

Insights into the genome of the De Donno strain of Xylella fastidiosa

Annalisa Giampetruzzi

PhD researcher













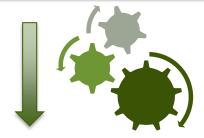
- 1) Whole-genome phylogeny based on single nucleotide polymorphisms (SNPs) and the study of the pan-genome of the 27 public available whole genome sequences of X. fastidiosa in 2016 (Giampetruzzi et al., 2017-Phytopatology)
- 2) Complete Genome Sequence of the Olive-Infecting Strain Xylella fastidiosa subsp. pauca De Donno (Giampetruzzi et al., 2017-Genome Announcement)
- 3) Updating of study of Whole-genome phylogeny(SNPs) and of the pan-genome of the 40 currently public available whole genome sequences of X.fastidiosa including Xylella fastidiosa subsp.pauca De Donno (unpublished)

Whole-genome phylogeny based on single nucleotide polymorphisms (SNPs)

TABLE 1. List and general features of Xylella fastidiosa strains used in the study*

Collection Accession number Plasmids accession

In 2016, 27 *X. fastidiosa* genomes were available in the NCBI genomes database



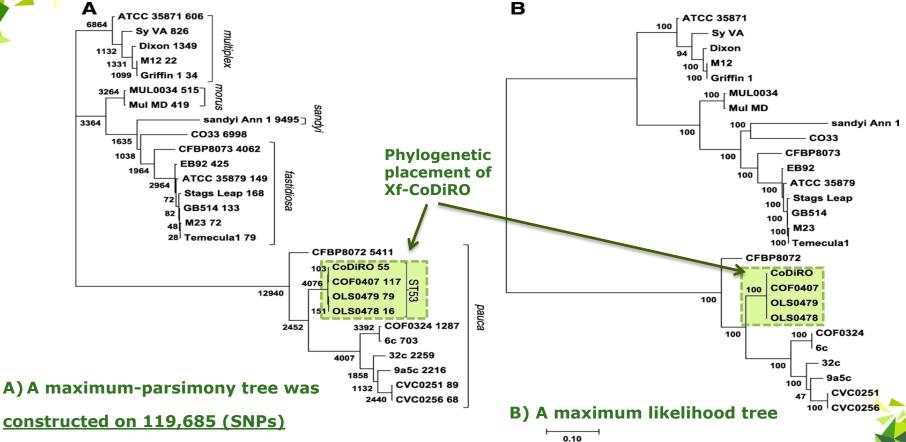
kSNP 3.02 software,

an alignment-free sequence analysis tool, it identifies the pan-genome SNPs in a set of genome sequences, and estimates phylogenetic trees based upon those SNPs (Gardner et al., 2015)

| Strain | Host plant | Origin | Collection date | Size (Mb)/CDS | GC% | Accession number (chromosomes/WGS) | Plasmids accession number | Citation or release date | | |
|-------------------|-----------------------------|----------------------------------------------------------------------|-----------------|---------------------------------------|----------------|---------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------|--|--|
| 0a5c | Citrus sinensis | São Paulo, Brazil | 1992 | 2.73175/2,982 | 52.64 | NC002488.3 | pXF1.3: NC002489.3; pXF51: NC002490.1 | Simpson et al. 2000 | | |
| Dixon | Prunus dulcis | California, USA | na | 2.62233/2,914 | 52 | AAAL02 | pxF51. NC002490.1 | 7/10/2002 | | |
| Temecula 1 | | | 1998 | | 51.80 | NC004556.1 | pXFPD1.3: | | | |
| remecuiar | Vitis vinifera | California, USA | 1998 | 2.52115/2,757 | 51.80 | NC004556.1 | | Van Sluys et al. | | |
| 11202 | 22 12 2002-1 | manuación an lacard | 0/2002/2012 | 21022222222 | | 202222222 | NC004554.1 | 2003 | | |
| M12 | P. dulcis | California, USA | 2003 | 2.47513/2,706 | 51.9 | NC010513.1 | received the transfer of the second | Chen et al. 201 | | |
| M23 | P. dulcis | California, USA | 2003 | 2.57399/2,803 | 51.76 | NC010577.1 | pXFAS01: NC010579.1 | Chen et al. 201 | | |
| GB514 | Vitis vinifera | Texas, USA | na | 2.51738/2,835 | 51.77 | NC017562.1 | Unnamed: NC017561.1 | 9/23/2010 | | |
| EB92.1 | Sambuccus nigra | USA | 1992 | 2.47543/2,750 | 51.5 | AFDJ01 | - 2 | Zhang et al. 20 | | |
| ATCC 35871 | P. salicina | Georgia, USA | na | 2.41626/2,676 | 51.7 | AUAJ01 | <u> </u> | 7/15/2013 | | |
| Griffin-1 | Quercus rubra | Georgia, USA | 2006 | 2.38731/2,724 | 51.7 | AVGA01 | 52 4 | Chen et al. 201 | | |
| 32c | Coffea sp. | São Paulo, Brazil | na | 2.60755/2,915 | 52.5 | AWYH01 | - 0 | Alencar et al. 20 | | |
| ic | Coffea sp. | São Paulo, Brazil | na | 2.606/2,916 | 52.35 | AXBS01 | pxF6c: | Alencar et al. 20 | | |
| | COMPAN OF | | | TOWNS CHOOS | | | NZAXBS01000046.1 | SCHOOL COST CONTRACTOR | | |
| Mul-MD | Morus alba | Maryland, USA | 2011 | 2.52055/2,838 | 51.6 | AXDP01 | _ | Guan et al. 201 | | |
| Ann-1 | Nerium oleander | USA | na | 2.78091/3,162 | 52.07 | NZCP006696.1 | Unnamed1: | 6/6/2014 | | |
| | | | | Company of the Company of the Company | | | NZCP006697.1 | | | |
| MUL0034 | M. alba | USA | na | 2.66658/2,974 | 51.97 | NZCP006740.1 | Unnamed2: NZCP006739.1 | 6/6/2014 | | |
| Sycamore Sy-VA | Platanus occidentalis | Virginia, USA | 2002 | 2.47588/2,758 | 51.6 | JMHP01 | 5 5 | Guan et al. 201 | | |
| ATCC 35879 | Vitis vinifera | Florida, USA | 1987 | 2.52233/2758 | 51.8 | JQAP01 | <u></u> | 10/21/2014 | | |
| CoDiRO | Olea europaea | Italy | 2013 | 2.54293/2,816 | 51.96 | JUJW01 | Unnamed: | Giampetruzzi | | |
| | (Vinca minor) | | | | | | NZCM003178.1 | et al. 2015a | | |
| 2033 | Coffea arabica | Costa Rica | 2015 | 2.68193 | 51.7 | LJZW01 | ₩. | Giampetruzzi et al. 2015b | | |
| CFBP8072 | Coffea arabica | Ecuador | 2012 | 2.49666/2,763 | 51.9 | LKDK01 | | Jacques et al. 20 | | |
| CFBP8073 | Coffea canephora | Mexico | 2012 | 2.58215/2,840 | 51.6 | LKES01 | 200 | Jacques et al. 20 | | |
| DLS0479 | Nerium oleander | Costa Rica, San | 2012 | 2.53996/2,827 | 51.93 | LRVH01 | pXF-P1.COF0407: | 2/5/2016 | | |
| JL304/9 | Nerum oleunder | Jose Province | 2011 | 2.33990/2,027 | 31.93 | LKVHOI | NZCM003743.1; | 2/3/2010 | | |
| CVC0256 | Citrus sinensis | São Paulo, Brazil | 1999 | 2.70214/3,045 | 52.51 | LRVF01 | pXF-P4.COF0407: NZCM003744.1; pXF-P5.COF0407: NZCM003745.1; pXF-RC.COF0407: NZCM003746.1 pXF-BHR.CVC0256: NZCM003749.1; pXF-P1.CVC0256: NZCM003749.1; pXF-P4.CVC0256: | 2/5/2016 | | |
| DLS0478 | Nerium oleander Coffea sp. | Costa Rica, San Jose Province Costa Rica, San Jose Province | 2011 | 2.55541/2,861 2.53847/2,837 | 51.91 51.84 | LRVI01 | NZCM003750.1; pXF-Ps. CVC0256; NZCM003751.1 pXF-P1.0LS0478; NZCM003752.1; pXF-P4.0LS0478; NZCM003753.1 pXF-P1.0LS0479; NZCM003762.1; pXF-P4.0LS0479; NZCM003763.1; pXF-PS.0LS0479; NZCM003764.1; pXF-PS.0LS0479; | 2/5/2016 | | |
| Stag's Leap | Vitis vinifera | California, USA | 2005 | 2.5108/2,755 | 51.7 | LSMJ01 | NZCM003765.1 | Chen et al. 201 | | |

SNP analysis of 27 X. fastidiosa genomes

Genetic relationships among ST53 isolates and Xylella fastidiosa subspecies



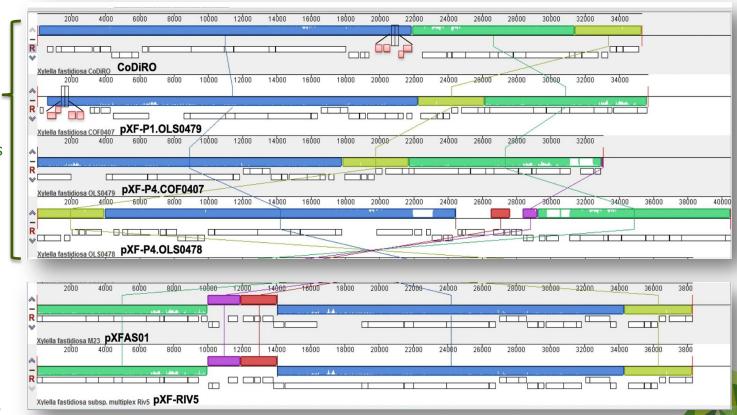
Genomic elements characterizing CoDiRO and Costa Rican isolates: a conjugative plasmid (35kb)

Progressive Mauve multiple alignment of plasmids

The four ST53 isolates

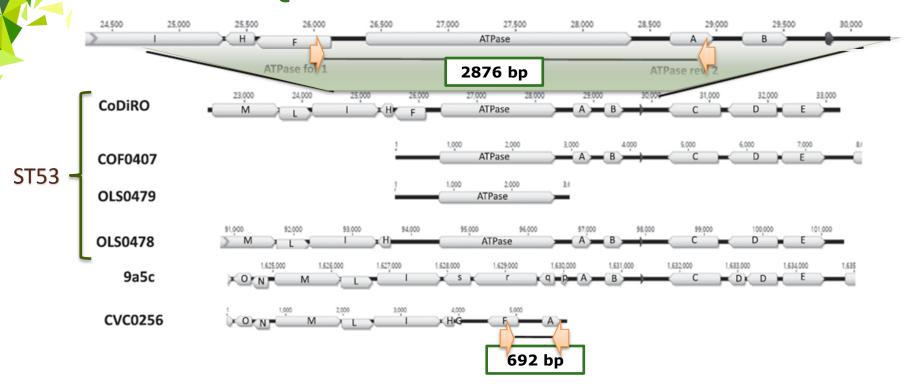
The ParD/ParE containing sequence was additionally present in CoDiRO and pXF.P1.OLS0479 (Xf strain COF0407) and indicated by pink boxes.

The same type of conjugative plasmid (35kb) shared by the four ST53 isolates was already described in the *Xf* multiplex strain: **pXFAS01, and pXF-RIV5**.



PANGENOME ANALYSIS OF XYLELLA FASTIDIOSA (GET_HOMOLOGUES software) Contreras-Moreira B et al.,2013 Pan-genome tree; gower dist. Temecula1 ATCC 35879 Stags Lear 889 754 6869 CFBP8073 MUL0034 Mul MD sandyi Ann ATCC_35871 Sy_VA Griffin 1 total clusters = 6.869 cloud shell number of gene clusters soft core core 7:FBP8072 OLS0479 COF0407 CODIMO OLS0478 COF0324 6 6 9a56 9a56 32c CVC0255 M112 Dixon Griffin_1 200 2668 cloud in 1-2 genomes Heatmap and dendrogram 2680 shell in 3-24 genomes obtained from the average 1521 soft core in 25-26 genomes nucleotide identity matrix based 1269 core in all 27 genomes number of genomes in clusters (occupancy) on the CDSs composition European Conference on Xylella fastidiosa 2017: finding answers to a global problem

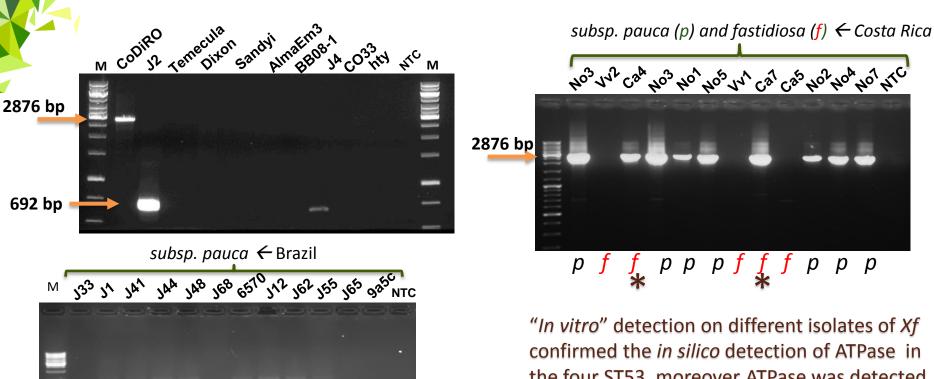
GENES UNIQUE TO THE ST53 CLADE



In silico identification of a gene encoding a putative histidine kinase-like ATPase,



VITRO" DETECTION OF GENE CODING A PUTATIVE ATPase



692bp

European Conference on Xylella fastidiosa 2017: finding answers to a global problem

confirmed the *in silico* detection of ATPase in the four ST53, moreover ATPase was detected in two (*) ST21 isolates (coffee plants) of the subspecies fastidiosa from Costa Rica

1) CONCLUSIONS

- Maximum-parsimony and maximum likelihood trees constructed using the SNPs and the pan-genome data distinguished the subsp. fastidiosa, multiplex, pauca, sandyi, and morus and groups the Italian and three Costa Rican ST53 isolates in a compact clade that diverges from the South American pauca isolates.
- ➤ Gene encoding a putative histidine kinase-like **ATPase was identified** only in the clade of **the Italian and three Costa Rican ST53 isolates**, and it will be further characterized ...
- ➤The work described, represented a great opportunity of exchanging experiences, materials and methods among institutions of different countries involved in the EU projects,

Phytopathology • 2017 • 107:816-827 • http://dx.doi.org/10.1094/PHYTO-12-16-0420-R

Bacteriology

e-Xtra*

Genome-Wide Analysis Provides Evidence on the Genetic Relatedness of the Emergent *Xylella fastidiosa* Genotype in Italy to Isolates from Central America

Annalisa Giampetruzzi, Maria Saponari, Giuliana Loconsole, Donato Boscia, Vito Nicola Savino, Rodrigo P. P. Almeida, Stefania Zicca, Blanca B. Landa, Carlos Chacón-Diaz, and Pasquale Saldarelli

DISSPA-UNIBA
CNR-IPSP Bari
University of California, Berkeley
CSIC-Córdoba, Spain
CIET, Universidad de Costa Rica

TOPICS OF PRESENTATION

- 1) Whole-genome phylogeny based on single nucleotide polymorphisms (SNPs) and the study of the pan-genome of the 27 public available whole genome sequences of X. fastidiosa in 2016 (Giampetruzzi et al., 2017-Phytopatology)
- 2) Complete Genome Sequence of the Olive-Infecting Strain Xylella fastidiosa subsp. pauca De Donno (Giampetruzzi et al., 2017-Genome Announcement)
- 3) Updating of study of Whole-genome phylogeny(SNPs) and of the pan-genome of the 40 currently public available whole genome sequences of *X.fastidiosa* including *Xylella fastidiosa* subsp.pauca De Donno (unpublished data)

January 2014

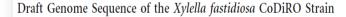
"De Donno" olive tree (40.011389 N 18.048056 E)

Isolate from the De Donno tree was used for the assembling of the first version of the **Draft** Genome released in the 2015:









Annalisa Giampetruzzi,^a Michela Chiumenti,^a Maria Saponari,^a Giacinto Donvito,^b Alessandro Italiano,^b Giuliana Loconsole,^a Donato Boscia,^a Corrado Cariddi,^c Giovanni Paolo Martelli,^c Pasquale Saldarelli^a

Institute for Sustainable Plant Protection, National Research Council (CNR), Bari, Italy^a, Department of Physics, University of Bari Aldo Moro, Bari, Italy^a, Department of Soil, Plant and Food Sciences. University of Bari Aldo Moro, Bari, Italy^a, Department of Soil,

A.G. and M.C. contributed equally to this work

l∐gen⊕me∠

Journals.ASM.org

We determined the draft genome sequence of the Xylella fastidiosa CoDiRO strain, which has been isolated from olive plants in southern Italy (Apulia). It is associated with olive quick decline syndrome (OQDS) and characterized by extensive scorching and desiccation of leaves and twigs.



Complete Genome Sequence of the Olive-Infecting Strain *Xylella fastidiosa* subsp. *pauca* De Donno

Annalisa Giampetruzzi,^a Maria Saponari,^b Rodrigo P. P. Almeida,^c Salwa Essakhi,^b Donato Boscia,^b Giuliana Loconsole,^a Pasquale Saldarelli,^b

Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Bari, Italy^a; Institute for Sustainable Plant Protection, National Research Council (CNR), Bari, Italy^a; Department of Environmental Science, Policy and Management, University of California, Berkeley, Berkeley, California, USA^c





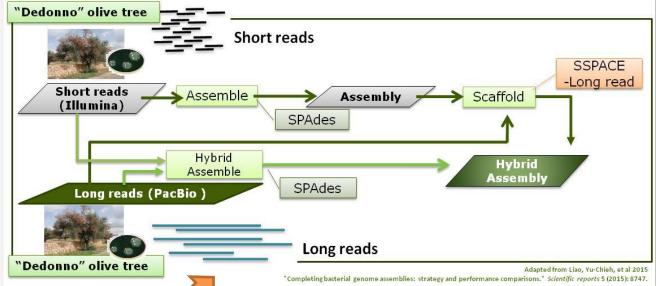
Vincent J. Coates Genomics Sequencing Laboratory at the University of California (UC), Berkeley



HiSeq 4000 Illumina platform and PacBio RSII platform

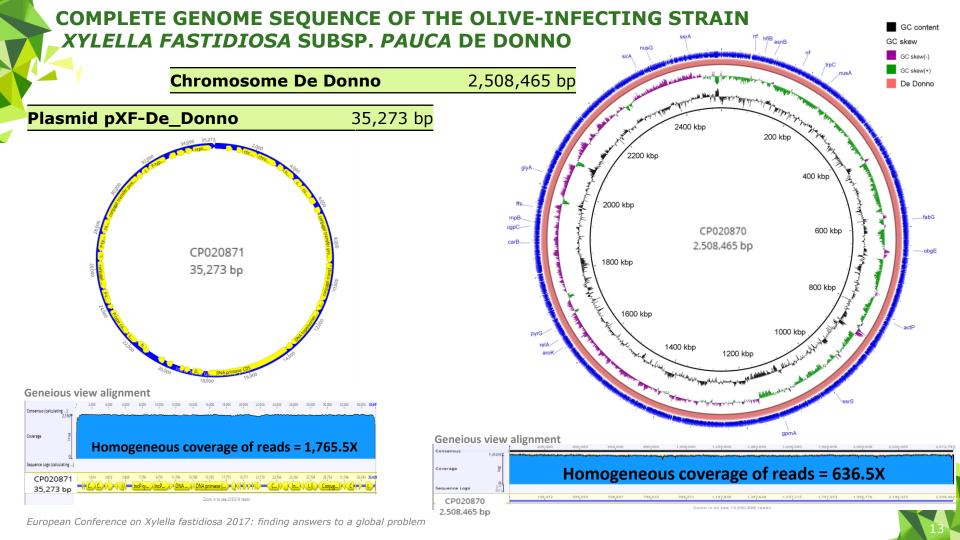
5,700,601 (2 × 150-bp) **fastq paired reads**

ASSEMBLY STRATEGIES

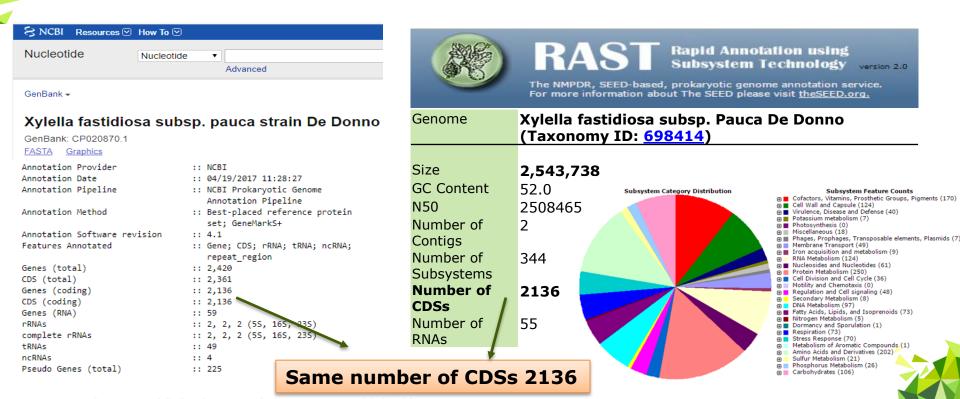


105,585 fastq reads, mean length of 8,527 bp (longest read, 56,602 bp)





PROKARYOTIC GENOME ANNOTATION: PGAP and RAST



FRAMESHIFTS IN HEMAGGLUTININ CDSs

Dal Donno chromosome

Frameshifted hemoagglutinin-like genes

2.508.46 200.000 400.000 600,000 800.000 1.000.000 1,200,000 1.400.000 1.600.000 1.800.000 2.000.000 2,200,000 B9J09_03955 B9J09 10079 B9J09_11875 AR069663.1 B9J09_03950 hemagglutinin hypothetical protein Name: hemagglutinin CDS Name: hemagglutinin CDS Name: hemagglutinin CDS 3) Length: 10,195

PGAP annotation description by NCBI

1) Length: 8,518

Interval: 864,138 -> 872,655 locus tag: B9J09 03935 similar to AA sequence: RefSeq:WP 010895189.1

note: frameshifted; internal stop; incomplete; partial on complete genome; missing stop, pseudo:

"Frameshifts in hemoagglutinin-like genes may impair their functions making the bactera more motile and virulent (Pierry, PM et al 2012; MR Guilhabert and BC Kirkpatrick 2005)

2) Length: 10,024

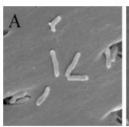
Interval: 2,079,763 -> 2,069,740

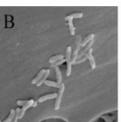
locus tag: B9J09 10075 similar to AA sequence: RefSeq:WP 012382593.1

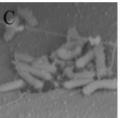
note: frameshifted; pseudo:

Interval: 2,500,920 -> 2,490,726

locus tag: B9J09 11875 similar to AA sequence: RefSeq:WP 010895189.1 note: **frameshifted**; pseudo:







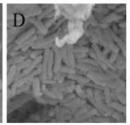


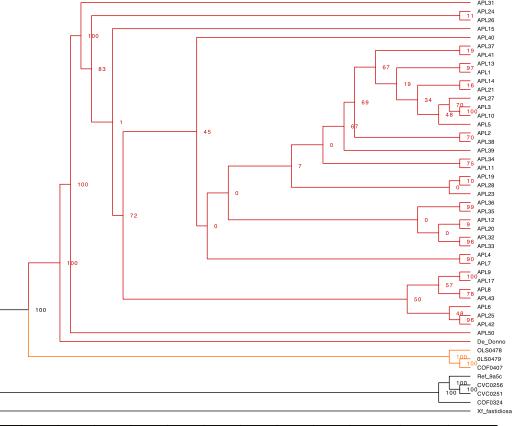
Fig. 5. Model of possible mechanisms involved in Xylella fastidiosa adhesion to xylem vessels of grapevines. A, X, fastidiosa bacteria attach to the surface, probably using fimbrial and nonfimbrial adhesins other than FimA, FimF, and hemagglutinins (this study, Feil et al. 2003); B, HxfA, HxfB, and other adhesins mediate secondary contact between X. fastidiosa cells, which leads to C, microcolony formation. Based on our results, hemagglutinins appear to be important mediators for cell-cell aggregation. D, Bacterial cells finally aggregate to each other via hemagglutinins HxfA and HxfB, fimbriae, and exopolysaccharides to form matured biofilms within the xylem vessels (this study, Feil et al. 2003).

CONCLUSIONS

Complete genome of the Xf strain De Donno was been used as a reference for the typing by WGS (SNP based) of all 40 isolates that were sampled in the outbreak of Apulia (NGS task Bari/Berkeley)

Genetic similarity among Apulian isolates

Number of SNPs in comparison to the Apulian strain De Donno:



| Isolates | APL1 | APL2 | APL3 | APL4 | APL5 | APL6 | APL7 | APL8 | APL9 | APL10 | APL11 | APL12 | APL13 | APL14 | APL15 | APL17 | APL19 | APL20 | APL21 | APL23 | APL24 |
|----------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|---------|---------|---------|
| Number of SNPs | 135 | 144 | 158 | 141 | 146 | 148 | 157 | 144 | 133 | 160 | 152 | 159 | 135 | 136 | 131 | 141 | 151 | 144 | 150 | 144 | 139 |
| Isolates | APL25 | APL26 | APL27 | APL28 | APL31 | APL32 | APL33 | APL34 | APL35 | APL36 | APL37 | APL38 | APL39 | APL40 | APL41 | APL42 | APL43 | APL50 | COF0407 | OLS0478 | OLS0479 |
| Number of SNPs | 134 | 143 | 157 | 157 | 142 | 142 | 142 | 136 | 146 | 141 | 145 | 145 | 156 | 152 | 145 | 141 | 146 | 126 | 172 | 192 | 155 |

TOPICS OF PRESENTATION

- 1) Whole-genome phylogeny based on single nucleotide polymorphisms (SNPs) and the study of the pan-genome of the 27 public available whole genome sequences of X. fastidiosa in 2016 (Giampetruzzi et al., 2017-Phytopatology)
- 2) Complete Genome Sequence of the Olive-Infecting Strain Xylella fastidiosa subsp. pauca De Donno (Giampetruzzi et al., 2017-Genome Announcement)
- Updating of study of Whole-genome phylogeny(SNPs) and of the pan-genome of the 40 currently public available whole genome sequences of X.fastidiosa including Xylella fastidiosa subsp.pauca De Donno (unpublished data)

Updating of Whole-genome phylogeny based on SNPs and of pangenome analysis of Xf

In 2017, 40 *X.fastidiosa* genomes becomes available in the NCBI genome database



kSNP

https://www.ncbi.nlm.nih.gov/genome/genomes/173





GET_HOMOLOGUES

27 genomes

(Giampetruzzi et al 2017):

COF0324 9a5c

Temecula1

M12

M23

EB92.1

Dixon

ATCC 35871 32

Ann-1

MUL0034

Mul-MD

sycamore Sy-VA ATCC 35879

CoDiRO

CO33

OLS0479

CVC0256 OLS0478

OLS0478 CVC0251

COF0407

Stag's Leap

CFBP8072 CFBP8073

6c

Griffin-1 GB514 13 Genomes available

In 2017

BB01

11399

DSM 10026

CFBP8418 CFBP8417

CFBP8416

Fb7

3124

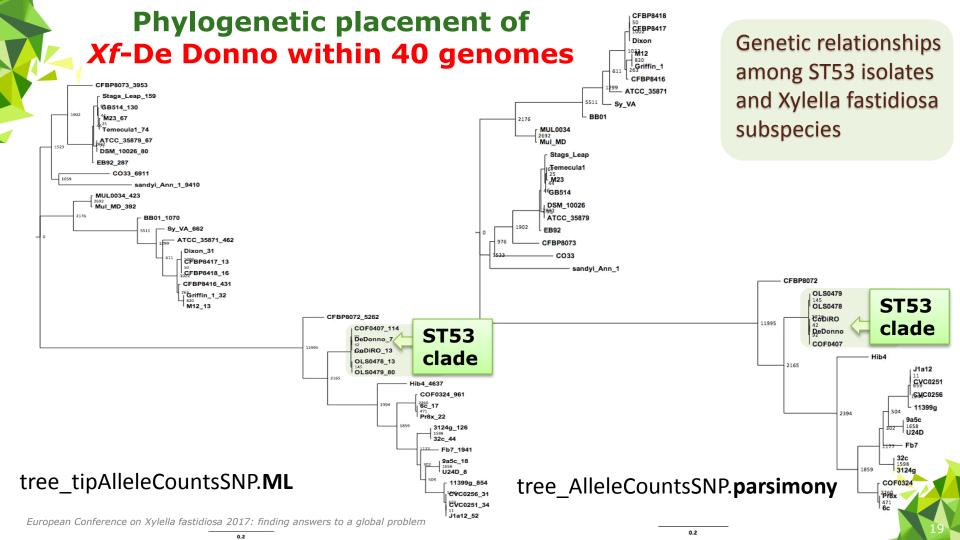
J1a12

U24D

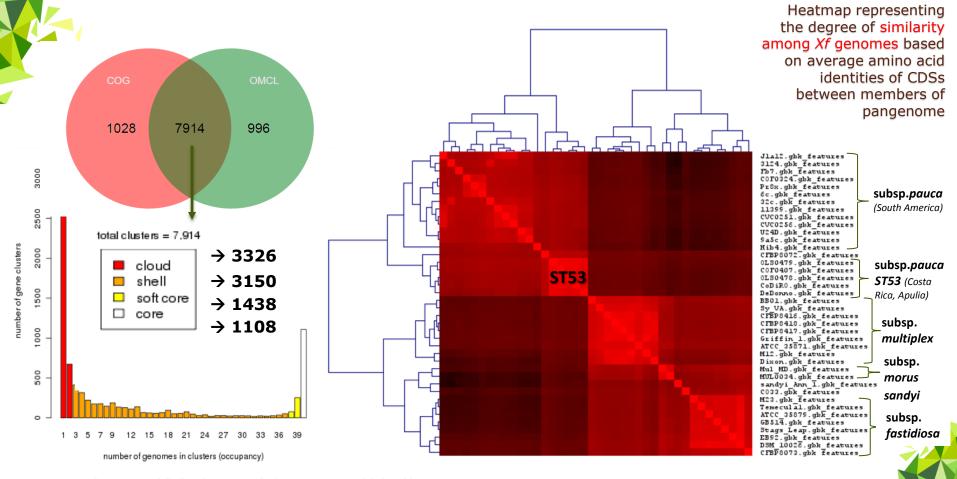
Pr8x Hib4

<u>De Donno</u>





Updating of PANGENOME ANALYSIS OF XYLELLA FASTIDIOSA



3) CONCLUSIONS

- The results of the updating of whole-genome phylogenies based on SNPs and of pangenome analysis of Xf genomes confirmed the **phylogenetic placement of "De Donno"** and the ST53 clade previously published.
- Gene encoding a putative histidine kinase-like ATPase, identified only in the clade of the Italian and three Costa Rican ST53 isolates, still remains unique to that clade.



Thanks to

CSIC

Blanca



CARLOS CHACÓN DIAZ













Di.S.S.P.A.

Prof.Vito Nicola Savino Giuliana Loconsole













Shana McDevitt **UC** Berkeley (QB3-UCB)

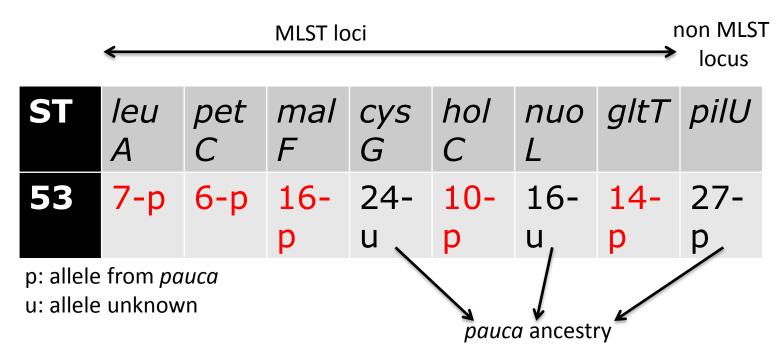


Add slides





Xylella fastidiosa from olive is a ST53 phylotype



Nunney et al. 2014. The complex biogeography of the plant pathogen *Xylella fastidiosa*: genetic evidence of introductions and subspecific introgression in Central America. PlosONE

Loconsole et al. 2016. Intercepted isolates of *Xylella fastidiosa* in Europe reveal novel genetic diversity. Eur. J. Plant Path.

Figure 6. Giampetruzzi Phytopathology

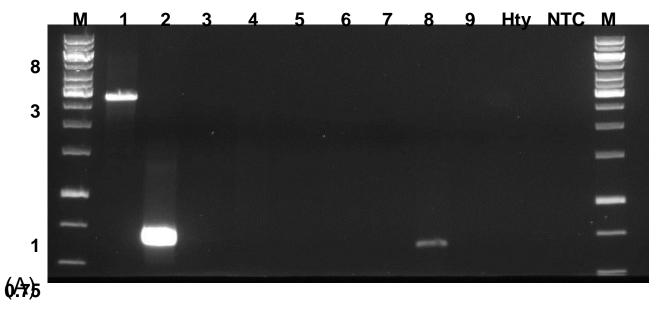


Fig. 6. Gel electrophoresis showing the products of PCR amplications of different *X. fastidiosa* subspeces with primers ATPase for 1 / ATPase rev2. Amplifications were from bacterial DNA of isolates: (A) CoDiRO (lane 1), J2 (lane 2), Temecula (lane 3), Dixon (lane 4), Sandyi (lane 5), AlmaEm3 (lane 6), BB08-1 (lane 7), J4 (lane 8); CO33 (lane 9), total DNA from xylem of healthy olive tree (lane Hty) and no template control (lane NTC); Generuler 1kb DNA ladder, Thermo Scientific (lane M). (B): No3 (lane 1), Vv2 (lane 2), Ca4 (lane 3), No3 (lane 4), No1 (lane 5), No5 (lane 6), Vv1 (lane 7), Ca7 (lane 8), Ca5 (lane 9), No2 (lane 10), No4 (lane 11), No7 (lane 12) and no template control (lane NTC); O'Gene-rulerTM DNA ladder mix, Fermentas, St Leon-Rot, Germany (lane M).(C): (C): J33 (lane 1), J1 (lane 2), J41 (lane 3), J44 (lane 4), J48 (lane 5), J68 (lane 6), 6570 (lane 7), J12 (lane 8), J62 (lane 9), J55 (lane 10), J65 (lane 11), 9a5c (lane 12) and no template control (lane NTC). Relevant bands of the Generuler 1kb DNA ladder (lane M; Thermo Scientific) are reported.

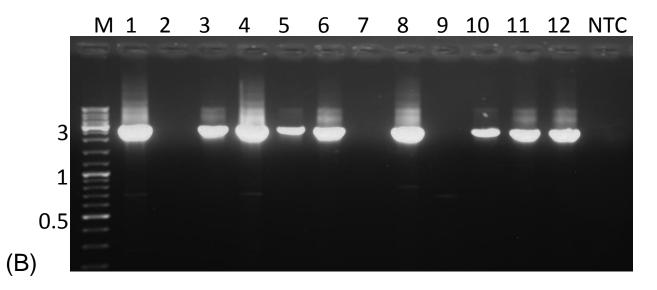
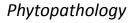


Fig. 6. Gel electrophoresis showing the products of PCR amplications of different *X. fastidiosa* subspeces with primers ATPase for 1 / ATPase rev2. Amplifications were from bacterial DNA of isolates: (A) CoDiRO (lane 1), J2 (lane 2), Temecula (lane 3), Dixon (lane 4), Sandyi (lane 5), AlmaEm3 (lane 6), BB08-1 (lane 7), J4 (lane 8); CO33 (lane 9), total DNA from xylem of healthy olive tree (lane Hty) and no template control (lane NTC); Generuler 1kb DNA ladder, Thermo Scientific (lane M). (B): No3 (lane 1), Vv2 (lane 2), Ca4 (lane 3), No3 (lane 4), No1 (lane 5), No5 (lane 6), Vv1 (lane 7), Ca7 (lane 8), Ca5 (lane 9), No2 (lane 10), No4 (lane 11), No7 (lane 12) and no template control (lane NTC); O'Gene-rulerTM DNA ladder mix, Fermentas, St Leon-Rot, Germany (lane M).(C): (C): J33 (lane 1), J1 (lane 2), J41 (lane 3), J44 (lane 4), J48 (lane 5), J68 (lane 6), 6570 (lane 7), J12 (lane 8), J62 (lane 9), J55 (lane 10), J65 (lane 11), 9a5c (lane 12) and no template control (lane NTC). Relevant bands of the Generuler 1kb DNA ladder (lane M; Thermo Scientific) are reported.



J33

Figure 6. Giampetruzzi

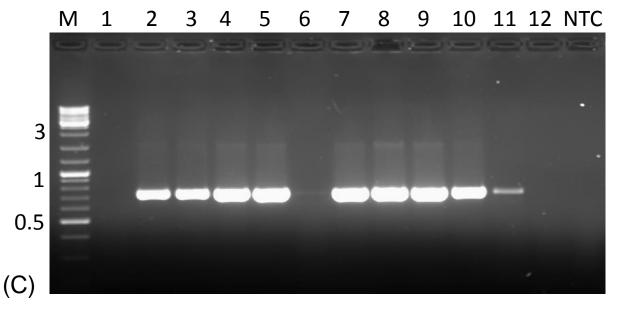


Fig. 6. Gel electrophoresis showing the products of PCR amplications of different *X. fastidiosa* subspeces with primers ATPase for 1 / ATPase rev2. Amplifications were from bacterial DNA of isolates: (A) CoDiRO (lane 1), J2 (lane 2), Temecula (lane 3), Dixon (lane 4), Sandyi (lane 5), AlmaEm3 (lane 6), BB08-1 (lane 7), J4 (lane 8); CO33 (lane 9), total DNA from xylem of healthy olive tree (lane Hty) and no template control (lane NTC); Generuler 1kb DNA ladder, Thermo Scientific (lane M). (B): No3 (lane 1), Vv2 (lane 2), Ca4 (lane 3), No3 (lane 4), No1 (lane 5), No5 (lane 6), Vv1 (lane 7), Ca7 (lane 8), Ca5 (lane 9), No2 (lane 10), No4 (lane 11), No7 (lane 12) and no template control (lane NTC); O'Gene-rulerTM DNA ladder mix, Fermentas, St Leon-Rot, Germany (lane M).(C): (C): J33 (lane 1), J1 (lane 2), J41 (lane 3), J44 (lane 4), J48 (lane 5), J68 (lane 6), 6570 (lane 7), J12 (lane 8), J62 (lane 9), J55 (lane 10), J65 (lane 11), 9a5c (lane 12) and no template control (lane NTC). Relevant bands of the Generuler 1kb DNA ladder (lane M; Thermo Scientific) are reported.

pm_pierry@msn.comKeywords: Phytopathogen, Xylella fastidiosa, Leaf Scald, Pyrosequencing, Comparative genomics. Xylella fastidiosa is a non-flagellated Gram-negative bacterium, of the Gamma-proteobacteria subgroup, that colonizes the xylem of many cultivated and wild plants, eventually causing economically important diseases. Genomes of X. fastidiosa strains isolated from different hosts have been completely or partially sequenced and, comparative genomics studies associated with functional genomics and molecular genetics approaches have enabled detailed studies of mechanisms potentially relevant to disease development. Except for strain 9a5c, all other publicly available genome sequences are from strains isolated in North America. Here we describe the genome pyrosequencing of strains Pr8x and Hib4 isolated, respectively, from plum and hibiscus. Global nucleotide sequence comparison show extensive collinearity between both genomes, greater than with strain 9a5c. Strain Pr8x has a main chromosome of 2,666,242 bp and one plasmid, pXF39, of 39,580 bp which presents similarities with pXF51 from the reference strain 9a5c. Hib4 shows the largest chromosome (2,813,297 bp) and the largest plasmid (pXF64 with 64,251 bp) described for X. fastidiosa. pXF64 shows extensive similarity with plasmid pBVIE04 of Burkholderia vietnamensis G4 strain and is described for the first time in X. fastidiosa. This plasmid presents all repertoire for conjugal transfer, besides hypothetical proteins. Comparative analyzes of these genomes have identified several differences that may be correlated with the phenotypes displayed by these strains. Both genomes have shown some shared differences when compared to the reference genome from strain 9a5c. Two absent CDS were observed, coding for an arginine deaminase and HicB protein. The Pr8x genome showed other absent CDS: a toxin/antitoxin system (HigB/HigA) and a cluster of CDS related to conjugal transfer proteins (trb genes). Other CDS have shown deletions and polymorphisms that may impair their functions in both genomes, like "hemagglutinin-like" proteins, a surface protein and hemolysin-like proteins. For Pr8x, additional CDS, coding for PilY1 and PilQ are discrepant, as well as a fimbrial adhesin precursor and ChpA in Hib4 genome. Finally, we highlight that in both genomes the CDS coding for a polygalacturonase precursor was found intact, without the nucleotide insertion observed in other South-American strains, which would generate a truncated protein; thus, this important enzyme must be functional in these strains, as observed in North-American strains. Therefore, genome sequencing of these Xylella strains isolated from different plant hosts brings new information that might explain host specificity and disease development. Financial Support: CNPq, FINEP and FAPESP.



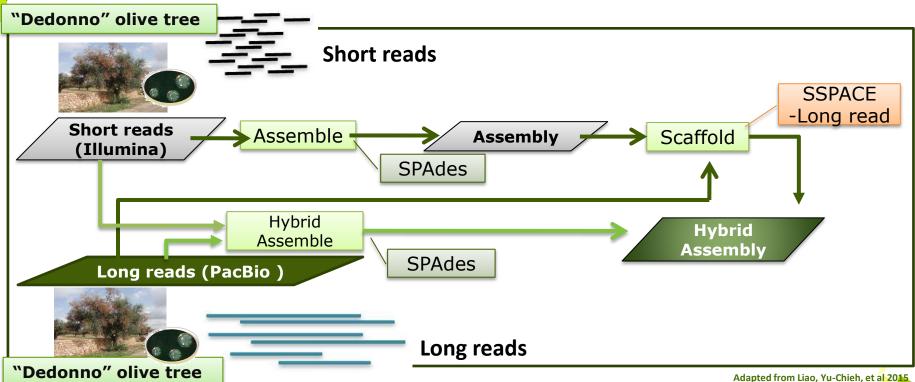
PROPHAGE REGIONS ANNOTATION



Total: 7 prophage regions have been identified, of which 5 regions are intact, 1 regions are incomplete, and 1 regions are questionable.

| Region | Region Length | Length Completeness Score # | | | Region Position | Most Common Phage | GC % | Details | |
|--------|-----------------------------------------------------------|-----------------------------|-----|---------|--------------------------------|-----------------------------------|--------|---------|--|
| | | | | De_Donn | o Xylella fastidiosa subsp. pa | auca | | | |
| 1 | 17.7Kb | incomplete | 60 | 19 | 925482-943270 ① | PHAGE_Cyanop_S_RIM50_NC_031242(6) | 58.72% | Show ① | |
| 2 | 5.9Kb | intact | 107 | 8 | 1130312-1136279 ① | PHAGE_Stenot_phiSMA9_NC_007189(5) | 44.44% | Show @ | |
| 3 | 29Kb | intact | 128 | 38 | 1135598-1164639 ① | PHAGE_Stenot_phiSMA7_NC_021569(6) | 43.70% | Show @ | |
| 4 | 48.4Kb | intact | 150 | 51 | 1176535-1225033 ① | PHAGE_Xylell_Xfas53_NC_013599(32) | 54.68% | Show © | |
| 5 | 47.9Kb | intact | 100 | 69 | 1289895-1337865 ① | PHAGE_Pseudo_JBD69_NC_030908(27) | 55.29% | Show @ | |
| 6 | 60.6Kb | intact | 110 | 56 | 1365616-1426305 ① | PHAGE_Pseudo_NP1_NC_031058(25) | 54.19% | Show © | |
| 7 | 9Kb | questionable | 85 | 13 | 1734875-1743918 ① | PHAGE_Stenot_phiSMA7_NC_021569(4) | 43.51% | Show ① | |
| Questi | (score > 90) ionable (score 70-9 plete (score < 70) | 0) | | | | | | | |
| | | | | 1 | 23 4 5 6 | 7 | | | |
| | | | | 9236 | 7 23W | 1738/Z | | | |

ASSEMBLY STRATEGIES



"Completing bacterial genome assemblies: strategy and performance comparisons." Scientific reports 5 (2015): 8747