OnLine Journal of Biological Sciences

Original Research Paper

Comparative Genomics of *Xylella fastidiosa* Subsp. Pauca and Insights on its Current and Potential Movements in Ecuador

[1]Carlos Bolanos-Carriel, [2]Eliana Granja Guerra, [3]Jaris Veneros, [3]Grobert A. Guadalupe and [3,4]Ligia García

[1]Facultad de Ciencias Agrícolas, Laboratorio de Microbiología y Fitopatología, Universidad Central del Ecuador, Av. Unidad Educativa, Tumbaco, Pichincha, Ecuador
[2]Facultad de Ciencias Agropecuarias y Recursos Naturales, Universidad Técnica del Cotopaxi, Barrio Salache S/N, Latacunga, Ecuador
[3]Instituto de Investigación Para el Desarrollo Sustentable de Ceja de Selva (INDES-CES), Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas, 342 Higos Urco, Chachapoyas, Perú
[4]Facultad de Ingeniería Zootecnista, Agronegocios y Biotecnología Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas, 342 Higos Urco, Chachapoyas, Perú

Article history
Received: 27-06-2024
Revised: 15-08-2024
Accepted: 28-08-2024

Corresponding Author:
Ligia García
Instituto de Investigación Para el Desarrollo Sustentable de Ceja de Selva (INDES-CES), Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas, 342 Higos Urco, Chachapoyas, Perú
Email: ligia.garcia@untrm.edu.pe

Abstract: *Xylella fastidiosa* subsp. Pauca causes Coffee Leaf Scorch (CLS). It is a major threat to coffee production in most South and Central American countries. *X. fastidiosa* is prone to intersubspecific recombination, and it is believed that this species became pathogenic in citrus and coffee due to recent mutation events and gene flow between closely related strains. CLS epidemics have not been reported in either Ecuador, Perú, or Colombia. In this research, we conducted a comparative genomic analysis of *X. fastidiosa* subsp. Pauca sequence type (ST-74) (strain CFBP 8072) isolated from Ecuadorian coffee trees. The phylogenetic analysis showed that the whole genome sequences clustered with strains *X. fastidiosa* subsp. Pauca 9a5c, and 3124 (ST-16) with an average nucleotide identity of above 98%. Pangenome analysis detected over 10% (1219/11989) genes in singleton families. There is a high risk of CLS outbreaks due to the potential replacement of *Xylella fastidiosa* subsp. Pauca strains with unknown aggressiveness in new environmental conditions. Anthropogenic movements of plant and seed material could lead to a substantial spread of a pathogen across a region, and inadvertent consequences are the threat of movement and establishment of a new pathogen, unpredictable yield loss, increase in the cost of production and re-planting, and the loss and abandonment of orchards.

Keywords: Coffee Leaf Scorch, Citrus Variegated Chlorosis, Olive Quick Decline, *Caribovia Coffeicola*, *Xylella fastidiosa*

Introduction

Coffee (*Coffea arabica* L. and *Coffea canephora* Pierre ex Froehner) is a crop of global economic importance, produced in over 80 (Melese and Kolech, 2021) and generating approximately 120 million jobs worldwide. With annual sales surpassing US 90 billion dollars, it is a crucial player in the global economy (Naik *et al*., 2021). Coffee is part of the botanical family *Rubiaceae* and its origin and cultivation can be traced back to southwestern Ethiopia, around 2500 years ago (Aga, 2005; Anthony *et al*., 2002; Melese and Kolech, 2021). The two main species in commercial cultivation are *Coffea arabica* (70% of the global planted area) and *Coffea canephora* (30% of the global planted area) (Bunn *et al*., 2015; Labouisse *et al*., 2008; Philippe *et al*., 2008). *C. canephora* is a diploid species, while *C. arabica* is tetraploid, however, both species originated from native undomesticated diploids (López-Gartner *et al*., 2009; Mindell, 2006).

Coffee Leaf Scorch (CLS) or "crespera" is caused primarily by the bacterium *Xylella fastidiosa* subsp. Pauca (Beretta, 1996; De Lima *et al*., 1998). This bacterium was first reported in Brazil in 1995 and has since been linked to outbreaks of CLS in South and Central America and the Caribbean basin (Rodríguez *et al*., 2001; Lacava *et al*., 2004; Li *et al*., 2001; Paradela-Filho *et al*., 1995). In Puerto Rico, symptoms of CLS have been associated with positive ELISA tests, as well as an abundance of hemipteran vectors (Brodbeck *et al*., 2017; Rico *et al*., 2015). Also, in Puerto Rico, the most likely potential vector of *X. fastidiosa* was *Caribovia coffee cola* Dozier (Rico *et al*., 2015). In

practice, it has been present in North and South America for a century and was previously restricted to that region of the world. However, despite quarantine measures, it has been spreading in Europe around the Mediterranean (Toussaint and Piñeiro, 2024). Up to date, CLS has not been reported in Ecuador or in Colombia. In Colombia, the third-largest producer in the world, coffee has major economic and social importance. In particular, the case of *Xylella fastidiosa* is of concern as it is considered one of the most dangerous plant-pathogenic bacteria worldwide (Mindell, 2006; Toussaint and Piñeiro, 2024).

Symptoms of coffee leaf scorch include apical necrosis, leaf curling, witches' brooms, and canopy dieback (De Lima *et al*., 1998; Rocha *et al*., 2010). *X. fastidiosa* subsp. Pauca has been linked to the spread of the epidemic of olive quick decline in Italy; a disease also known as "Complesso del Disseccamento Rapido dell Olivo" (CoDiRO) (Saponari *et al*., 2013). The CoDiRO strain was introduced to the Apulian province of Lecce (southeastern Italy) in coffee trees from Central America (Marcelletti and Scortichini, 2016). In 2012, four coffee plants imported from Mexico to France tested positive for *X. fastidiosa* subsp. *fastidiosa* and subsp. sandyi (Jacques *et al*., 2016). Despite being a major producer of the coffee region, Ecuador has not reported any outbreaks of CLS; however, the strain CBFP8072 of *X. fastidiosa* subsp. Pauca has been isolated from coffee plants of Ecuadorian origin.

X. fastidiosa is gram-negative and grows exclusively in the xylem of plants (Bleve *et al*., 2024; Velasco-Amo *et al*., 2024). *X. fastidiosa* is smaller (0.2-0.5 by 1.0-4.0 μm) compared to other pathogenic bacteria and belongs to the family *Xanthomonadaceae* (Rico *et al*., 2015). Several hypotheses have been proposed to explain the pathogenicity of *X. fastidiosa*, with the main one being the accumulation of the bacterium and its exudates in the plant's xylem vessels, thereby blocking the flow of water and minerals from reaching the leaves and upper plant organs (Simpson *et al*., 2000). The bacterium's production of Cell Wall-Degrading Enzymes (CWDE) may also play a role in its pathogenicity and plant cell colonization. Genes involved in plant cell wall degradation, such as beta-1,4-endoglucanases, xylanases, and xylosidases, have been identified in the *X. fastidiosa* genome (Rapicavoli *et al*., 2018; Roper, 2006; Roper *et al*., 2007). Despite efforts to eradicate and control *X. fastidiosa*, it has caused significant economic losses and changes in the rural landscapes of Italy, France, and Spain. Diagnostic methods for the bacterium lack specificity and reproducibility, making it difficult to effectively control its spread (Toussaint and Piñeiro, 2024).

Previous studies have shown that *X. fastidiosa* can infect both coffee and citrus trees, with genetic elements from multiple *X. fastidiosa* subspecies present in *X. fastidiosa* subsp. Pauca (Vanhove *et al*., 2019). Due to the close proximity of citrus and coffee crops and the presence of shared sharpshooter vectors, it is possible for a coffee strain of *X. fastidiosa* to gain the ability to infect citrus (Chatterjee *et al*., 2008; Li *et al*., 2001). The bacterium is transmitted by different insect 77 vectors, being the most important of the members of the Cicadellidae (De Lima *et al*., 1998). *X. fastidiosa* contains several strains differing in host range (Baldi and La Porta, 2017), pathogenicity, DNA similarity, and nutritional requirements, which determine the grade of fastidiousness for isolation and cultivation outside the hosts (Koide *et al*., 2004). *X. fastidiosa* is responsible for several diseases in different fruit trees and other species. Symptoms of infection include leaf chlorosis, leaf scorch, wilting, and finally plant death (Castro *et al*., 2021). *X. fastidiosa* also colonizes several plant species without causing symptoms. These hosts are a reservoir for the transmission of the pathogen. *Xylella fastidiosa* is subdivided into six subspecies (Pauca, sandyi, multiplex, *fastidiosa*, Morus, and tashke (Brodbeck *et al*., 2017; De Lima *et al*., 1998; Lacava *et al*., 2004; Saponari *et al*., 2013), based on DNA-DNA hybridization. This study aimed to compare the genome of *Xylella fastidiosa* subsp. Pauca strains CFBP8072 to two other Pauca strains, one causing citrus variegated chlorosis (9a5C) and another causing coffee leaf scorch (3124). Recently, high-quality draft genome sequences were obtained for eight strains of *Xylella fastidiosa* subsp. Pauca was isolated from symptomatic plants across South America. Of these strains, J1a12, B111, 3124, U24D, XRB, Pr8x, and Hib4 were isolated in Brazil, while strain Fb7 was isolated in Argentina. These genome sequences provide valuable insights into the genetic diversity and pathogenicity of *X. fastidiosa* sunsp. Pauca in different regions contributes to a better understanding of its spread and potential control strategies in affected crops (Marque-Pierry *et al*., 2020). The study aimed to determine the origin and diversity of the pathogen in Ecuador and to provide insight into its current and potential movements.

Materials and Methods

Collection of material for sequencing

Sequence reads of *Xylella fastidiosa* subsp. Pauca used in this study belonged to the CoDiRO strain (SRR1707402), strain 9a5C (GCA-000006725.1) from citrus, CBFP8072 (SRR8501430) from coffee trees of Ecuador, a CLS strain 3124 (CP009829.1), *Xylella fastidiosa* Hib4 (GenBank: CP009885.1) from hibiscus of Brazil, and *X. taiwanensis (*SRR16971272). Genomic sequences were retrieved from the SRA database of the National Center for Biotechnology Information, U.S. National Library of Medicine. Sequencing approaches consisted of 454 and Illumina platforms.

Processing Sequences

Processing of sequences and analysis of genomic information from strains of *Xylella fastidiosa* subsp. Pauca was carried out with KBase (Allen *et al*., 2017). All the data, analyses, and outputs from the different applications are available under the "Xf" interactive digital notebook (https://kbase.us/n/70410/57/).

Reads were quality assessed using FASTQC. Sequences flagged as poor quality, sequence length, % GC content, as well as duplication for every sequence were analyzed. Reads were assembled with SPAdes, using the deBrujin graph assembly algorithm (Li *et al*., 2002). Multiple deBruijn graphs were constructed while detecting and removing chimeric reads. Distances between the k-mers were estimated for mapping the edges of the assembly graph. SPAdes contig was deposited at Kbase under the name *Xf_CFBP8072EC.fa_assembly*. Genome annotation was performed using PROKA (v1.14.5).

Screening of Pangenome

Ortholog and paralog sequences were grouped with OrthoMCL using a genome-scale algorithm. Groups sharing by two or more genomes, as well as groups representing species-specific gene expansion families were annotated. The output of OrthoMCL was a pangenome object, with a pangenome summary, shared homolog families, and a list of homologous protein clusters predicted by OrthoMCL. A pangenome circle plot was used to view the overlapping membership of genes against a base genome. First 9a5C was used to compare against 3124, CBFP8072, Hib4, and *X. taiwanensis*. A second run was done using CBFP8072 as the base genome against the other four genomes.

The core ortholog set consisted of at least one gene from the ortholog set with a gene in each of the genomes. Singletons corresponded to genes with no sequence homology to genes in any other genomes. These are categorized as *Singletons*. For pangenome comparison, two base genomes were used to order the ortholog clusters into a ring first using *X. fastidiosa* subsp. Pauca 9a5c and second using CBFP8072 as base genome. The pre-calculated pangenome object was visualized showing the orthology relationships between the genes. A species phylogenetic tree was conducted using 22 full genomes belonging to the *Xanthomonadales* using View Tree-v1.4.0. Whole-genome Average Nucleotide Identity (ANI) was identified with FASTANI-v0.1.3.

Results and Discussion

The National Center for Biotechnology Information (NCBI) database contains over 80 fully sequenced whole genomes representing all six subspecies of *X. fastidiosa*. These genomes exhibit sizes ranging from 2.4-2.7 Mb, a G+C content of 51-52 mol%, contig numbers varying from 1 to over 400, ambiguous base counts spanning from 0 to over 300, and overall completeness that typically exceeds 99% (Trkulja *et al*., 2022). At least 12 of them belong to the *Xylella fastidiosa* subsp. Pauca and are deposited in the SRA database.

To analyze genomic information from strains of *Xylella fastidiosa* subsp. Pauca genomic analyses were carried out with KBase (Allen *et al*., 2017). All the data, analyses, and outputs from the different applications are available under the "Xf" interactive digital notebook (https://kbase.us/n/70410/57/).

The NCBI-SRA accession SSR8501430 contains the genome sequence of CBFP807, a strain of *X. fastidiosa* subsp. Pauca is isolated from a coffee tree of Ecuadorian origin. Our SPAdes assembly of this genome generated a single contig with 52.57% GC content and a length of 224,836 bp. This assembly exhibits homology with 40 genomes, sharing an average nucleotide identity ANI <15%. The annotated assembly comprises 2399 predicted genes, with 97.9% (2349 out of 2399) identified as protein-coding genes. Among these, 1338 genes were associated with hypothetical functions, while 767 genes were assigned Enzyme Commission (EC) numbers, providing insights into specific enzymatic activities. The average protein length within this dataset was 278 amino acids, and 642 genes were annotated with Seed Subsystem Ontology, further enhancing our understanding of their functional roles.

New insights into the recombination and evolutionary history of *X. fastidiosa* were gained from the analysis of 72 genomes via assembly, annotation, and pangenome analysis (Vanhove *et al*., 2019), confirming that *X. fastidiosa* subsp. Pauca contains genetic elements from *X. fastidiosa* subsp. Multiplex and from *X. fastidiosa* subsp. *fastidiosa*, suggesting a Central American origin. Strains from citrus can infect and reproduce in coffee. Additionally, there is no natural admixture of *X. fastidiosa* strains isolated from coffee and citrus (Francisco *et al*., 2017) Due to citrus and coffee are sympatric crops, with many sharpshooter species in common, and both are infected by closely related *X. fastidiosa* strains, it is possible that at some point a strain from coffee gain ability to infect citrus. STs infecting coffee, citrus, and olives are presented in Table (1).

Sequence types of *X. fastidiosa* subsp. Pauca retrieved from the isolates database and the European food safety authority-sequence types: *X. fastidiosa* isolates database.

Table. 1: Sequence types of *Xylella fastidiosa* subsp. Pauca infecting *Coffea* sp., *Citrus* sp., and Olea Europea

Host	Sequence types	Origin
Coffea sp.	53,73	Costa Rica
	11,14,16, 53,66,68,72,76	Brazil
	74	Ecuador
Citrus sp.	11,12,13,64,65	Brazil
	69	Argentina

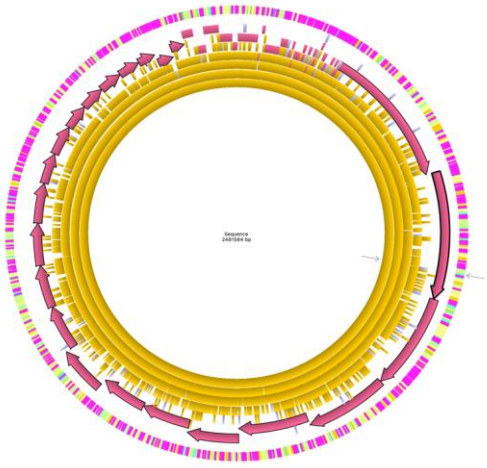


Fig. 1: Genome Assembly of *Xylella fastidiosa* strain CBFP8072 sequence type 74, showing Open Reading frames (yellow internal ring), annotated repeats (pink arrows), and restriction sites (external ring)

The tree included *X. taiwanensis* which clustered separately compared to the genome sequences of *X. fastidiosa* subsp. Pauca 9a5c, CFBP-8072 (ST 74) (Fig. 1), Hib4 (ST 70), and 3124 (ST-16). Tolocka *et al*. (2017) detected a cluster of ST74 (CBFP8072) and ST53 (Olive-Italy). Hib4 (ST70), an *X. fastidiosa* strain isolated from Hibiscus of Brazil, was close to CBFP8072 (Coffee-Ecuador), showing that strains are associated with different hosts from wide geographic regions of America Fig. (2).

Pan-genome refers to the complete set of genes within a species, a term employed in the context of bacteria and archaea. This concept is particularly relevant in these microorganisms due to the significant variations in gene content observed among closely related strains (Tettelin *et al*., 2005). Pangenome analysis detected over 10% (1219/11989) genes in singleton families. The highest number of singletons per family was for *Xylella taiwanensis* (2316 total genes). Genes in homologs varied from 2486-2663 (https://kbase.us/n/70410/57/).

This indicates a significant portion of unique genetic material within the studied population. The presence of many singleton genes suggests high genetic diversity or unique adaptations within the population. Figure (3) shows that there are more base singletons detected when CBFP8072 is used as the core genome Fig. (3B) than using 9a5c as the core genome Fig. (3A).

Marcelletti and Scortichini (2016) presented a consensus tree for 27 *X. fastidiosa* strains, clustering strains from *Coffea* sp. (Minas Gerais Brazil-COF0324), from *Citrus x sinensis* (Sao Paulo Brazil-9a5c, CVC0251, and CVC 0256), *Coffea arabica* from Ecuador-CFBP8072, San Jose Costa Rica-COF0407, and the CoDiRo strain from *Olea europea* (Apulia Italy). Vanhove *et al*. (2019) determined the relative recombination rate of 72 *X. fastidiosa* genomes, determining a high effect of recombination as a major driver of shifts among hosts, through comparison of core genome alignments. Strains of *Xylella fastidiosa* subsp. Pauca found in citrus exhibited genetic elements acquired from strains affecting coffee plants, as well as genetic elements from both *X. fastidiosa* subsp. *fastidiosa* and *X. fastidiosa* subsp. Multiplex. Genetic relationships and phylogenetic clade analysis revealed the significance of recombination in shaping *X. fastidiosa* genomes (Vanhove *et al*., 2019). Uceda Campos *et al*. conducted a comparative analysis of 94 publicly accessible whole-genome sequence assemblies of *X. fastidiosa* strains unveiled a pangenome consisting of 4549 orthologous CDSs (Coding DNA Sequences) and a core genome comprised of 954 CDSs (Uceda-Campos *et al*., 2022). We detected fewer total ortholog genes, while the size of the pangenome is roughly proportional to other published studies Table (2).

In 2012, in France, the presence of CLS symptoms was confirmed in four confined glasshouse coffee plants, including *Coffea arabica* and *C. canephora*. Three strains of *X. fastidiosa* were isolated from these plants, confirming an initial diagnosis based on immunofluorescence. Characterization through multiplex PCR and multilocus sequence analysis/typing (MLSA-MLST) based on seven housekeeping genes revealed a new Sequence Type of *X. fastidiosa* subsp. Pauca (ST-74) with novel alleles at two loci. For CFBP8072 (Jacques *et al*., 2016). Up-to-date, ST4 is the only strain of *X. fastidiosa* subsp. Pauca is isolated from Ecuador.

ST74 from *X. fastidiosa* strain CFBP8072 have a circular genome of length: 2 481 584 nt, GC content: 52.57%, ssDNA molecular weight: 767025260.99 Da, extinction coefficient: 23610954050 L/ (mol * cm), µg/OD260: 32.49. dsDNA has a Molecular weight: of 1533429691.83 Da, Extinction coefficient: of 39325004512 L/ (mol * cm), µg/OD260: 38.99. The assembly has annotated 3571 repeats spread through one contig.

The sequence types of *Xylella fastidiosa* subsp. Pauca exhibits regional and host-specific variations. Strains originating from Costa Rica are classified as Sequence Type (ST) 53 and 73 (Denancé *et al*., 2019). Sequence types sharing the same host origin pose a potential risk of transmission between hosts (Tolocka *et al*., 2017).
The phylogenetic tree was obtained from 22 genomes of the Clusters of Orthologous Groups (COG) gene families.

The tree included *X. taiwanensis* which clustered separately compared to the genome sequences of *X. fastidiosa* subsp. Pauca 9a5c, CFBP-8072 (ST 74), Hib4 (ST 70), and 3124 (ST-16). Tolocka *et al*. (2017) detected a cluster of ST74 (CBFP8072) and ST53 (Olive-Italy). Hib4 (ST70), an *X. fastidiosa* strain isolated from Hibiscus of Brazil, was close to CBFP8072 (Coffee-Ecuador), showing that strains are associated with different hosts from wide geographic regions of America Fig. (2).

Table 2: Comparative analysis of published pangenomes using different bioinformatic frameworks to obtain orthologous Coding DNA Sequences (CDSs) and core genomes

Study	Orthologs	Core genomes	Bioinformatic Framework	Reference
Comparative analyses of 94 publicly available whole-genome sequence assemblies of *X. fastidiosa*	4549	954	Gene Tags Assessment by Comparative Genomics (GTACG)	Uceda-Campos *et al*. (2022)
Analysis of 72 *X. fastidiosa* genomes, including representatives of the five currently described subspecies	4747	622	Roary	Vanhove *et al*. (2019)
Comparative genomic of 27 *X. fastidiosa* genomes	6869	899	Get Homologs/COG	Giampetruzzi *et al*. (2015)
This study	3611	576	Ortho MCL	

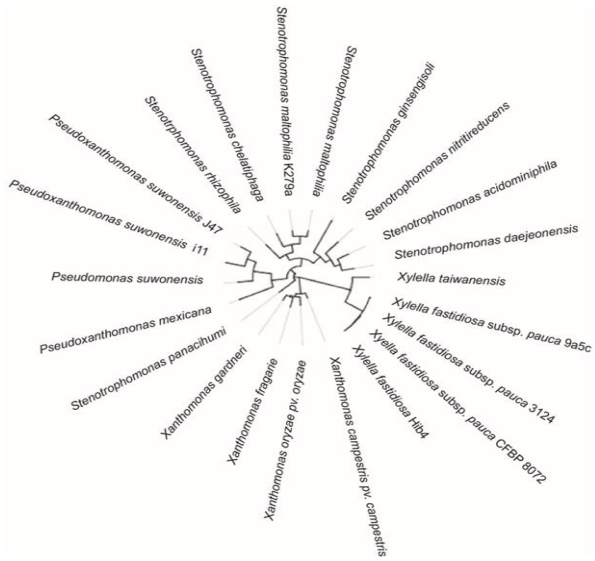
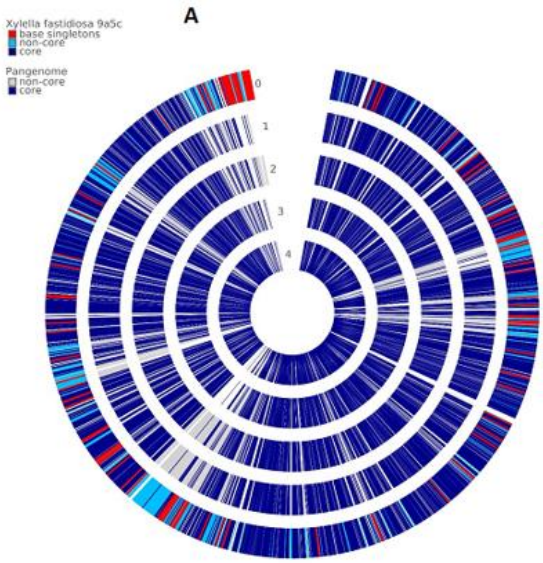


Fig. 2: Phylogenetic tree of 22 genomes publicly available within the *Xanthomonadaceae*



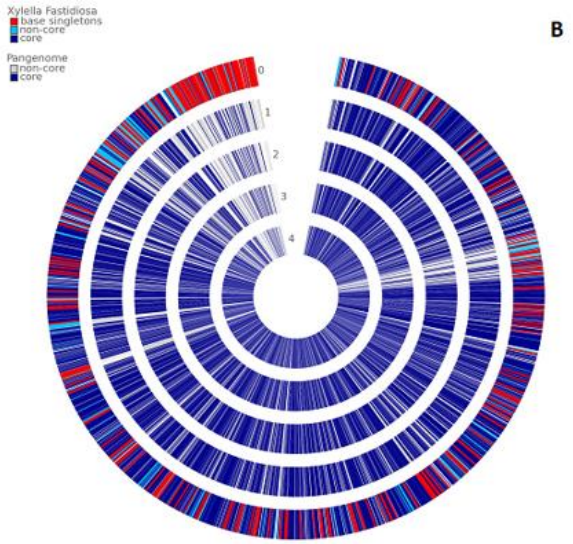


Fig. 3: Pangenome analysis of *Xylella fastidiosa* subsp. Pauca: (A) base genome (0): strain 9a5c, genome 1 strain Hib4, genome 2 strain 3124, genome 3 strain CFBP8072, and genome 4 *Xylella taiwanensis* (B) (A) base genome (0): CBFP8072, genome 1 strain 9a5c, genome 2 strain 3124, genome 3 strain Hib4, and genome 4 *Xylella taiwanensis*

Pan-genome refers to the complete set of genes within a species, a term employed in the context of bacteria and archaea. This concept is particularly relevant in these microorganisms due to the significant variations in gene content observed among closely related strains (Tettelin *et al*., 2005). Pangenome analysis detected over 10% (1219/11989) genes in singleton families. The highest number of singletons per family was for *Xylella taiwanensis* (2316 total genes). Genes in homologs varied from 2486-2663 (https://kbase.us/n/70410/57/).

This indicates a significant portion of unique genetic material within the studied population. The presence of many singleton genes suggests high genetic diversity or unique adaptations within the population. Figure (3) shows that there are more base singletons detected when CBFP8072 is used as the core genome Fig. (3B) than using 9a5c as the core genome Fig. (3A).

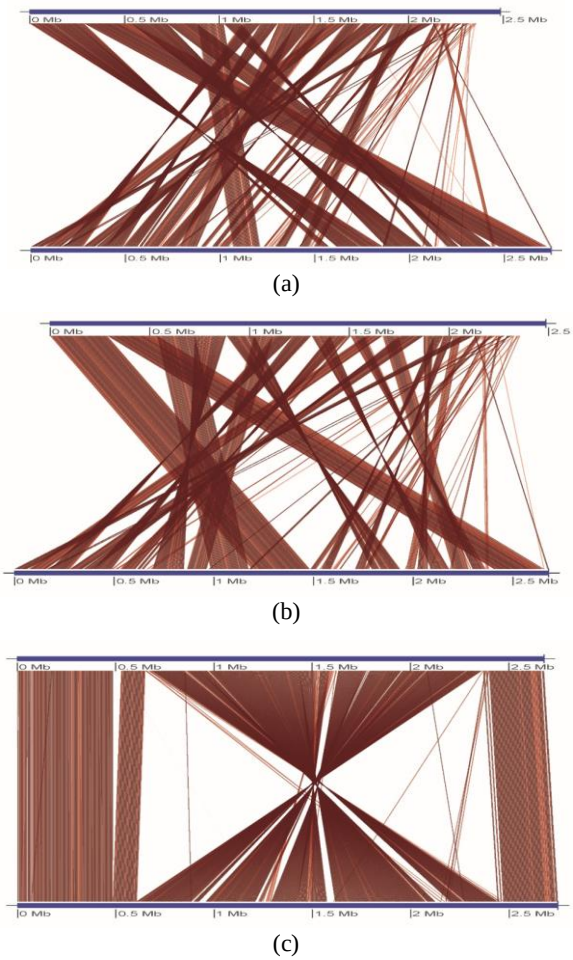
Marcelletti and Scortichini (2016) presented a consensus tree for 27 *X. fastidiosa* strains, clustering strains from *Coffea* sp. (Minas Gerais Brazil-COF0324), from *Citrus x sinensis* (Sao Paulo Brazil-9a5c, CVC0251, and CVC 0256), *Coffea arabica* from Ecuador-CFBP8072, San Jose Costa Rica-COF0407, and the CoDiRo strain from *Olea europea* (Apulia Italy). Vanhove *et al*. (2019) determined the relative recombination rate of 72 *X. fastidiosa* genomes, determining a high effect of recombination as a major driver of shifts among hosts, through comparison of core genome alignments. Strains of *Xylella fastidiosa* subsp. Pauca found in citrus exhibited genetic elements acquired from strains affecting coffee plants, as well as genetic elements from both *X. fastidiosa* subsp. *fastidiosa* and *X. fastidiosa* subsp. *Multiplex*. Genetic relationships and phylogenetic clade analysis revealed the significance of recombination in shaping *X. fastidiosa* genomes (Vanhove *et al*., 2019). Uceda Campos *et al*. conducted a comparative analysis of 94 publicly accessible whole-genome sequence assemblies of *X. fastidiosa* strains unveiled a pangenome consisting of 4549 orthologous CDSs (Coding DNA Sequences) and a core genome comprised of 954 CDSs (Uceda-Campos *et al*., 2022). We detected fewer total ortholog genes, while the size of the pangenome is roughly proportional to other published studies Table (2).

The analysis of whole-genome Average Nucleotide Identity (ANI) showed strains 9a5c and 3124 sharing homologies of 98.43 and 98.37%, respectively with strain CFBP 8072 (isolated from Ecuadorian coffee trees). The highest number of orthologous gene pairs shared were between *X. fastidiosa* subsp. Pauca strains 9a5c (CVC strain) and 3124 (CLS strain) (ANI 99.36%) Fig. (4). Both strains are from the region of Sao Paulo.

Quantifying losses due to CLS is challenging; however, in Brazil, during a CLS epidemic it was reported approximately 30% of yield loss and incidence in all areas of production (Li *et al*., 2001). Vector abundance and prevalence of symptoms are higher in high-altitude locations compared to lower-altitude locations (Brodbeck *et al*., 2017; Rico *et al*., 2015). Although wet and rainy conditions can impact favorably the endophyte community of coffee, as well as potential vectors, symptoms of CLS are more evident in the dry and cold season. Artificial cross-infection assays with Coffee and Citrus strains gave no long-term infections (Francisco *et al*., 2017). In the field, strains of CLS appear to remain in the same host with no cross-infection with Citrus (Lopes *et al*., 2009).

During epidemics of CVC in Brazil, the secondary spread (tree by tree) produced major outbreaks in whole production regions (Gottwald *et al*., 2007). Once the pathogen is established in an orchard it is very difficult to eliminate. *X. fastidiosa* strains isolated from citrus can infect coffee, but not the other way around. Coffee leaf scorch *X. fastidiosa* strains were not recovered from inoculated citrus plants without regard to the bacterial load (10^4-10^9 colony forming units / mL^{-1}) (Simpson *et al*., 2000). Also, these authors detected a positive linear relationship between inoculum concentration and the proportion of coffee plants infected by *X. fastidiosa* strain isolated from citrus. *X. fastidiosa* is mainly transmitted through vectors. Lopes *et al*. (2009), detected high specificity of coffee and citrus strains to remain in their hosts; therefore, the spreading from coffee to citrus and vice versa could be limited.

There is a high risk of CLS contamination and the replacement of strains of *Xylella fastidiosa* subsp. Pauca. Until 2015, the Netherlands Food and Consumer Product Safety Authority 195 considered Ecuador as free from *X. fastidiosa* (NVWA, 2024). In 2016, the strain CBFP8072 of *X. fastidiosa* subsp. Pauca, ST-74, was isolated from coffee samples collected from orchards in Ecuador (Marcelletti and Scortichini, 2016) It is unknown if ST-74 is endemic, its aggressiveness towards citrus and coffee cultivars under the environmental conditions of this region, and the impact of changes in *X. fastidiosa* subsp. Pauca populations for Ecuadorian coffee and citrus industries.



(a)

(b)

(c)

Fig. 4: Visualization of orthologous maps between Xylella fastidiosa subsp. pauca strains: (A) CFBP8072 vs 9a5c, (B) CFBP8072 vs 3124 and (C) 9a5c vs 3124

Xylella fastidiosa subsp. Pauca is a significant threat to plant health worldwide, not only because of its current quarantine status but also because of its historical repercussions on commercial crops such as grapes and citrus in America. *X. fastidiosa* subsp. Pauca could cause epidemics of CVC and CLS and has the potential to devastate coffee and citrus orchards in South America and Central America, causing significant economic losses. However, the toll is even higher in terms of social impact, and the effect that CLS may have on human mobility to the Central and South American populations. *X. fastidiosa* subsp. Pauca has been detected mainly in South America (Brazil). The arrival of *X. fastidiosa* strains that can cause CLS and CVC epidemics can devastate coffee and citrus industries in regions where the pathogen is novel, and its aggressiveness is unknown towards the cultivars planted in those areas.

Conclusion

In conclusion, *X. fastidosa* subsp. Pauca strain CBFP8072 has a significant portion of unique genetic material. The presence of many singleton genes suggests high genetic diversity or unique adaptations within the strain. Pangenome analysis shows that there are more base singletons detected when CBFP8072 is used as the core genome. Even though, CLS epidemics have not been reported in either Ecuador, Perú, or Colombia., there is a high risk of CLS outbreaks due to the potential replacement of *Xylella fastidiosa* subsp. Pauca strains with unknown aggressiveness in new environmental conditions.

Acknowledgment

Carlos Andrés Bolaños Carriel is a scholar of the National Secretariat of Higher Education of Ecuador. The author is indebted to the Ohio Supercomputing Center and Mike Sovic from OSU for their bioinformatic support. The authors appreciate and acknowledge the support of the Instituto de Investigación para el Desarrollo Sustentable de Ceja de Selva (INDES-CES) of the Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas (UNTRM). CEINCAFE and CoffeSmart Projects.

Funding Information

The study was funded through the Instituto de Investigación para el Desarrollo Sustentable de Ceja de Selva (INDES-CES) in the project named “Creación de los Servicios del Centro de Investigación, innovación y Transferencia Tecnológica de Café de la Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas” (C.U.I. N° 2317883-CEINCAFÉ) and Coffe-Smart Project.

Data Availability Statement

All the data, analyses, and outputs from the different applications are available under the "Xf” interactive digital notebook (https://kbase.us/n/70410/57/).

Author’s Contributions

Carlos Bolanos-Carriel: Mad a mayor contribution to the conduct of the research and data acquisition.

Eliana Granja Guerra: Coordinate the data analysis and contributed to written of the manuscript.

Jaris Veneros and Grobert A. Guadalupe: Critically reviewed and provided substantial edits to the manuscript.

Ligia García: Create concept and designed, monitor the progress of research from start to finished and ensure that the entire research process goes according to planed, analysis and interpretation of data.

Ethics

Authors should address any ethical issues that may arise after the publication of this manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Reference

Aga, E. (2005). Molecular Genetic Diversity Study of Forest Coffee Tree (Coffea arabica L.) Populations in Ethiopia: *Implications for Conservation and Breeding* (79th Ed., Vol. 2005). Department of Crop Science, Swedish University of Agricultural Sciences.

Allen, B., Drake, M., Harris, N., & Sullivan, T. (2017). Using KBase to Assemble and Annotate Prokaryotic Genomes. *Current Protocols in Microbiology*, *46*(1), 1E – 13. https://doi.org/10.1002/cpmc.37

Anthony, F., Combes, M., Astorga, C., Bertrand, B., Graziosi, G., & Lashermes, P. (2002). The Origin of Cultivated Coffea Arabica L. Varieties Revealed by AFLP and SSR Markers. *Theoretical and Applied Genetics*, *104*(5), 894–900. https://doi.org/10.1007/s00122-001-0798-8

Baldi, P., & La Porta, N. (2017). *Xylella fastidiosa*: Host Range and Advance in Molecular Identification Techniques. *Frontiers in Plant Science*, *8*, 944. https://doi.org/10.3389/fpls.2017.00944

Beretta, G, M. J. (1996). First Report of *Xylella fastidiosa* in Coffee. *Plant Disease*, *80*, 821. https://doi.org/10.1094/pd-80-0821d

Bleve, G., Trivellin, N., Chirizzi, D., Tarantini, A., Orlandi, V. T., & Milano, F. (2024). Sensitivity of *Xylella fastidiosa* Subsp. Pauca Salento-1 to light at 410 nm. *Photochemical & Photobiological Sciences*, *23*(4), 793–801. https://doi.org/10.1007/s43630-024-00556-z

Brodbeck, B. V., Andersen, P. C., Oden, S., Mizell, R. F., McKamey, S. H., & Zapata, M. (2017). The Distribution of Cicadellinae Leafhoppers and Other Auchenorrhyncha on Coffee and Citrus in Puerto Rico. *Environmental Entomology*, *46*(3), 511–520. https://doi.org/10.1093/ee/nvx086

Bunn, C., Läderach, P., Ovalle Rivera, O., & Kirschke, D. (2015). A Bitter Cup: Climate Change Profile of Global Production of Arabica and Robusta Coffee. *Climatic Change*, *129*(1), 89–101. https://doi.org/10.1007/s10584-014-1306-x

Rodríguez, C. M., Obando, J. J., Villalobos, W., Moreira, L., & Rivera, C. (2001). First Report of *Xylella fastidiosa* Infecting Coffee in Costa Rica. *Plant Disease*, *85*(9), 1027. https://doi.org/10.1094/pdis.2001.85.9.1027a

Castro, C., DiSalvo, B., & Roper, M. C. (2021). *Xylella fastidiosa*: A Reemerging Plant Pathogen that Threatens Crops Globally. *PLOS Pathogens*, *17*(9), e1009813. https://doi.org/10.1371/journal.ppat.1009813

Chatterjee, S., Almeida, R. P. P., & Lindow, S. (2008). Living in two Worlds: The Plant and Insect Lifestyles of *Xylella fastidiosa*. *Annual Review of Phytopathology*, *46*(1), 243–271. https://doi.org/10.1146/annurev.phyto.45.062806.094342

De Lima, J. E. O., Miranda, V. S., Hartung, J. S., Brlansky, R. H., Coutinho, A., Roberto, S. R., & Carlos, E. F. (1998). Coffee Leaf Scorch Bacterium: Axenic Culture, Pathogenicity, and Comparison with *Xylella fastidiosa* of Citrus. *Plant Disease*, *82*(1), 94–97. https://doi.org/10.1094/pdis.1998.82.1.94

Denancé, N., Briand, M., Gaborieau, R., Gaillard, S., & Jacques, M.-A. (2019). Identification of Genetic Relationships and Subspecies Signatures in *Xylella fastidiosa*. *BMC Genomics*, *20*(1), 239. https://doi.org/10.1186/s12864-019-5565-9

Francisco, C. S., Ceresini, P. C., Almeida, R. P. P., & Coletta-Filho, H. D. (2017). Spatial Genetic Structure of Coffee-Associated *Xylella fastidiosa* Populations Indicates that Cross Infection Does Not Occur with Sympatric Citrus Orchards. *Phytopathology*, *107*(4), 395–402. https://doi.org/10.1094/phyto-08-16-0300-r

Giampetruzzi, A., Loconsole, G., Boscia, D., Calzolari, A., Chiumenti, M., Martelli, G. P., Saldarelli, P., Almeida, R. P. P., & Saponari, M. (2015). Draft Genome Sequence of CO33, A Coffee-Infecting Isolate of *Xylella fastidiosa*. *Genome Announcements*, *3*(6), 10–1128. https://doi.org/10.1128/genomea.01472-15

Gottwald, T. R., Graça, J. V. da, & Bassanezi, R. B. (2007). Citrus Huanglongbing: The Pathogen and Its Impact. *Plant Health Progress*, *8*(1), 31. https://doi.org/10.1094/php-2007-0906-01-rv

Jacques, M.-A., Denancé, N., Legendre, B., Morel, E., Briand, M., Mississipi, S., Durand, K., Olivier, V., Portier, P., Poliakoff, F., & Crouzillat, D. (2016). New Coffee Plant-Infecting *Xylella fastidiosa* Variants Derived Via Homologous Recombination. *Applied and Environmental Microbiology*, *82*(5), 1556–1568. https://doi.org/10.1128/aem.03299-15

Koide, T., Zaini, P. A., Moreira, L. M., Vêncio, R. Z. N., Matsukuma, A. Y., Durham, A. M., Teixeira, D. C., El-Dorry, H., Monteiro, P. B., da Silva, A. C. R., Verjovski-Almeida, S., da Silva, A. M., & Gomes, S. L. (2004). DNA Microarray-Based Genome Comparison of a Pathogenic and a Nonpathogenic Strain of *Xylella fastidiosa* Delineates Genes Important for Bacterial Virulence. *Journal of Bacteriology*, *186*(16), 5442–5449. https://doi.org/10.1128/jb.186.16.5442-5449.2004

Labouisse, J.-P., Bellachew, B., Kotecha, S., & Bertrand, B. (2008). Current Status of Coffee (Coffea arabica L.) Genetic Resources in Ethiopia: Implications for Conservation. *Genetic Resources and Crop Evolution*, *55*(7), 1079–1093. https://doi.org/10.1007/s10722-008-9361-7

Lacava, P. T., Araujo, W.L., Marcon, J., Maccheroni, W., & Azevedo, J. L. (2004). Interaction between endophytic bacteria from citrus plants and the phytopathogenic bacteria Xylella fastidiosa, causal agent of citrus-variegated chlorosis. *Letters in Applied Microbiology*, *39*(1), 55–59. https://doi.org/10.1111/j.1472-765X.2004.01543.x

Li, W.-B., Pria, W. D., Teixeira, D. C., Miranda, V. S., Ayres, A. J., Franco, C. F., Costa, M. G., He, C.-X., Costa, P. I., & Hartung, J. S. (2001). Coffee Leaf Scorch Caused by a Strain of *Xylella fastidiosa* from Citrus. *Plant Disease*, *85*(5), 501–505. https://doi.org/10.1094/pdis.2001.85.5.501

Li, W.-B., Zhou, C.-H., Pria, W. D., Teixeira, D. C., Miranda, V. S., Pereira, E. O., Ayres, A. J., He, C.-X., Costa, P. I., & Hartung, J. S. (2002). Citrus and Coffee Strains of *Xylella fastidiosa* Induce Pierce's Disease in Grapevine. *Plant Disease*, *86*(11), 1206–1210. https://doi.org/10.1094/pdis.2002.86.11.1206

Lopes, J. R. S., Daugherty, M. P., & Almeida, R. P. P. (2009). Context-Dependent Transmission of a Generalist Plant Pathogen: Host Species and Pathogen Strain Mediate Insect Vector Competence. *Entomologia Experimentalis et Applicata*, *131*(2), 216–224. https://doi.org/10.1111/j.1570-7458.2009.00847.x

López-Gartner, G., Cortina, H., McCouch, S. R., & Moncada, M. D. P. (2009). Analysis of Genetic Structure in A Sample of Coffee (Coffea arabica L.) Using Fluorescent SSR Markers. *Tree Genetics & Genomes*, *5*(3), 435–446. https://doi.org/10.1007/s11295-008-0197-2

Marcelletti, S., & Scortichini, M. (2016). *Xylella fastidiosa* CoDiRO Strain Associated with the Olive Quick Decline Syndrome in Southern Italy Belongs to a Clonal Complex of the Subspecies Pauca that Evolved in Central America. *Microbiology*, *162*(12), 2087–2098. https://doi.org/10.1099/mic.0.000388

Marque-Pierry, P. M., de Santana, W. O., Kitajima, J. P., Martins-Junior, J., Zaini, P. A., Uceda-Campos, G., Feitosa-Junior, O. R., Pessoa, P. I. S., Coletta-Filho, H. D., de Souza, A. A., Machado, M. A., Gesteira, A. da S., Martins, L. F., Amaral, M. S., Beckedorff, F. C., de Almeida, L. G. P., de Vasconcelos, A. T. R., Verjovski-Almeida, S., Setubal, J. C., & da Silva, A. M. (2020). High-Quality Draft Genome Sequence Resources of Eight *Xylella fastidiosa* Strains Isolated from Citrus, Coffee, Plum, and Hibiscus in South America. *Phytopathology*, *110*(11), 1751–1755. https://doi.org/10.1094/phyto-05-20-0162-a

Melese, Y. Y., & Kolech, S. A. (2021). Coffee (Coffea arabica L.): Methods, Objectives, and Future Strategies of Breeding in Ethiopia—Review. *Sustainability*, *13*(19), 10814. https://doi.org/10.3390/su131910814

Mindell, D. (2006). *The Evolving World: Evolution in Everyday life*. David P. Mindell.

Naik, B. J., Kim, S.-C., Seenaiah, R., Basha, P. A., & Song, E. Y. (2021). Coffee Cultivation Techniques, Impact of Climate Change on Coffee Production, Role of Nanoparticles and Molecular Markers in Coffee Crop Improvement, and Challenges. *Journal of Plant Biotechnology*, *48*(4), 207–222. https://doi.org/10.5010/jpb.2021.48.4.207

NVWA. (2024). *EU list of countries free of Xylella fastidiosa (English)*. https://www.nvwa.nl/documenten/plant/plantziekte-en-plaag/plantziekte/xyllela/eu-list-of-countries-registered-as-being-free-from-xylella-fastidiosa

Paradela-Filho, O., Sugimori, M. H., Ribeiro, I. J., Machado, M. A., Laranjeira, F. F., Garcia, A., & Beriam, L. O. (1995). Primeira Constataçãoem Cafeeiro no Braose Variegada doscitros Science Open. *Laranja*, *16*(2), 135–136.

Philippe, L., Benoít, B., & Hervé, E. (2008). Breeding Coffee (Coffea arabica) for Sustainable Production. In S. M. Jain & P. M. Priyadarshan (Eds.), *Breeding Plantation Tree Crops* (pp. 525–543). Springer New York. https://doi.org/10.1007/978-0-387-71201-7_14

Rapicavoli, J., Ingel, B., Blanco-Ulate, B., Cantu, D., & Roper, C. (2018). *Xylella fastidiosa*: An Examination of a re-Emerging Plant Pathogen. *Molecular Plant Pathology*, *19*(4), 786–800. https://doi.org/10.1111/mpp.12585

Rico, Bolaños, C., Zapata, M., Brodbeck, B., Andersen, P., Wessel-Beaver, L., & Estévez de Jensen, C. (2015). Spatial Distribution of Coffee Trees (Coffea arabica L.) Potentially Diseased with Coffee leaf Scorch Caused by *Xylella fastidiosa* In Puerto. *The Journal of Agriculture of the University of Puerto Rico*, *99*(2), 157–165. https://doi.org/10.46429/jaupr.v99i2.3031

Rocha, J. G., Zambolim, L., Zambolim, E. M., Ribeiro do Vale, F. X., Junior, W. C. J., & Filho, A. B. (2010). Quantification of Yield Loss Due to Coffee Leaf Scorch. *Crop Protection*, *29*(10), 1100–1104. https://doi.org/10.1016/j.cropro.2010.04.011

Roper, M. C. (2006). *The Characterization and Role of Xylella fastidiosa Plant Cell Wall Degrading Enzymes and Exopolysaccharide in Pierce's Disease of Grapevine*. University of California, Davis.

Roper, M. C., Greve, L. C., Warren, J. G., Labavitch, J. M., & Kirkpatrick, B. C. (2007). *Xylella fastidiosa* Requires Polygalacturonase for Colonization and Pathogenicity in Vitis Vinifera Grapevines. *Molecular Plant-Microbe Interactions*, *20*(4), 411–419. https://doi.org/10.1094/mpmi-20-4-0411

Saponari, M., Boscia, D., Nigro, F., & Martelli, G. P. (2013). Identification of DNA sequences related to Xylella fastidiosa in oleander, almond and olive trees exhibiting leaf scorch symptoms in Apulia (southern Italy). *Journal of Plant Pathology*, *95*(3), 668. http://sipav.org/main/jpp/index.php/jpp/article/view/2986

Simpson, A. J. G. (2000). The Complete Genome Sequence of the Plant Pathogen *Xylella Fastidiosa*. *Biochemical Society Transactions*, *28*(5), A102–A102. https://doi.org/10.1042/bst028a102b

Tettelin, H., Masignani, V., Cieslewicz, M. J., Donati, C., Medini, D., Ward, N. L., Angiuoli, S. V., Crabtree, J., Jones, A. L., Durkin, A. S., DeBoy, R. T., Davidsen, T. M., Mora, M., Scarselli, M., Margarit y Ros, I., Peterson, J. D., Hauser, C. R., Sundaram, J. P., Nelson, W. C., Fraser, C. M. (2005). Genome analysis of multiple pathogenic isolates of *Streptococcus agalactiae*: Implications for the microbial "pan-genome." *Proceedings of the National Academy of Sciences*, *102*(39), 13950–13955. https://doi.org/10.1073/pnas.0506758102

Tolocka, P. A., Mattio, M. F., Paccioretti, M. A., Otero, M. L., Roca, M. E., Guzmán, F. A., & Haelterman, R. M. (2017). *Xylella Fastidiosa* Subsp. Pauca ST69 in Olive in Argentina. *Journal of Plant Pathology*, *99*(3), 803.

Toussaint, B., & Piñeiro, M. (2024). *Overview and Outlook of Phage Therapy and Phage Biocontrol JRC Science for Policy Report*. https://doi.org/doi:10.2760/71288

Trkulja, V., Tomić, A., Iličić, R., Nožinić, M., & Milovanović, T. P. (2022). *Xylella fastidiosa* in Europe: From the Introduction to the Current Status. *The Plant Pathology Journal*, *38*(6), 551–571. https://doi.org/10.5423/ppj.rw.09.2022.0127

Uceda-Campos, G., Feitosa-Junior, O. R., Santiago, C. R. N., Pierry, P. M., Zaini, P. A., de Santana, W. O., Martins-Junior, J., Barbosa, D., Digiampietri, L. A., Setubal, J. C., & da Silva, A. M. (2022). Comparative Genomics of *Xylella fastidiosa* Explores Candidate Host-Specificity Determinants and Expands the Known Repertoire of Mobile Genetic Elements and Immunity Systems. *Microorganisms*, *10*(5), 914. https://doi.org/10.3390/microorganisms10050914

Vanhove, M., Retchless, A. C., Sicard, A., Rieux, A., Coletta-Filho, H. D., De La Fuente, L., Stenger, D. C., & Almeida, R. P. P. (2019). Genomic Diversity and Recombination among *Xylella fastidiosa* Subspecies. *Applied and Environmental Microbiology*, *85*(13), e02972-18. https://doi.org/10.1128/aem.02972-18

Velasco-Amo, M. P., Arias-Giraldo, L. F., & Landa, B. B. (2024). Complete Genome Assemblies of Several *Xylella Fastidiosa* Subspecies Multiplex Strains Reveals High Phage Content and Novel Plasmids. *Phytopathologia Mediterranea*, *63*(1), 15–23. https://doi.org/10.36253/phyto-14931