Genomic insights into the evolution of pathogenicity in a new walnut-associated *Xanthomonas* species

Leonor Martins[1,2], **Camila Fernandes**[1,2,3], **Jochen Blom**[4], **Joël F. Pothier**[5], **Fernando Tavares**[1,2]

E-mail: leonor.martins@cibio.up.pt

1 – CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO, Laboratório associado, Universidade do Porto, Portugal

2 – FCUP, Faculdade de Ciências, Departamento de Biologia, Universidade do Porto, Portugal

3 – INIAV, Instituto Nacional de Investigação Agrária e Veterinária, Oeiras, Portugal

4 – Bioinformatics and Systems Biology, Justus-Liebig-University Giessen, Germany

5 – Environmental Genomics and Systems Biology Research Group, Institute for Natural Resource Sciences, Zurich University of Applied Sciences (ZHAW), Wädenswil, Switzerland

Introduction

Xanthomonas arboricola pv. *juglandis* (*Xaj*) is a widespread threat to walnut orchards, causing severe economic losses worldwide [1][2]. The rise of novel plant-pathogenic strains threatens crops and trees, urging a deeper understanding of the evolutionary forces shaping adaptation to pathogenicity [3]. Particularly for *Xanthomonas* genus, recombination and horizontal gene transfer (HGT) continuously drive the evolution of pathogenic strains [4]. Type III effectors (T3E) are proteins involved in host pathogenicity and are released into the plant cell through the Type III Secretion System (T3SS) [5].

Recently, we observed the frequent occurrence of pathogenic and non-pathogenic xanthomonad lineages co-colonizing the same walnut host, suggesting that a sympatric lifestyle may contribute to genetic trade-offs related to pathogenicity in *Xanthomonas* [5]. Comparative genomics and Average Nucleotide Identity (ANI) of five co-colonizing *Xanthomonas* strains revealed that three strains belong to a novel walnut-associated *Xanthomonas* species (CPBF 367, CPBF 424, CPBF 426) and show different pathogenicity phenotypes.

The aim of this study is to understand the evolutionary patterns leading to the emergence of a novel *Xanthomonas* strain pathogenic on walnut (CPBF 424).

Material and Methods

The prediction of T3SS and T3E homologues was carried out by BLAST, aligning the genomic sequences of the strains with a database of protein sequences reported to be related with virulence and pathogenicity of *Xanthomonas* pathogens (e-value < 1e-10, Query coverage ≥ 40%, identity ≥ 70%).

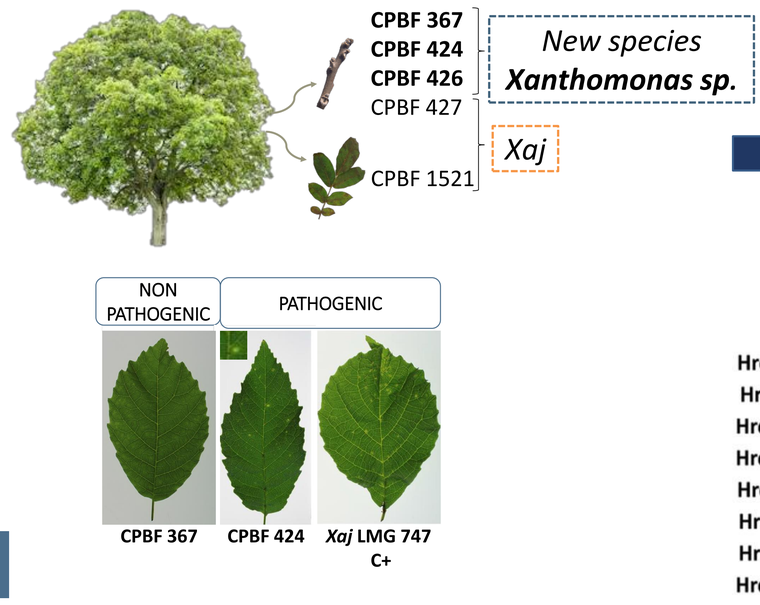
Analyses for recombination of the T3SS cluster was accomplished using the whole genome alignment tool MAUVE [6] from Geneious 9.1.7 (http://www.geneious.com). T3SS cluster structure and organization was assembled using the genome browser tool of EDGAR [7][8].

The strains used in this work are deposited in commercially available collections:

- ✓ **CPBF 424^T** [9] = LMG 31037= CCOS 1891 = NCPPB 467
- ✓ **CPBF 1521** [10] =LMG 31040 = CCOS 1894 = NCPPB 4676
- ✓ **CPBF 367** = LMG 31036 = CCOS 1890
- ✓ **CPBF 426** = LMG 31038 = CCOS 1892
- ✓ **CPBF 427** = LMG 31039 = CCOS 1893

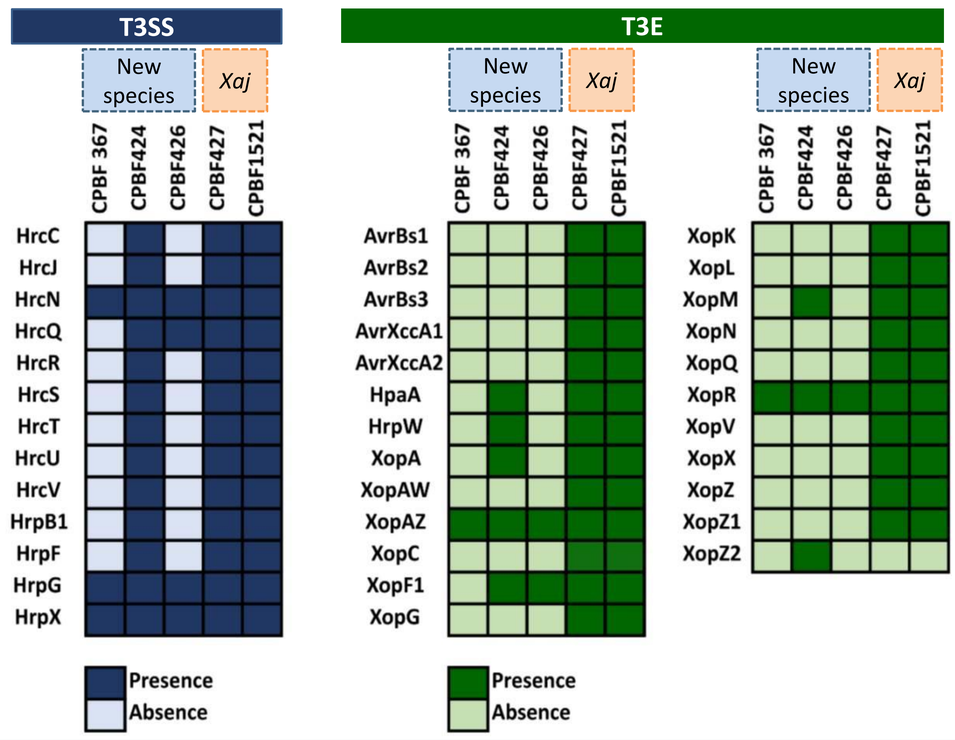
References

[1]Frutos D. & López G. 2012. DOI: 10.4454/jpp.v94i1sup.007;
[2]Lamichhane JR. 2014 DOI: 10.1094/PDIS-08-14-0831-FE;
[3] Meline V. et al.2019 DOI: 10.1111/mpp.12737
[4] Merda et al. 2016 DOI: 10.1111/1758-2229.12397
[5] Hajri et al. 2009 DOI: 10.1371/journal.pone.0006632
[6] Fernandes C. et al. 2018a bioRxiv:397703.
[7] Darling AC. et al. 2004 DOI: 10.1101/gr.2289704
[8] Blom J. et al. 2009 DOI: 10.1186/1471-2105-10-154
[9] Blom J. et al. 2016 DOI: 10.1093/nar/gkw255
[10] Fernandes C. et al. 2018b DOI: 10.1128/mra.00921-18
[11] Fernandes C. et al. 2018c DOI: 10.1128/mra.00887-18



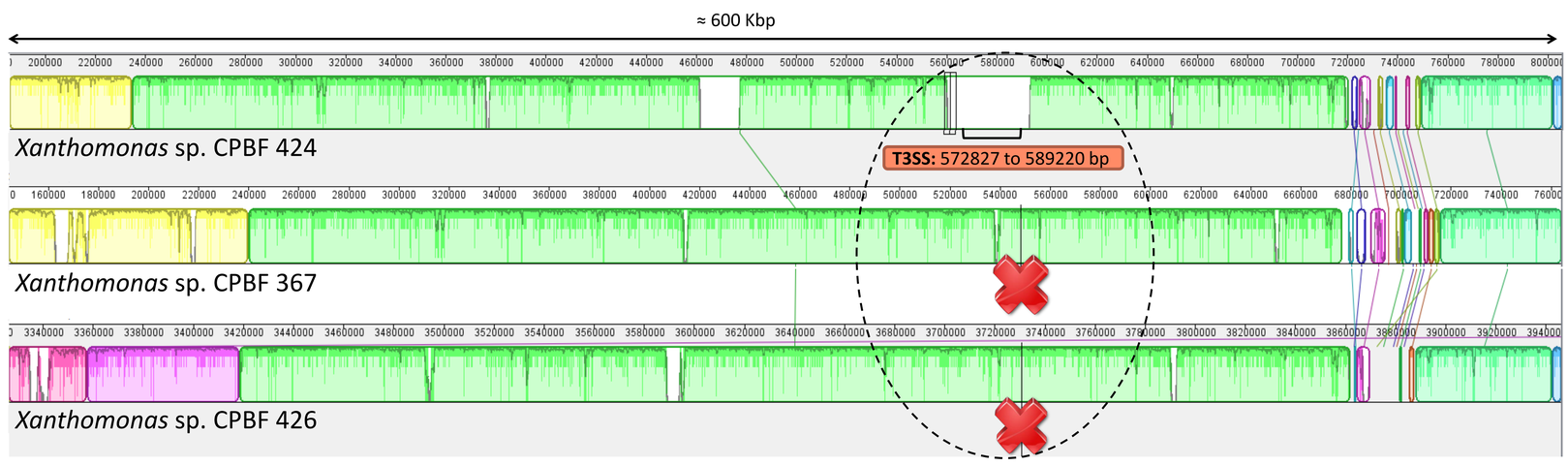
→ **What makes CPBF 424 pathogenic?**

→ **Understand the evolutionary pathway of these strains**

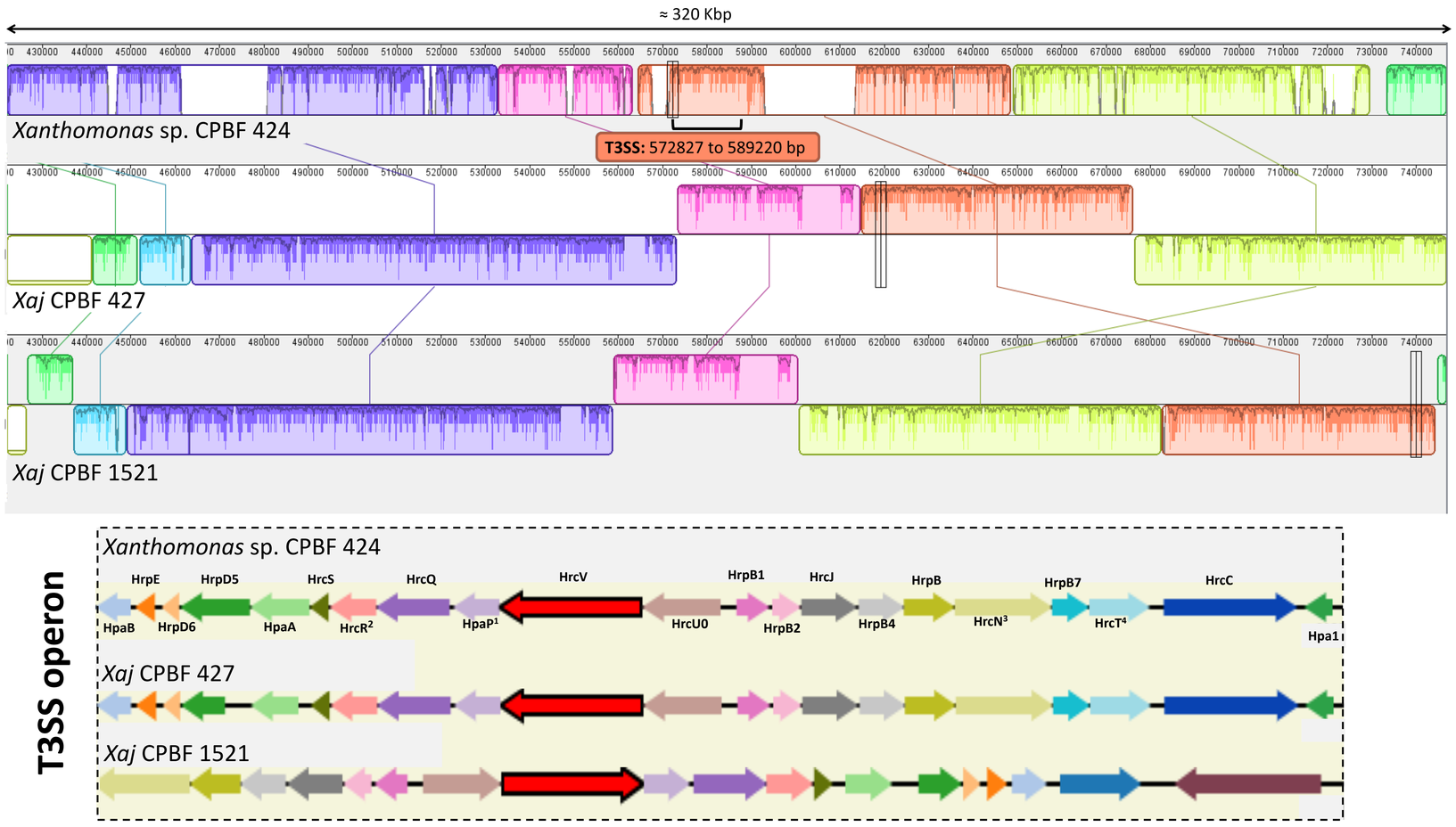


Synteny analysis

→ T3SS **present** in CPBF 424 is **absent** in nonpathogenic strains CPBF 367 and CPBF 426



→ High synteny **suggests a common ancestor** between *Xaj* and *Xanthomonas* sp. CPBF 424



Acknowledgements

This research was co-financed by the European Structural & Investment Funds (ESIFs) through COMPETE 2020; and by National Funds through FCT- Fundação para a Ciência e Tecnologia, within the framework of the project EVOXANT (PTDC/BIA-EVF/3635/2014-POCI-01-0145-FEDER-016600) and by the COST action EuroXanth-CA16107 Leonor Martins is supported by a FCT fellowship (SFRH/BD/137079/2018).

Conclusions

- ✓ The absence of T3SS and T3E genes support the nonpathogenic phenotype of CPBF 367 and CPBF 426.
- ✓ Strain CPBF 424 is a particullarly interesting model for studying pathogenicity and virulence determinants, as this strain is able to cause disease in walnut with a smaller T3E repertoire than the typical *Xaj* strains.
- ✓ The high synteny observed for the T3SS flanking regions in pathogenic CPBF 424 and nonpathogenic CPBF 367 and CPBF 426 strains and the considerable loss of genes of T3SS operon in the nonpathogenic strains (CPBF367 and CPBF426) raises the hypothesis of an adaptation to an epiphytic lifestyle.
- ✓ Interestingly, the T3SS cluster organization of CPBF 424 remained very similar to the one present in CPBF 427, one of the *Xaj* strains isolated from the same tree.