



# Type III virulence effectors in *Pseudomonas syringae* pathovar *lachrymans* strain 814/98



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## Introduction

Current genomics technologies provide means for comparative genome analysis from which conclusions related to the virulence of the pathogens may be drawn. One of the virulence compounds in plant pathogens are virulence effectors. The genomes of seven strains belonging to *Pseudomonas syringae* pathovar *lachrymans*, that is causing angular leaf spot disease in cucumber (Figure 1), have been sequenced, assembled and are available as drafts at NCBI. However, only two strains, MAFF301315 and MAFF302278 were compared to other strains of *P. syringae* by Baltrus et al. (2011). These strains, unlike other *P. syringae* stains, have only a low percentage of Type Three Effectors (TTEs).

In this study we used bioinformatics approach and analyzed the presence of TTEs in *P. syringae* strains representing different pathovars with a special focus on strains belonging to pathovar *lachrymans* - and mainly on highly virulent strain 814/98 sequenced recently at our Department.



Fig 1. Angular leaf spot symtopms on 3 different cucumber varieties caused by *Pseudomonas syringae* pv. *lachrymans*

## Materials and Methods

**Identification of TTEs.** In order to identify TTEs a dataset of 23 *Pseudomonas* spp. genome sequences was constructed. It consisted the genome of strain 814/98 sequenced at our Department and genomes of other strains of pathovar *lachrymans* and strains belonging to *P. syringae* or *P. savastanoi* pathovars available at NCBI (<http://blast.ncbi.nlm.nih.gov>). The genome sequence of each strain was surveyed with tBLASTn algorithm in CLC Genomic Workbench 9.0 (CLC Bio, Aarhus, Denmark). The set of known effectors was constructed based on an extended table of the Baltrus et al. (2011) concept and on validated TTEs family members deposited on PPI website (<http://www.pseudomonas-syringae.org/>). A strain was considered to possess an effector if a majority of the protein sequences had significant BLAST hits ( $<1e-5$ ) with an identity of at least 90%).

**Strains grouping.** The binary matrix of the presence or absence of TTEs for *Pseudomonas* spp. set was constructed. Based on the TTEs genes presence the dendrogram and Venn diagram were constructed. The TTEs comparison between the three fully sequenced genomes, i.e.: *P. syringae* pv. *tomato* DC3000, *P. syringae* pv. *syringae* B728a, and *P. syringae* pv. *phaseolicola* 1448A with *P. syringae* pv. *lachrymans* strains 814/98 and 3988, which are genetically distant, allowed to show differences between strains.

## Results

Out of 90 TTEs known for plant pathogenic bacteria, 78 were present in the genomes of 23 *Pseudomonas* spp. strains. Strains representing pathovar *lachrymans* possessed different content of TTEs and based on it were divided into two groups (Figure 2). The first group consisted of five *lachrymans* strains, i.e.: 107, 98A-744, YM7902, 814/98 and MAFF301315. The second group of different TTEs content consisted of two *lachrymans* strains 3988 and MAFF302278.

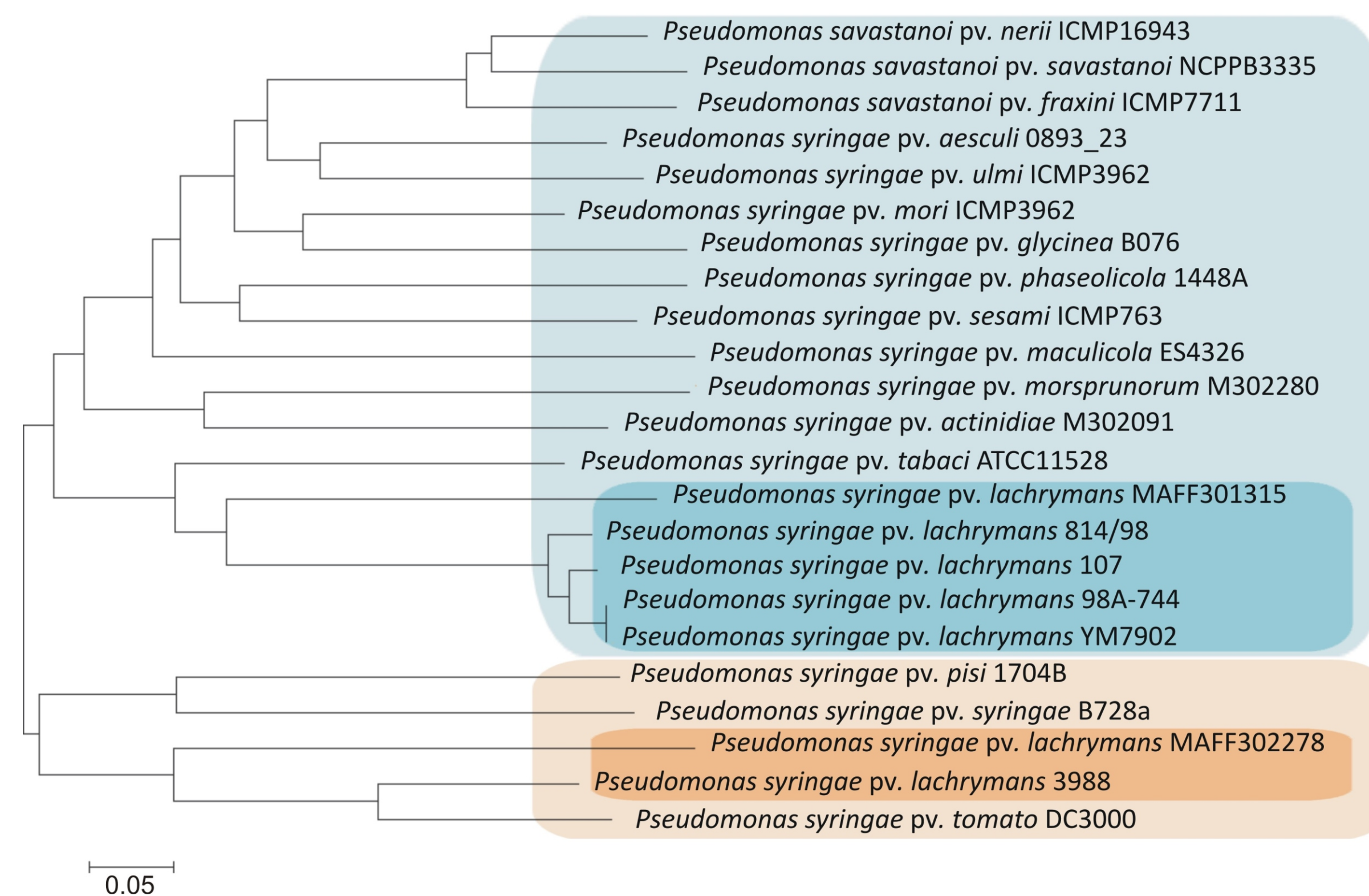


Fig. 2. A dendrogram of relationships among phytopathogenic strains of *Pseudomonas* spp. based on the TTEs presence, two distinct groups of *lachrymans* strains are indicated in darker colour.

After TTEs analysis in strains representing different pathovars we concluded that a core set of 6 TTEs: *avrE1*, *hopI1*, *hopM1*, *hopAA1*, *hopAF1*, and *hopAH1* is conserved among many plant pathogenic *P. syringae* strains, including reference strains *P. syringae* pv. *tomato* DC3000, *P. syringae* pv. *syringae* B728a and *P. syringae* pv. *phaseolicola* 1448A. In addition to the conserved TTEs set, each genome contained several unique TTEs genes.

We compared TTEs of two strains 814/98 and 3988, representing two distinct groups of *lachrymans* strains. The strain 814/98 contained: the core set of TTEs, 10 TTEs common for all *lachrymans* strains: *avrPto1*, *hopE1*, *hopA1*, *hopV1*, *hopAB3*, *hopAE1*, *hopAG1*, *hopI1*, *hopAS1*, *hopAZ1* (bolded and underlined in Figure 3A) and additional 8 TTEs present only in 814/98, i.e.: *avrD1*, *avrPto4*, *hopW1*, *hopZ4*, *hopAR1*, *hopAW1*, *hopBD1* and *hopBK1*. Different TTEs repertoire was found for strain 3988 representing the second group of *lachrymans* strains. It contained: six core TTEs and 23 hop weffectors present in *P. syringae* pv. *tomato* DC3000 (bolded and underlined in Figure 3B). Interestingly, there are two *hop* genes, *hopAW1* and *hopBD1*, that are present in both *lachrymans* groups, but absent in other pathovars and are unique virulence *lachrymans* effectors.

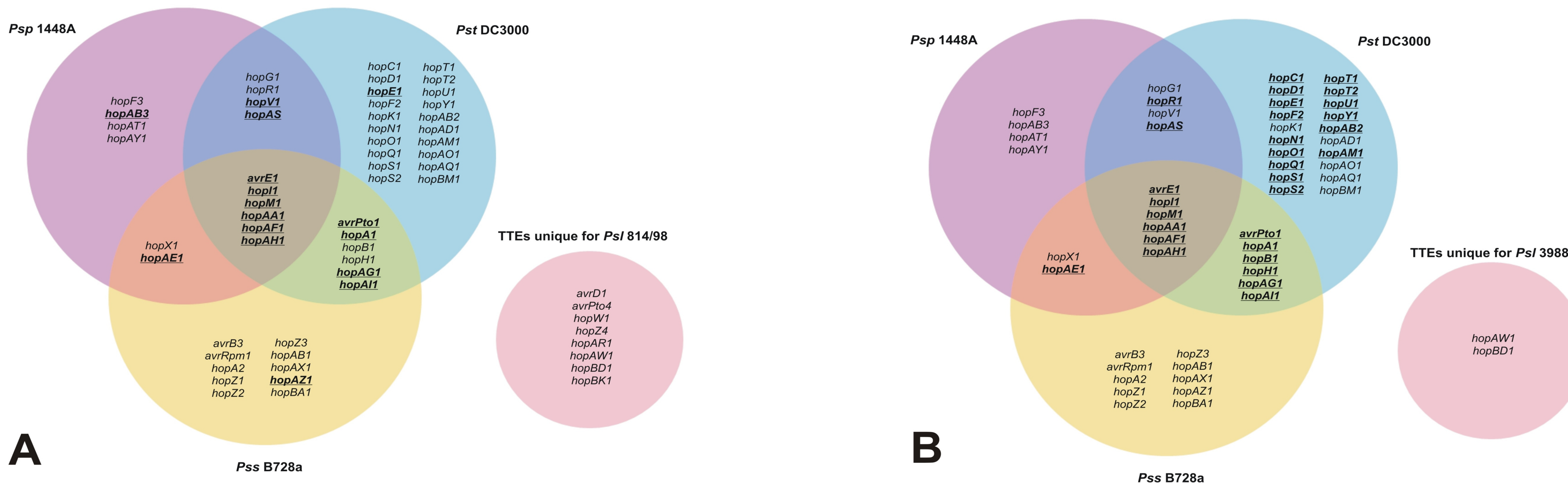


Fig. 3. TTEs in *P. syringae* pv. *tomato* DC3000, *P. syringae* pv. *syringae* B728a, and *P. syringae* pv. *phaseolicola* 1448A reference strains compared with TTEs repertoire of strain 814/98 (A) and 3988 (B) that are representing genetically distinct *P. syringae* pv. *lachrymans* group of strains.

## Conclusions

- 1.The analysis of TTEs in the 814/98 genome resulted in identification of 24 effectors which are members of different TTEs families, e.g. *avrE*, *hopAA*, *hopM*, *hopI*.
- 2.The effectors present in strain 814/98 belong either to the core TTEs or to the flexible TTEs that are strain diverse in sequence.
- 3.This study indicates a heterogeneous distribution of TTEs within *P. syringae* - the differences between two groups of pathovar *lachrymans* strains indicate that they may have evolved separately from different *Pseudomonas* species.

## Acknowledgements

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## Literature

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