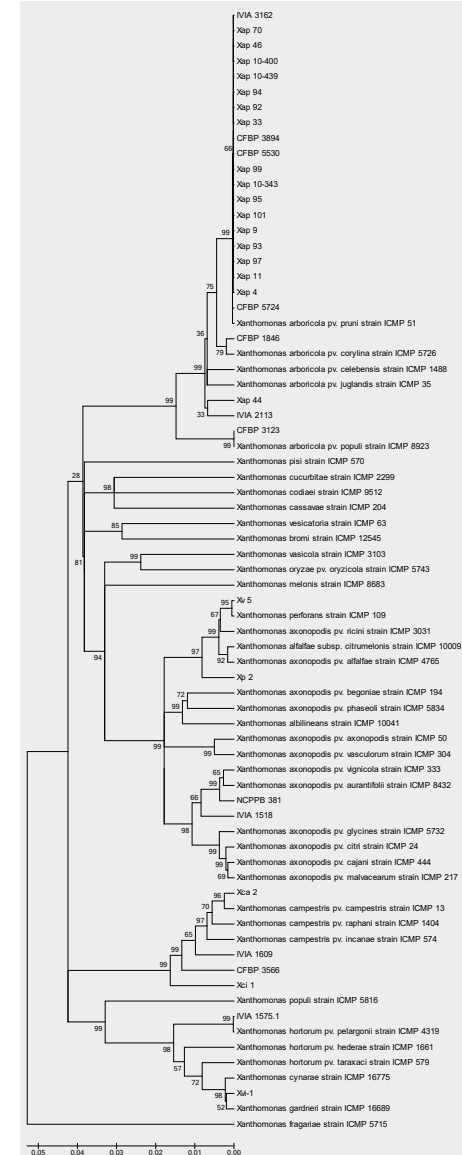
Xanthomonas arboricola pv. *pruni*: precise identification...and virulence factors



Xanthomonas arboricola pv. pruni in Spain



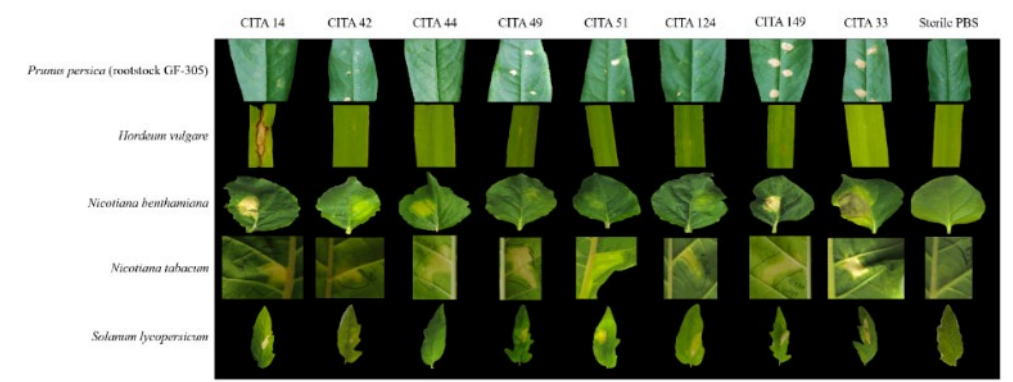
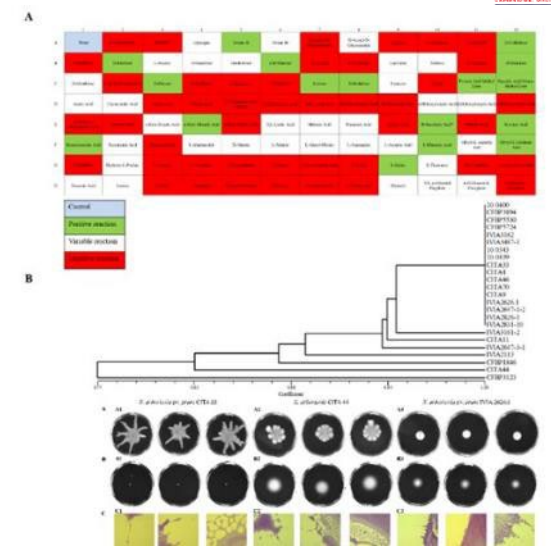
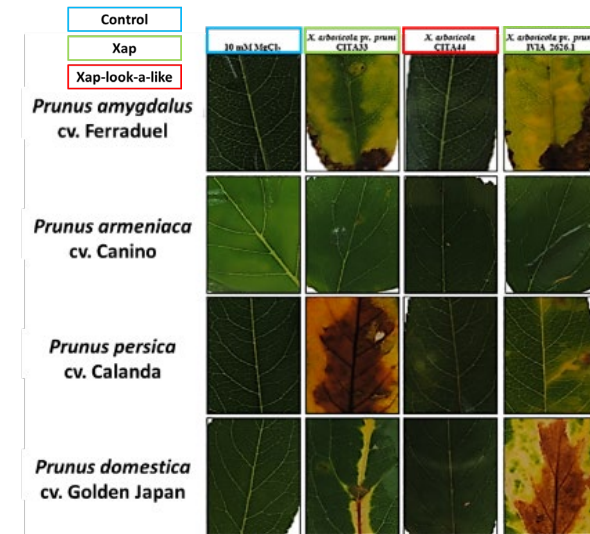
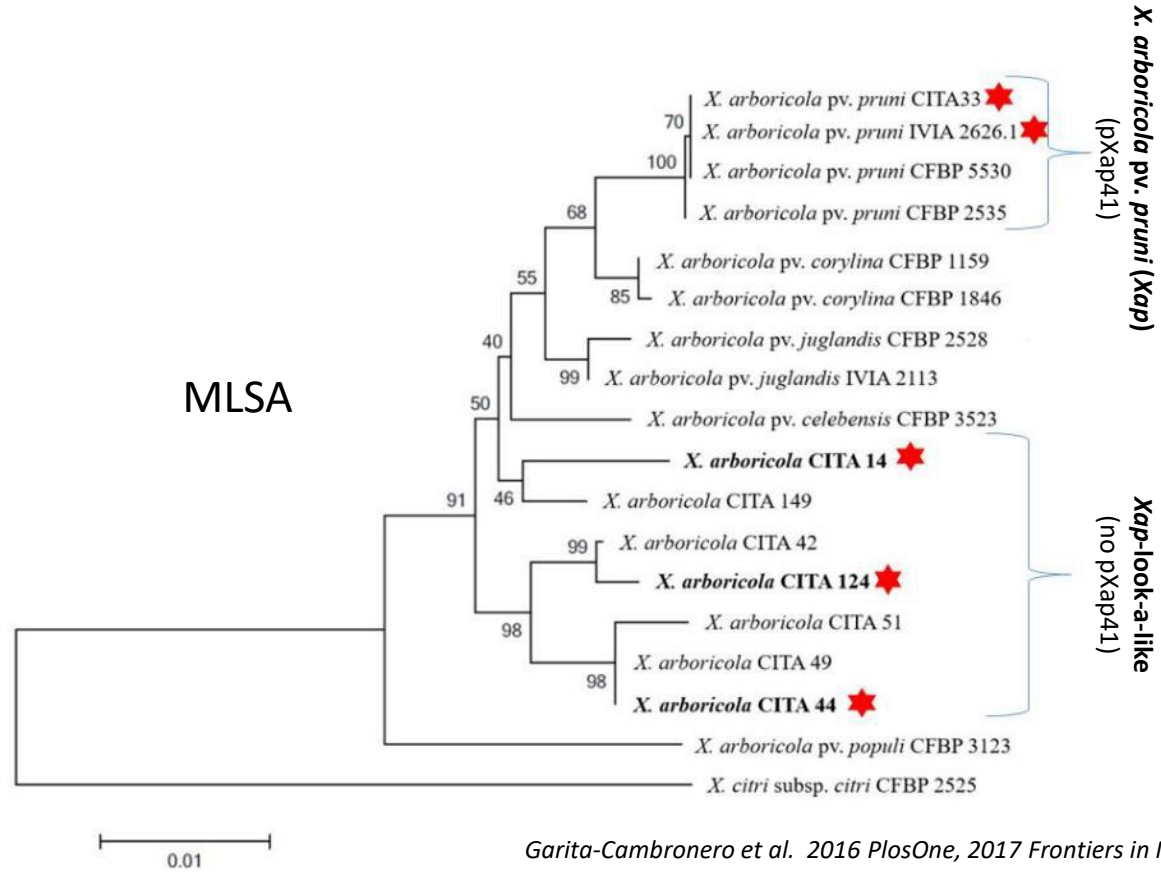
- Disease: Bacterial spot of stone fruits and almond
- Gram negative, Xanthomonadacea, *Xanthomonas arboricola*
- EPPO A2 List
- RNQP (Regulated Non-Quarantine Pest) ‘a non-quarantine pest whose presence in plants for planting affects the intended use of those plants with an economically unacceptable impact and which is therefore regulated within the territory of the importing contracting party
- Host plants: *Prunus* spp.
- Apricot, plum, peach, Japanese plum, cherry
- Serious disease:
 - ✓ Difficult control
 - ✓ Easy dispersion
 - ✓ Economic losses
- Existence of strains not classified by molecular techniques as Xap (Xap look-a-like)



First identification in Spain: specificity of PCR reactions?

The onset of Bacterial Spot in Spain...

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

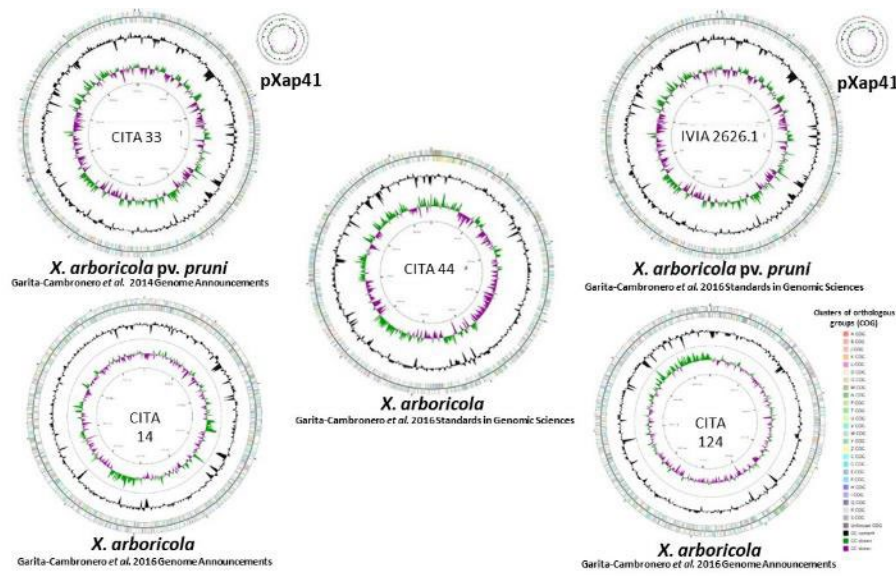


Main differences:

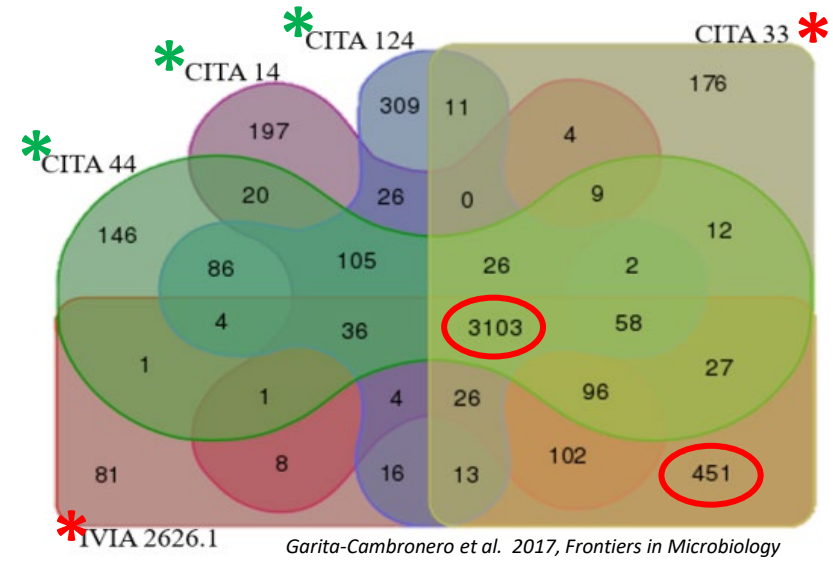
- Pathogenicity/virulence
- Motility
- Chemotaxis
- Carbon source compounds

A few strains were misidentified...

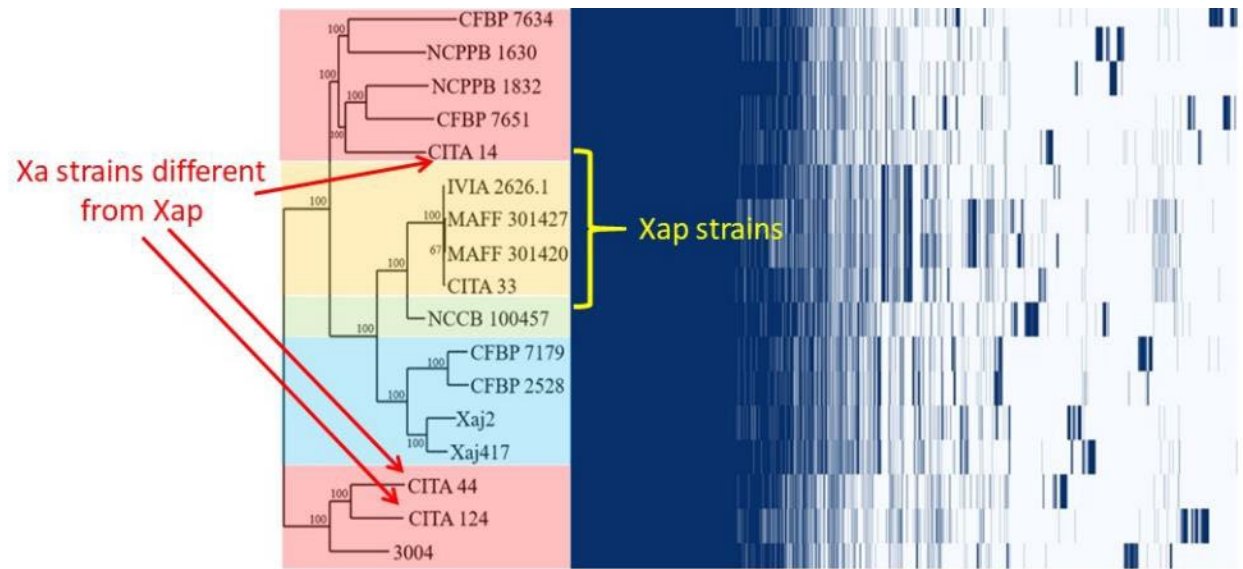
Genomes of some *X. arboricola* isolated from *Prunus* species



* Xap
* Xap-look-a-like



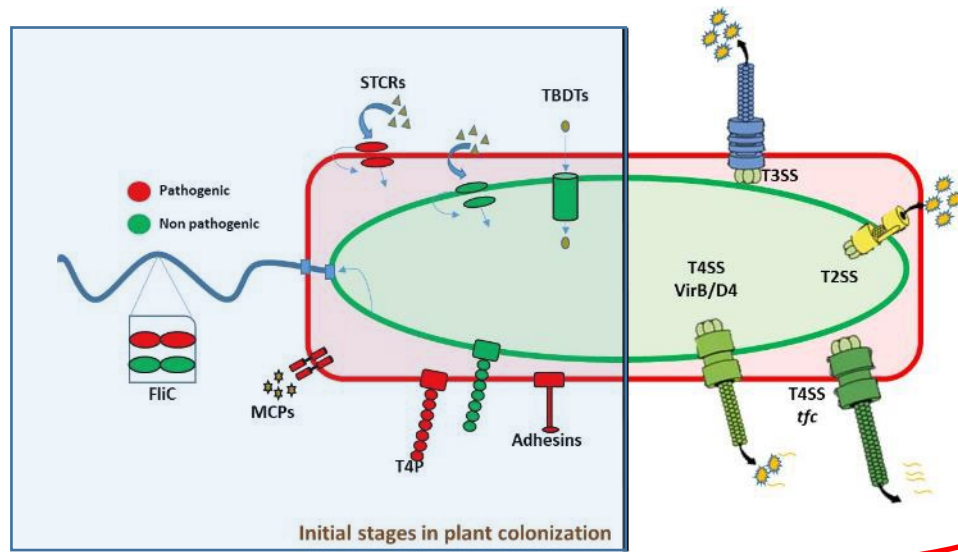
Garita-Cambronero et al. 2017, *Frontiers in Microbiology*



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

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

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



ENVIRONMENTAL SENSORS

MCPs (methyl accepting chemotaxis proteins)

- 11/26 genes described in *Xanthomonas*
- One specific gene in pathogenic strains of *Prunus* (CAJ23610.1)

TBDTs (tonB-dependent transporters)

- 17/28 genes described in *Xanthomonas*
- 3 specific genes in non pathogenic strains of *Prunus* (NP_635700.1, NP_635515.1 y NP_639391.1)

STCRs (Sensors of the two-component regulatory system)

- 60/86 genes described in *Xanthomonas*
- 2 specific genes in pathogenic strains of *Prunus* (XAC0136, XAC1345)
- 4 specific genes in non pathogenic strains (AAM36681.1 (*tspO*), AAM35218.1 (*baeS*), AAM37649.1 (*smeS*), NP_637535.1)

FIMBRIAL ADHESINS: TYPE IV PILUS

T4P

Adhesins

25/31 genes described in *Xanthomonas*

- Six specific gene in pathogenic strains of *Prunus*

NON FIMBRIAL ADHESINS

Adhesins

6/10 adhesins described in *Xanthomonas*

- specific Hemagglutinin *fhaB2* (CAJ23538.1) in pathogenic strains of *Prunus*

FLAGELLA

Aspartic acid by Valine in pathogenic strains in aa 43

immune signaling and defence responses

Matzke 2003

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

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

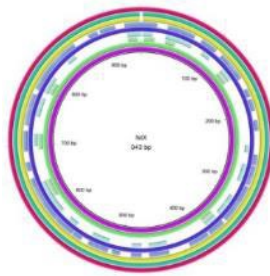
Taxa	Strain	Result ABC	Result xopE3	Blind identification
Agrobacterium tumefaciens	Agrob-6	-	-	unknown
Agrobacterium tumefaciens	Agrob-9	-	-	unknown
Agrobacterium tumefaciens	IVIA 1245-80	-	-	unknown
Agrobacterium tumefaciens	IVIA 2304-10	-	-	unknown
Pantoea	IVIA 2261-1	-	-	unknown
Pseudomonas syringae 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 col7	+	-	unknown
X. arboricola pv. corylina	CFBP 1846	+	-	unknown
X. arboricola pv. corylina	IVIA 3978	+	-	unknown
X. arboricola pv. corylina	RHP-308	+	-	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 2899-1	-	+	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
Curtobacterium sp.	EP-2.2	-	-	unknown
Pantoea sp.	EP-14.1	-	-	unknown
Microbacterium sp.	EP-16.1	-	-	unknown
Sphingomonas sp.	EP-16.2	-	-	unknown
Pseudoclavibacter sp.	EP-16.4	-	-	unknown
Terrabacter sp.	EP-16.6	-	-	unknown
Pseudomonas sp.	EP-17.1	-	-	unknown
Curtobacterium sp.	EP-18.1	-	-	unknown
Rhodococcus sp.	21/14-7.B3	-	-	unknown
Pseudomonas sp.	21/14-12.B8	-	-	unknown

		XopE3	
		+	-
fstx	-		
	+	Xap 54/54*	Xap-look-a-like 7/7

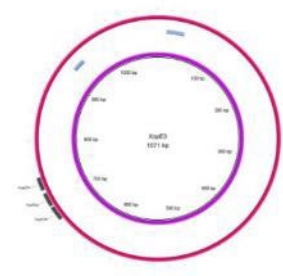
*Non desire PCR + in from *Xanthomonas citri* subsp. *citri*

		XopE3	
		+	-
ABC	+	Xap	Xap-a-like
	-	X. spp	No X

fstx ABC

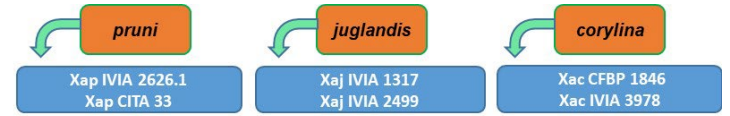


xopE3

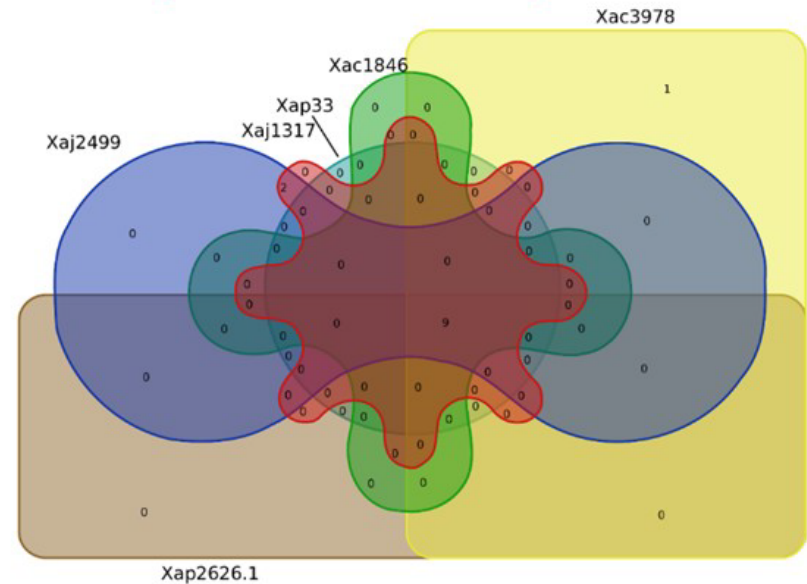
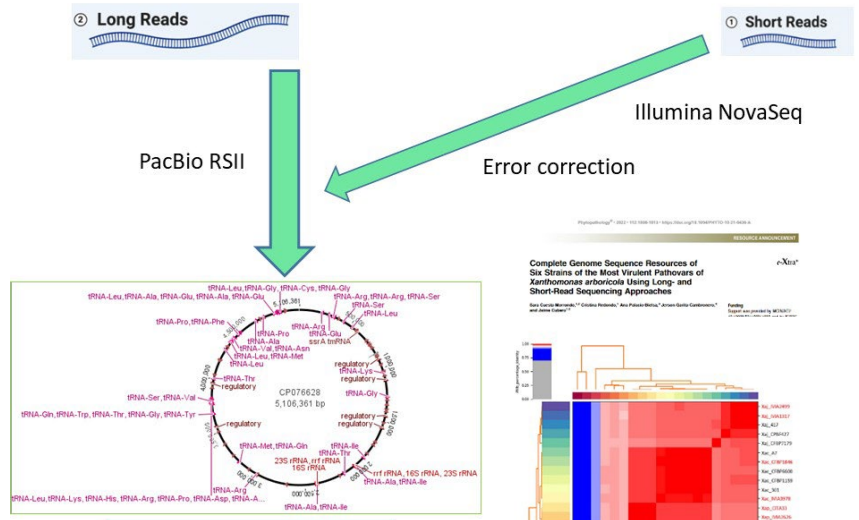


A combination is the best solution

Genomics *X. arboricola* pvs. *pruni* (Xap), *juglandis* (Xaj) and *corylina* (Xac)

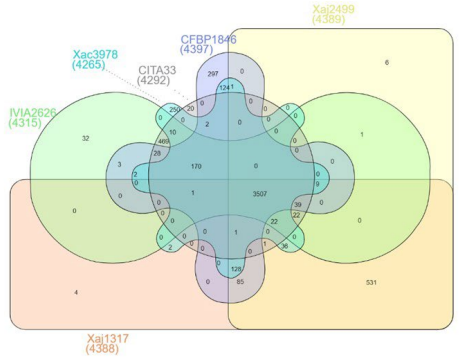


Example of primer design on TonB transporter genes



Common and non common elements

Names	total	elements
Xac1846	9	XAC3613
Xac3978		XAC3620
Xaj1317		XCC1719
Xaj2499		XCC3635
Xap2626.1		XCC4162
Xap33		XAC3077
		XAC3498
		XAC3201
		XCC3595
Xaj1317	2	XCC2046
Xaj2499		XCC4237
Xac3978	1	XCC2867



Summary of pathogenesis factors

Molecular Plant Pathology
 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

The same species, different pathogens means similar genomes!

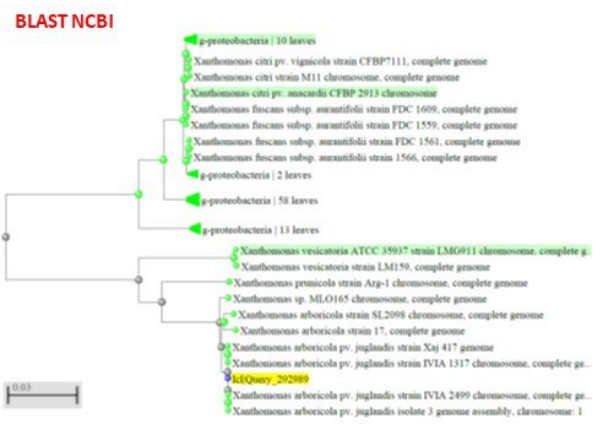
Differences in host range/virulence?

Diagnosis and detection markers

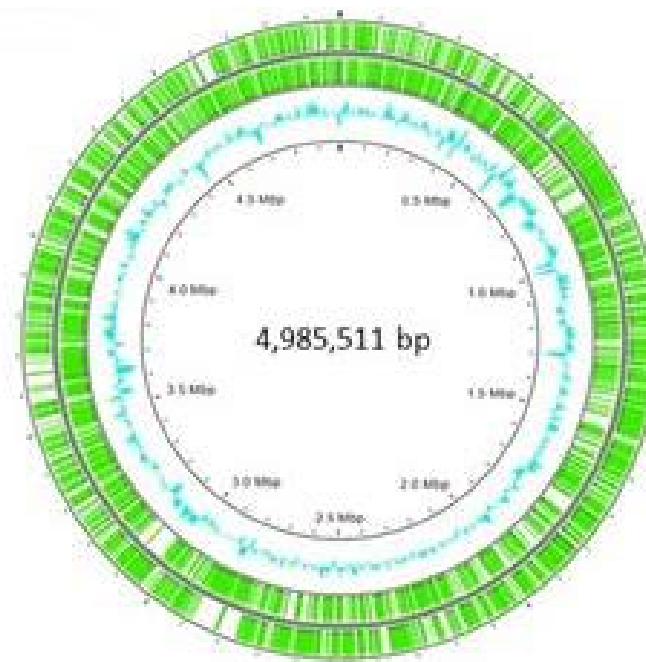
TBDT sequence

Xcc2046 en Xaj1317
 ACAAGCTTCATGTTTCAGGCCAAGATAGAGCTGGCGCCCTGCCGCTGGC GAAAACATCGGCTGCGCCTGCGTCCAGAAAGAAAGCTGCTGTACCA CACGTA
 GGCCATACCTCGCGCGCGTGGCATTGCGGACCTGCAGATCCACCGCTCCAGGCGGATGATGGAGTATCTGGCGTGA GATCCAGGCTGGCGGAAGCC
 CGCGTATTTCCCGCCAGGTTGCGCTCCAGATAGTAGTGC CGTTGCG GCG CGG CC CTGCATTCC CAAGCG CAGCG CAGCG GTCG GTCG GATCTCGA
 CGCCGACGTTGCTGATGTAACGCGCGGCTGGCGC CACTTCC GCG CGGAC CAGC GACACG CTGGCAT CGCG GTGCTC GCGTTC GATCTTTGCCCTC CTGG
 TACGCGTGCAGTGCCTATGCGTCCACTGCTGCG CCAAG CTGGC CATTGATCTGGC CATCCAC GCCA CGC CTGGCT GTCTTTC CAGGGTGA CGGTGGT
 GCGCGTGGCGGCATGTTGGAGACTTCGTTGGCGC CATCTGTTGC CACAC OGCAATGC GCG CCTGG CGCC CTGGAAAGCGGGTGAATTTTCATCG CCA
 GCTCCACGCGGATTTGTTGGAGCGGGCCGAC GTCC GCCTG CC CGG CGGTGAGTGAAG CCG GCG CGGTG GACC CGGTG CAG CAC CTGGAAAGCTG CGGC
 CCCAGTTGGCATAAGCGTTGGTGGTGGGGTGAAGGCGTAGACCCAGG CTCAGTTGGGCTGCTC GATGGTGC GTGACG CTGCA GCG CGC GCGGAA
 CGCCCGCGGCACTGCGGTGCG CGGTG CAAAGCG GTCCA CACGCTAGC CCG GCA C GATCTTCAGCG CCTC GGTGCG CTGATAGATGCG CCTGCA CGTA
 GGGGCCCAAGTTGTCGAAAGGATAGCGGCTGCTGTTGGATG CBTGCG CCG CGTGG CATTGAAA TCGGTGGG CTGCG CATAGCTGAAACGCT CGC GT
 CGGTAGGGGTTGCTGATGCTGCTGATGTTGATGCGCGCTCCAGGCTGA GCGTGTG CTGCT CTGCG CCAAGT CAGCGTGTGAG CATGC OGACCTGGT
 CTGCTCCAGATGCGCGCTGCGCTGCG CAGATTGCG CGGTGG GCAAGT CGCTGAA GATCCAC CGG CGGTG GTGCTGATGAC GGTGGAGTAAAGCTTG
 GTGCGGAAAAACAGCCGCTGCGCAGCTTAGATCCAGATGGACCG CAACTGG CGCATGTC GCG GTGCTGCG CC GTGCTGGCG GTTGGC CAGGTC CG
 AACTGCGACGCTGCTGCTGATTCGCTGCGCTCATGAAGC CGGGTTC GTGCG CTTGTTGATAGACG CGTG CGGTG CAG GCTGATGCG CATGCG
 TCGTGCAGGTTGCGGTGAGAACATTTGCGCCCAACGAATACTGCTGGAGGT GTGCTGGTGC GCG GTAGCCATC GGAAGC CTGCAC GCC GACGAAA
 GATGTTCTGCGCAAACCCGCTGCGTTGCGTCCAC GGC CAGCTG CGCATC GCGG GTGTTGAAAGCTGC CGTAGGTTAG GCG CGC GTGCGTGAATCG CC
 GCCTCGGCGGTGCGGCAAAATGATGTTGCGCGCATATTTGCGCAG GCG GTAGCG CGGATCATTTGGTGC CG CGCACTA CTTCGATGTAATCTGATTTCCA
 CGGGGAAACAGCATGTGATGAAGCGCTGTTGCACTGTTGAGCTGTTGCG GAGTGC CGTGCATCAG CGTCTTGTATGCG CATTGAGGTTAACCTTCCG CG
 TTGAAGGCGGAAAGCTGACCTTGCCTGATTCGGCGCTGCG CCG GTTTGGTGGTGC CTGGATGC CGGG CATCTGCG CCGAG CAGCT CCGAG CATAGG
 ACACGTTCTGCTCGATCTGATCGGCCCCCAAGCACT CCAC CGAGTCA GTACTGATGC GCGGT GAGTTGGC CTTGCT GATCTGCTGGCA CTAGG
 ACCTTGGCGAGCGTCAAGCGCAATCGGACGACT GTGCG CGTGC CTCAGG GAGCAG CAGAACGCG CGC CGAOC GCGCG CGC GAGAATGTGGATCTTC
 ATG (cadena reversa)

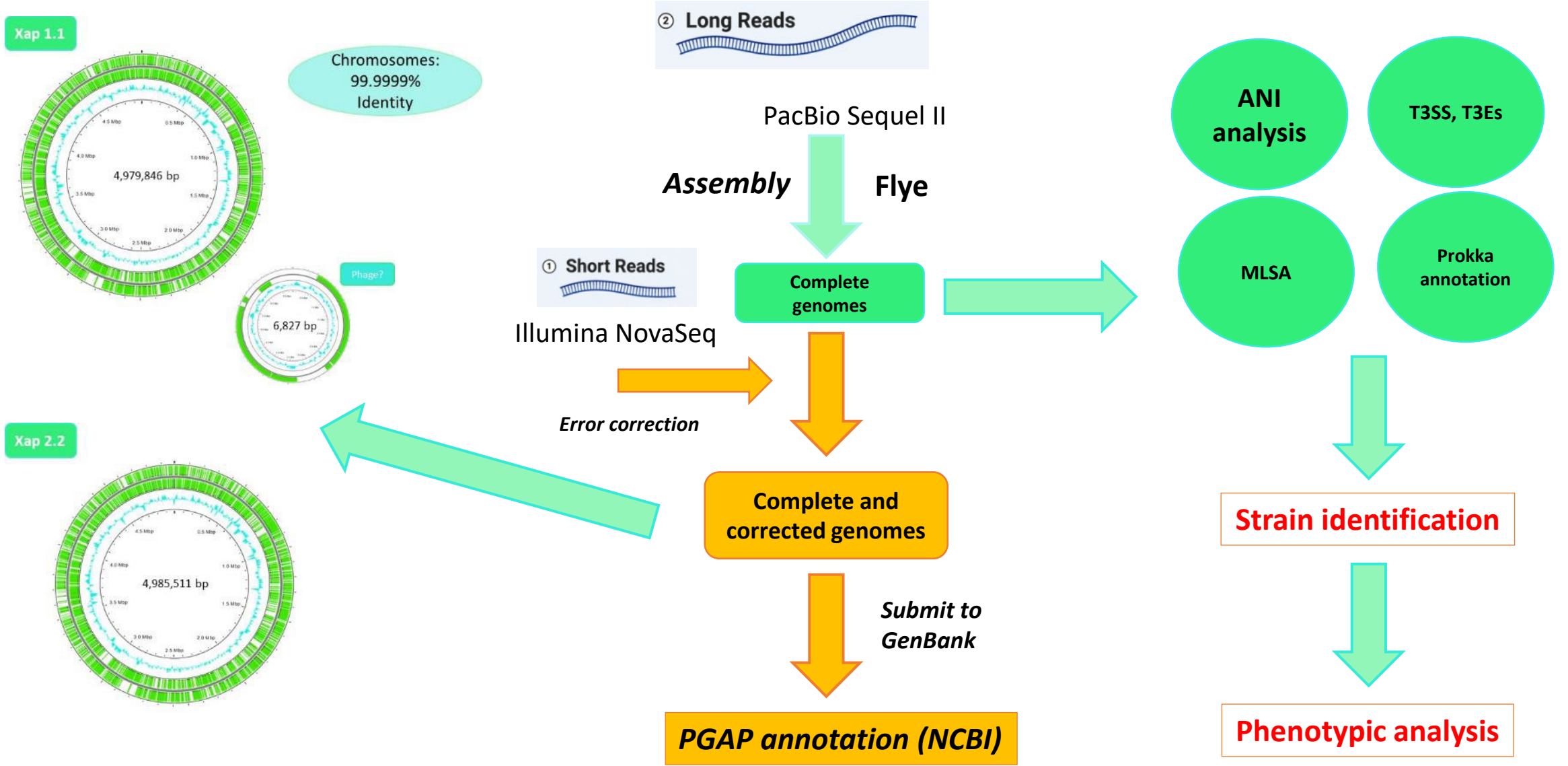
Phylogeny



Cherry *Xanthomonas arboricola* pv. *pruni* (?) in Montenegro: precise identification...and virulence factors

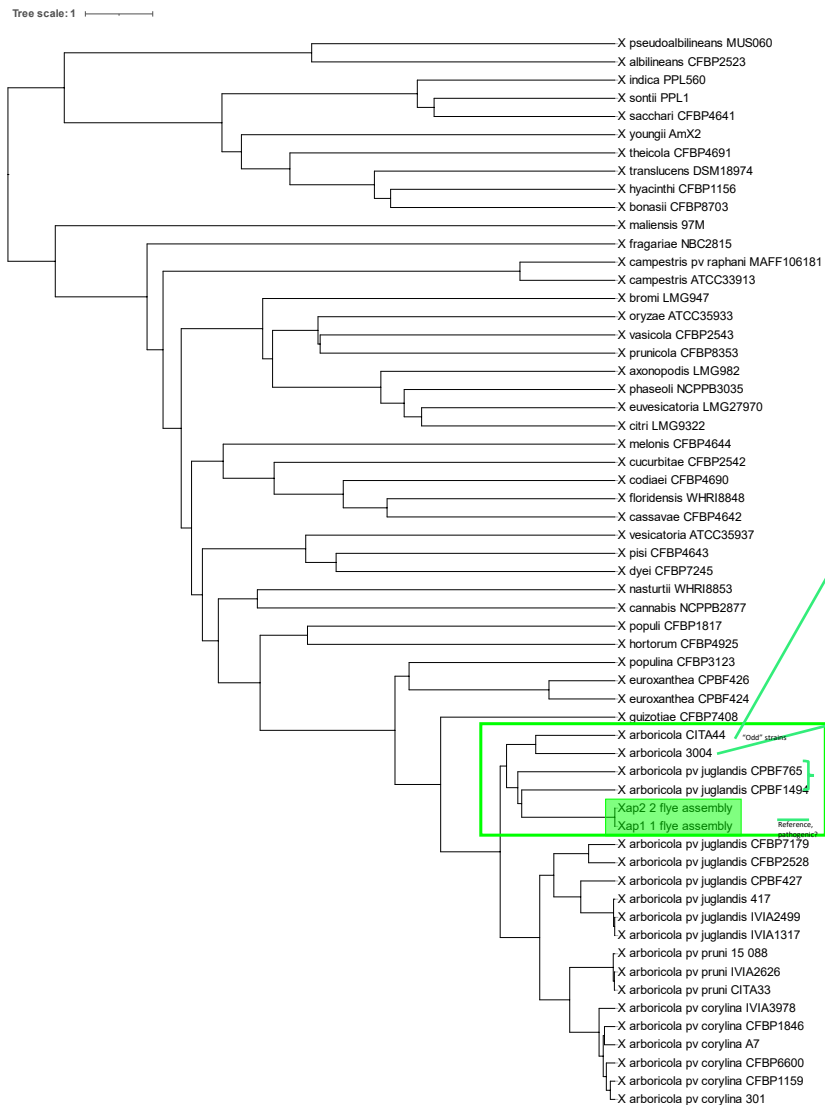


More genomics: Xap cherry strains in Montenegro?



ANI (Average Nucleotide Identity)

FastANI



Non-pathogenic *X. arboricola* isolated from *Prunus mahaleb*

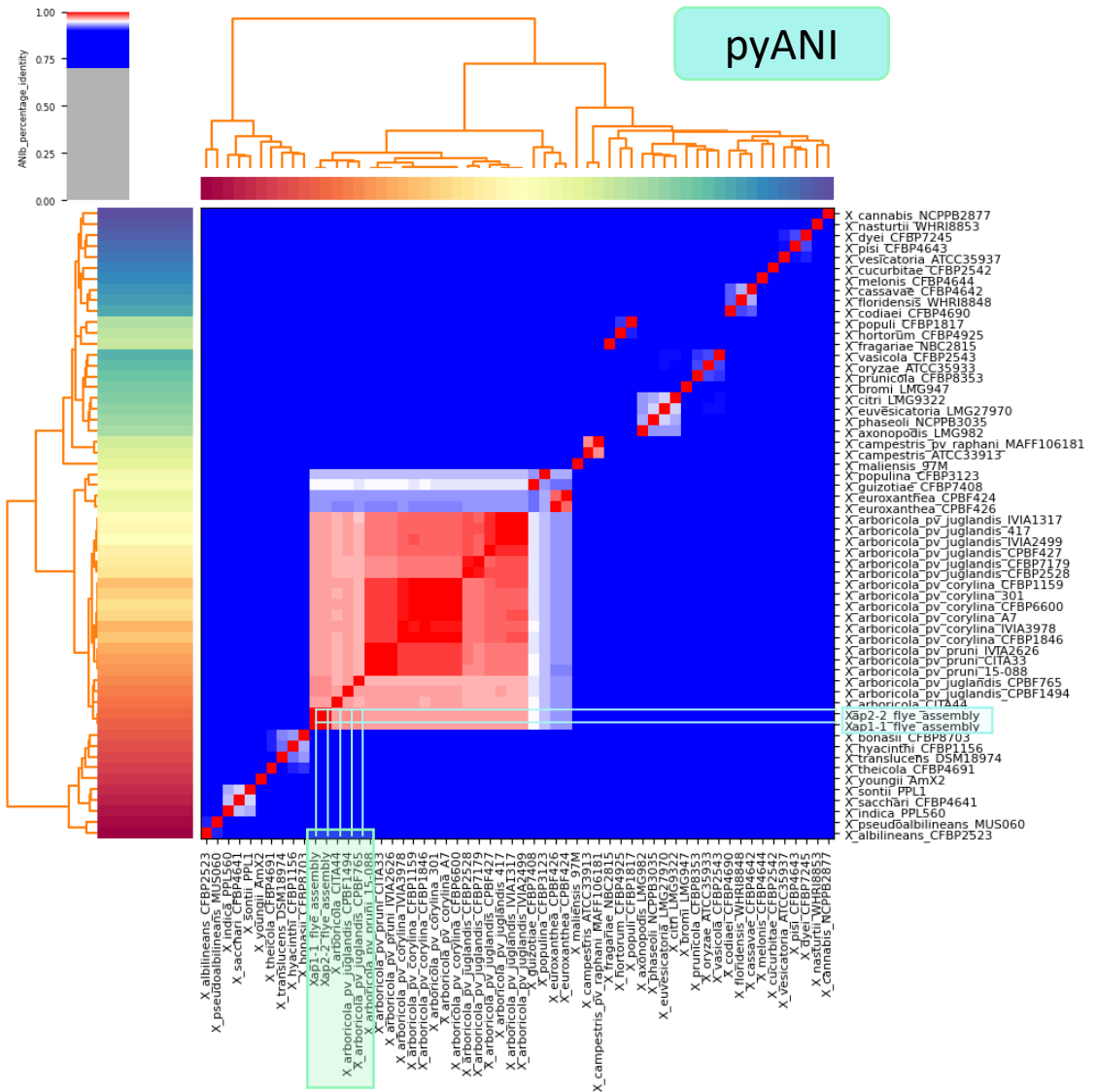


Low-pathogenic *X. arboricola* isolated from barley

Xaj strains isolated from pecan (*Carya illinoensis*)



pyANI



More genomics: Xap cherry strains in Montenegro?

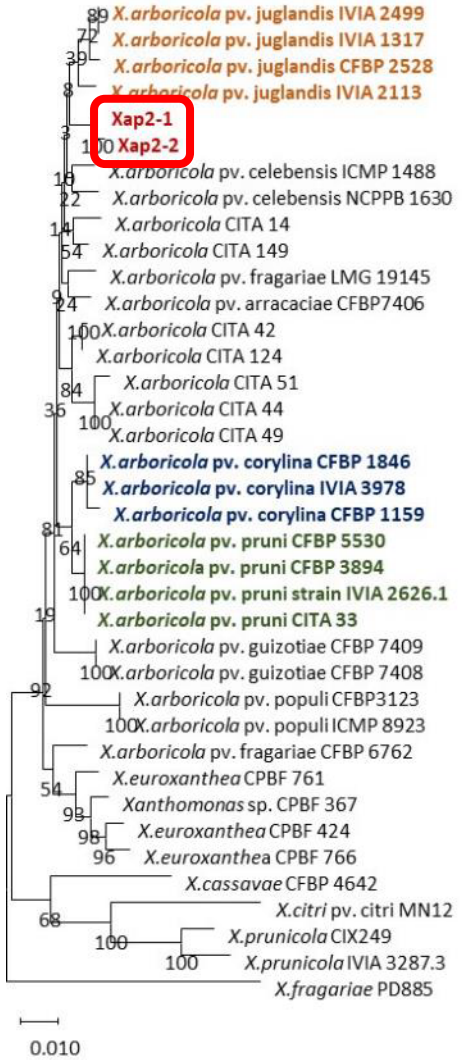
X. arboricola pv. *pruni* cherry tree strains (?????????)

From Montenegro

Analisis MLSA

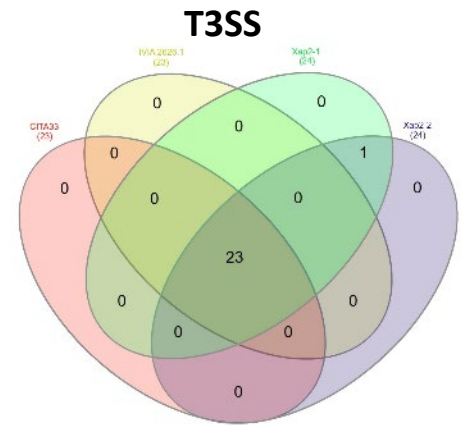
Genoma PacBio/Illumina

gyrB, *fyuA*,
dnaK, *rpoD*



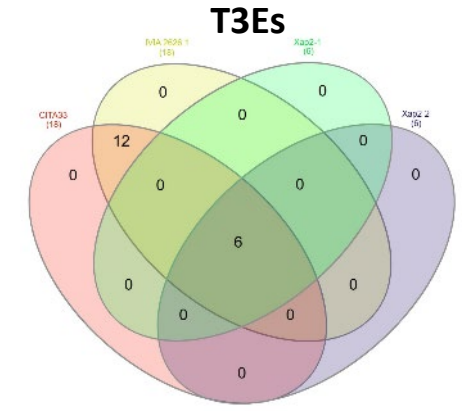
No Xap!

First analysis on type III secretion system



[Xap2-1] and [Xap2-2]:
XAC0404

T3SS identified

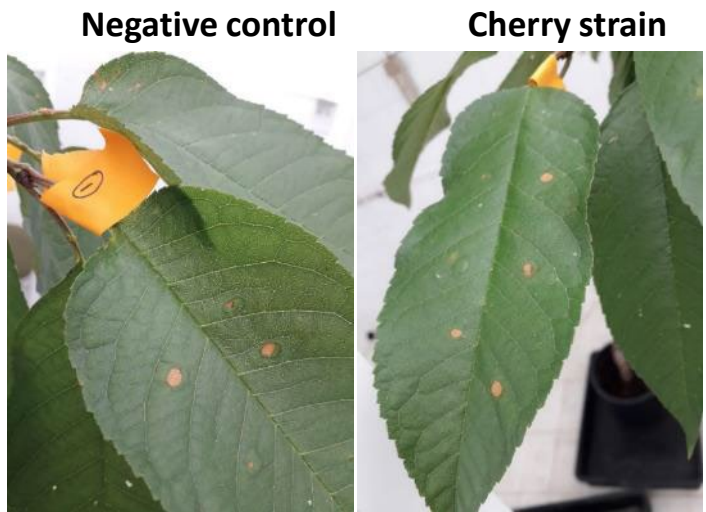
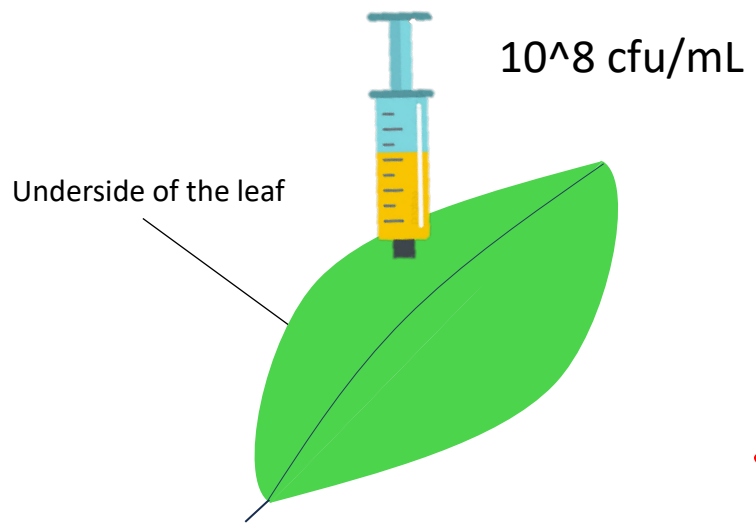


[CITA33] and [IVIA 2626.1]:
JF826917_1
JF826929_1
XAC2786
XAC3090
XAC3224
XAC3230
XAP_pXAP410054
XCAW_b00003
XCCB100_1770_AvrXccA2
XCV0572
XGA_2091
XOO2402

[CITA33] and [IVIA 2626.1] and [Xap1-1] and [Xap2-2]:
JJF826876_1
JQ595991_1
JQ595993_1
JQ595994_1
KT873949_1
XCC0052

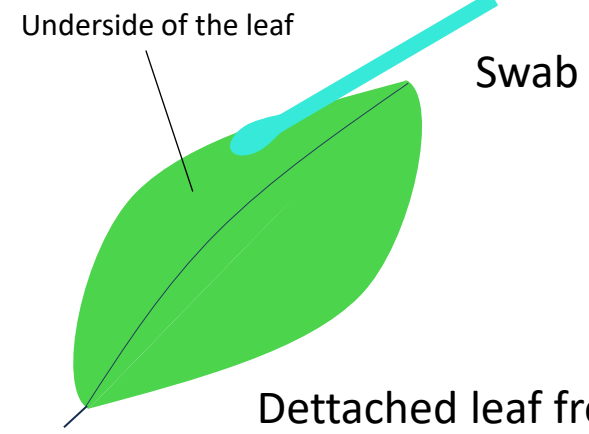
No Xap effectors

Plant inoculation: pathogenic strains?



No clear symptoms

NO Xap



Detached leaf from *Prunus* spp. GF677



10⁸ cfu/mL

Xap 1.1
Xap 2.2
Xap 2626: canonical Xap.
MgCl₂: Negative control.



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: bacterial detection disease 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



MPMI Vol. 27, No. 2, 2014, pp. 163-176 <http://dx.doi.org/10.1094/MPMI-09-13-0292-R>

e-Xtra*

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

Nelson A. Wulff,¹ Shujian Zhang,² João C. Setubal,³ Nalvo F. Almeida,⁴ Elaine C. Martins,¹ Ricardo Harakava,⁵ Dibyendu Kumar,⁶ Luiz Thiberio Rangel,² Xavier Foissac,⁷ Joseph M. Bové,² and Dean W. Gabriel¹

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,*}

Phytoparasitica (2012) 40:213-221
DOI 10.1007/s12690-012-0225-5

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 · Rosmarie Tedeschi · Netta Mozes-Daube · Ibolya Ember · Zoltan Acs · Maria Kolber · Einat Zchori-Fein · Daniele Daffonchio · Alberto Alma

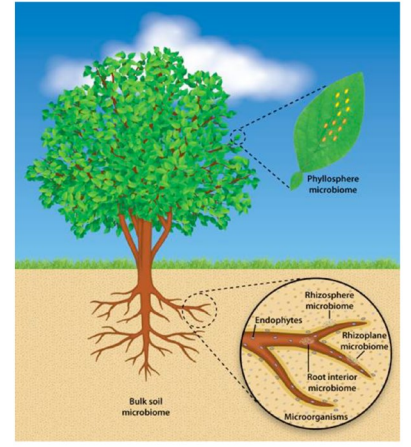
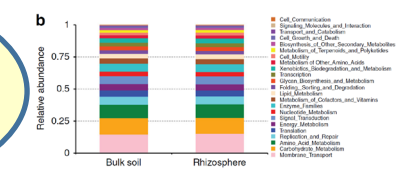
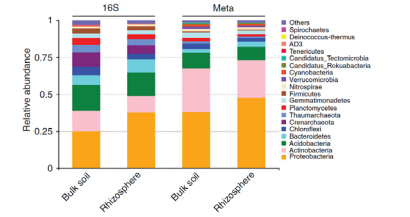
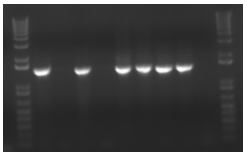
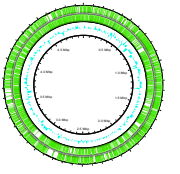
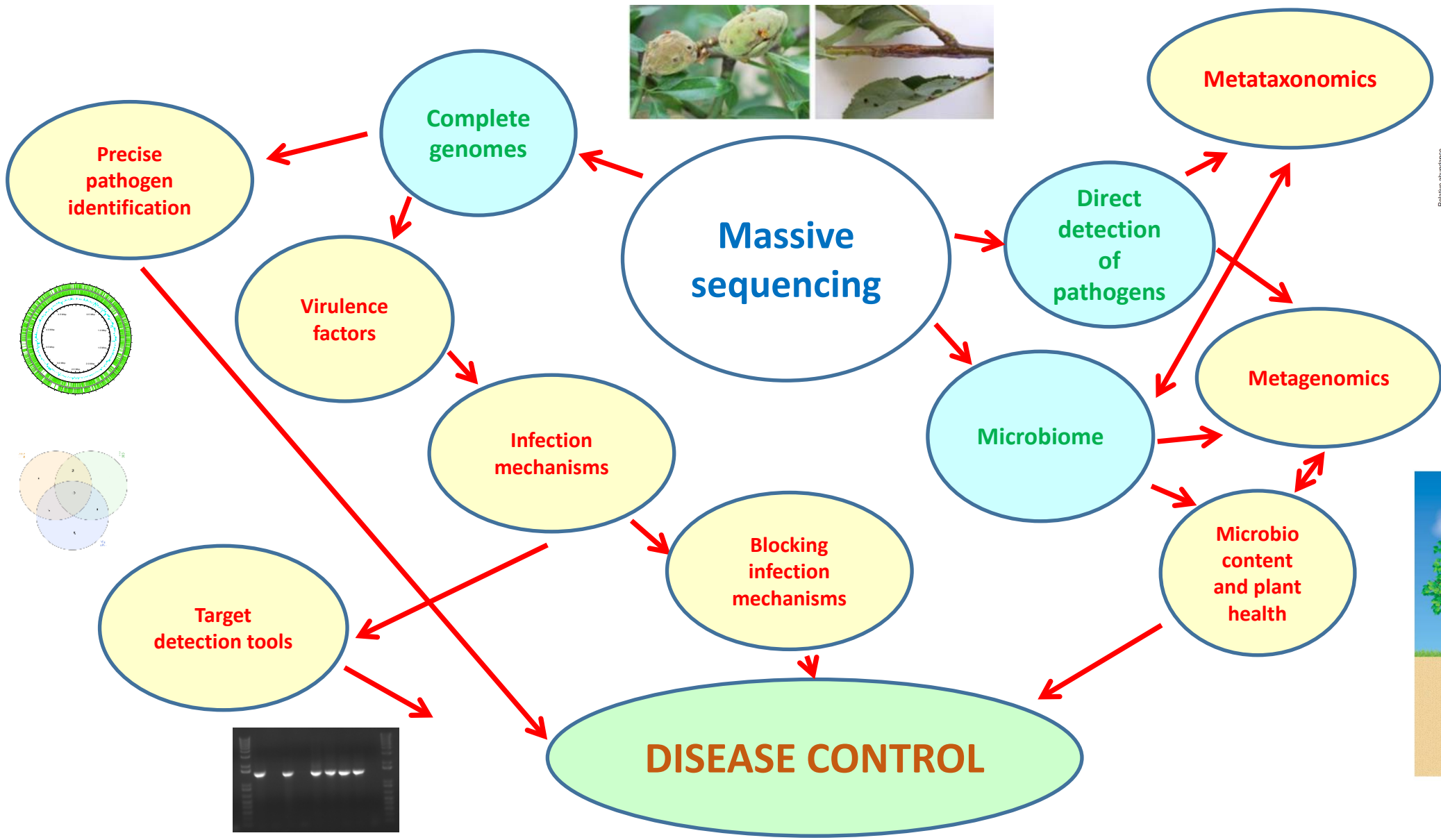


RESEARCH ARTICLE

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

Jinhui Wang¹, Minna Haapalainen¹, Thomas Schott², Sarah M. Thompson^{3,4}, Grant R. Smith^{3,5}, Anne I. Nissinen¹, Minna Puhonen¹

Summary of applications of massive sequencing in plant pathogenic bacteria



Some conclusions, reflections and takeaways

- Massive sequencing methods or NGS are nowadays an extremely valuable tool not only for research studies but also for applications directly related to disease control: precise pathogen identification, development of diagnostic methods...
- NGS offers significant advancements in understanding plant microbiota and diagnosing plant diseases

but... is it essential for diagnosis to determine all the viruses, bacteria, fungi, oomycetes, or viroids in a sample?

- Limitations related to data analysis, cost, sampling biases, microbial viability, and standardization is crucial for the broader adoption and effective utilization of HTS in plant disease diagnosis and management



Massive sequencing is a very valid tool but not there are different aspects to be considered...

Thanks for your attention

Bacteriology group at INIA/CSIC



 @XANTHERWOPj



Ralf Koebnik



Jerson-Garita Cambroner



Ana Palacio-Bielsa



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