Evolutionary switch from T4SS- to T6SS-mediated bacterial killing strategy in xanthomonads

P-330

2025 IS-MPMI Congress in Cologne (13-17 July)

Chloé Peduzzi[1*], Jules Butchacas[2,3], Veronica Roman-Reyna[5], François Nikis[1], Jonathan M. Jacobs[2,3], Ralf Koebnik[4] and Claude Bragard[1*]

*chloe.peduzzi@uclouvain.be, claude.bragard@uclouvain.be

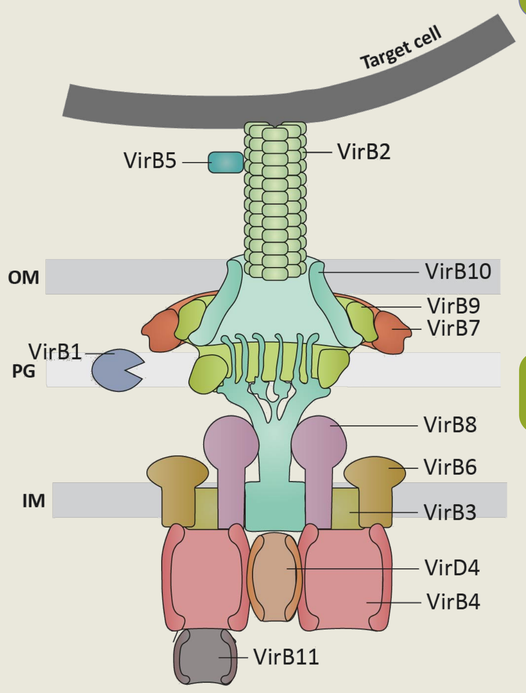
[1] Santé végétale, Earth & Life Institute, UCLouvain, Louvain-la-Neuve, Belgium
[2] Department of Plant Pathology, The Ohio State University, Columbus, OH 43210, U.S.A
[3] Infectious Diseases Institute, The Ohio State University, Columbus, OH 43210, U.S.A
[4] Plant Health Institute of Montpellier (PHIM), IRD, Montpellier, France
[5] Department of Plant Pathology and Environmental Microbiology, Pennsylvania State University, University Park, PA, USA

Background

1

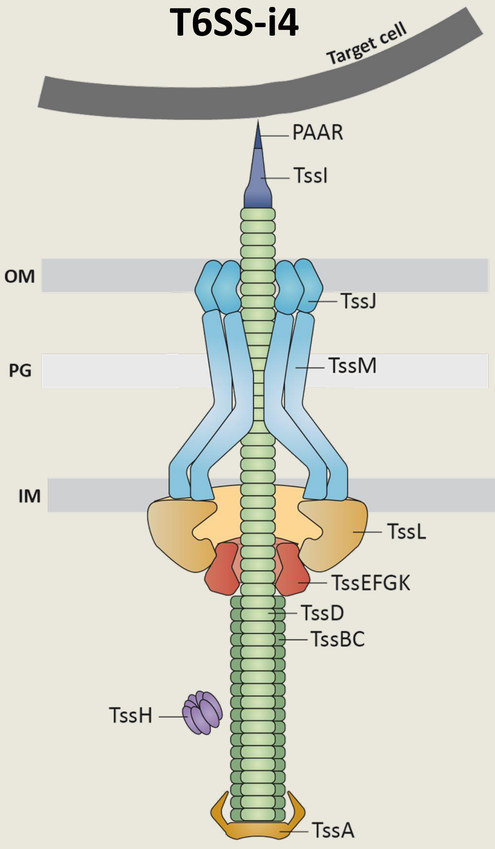
Two distinct contact-dependent competitive strategies have been identified within the *Xanthomonas* genus, the type IV (X-T4SS) and the type VI (T6SS) secretion systems [1-4]. These systems deliver toxic effectors into rival bacterial cells, promoting survival within their ecological niches. However, the ecological relevance for the evolution of two functionally convergent secretion systems in xanthomonads remains unclear. Here, we investigated their evolution and function in xanthomonads including the Bacterial Leaf Streak pathogen, *Xanthomonas translucens* (*Xt*) [5].

X-T4SS



Inspired from [Sgro, 2019]

T6SS-i4

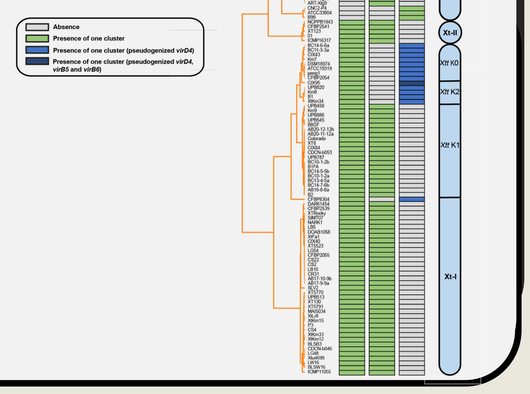


X-T4SS and T6SS-i4 display mutually exclusive distribution profiles in *Xt* strains

2

The tree is based on **Average Nucleotide Identity (ANI) [6]** among ***Xt* strains** and the table summarizes, for each strain, the presence or absence of X-T4SS, T6SS-i3 and i4 gene clusters.

Interestingly, X-T4SS was present when T6SS-i4 was absent and vice versa. Based on MCMCglmm analyses [7], we found a significant negative correlation between both presence of X-T4SS and T6SS-i4 loci.

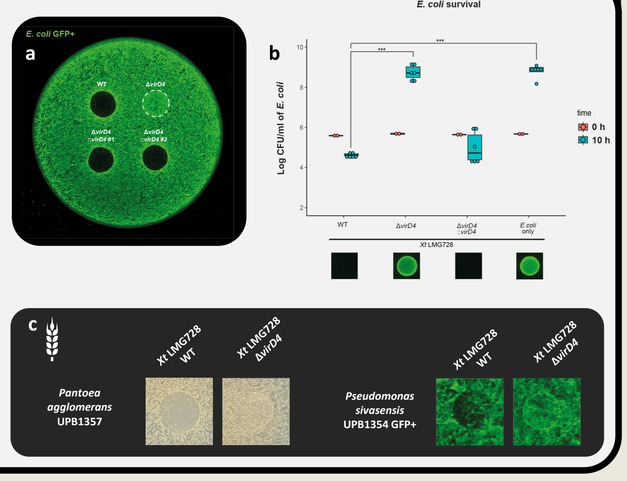


Xt X-T4SS is required for interbacterial competition

3

Competition assays using *Xt* LMG728 WT, and X-T4SS mutants against *E. coli, Pseudomonas* sp. or *Pantoea* sp.

(a, c) representative qualitative assay
(b) quantitative assay mixed in ratio 1:10 *E. coli*:*Xt*

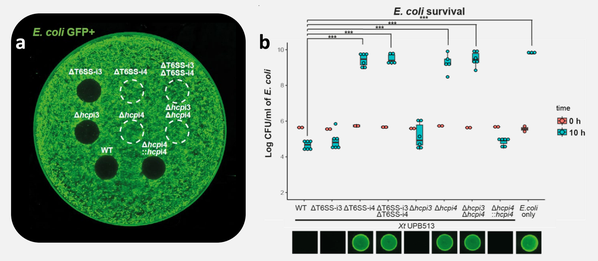


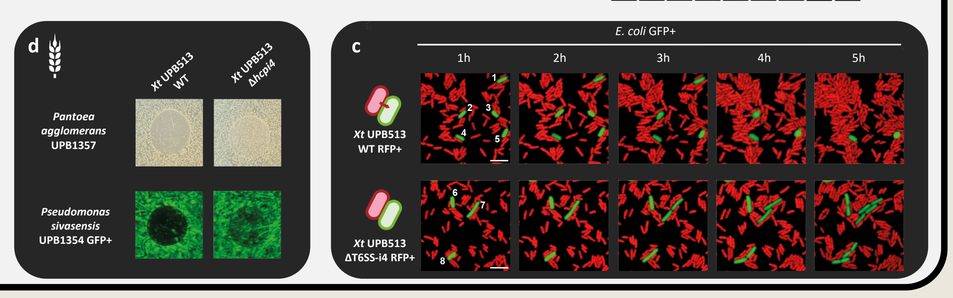
Xt T6SS-i4 is required for interbacterial competition

4

Competition assays using *Xt* UPB513 WT, and single and double mutants of T6SS-i3 and T6SS-i4 against *E. coli, Pseudomonas* sp. or *Pantoea* sp.

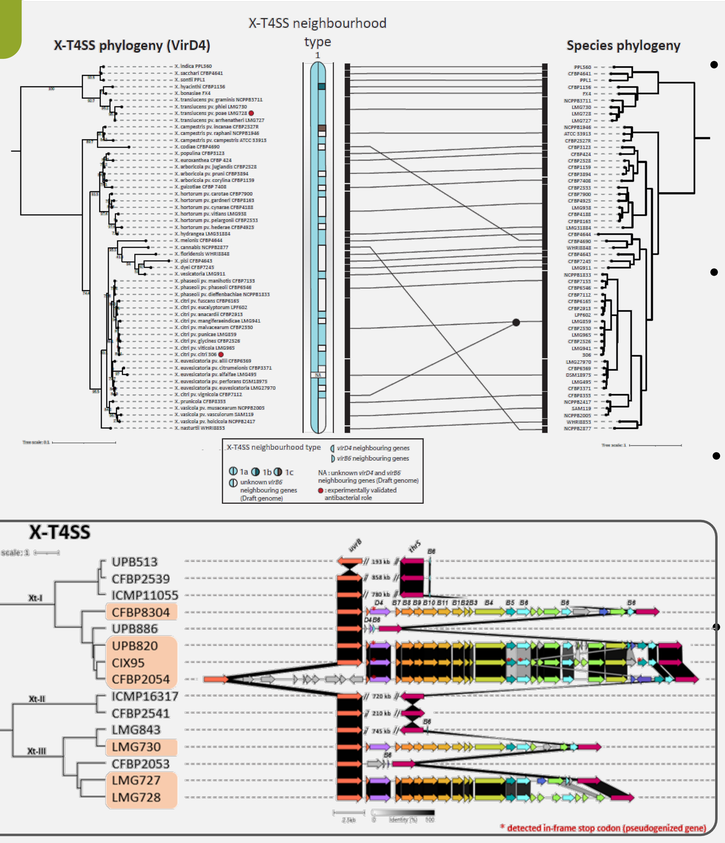
(a, d) representative qualitative assay
(b) quantitative assay mixed in ratio 1:10 *E. coli*:*Xt*
(c) time lapse





Loss of ancestral X-T4SS in xanthomonads

5



- X-T4SS gene phylogeny (VirD4) is **mostly congruent** with the species tree among xanthomonads.
- All X-T4SS gene clusters share the **same chromosomal location** across all xanthomonads
- **Fragments of X-T4SS gene** were found in the corresponding location of X-T4SS-lacking *Xt* strains.

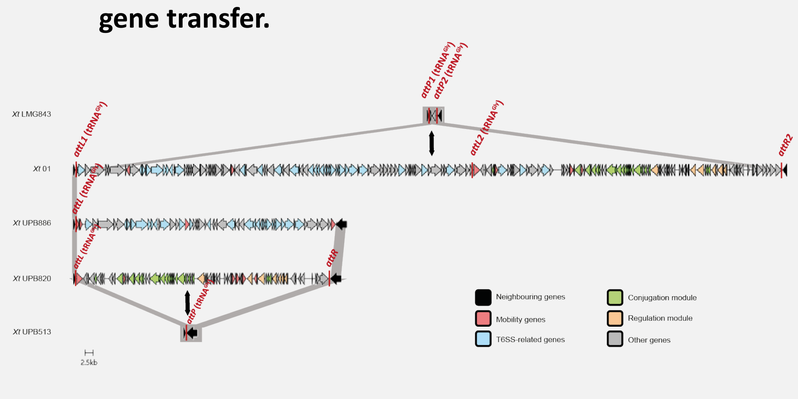
Some strains carry X-T4SS with a **pseudogenized *virD4***, but an otherwise intact cluster, suggesting the **T4SS pilus** may still form and may serve **new functions** as target cell adhesion, or cell motility [8].

Conclusion : X-T4SS was likely acquired ancestrally in *Xanthomonas* evolution, and subject to loss events in some lineages.

ICE are involved in T6SS-i4 distribution

6

- The T6SS-i4 gene phylogeny is **not congruent** with the species tree, and its clusters were **found at different genomic locations** across *Xanthomonas* lineages, suggesting **horizontal gene transfer.**



- In some cases, T6SS-i4 clusters are contained within **genomic islands (tRNA^{Gly} gene, *attL/ attR* sites).** Some strains also have Integrative and Conjugative Elements (ICE) with conjugative T4SS, in tandem with T6SS-i4, flanked by similar DRs, indicating T6SS-i4 may act as **cis-mobilizable elements (CIMEs) with the ICE.**

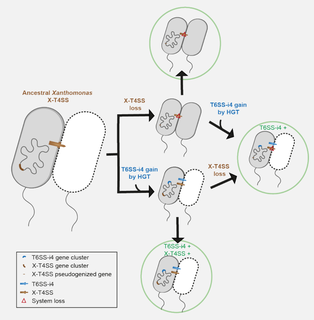
Evolutionary model for X-T4SS loss and T6SS-i4 gain

8

Our analyses support an evolutionary model in which the loss of the ancestral X-T4SS and the gain of T6SS-i4 result from **two possible scenarios :**

1) The introduction of T6SS-i4 drove X-T4SS loss

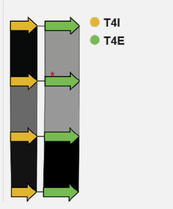
2) The presence of X-T4SS prevented the acquisition of T6SS-i4.



X-T4SS and T6SS-i4 effector arsenals

7

X-T4SS+	T6SS-i4-	LMG728	KM539_00435- KM539_00440
X-T4SS-	T6SS-i4+	UPB513	MZO50_RS02905-MZO50_RS02915
X-T4SS-	T6SS-i4+	Xtg2	HPGDLACB_03894-HPGDLACB_03895
X-T4SS-	T6SS-i4-	LMG726	KFS84_02910-KFS84_02915



The **X-T4SS effector degradation**, along with the **exclusive presence of T6SS-i4 effectors in T6SS-i4-possessing strains**, support the switch from X-T4SS- to T6SS-i4-mediated bacterial killing strategy in xanthomonads.

Acknowledgements:
C. Peduzzi is a fellow of the Fonds de Formation à la Recherche en Industrie et en Agriculture (FRIA – FNRS). This poster is based upon work from COST Action CA16107 EuroXanth, supported by COST (European Cooperation in Science and Technology).

[1] Alvarez-Martinez, C. E. *et al.* (2021). *Comput Struct Biotechnol J.*, 19, 279-302.
[2] Sgro, G. G. *et al.* (2019). *Front. Microbiol.*, 10, 1078.
[3] Souza, D. P. *et al.* (2015). *Nat. Commun.*, 6, 6453.
[4] Zhu, P.C. *et al.* (2020). *Res. Microbiol.*, 171(2), 64-73.
[5] Bragard, C. *et al.* (1995). *Appl. Environ. Microbiol.*, 61, 1020-1026.
[6] Goettelmann, F. *et al.* (2022). *Front. Microbiol.*, 12, 817815.
[7] Hadfield, J. D. J. *et al.* (2010). *Stat. Softw.*, 33, 1-22.
[8] Fronzes, R. *et al.* (2009). *Nat. Rev. Microbiol.*, *7*(10), 703-714.