

Csp1, A Cold-Shock Protein Homolog in *Xylella fastidiosa* is Involved in Stress Response and Biofilm Formation



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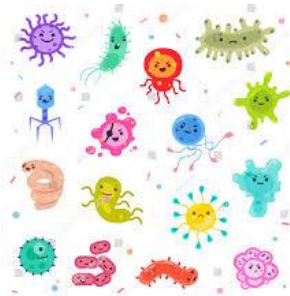
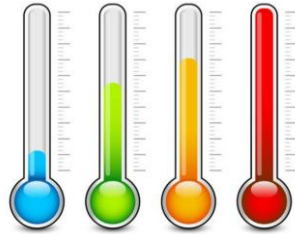
3rd European
conference on
*Xylella
fastidiosa*
2021



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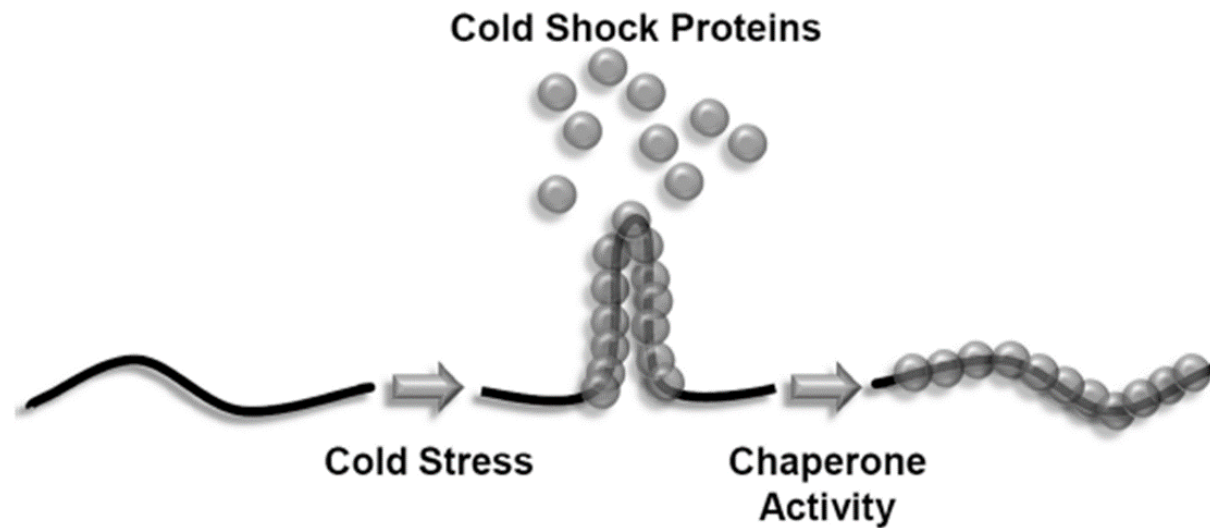


Bacteria encounter many environmental/host stressors



Bacteria cold shock-domain proteins (CSPs) are nucleic acid binding proteins that help bacteria adapt to stress

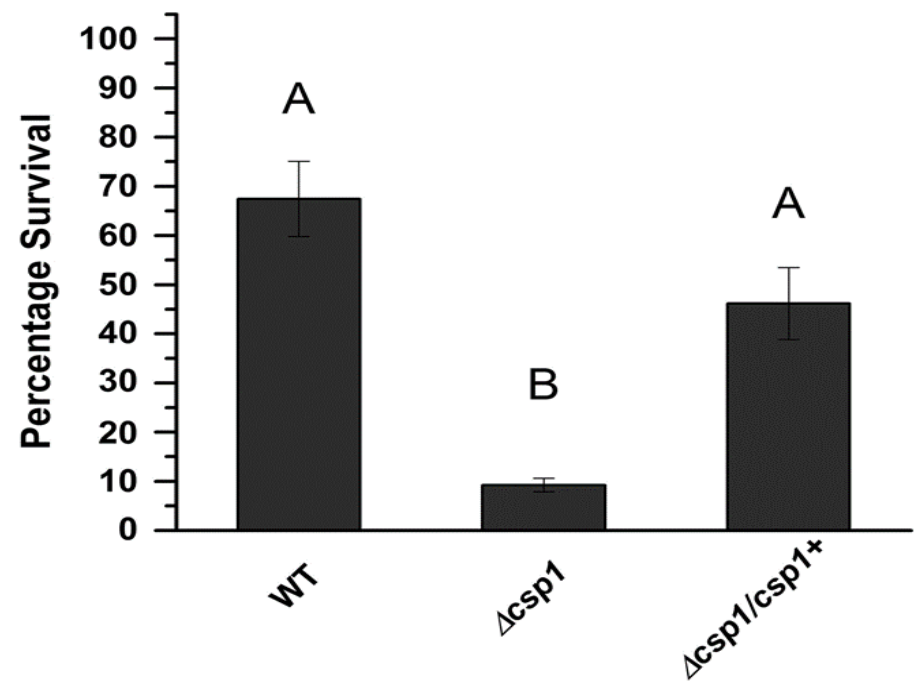
- Destabilizes secondary structures in target mRNA during cell stress
- Gene Expression: Transcriptional activator, transcriptional anti-terminator



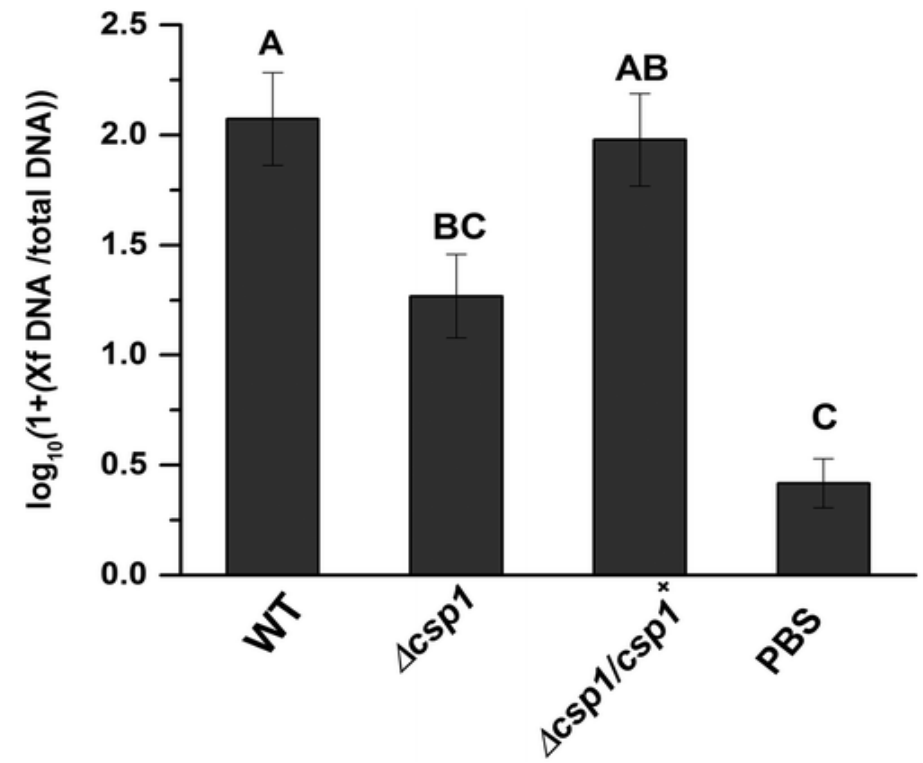
BACKGROUND

- Bacterial plant pathogen *Xylella fastidiosa* (Xf) has two **temperature-independent** CSP homologs, Csp1 & Csp2.
- Deleting *csp1* ($\Delta csp1$) in Xf strain Stag's Leap reduced osmotic stress tolerance *in vitro* and bacteria titer in grapevines ¹.

Osmotic Stress



Bacteria titer



1. Burbank, L. P., and Stenger, D. C. 2016. A Temperature-Independent Cold-Shock Protein Homolog Acts as a Virulence Factor in *Xylella fastidiosa*. Mol. Plant-Microbe Interactions®. 29:335–344

HYPOTHESIS:

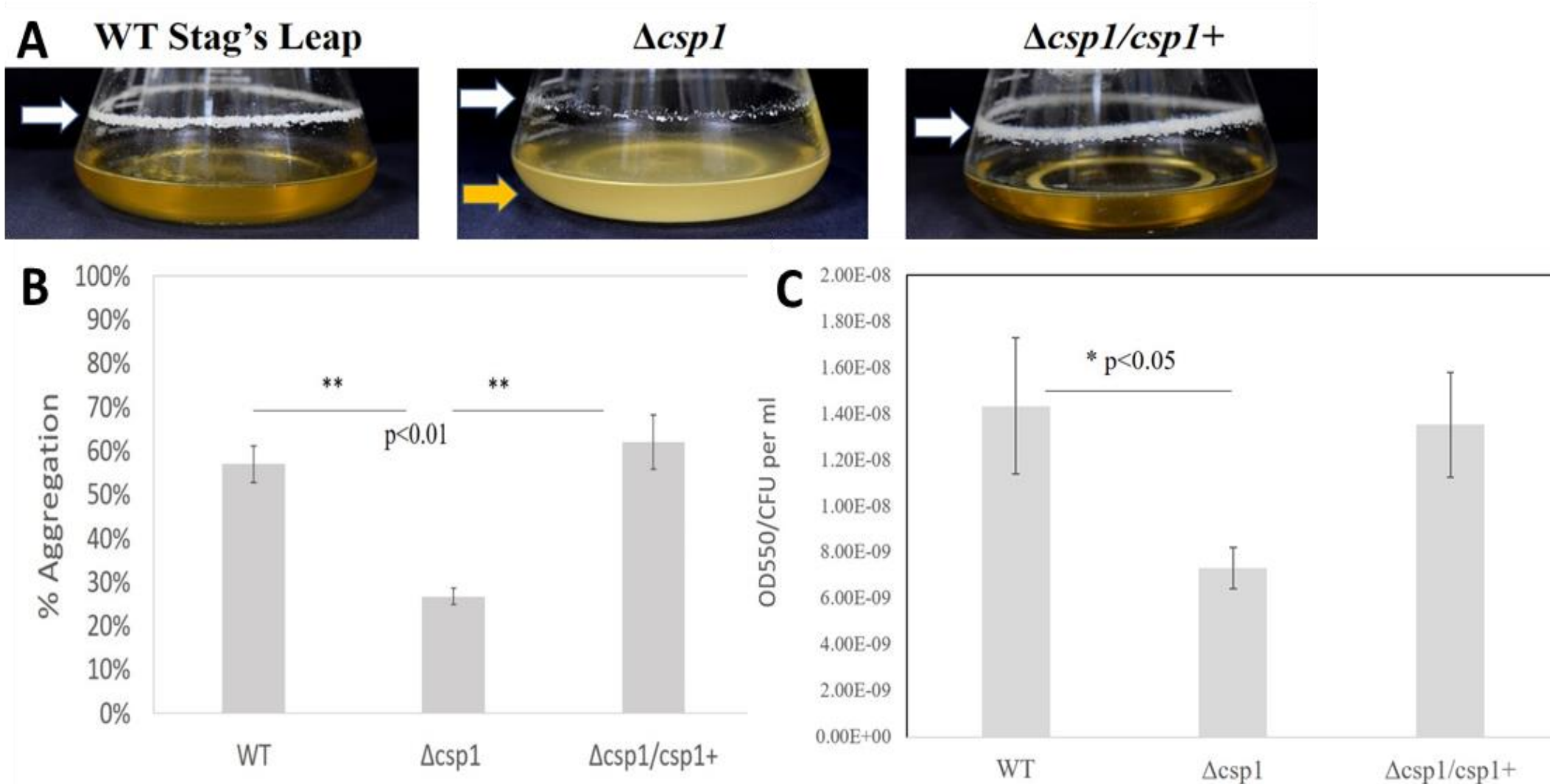
- Aside from general single-stranded nucleic acid binding activity, not much is known about the function(s) of Csp1.
- Studies in *E. coli* and other bacteria have shown Csps can alter gene expression via non-specific nucleic acid binding or binding gene promoter sequences².
- ***Xf* Csp1 may contribute to stress response & survival by influencing gene expression.**

OBJECTIVES:

- 1) Further characterize phenotypic differences between WT *Xf* Stag's Leap and $\Delta csp1$
- 2) Compare transcriptomes of WT and $\Delta csp1$ under standard growth conditions (28C)
- 3) Identify differentially expressed genes of interest and create *Xf* deletion mutants and complements

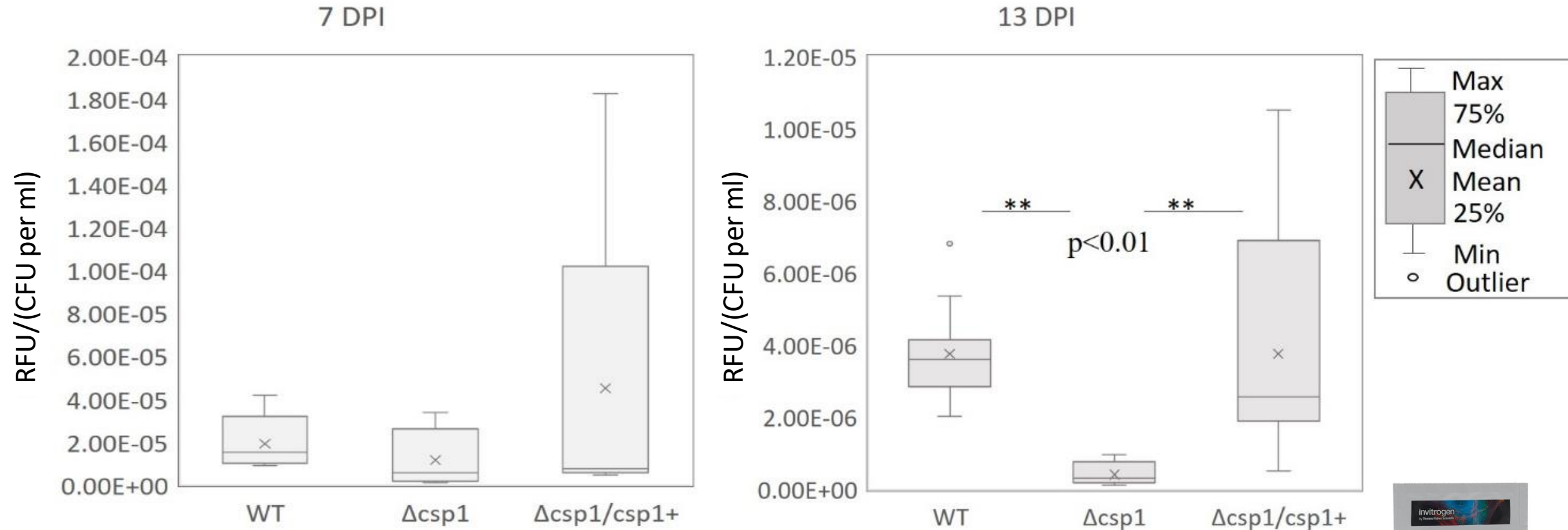
RESULTS

1. *Xf* $\Delta csp1$ showed reduced cellular aggregation and attachment



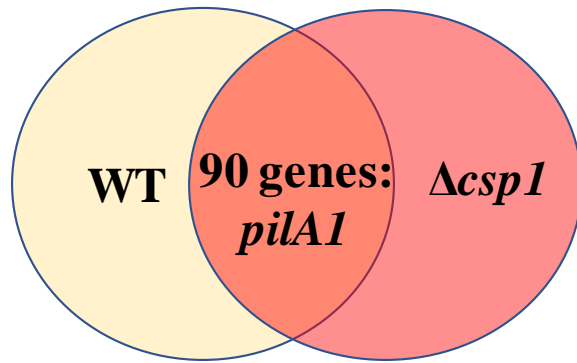
RESULTS

2. *Xf* $\Delta csp1$ showed reduced long-term survival *in vitro*



RESULTS

3) Several motility and attachment-related genes were differentially expressed in *Xf* $\Delta csp1$ at 28C

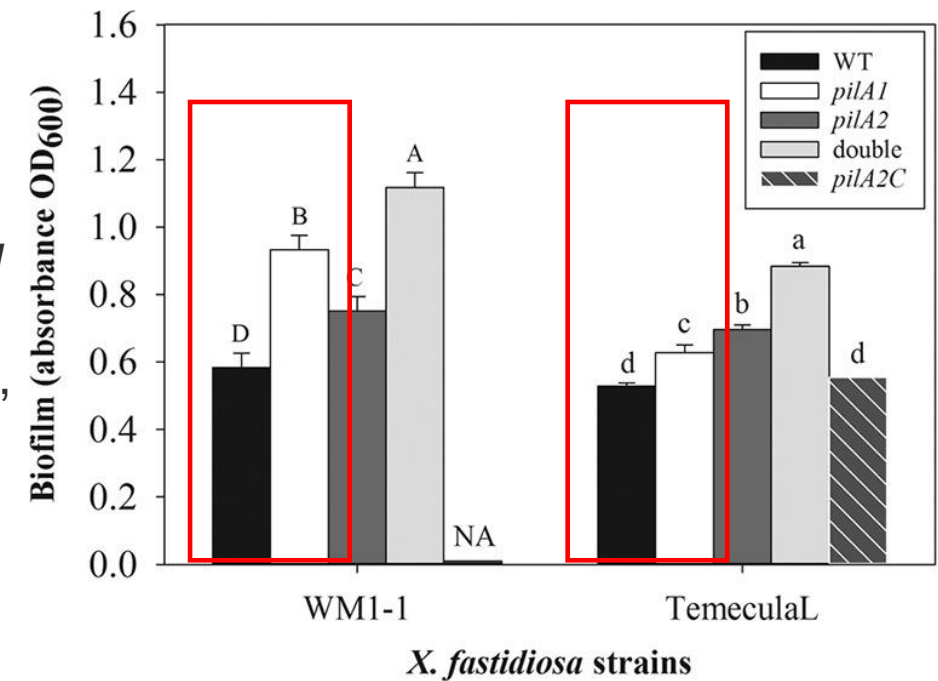


Transcripts per million

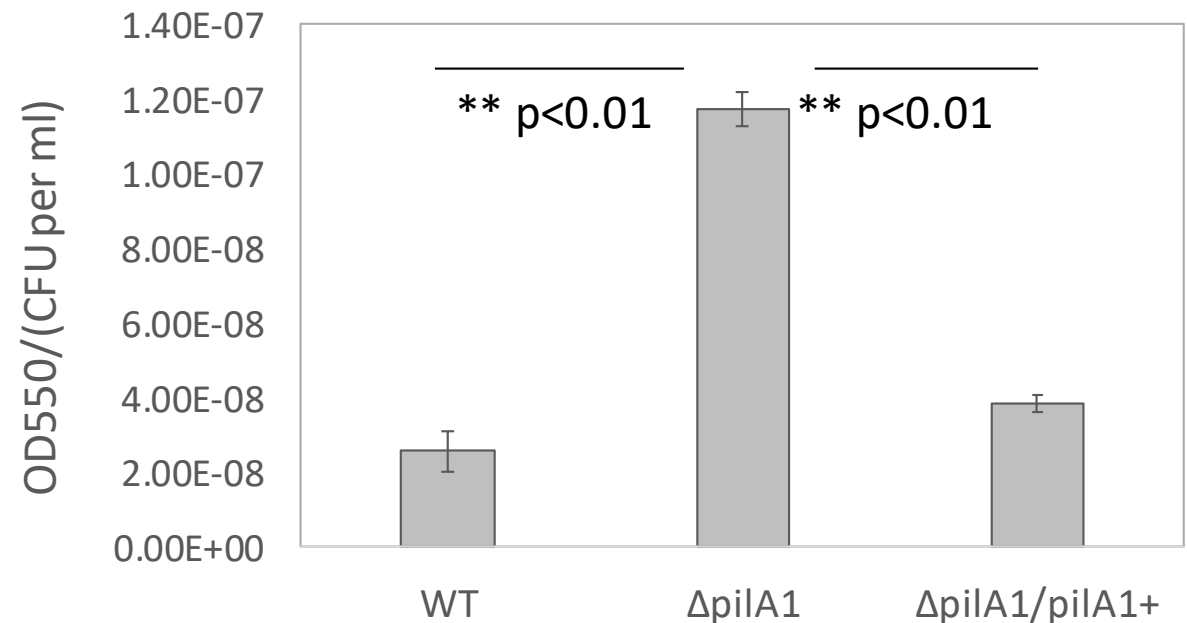
	WT	$\Delta csp1$
<i>pilA1</i> (PD1924)	202.45	892.96

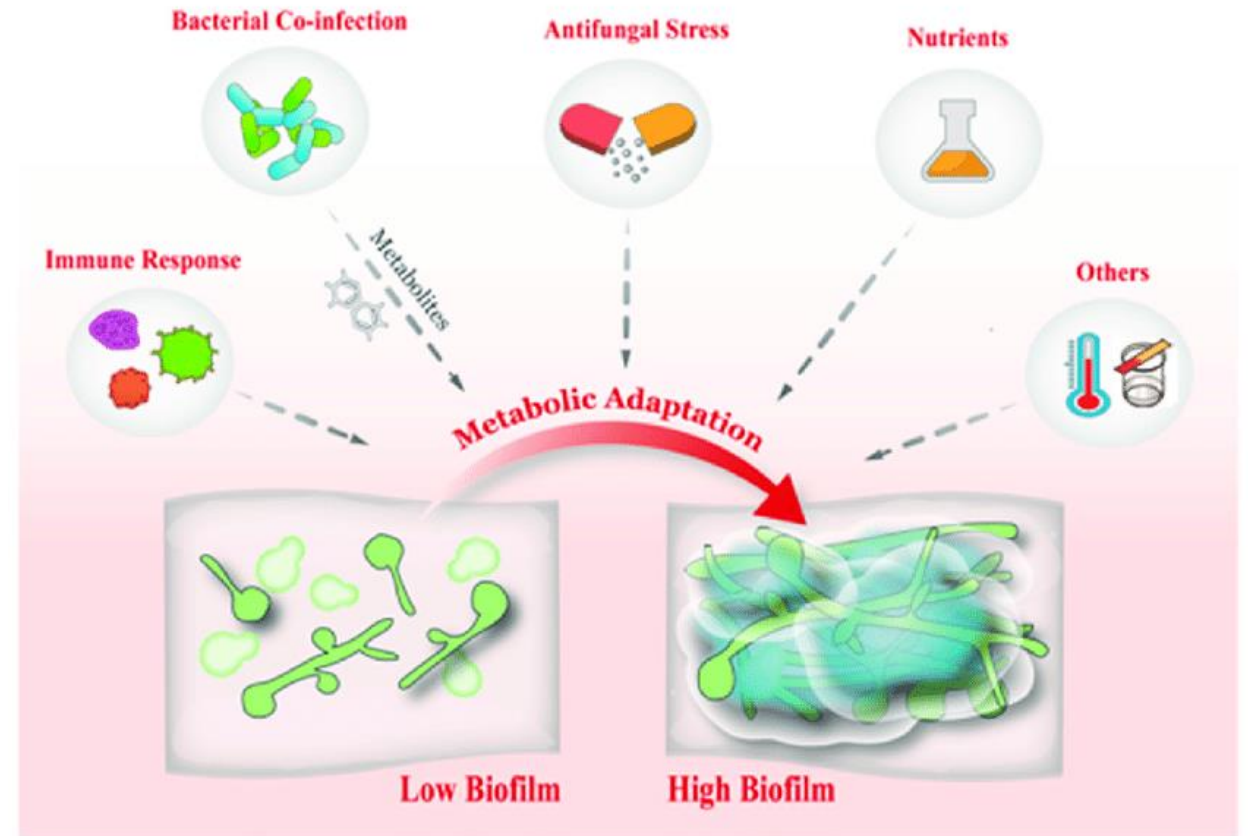
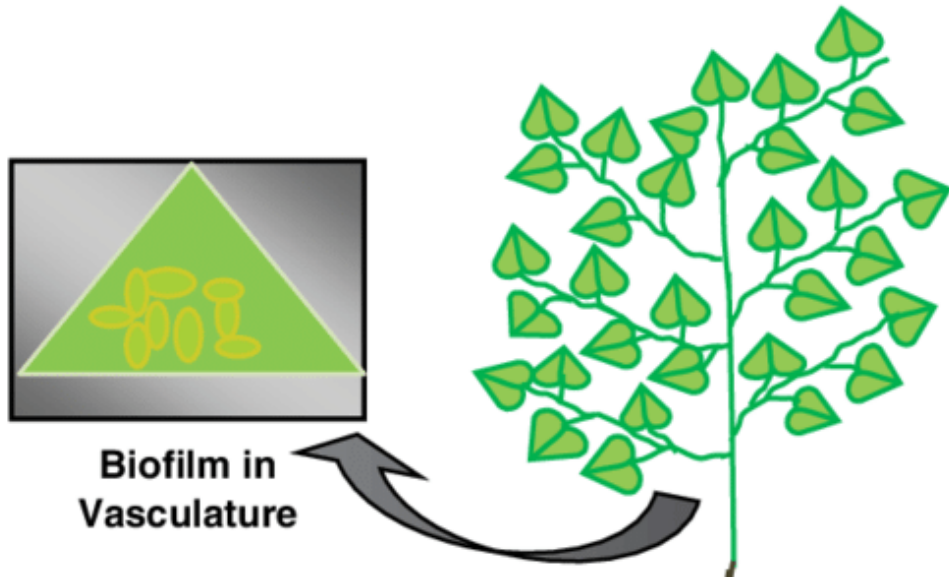
- PilA1: type IV pilin

Kandel et al. *Applied and environmental microbiology*, 84(18), e01167-18.



4) Stag's Leap $\Delta pilA1$ had increased biofilm formation





- Biofilms are essential to *Xf* vector and plant colonization.
- Bacteria in biofilms are more resistant to stressors.
- Csp1 may contribute to stress tolerance and colonization/survival by influencing expression of genes important for biofilm formation.

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Xylella Fastidiosa Active Containment Through a
multidisciplinary-Oriented Research Strategy



Poster link:

<https://zenodo.org/record/4678828#.YldUSvZFw2w>