



Corso di Dottorato in Scienze e  
Tecnologie Agrarie  
XXXII Ciclo

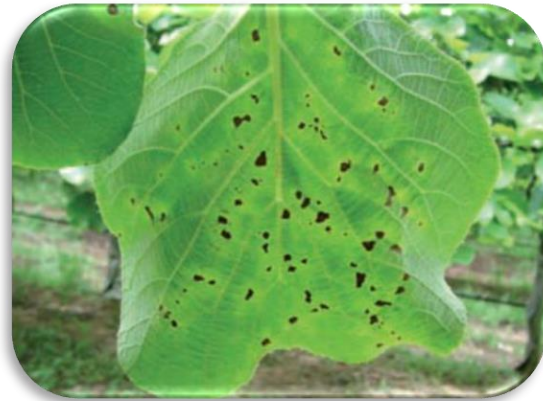
**COMPLEMENTARY RESEARCHES and  
BIOTECHNOLOGICAL INNOVATIVE APPROACHES  
TOWARDS ENVIRONMENTALLY-FRIENDLY CONTROL OF  
BACTERIAL DISEASES of PLANTS**

**Silvia Calamai**  
silvia.calamai@unifi.it

**TUTOR:** Prof.ssa Stefania Tegli  
**CO-TUTOR:** Prof. Stefano Biricolti



*Erwinia amylovora*



*Pseudomonas syringae pv. actinidiae*



*Xylella fastidiosa*

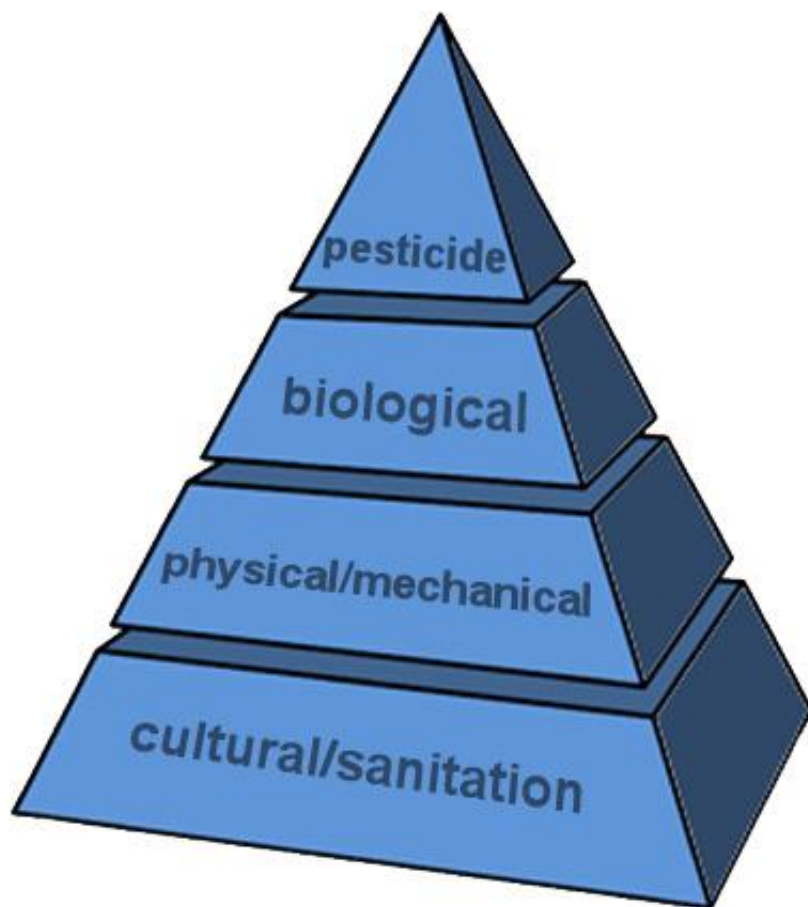




Intervention



Prevention



**"It doesn't seem to be covered in our  
invasive species management plan."**

# Control of Bacterial Plant Disease



## Nasty side effects of copper treatments:

- ✓ accumulation into the topsoil
- ✓ negative impact on soil biology
- ✓ negative impact on transformation/dynamic of nutrients
  - ✓ secondary pollution in watercourses
  - ✓ serious negative ecotoxicological effects

**...last but not least**

- ✓ increase of copper- and antibiotic-resistant bacteria



# Restriction of Copper Use



- Council Directive **91/414/EEC** of 15 July 1991 concerning the placing of plant protection products on the market.
- Commission Directive **2009/37/EC** of 23 April 2009 amending Council Directive 91/414/EEC to include chlormequat, copper compounds, propaquizafop, quizalofop-P, teflubenzuron and zeta-cypermethrin as active substances.
- Regulation (EC) No **1107/2009** of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.
- Commission Implementing Regulation (EU) No **540/2011** of 25 May 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the list of approved active substances.
- Commission Implementing Regulation (EU) No **541/2011** of 1 June 2011 amending Implementing Regulation (EU) No 540/2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the list of approved active substances.
- Commission Implementing Regulation (EU) **2015/232** of 13 February 2015 amending and correcting Implementing Regulation (EU) No 540/2011 as regards the conditions of approval of the active substance copper compounds.

## REASONED OPINION

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ADOPTED: 1 March 2018

doi: 10.2903/j.efsa.2018.5212

### **Review of the existing maximum residue levels for copper compounds according to Article 12 of Regulation (EC) No 396/2005**

## TECHNICAL REPORT

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APPROVED: 30 August 2018

doi:10.2903/sp.efsa.2018.EN-1486

### **Outcome of the consultation with Member States, the applicant and EFSA on the pesticide risk assessment for copper compounds copper(I), copper(II) variants namely copper hydroxide, copper oxychloride, tribasic copper sulfate, copper(I) oxide, Bordeaux mixture in light of confirmatory data**

## CONCLUSION ON PESTICIDES PEER REVIEW

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APPROVED: 20 December 2017

doi: 10.2903/j.efsa.2018.5152

### **Peer review of the pesticide risk assessment of the active substance copper compounds copper(I), copper(II) variants namely copper hydroxide, copper oxychloride, tribasic copper sulfate, copper(I) oxide, Bordeaux mixture**



**COMMISSION IMPLEMENTING REGULATION (EU) 2018/1981**

**of 13 December 2018**

renewing the approval of the active substances copper compounds, as candidates for substitution, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market, and amending the Annex to Commission Implementing Regulation (EU) No 540/2011

(Text with EEA relevance)

**Aim**

**COMPLEMENTARY RESEARCHES AND  
INNOVATIVE APPROACHES FOR THE SAFE AND  
ENVIRONMENTALLY-FRIENDLY CONTROL OF  
BACTERIA PLANT DISEASES**





## Gram-negative bacteria

*Pseudomonas savastanoi* pv. *nerii* Psn23



### Approach

Genomic

Physiological

### Studies

- ✓ MATE pumps
- ✓ IAA Homeostasis

## Gram-positive bacteria

*Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Cff)



### Approach

Genomic

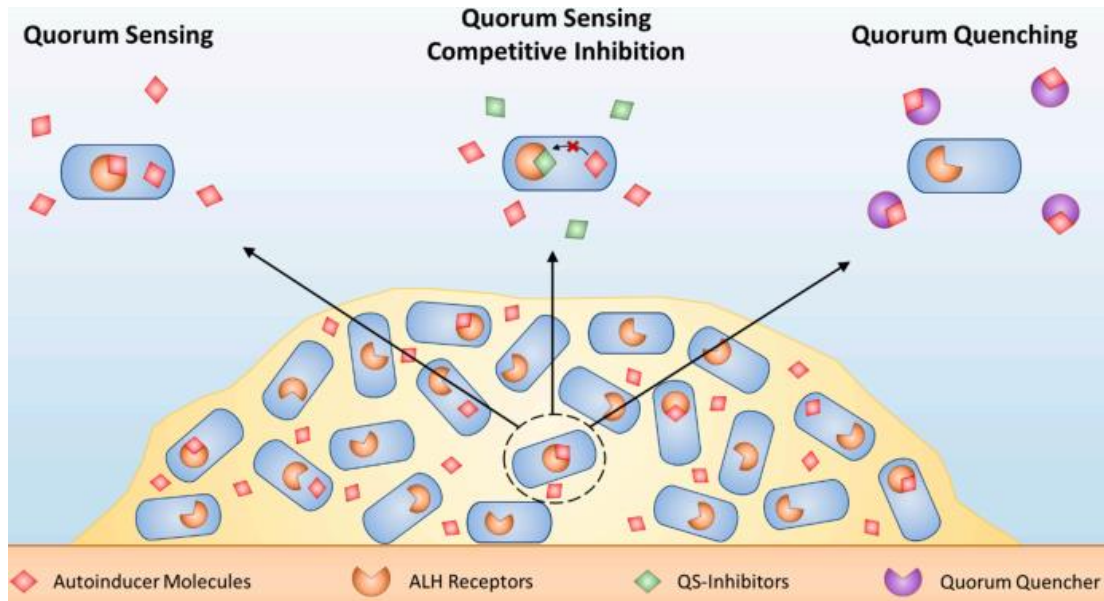
Epidemiological

### Studies

- ✓ Population Analysis
- ✓ Genomic Studies

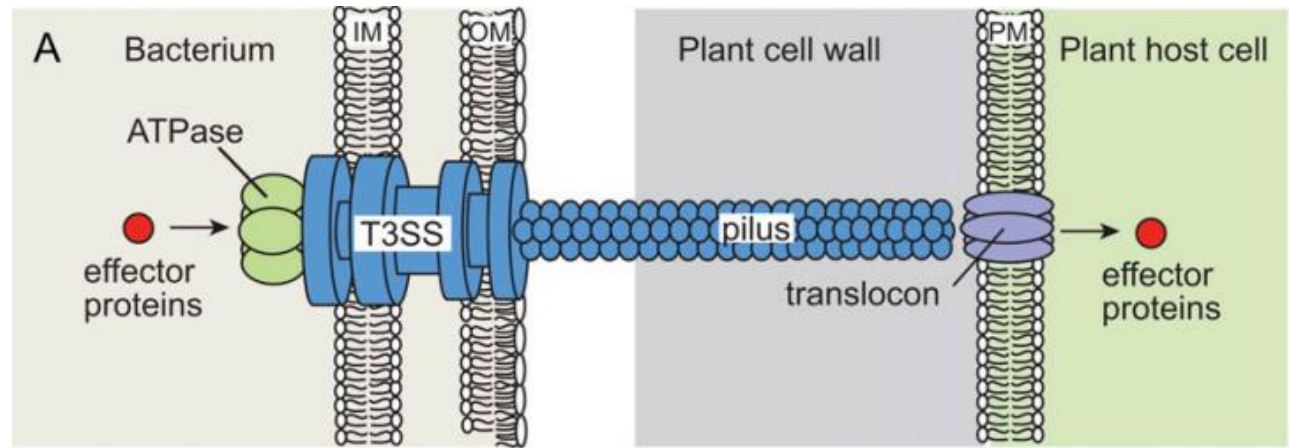
# *Pseudomonas savastanoi* pv. *nerii* Psn23

## Quorum Sensing



(Beitelshees *et al.*, 2018, *Materials*)

## Type Three Secretion System (TTSS)



(Buttner and He, 2009, *Plant Physiology*)

[PLoS One](#). 2016; 11(9): e0163357.

PMCID: PMC5036890

Published online 2016 Sep 26. doi: [10.1371/journal.pone.0163357](https://doi.org/10.1371/journal.pone.0163357)

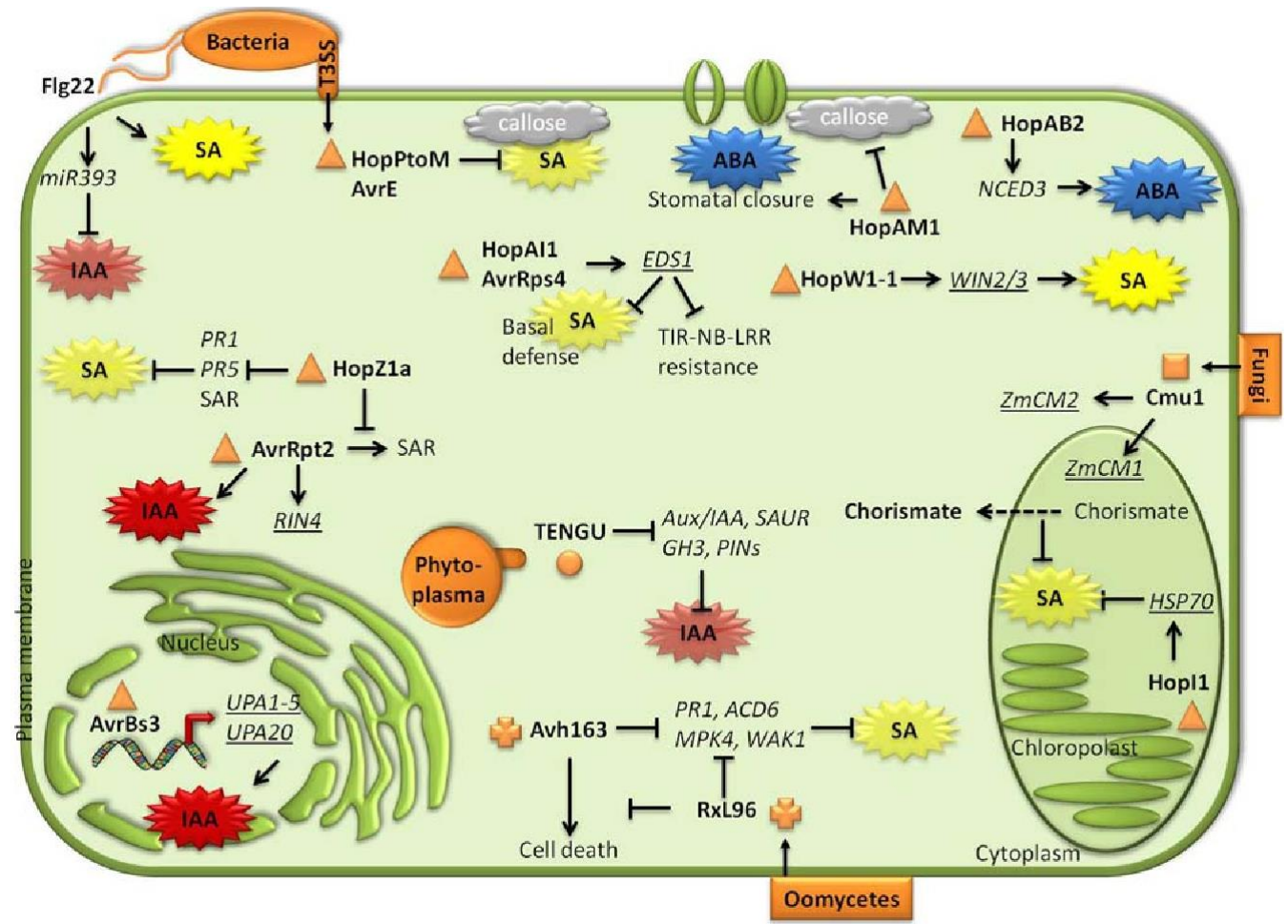
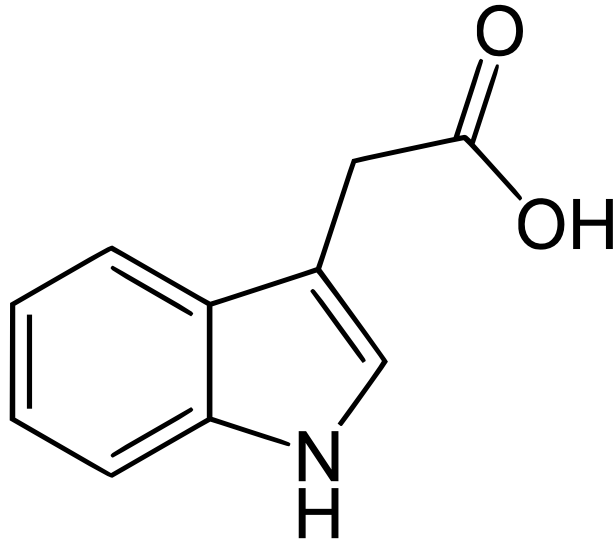
PMID: [27668874](https://pubmed.ncbi.nlm.nih.gov/27668874/)

## Global Analysis of Type Three Secretion System and Quorum Sensing Inhibition of *Pseudomonas savastanoi* by Polyphenols Extracts from Vegetable Residues

[Carola Biancalani](#),<sup>#1</sup> [Matteo Cerboneschi](#),<sup>#1</sup> [Francesco Tadini-Buoninsegni](#),<sup>#2</sup> [Margherita Campo](#),<sup>3,‡</sup>  
[Arianna Scardigli](#),<sup>4,‡</sup> [Annalisa Romani](#),<sup>4,‡</sup> and [Stefania Tegli](#)<sup>#1,\*</sup>

# Detecting alternative target

## Indol-3-acetic acid



**Research Note**

# **Auxin Production Is a Common Feature of Most Pathovars of *Pseudomonas syringae***

**Eric Glickmann,<sup>1</sup> Louis Gardan,<sup>2</sup> Sylvie Jacquet,<sup>2</sup> Shafik Hussain,<sup>2</sup> Miena Elasri,<sup>1</sup> Annik Petit,<sup>1</sup> and Yves Dessaux<sup>1</sup>**

<sup>1</sup>Institut des Sciences Végétales, Centre National de la Recherche Scientifique, Avenue de la Terrasse, F-91198 Gif sur Yvette Cedex; <sup>2</sup>Station de Pathologie Végétale, Institut National de la Recherche Agronomique, 42, rue Georges Morel, B.P. 57, F-49071 Beaucouzé Cedex, France

Accepted 3 November 1997.



ELSEVIER

## Research in Microbiology

Volume 167, Issues 9–10, November–December 2016, Pages 774–787

# Indole-3-acetic acid in plant–pathogen interactions: a key molecule for in planta bacterial virulence and fitness

Matteo Carboneschi <sup>a</sup>, Francesca Decorosi <sup>b</sup>, Carola Biancalani <sup>a</sup>, Maria Vittoria Orteni <sup>a</sup>, Sofia Macconi <sup>a</sup>,  
Luciana Giovannetti <sup>b</sup>, Carlo Viti <sup>b</sup>, Beatrice Campanella <sup>c</sup>, Massimo Onor <sup>c</sup>, Emilia Bramanti <sup>c</sup>, Stefania Tegli



RESEARCH ARTICLE

## Indole-3-acetaldehyde dehydrogenase-dependent auxin synthesis contributes to virulence of *Pseudomonas syringae* strain DC3000

Sheri A. McClerkin<sup>✉</sup>, Soon Goo Lee<sup>✉</sup>, Christopher P. Harper, Ron Nwumeh, Joseph M. Jez, Barbara N. Kunkel<sup>\*</sup>

Department of Biology, Washington University, St. Louis, Missouri, United States of America

✉ These authors contributed equally to this work.

\* [kunkel@wustl.edu](mailto:kunkel@wustl.edu)

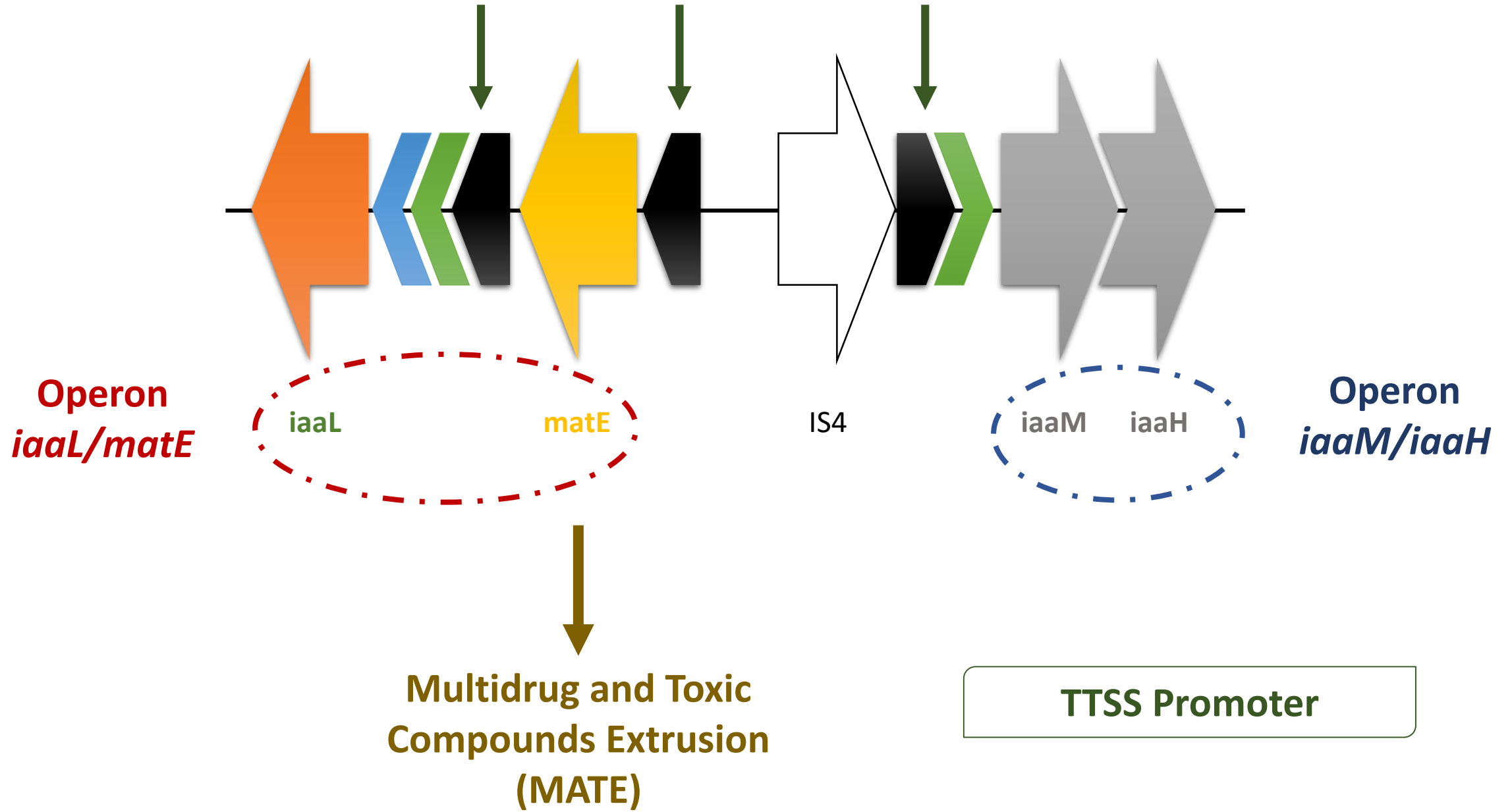


### Abstract

The bacterial pathogen *Pseudomonas syringae* modulates plant hormone signaling to promote infection and disease development. *P. syringae* uses several strategies to manipulate auxin physiology in *Arabidopsis thaliana* to promote pathogenesis, including its synthesis of indole-3-acetic acid (IAA), the predominant form of auxin in plants, and production of virulence factors that alter auxin responses in the host; however, the role of pathogen-derived auxin in *P. syringae* pathogenesis is not well understood. Here we demonstrate that *P. syringae* strain DC3000 produces IAA via a previously uncharacterized pathway and identify a novel indole-3-acetaldehyde dehydrogenase, AldA, that functions in IAA biosynthesis by

### OPEN ACCESS

**Citation:** McClerkin SA, Lee SG, Harper CP, Nwumeh R, Jez JM, Kunkel BN (2018) Indole-3-acetaldehyde dehydrogenase-dependent auxin synthesis contributes to virulence of *Pseudomonas syringae* strain DC3000. *PLoS Pathog* 14(1): e1006811. <https://doi.org/10.1371/journal.ppat.1006811>





Article

# A MATE Transporter is Involved in Pathogenicity and IAA Homeostasis in the Hyperplastic Plant Pathogen *Pseudomonas savastanoi* pv. *nerii*

Stefania Tegli <sup>1,\*</sup>, Lorenzo Bini <sup>1</sup>, Silvia Calamai <sup>1</sup>, Matteo Cerboneschi <sup>2</sup> and Carola Biancalani <sup>1</sup>

<sup>1</sup> Dipartimento di Scienze e Tecnologie Agrarie, Alimentari Ambientali e Forestali, Laboratorio di Patologia Vegetale Molecolare, Università degli Studi di Firenze, Via della Lastruccia 10, 50019 Sesto Fiorentino (Firenze), Italy; lorenzo.bini5@stud.unifi.it (L.B.); silvia.calamai@unifi.it (S.C.); carola.biancalani@unifi.it (C.B.)

<sup>2</sup> Next Genomics srl, Via Madonna del Piano, 6, 50019 Sesto Fiorentino (Firenze), Italy; matteo@nextgenomics.it

\* Correspondence: stefania.tegli@unifi.it

Received: 17 December 2019; Accepted: 18 January 2020; Published: 22 January 2020

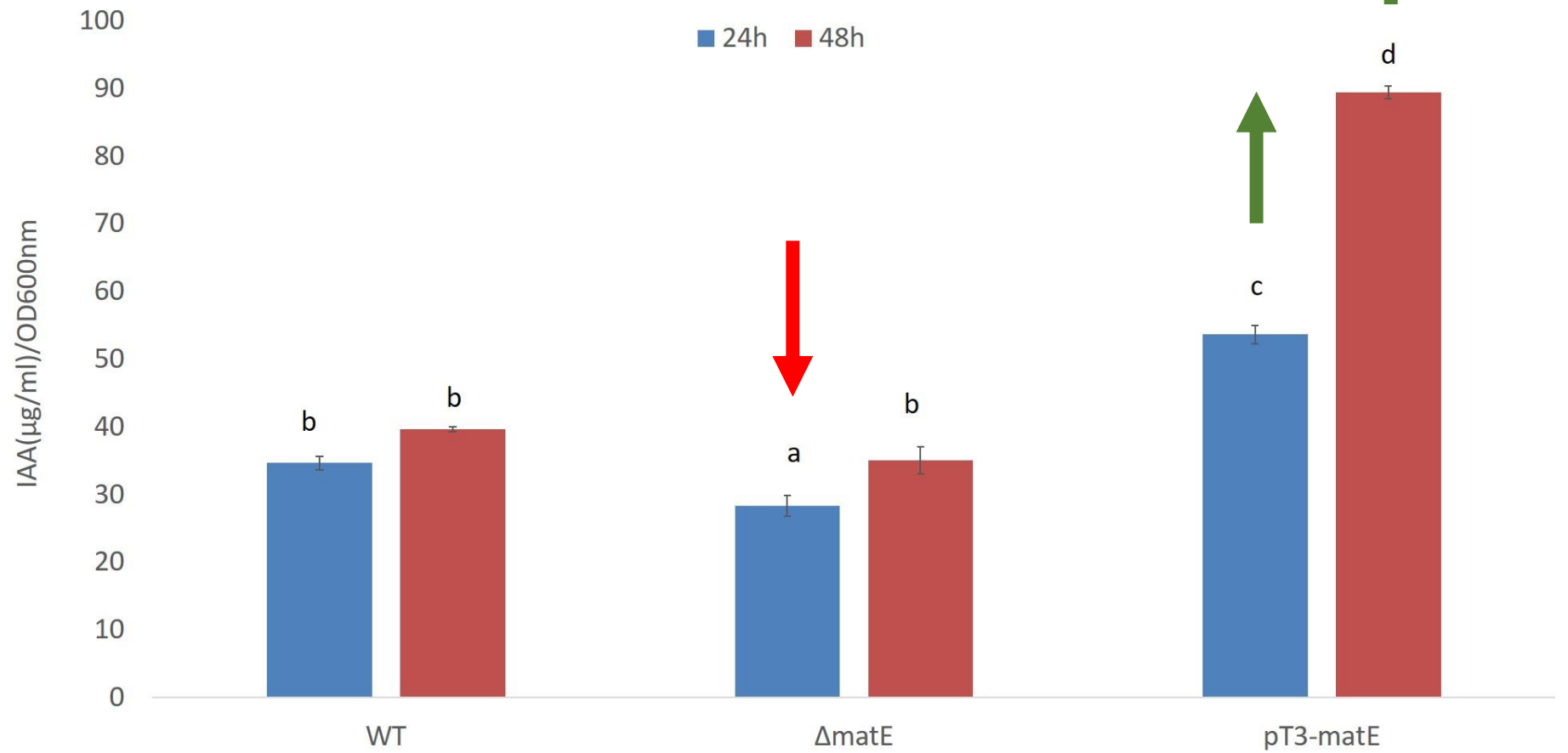


# Salkowski's assay

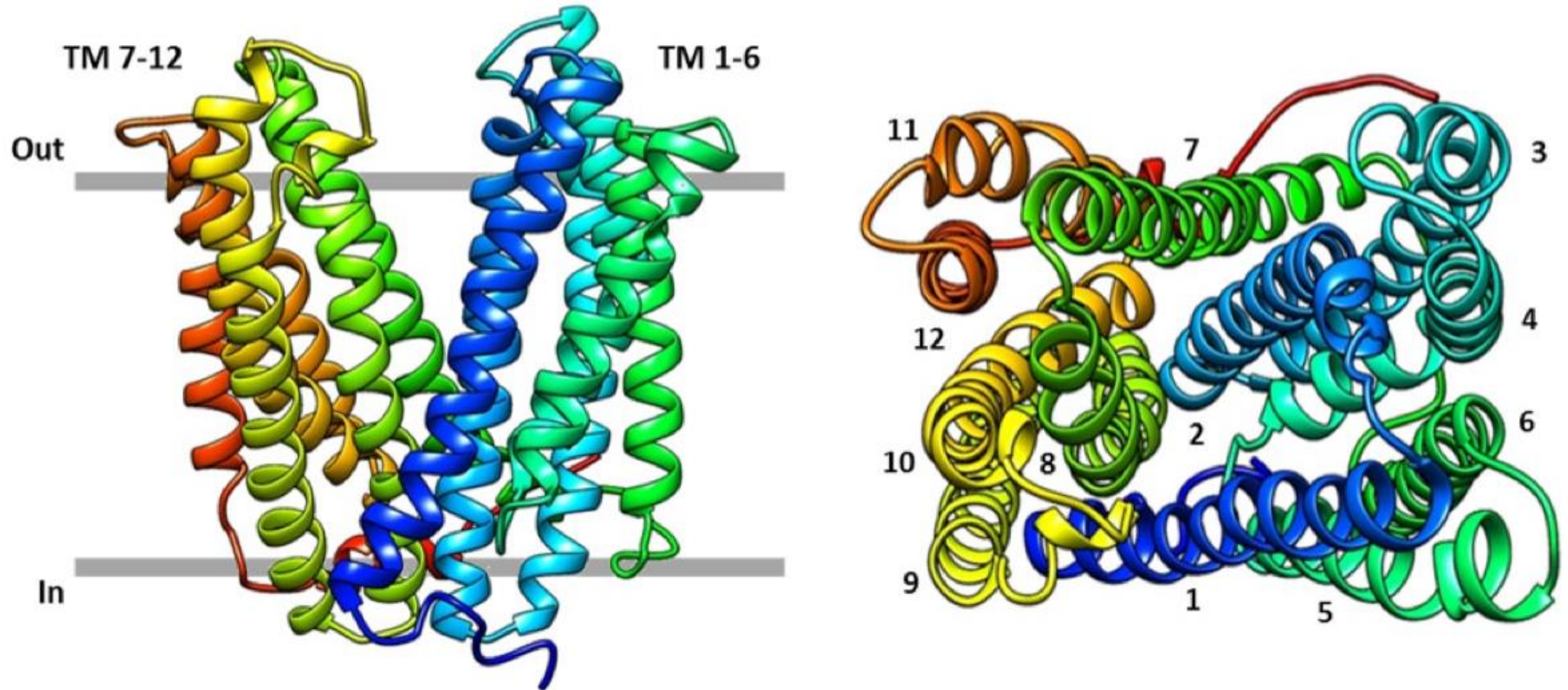
## Mutants

$\Delta$ matE

pT3\_matE



## Predicted structure of *Psn23* MatE



## Molecular Docking

Ligand	Energy	H T175	H Y200	V T170	V T173	V T175	V Y200
IAA-free	-102.5	0	0	0	0	-1.5	-28.5
IAA-lysine	-95.1	-5.9	0	-2.1	-3.5	-3.9	-16.8

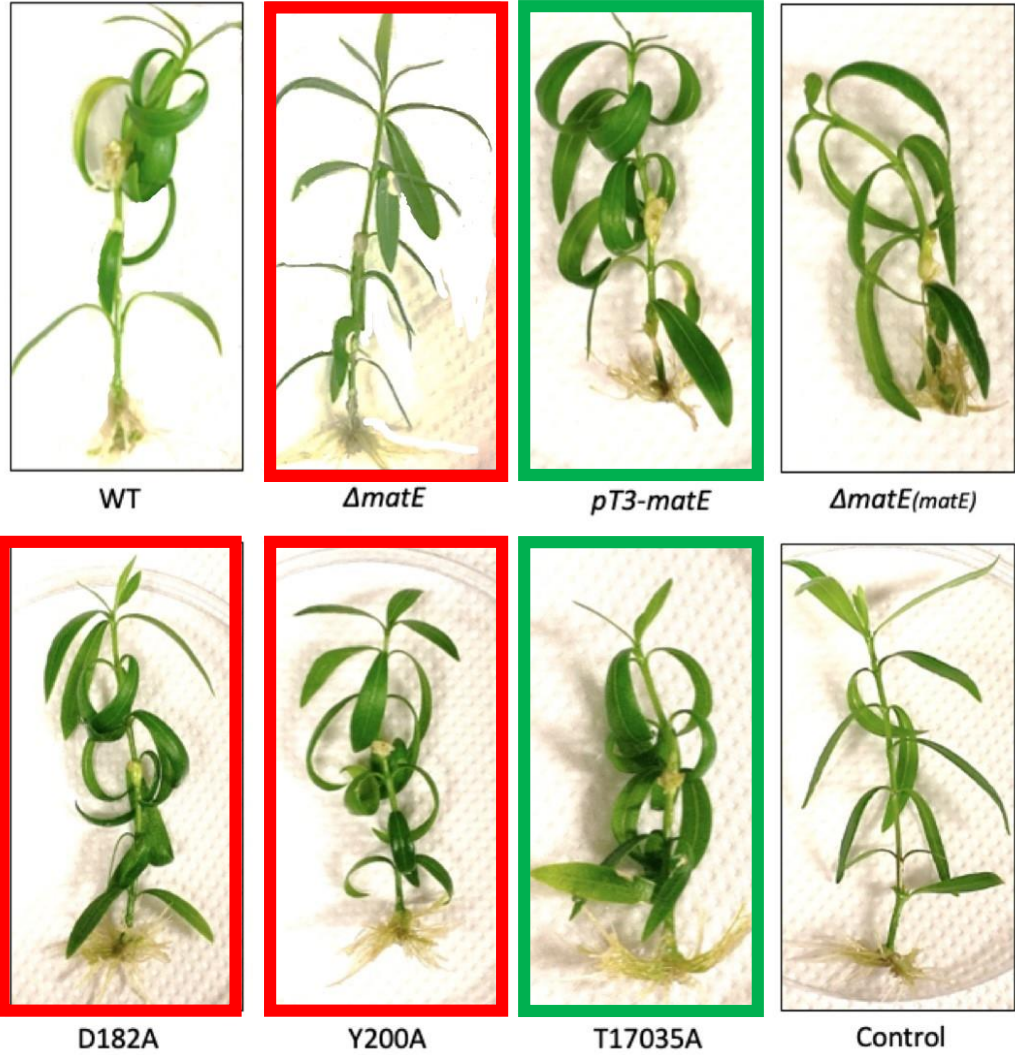
## Mutants

**D182A**

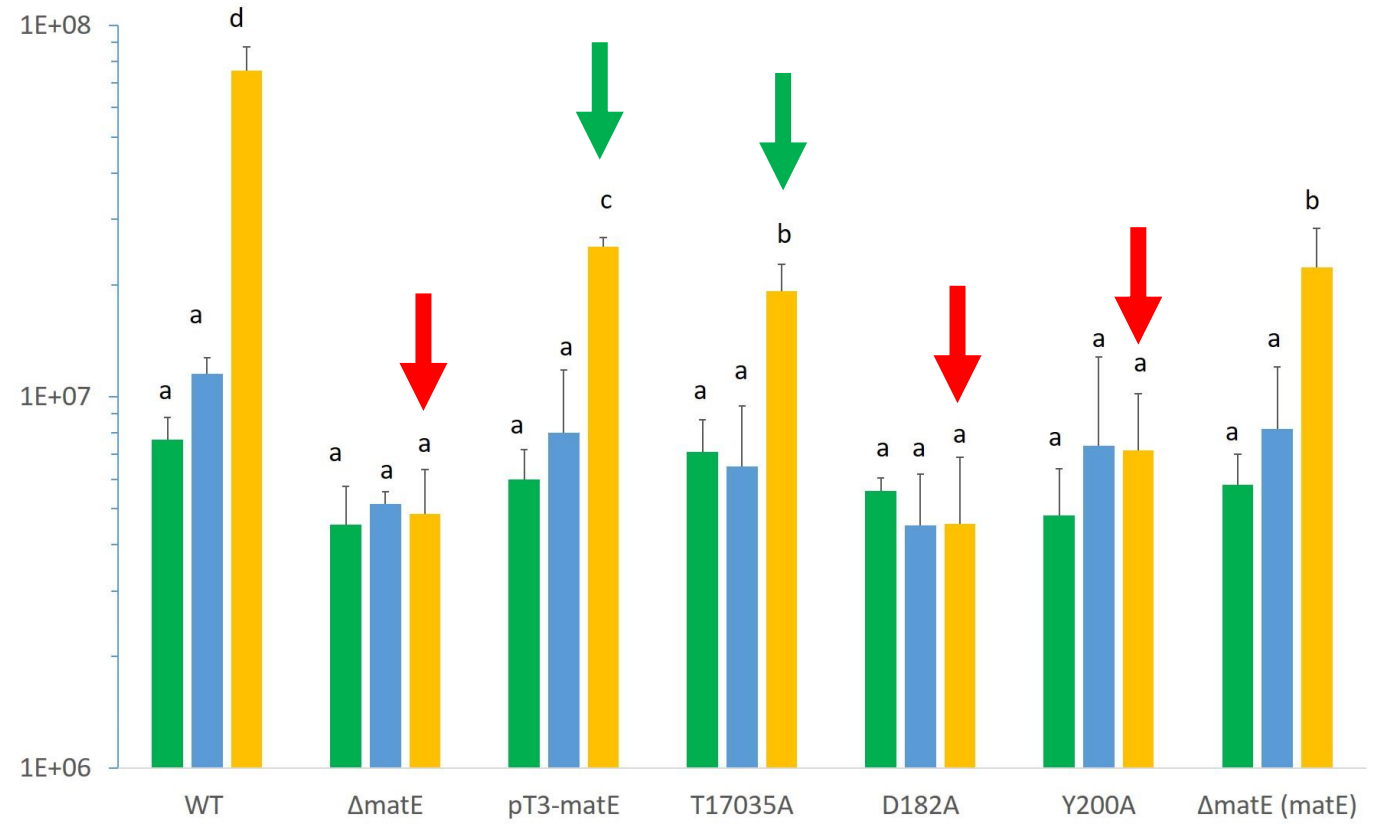
**Y200A**

**T17035A**

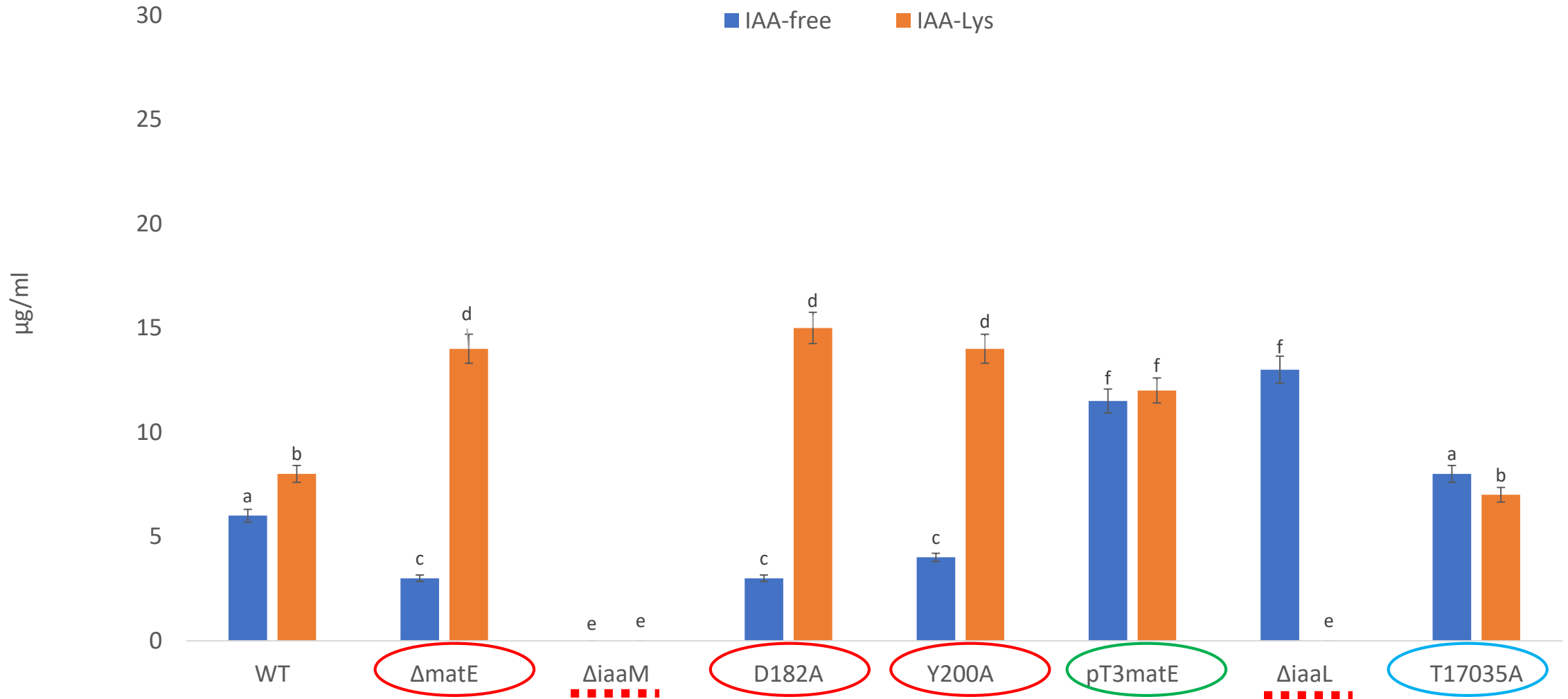
# Pathogenicity Trials



# Bacterial Growth *in planta*

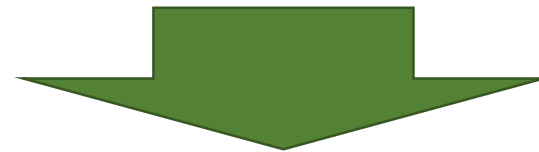
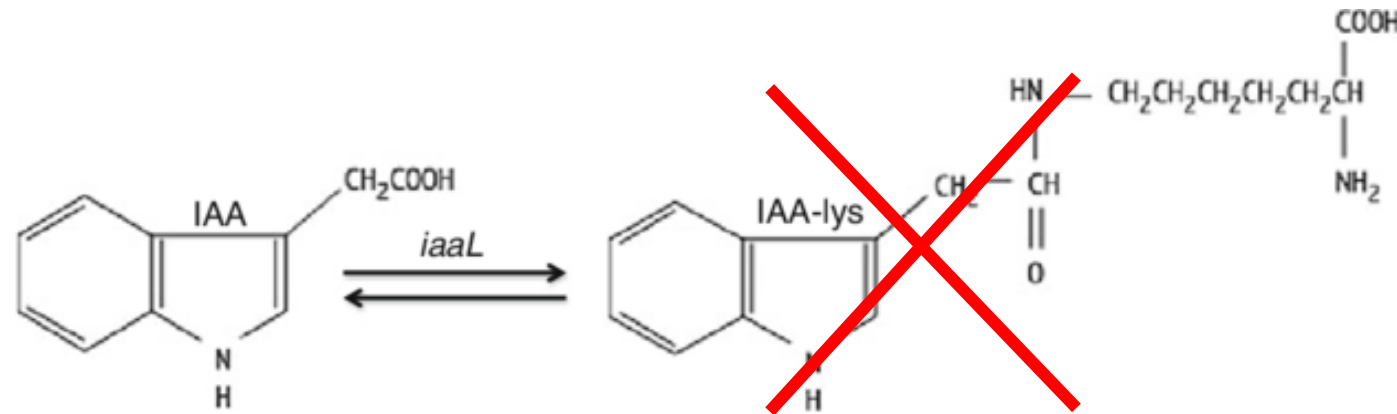


# IAA and IAA-Lys Quantification by HPLC-FLD



## Conclusion

- ✓ Psn23 MatE mediates IAA efflux and homeostasis



**Alternative Target for Bacterial Plant Disease Control**

## Gram-negative bacteria

*Pseudomonas savastanoi* pv. *nerii* Psn23



### Approach

Genomic

Physiological

### Studies

- ✓ MATE pumps
- ✓ IAA Homeostasis

## Gram-positive bacteria

*Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Cff)



### Approach

Genomic

Epidemiological

### Studies

- ✓ Population Analysis
- ✓ Genomic Studies

# *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Cff)

## Population Analysis

### Phenotypic and Molecular-Phylogenetic Analysis Provide Novel Insights into the Diversity of *Curtobacterium flaccumfaciens*

Ebrahim Osdaghi<sup>id</sup>,<sup>†</sup> S. Mohsen Taghavi, Silvia Calamai, Carola Biancalani, Matteo Cerboneschi,  
Stefania Tegli, and Robert M. Harveson

First and second authors: Department of Plant Protection, College of Agriculture, Shiraz University, Shiraz 71441-65186, Iran; third, fourth, fifth, and sixth authors: Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, Laboratorio di Patologia Vegetale Molecolare, Università degli Studi di Firenze, Via della Lastruccia 10, 50019 Sesto Fiorentino, Firenze, Italy; and seventh author: University of Nebraska, Panhandle Research & Extension Center, 4502 Ave. I., Scottsbluff 69361.

## Genomic Studies



**ICPM 2584**

**P990**

**Tom827**



# Population Analysis of *Curtobacterium flaccumfaciens*

Isolated from dry beans



→ **Virulent on dry beans**

Isolated from solanaceous

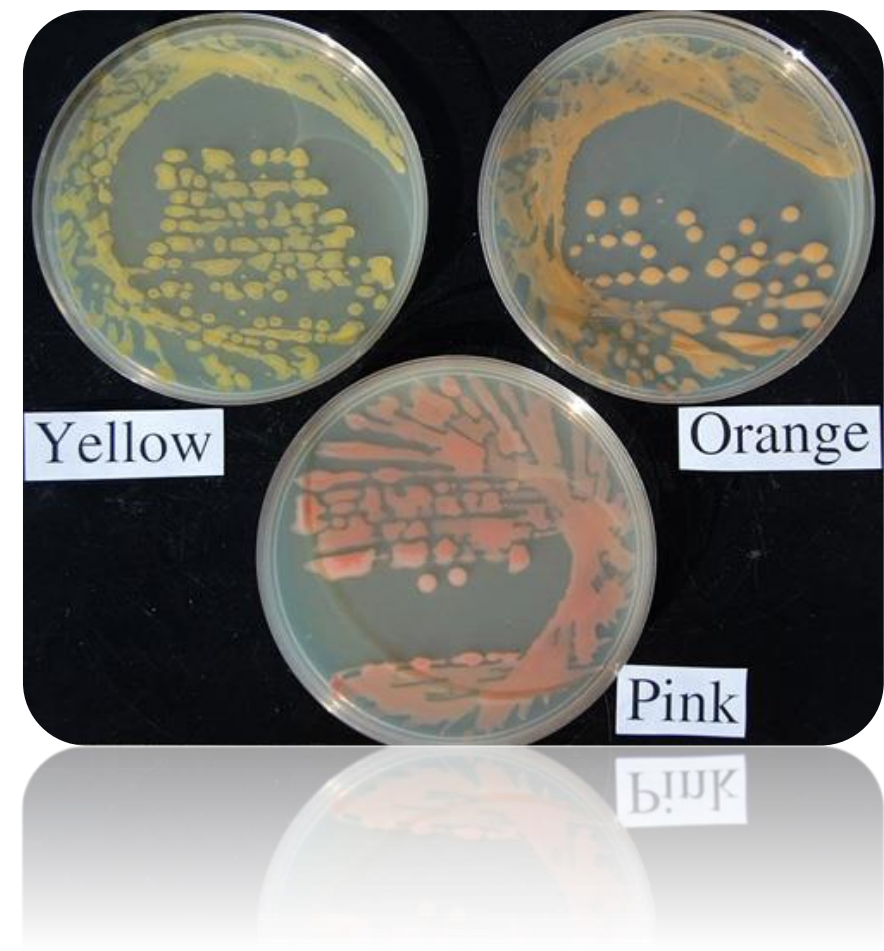
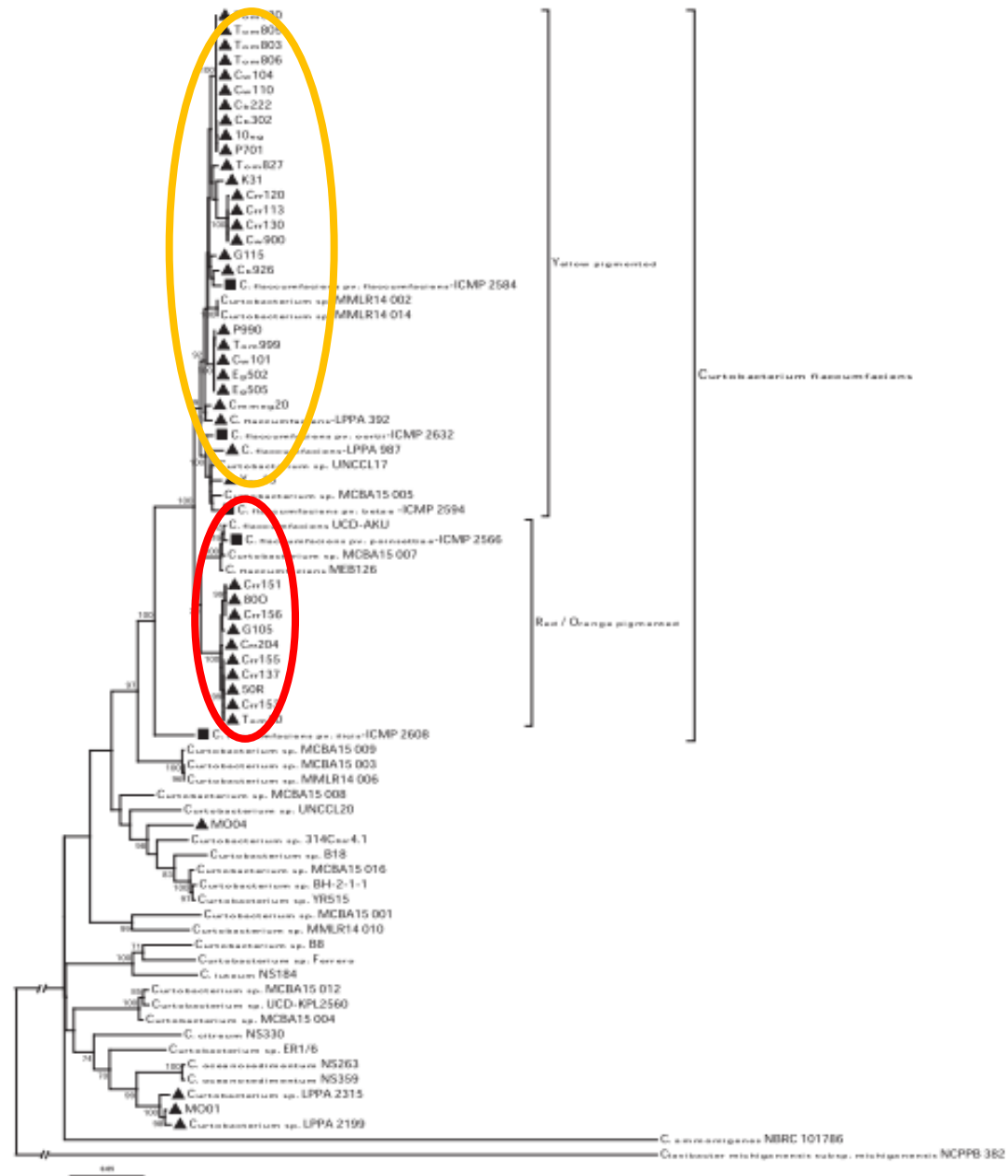


↗ **Virulent on dry beans**

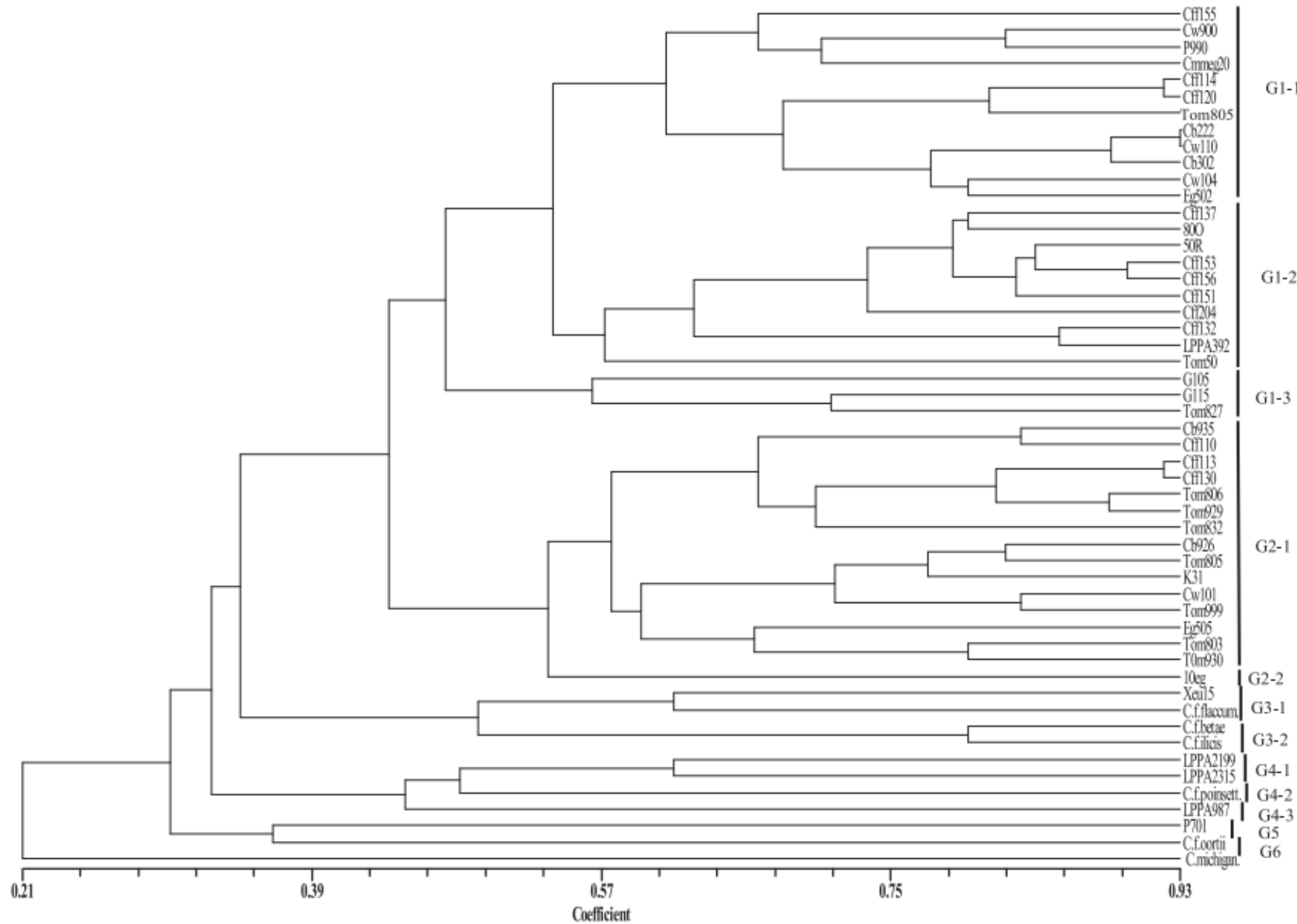
↘ **No virulent on dry beans**

- ✓ *Curtobacterium flaccumfaciens* pathovars
- ✓ *Clavibacter michiganensis* subsp. *michiganensis*

# Phylogenetic Analysis



# Genetic Diversity



***No genetic diversity between strains isolated from dry beans and solanaceous plants***

# Screening for Arsenite Resistance

TABLE 3. Growth rate of *Curtobacterium flaccumfaciens*, *Curtobacterium*-like, and *Clavibacter michiganensis* strains used in this study on different concentrations of sodium arsenite ( $\text{NaAsO}_2$ ) and sodium arsenate ( $\text{Na}_2\text{AsO}_4$ )<sup>a</sup>

Strain		Resistance to						Pathogenicity on common bean
		Sodium arsenite			Sodium arsenate			
		2 mM <sup>S</sup>	5 mM <sup>S</sup>	7 mM <sup>S</sup>	80 mM <sup>S</sup>	100 mM <sup>S</sup>	130 mM <sup>S</sup>	
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	10eg	10 <sup>2</sup> -10 <sup>4</sup>	≤10 <sup>2</sup>	≤10 <sup>2</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>2</sup> -10 <sup>4</sup>	+
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	50R	10 <sup>4</sup> -10 <sup>6</sup>	≤10 <sup>2</sup>	≤10 <sup>2</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>2</sup> -10 <sup>4</sup>	+
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	80O	10 <sup>2</sup> -10 <sup>4</sup>	≤10 <sup>2</sup>	≤10 <sup>2</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>2</sup> -10 <sup>4</sup>	+
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	Cb222	10 <sup>2</sup> -10 <sup>4</sup>	≤10 <sup>2</sup>	≤10 <sup>2</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>2</sup> -10 <sup>4</sup>	+
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	Cb302	10 <sup>2</sup> -10 <sup>4</sup>	≤10 <sup>2</sup>	≤10 <sup>2</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>2</sup> -10 <sup>4</sup>	+
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	Cb926	10 <sup>2</sup> -10 <sup>4</sup>	≤10 <sup>2</sup>	≤10 <sup>2</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>2</sup> -10 <sup>4</sup>	+
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	Cff110	10 <sup>2</sup> -10 <sup>4</sup>	≤10 <sup>2</sup>	≤10 <sup>2</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>2</sup> -10 <sup>4</sup>	+
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	Cff137	10 <sup>2</sup> -10 <sup>4</sup>	≤10 <sup>2</sup>	≤10 <sup>2</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>2</sup> -10 <sup>4</sup>	+
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	Cff151	10 <sup>2</sup> -10 <sup>4</sup>	≤10 <sup>2</sup>	≤10 <sup>2</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>2</sup> -10 <sup>4</sup>	+
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	Cff153	10 <sup>2</sup> -10 <sup>4</sup>	≤10 <sup>2</sup>	≤10 <sup>2</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>2</sup> -10 <sup>4</sup>	+
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	Cff155	10 <sup>2</sup> -10 <sup>4</sup>	≤10 <sup>2</sup>	≤10 <sup>2</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>2</sup> -10 <sup>4</sup>	+
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	Cff156	10 <sup>2</sup> -10 <sup>4</sup>	≤10 <sup>2</sup>	≤10 <sup>2</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>2</sup> -10 <sup>4</sup>	+
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	ICMP 2584 <sup>T</sup>	≤10 <sup>2</sup>	-	-	≤10 <sup>2</sup>	≤10 <sup>2</sup>	≤10 <sup>2</sup>	+
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	Cw101	10 <sup>2</sup> -10 <sup>4</sup>	≤10 <sup>2</sup>	≤10 <sup>2</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>2</sup> -10 <sup>4</sup>	+
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	Cw110	10 <sup>4</sup> -10 <sup>6</sup>	≤10 <sup>2</sup>	≤10 <sup>2</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>2</sup> -10 <sup>4</sup>	+
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	Eg502	10 <sup>2</sup> -10 <sup>4</sup>	≤10 <sup>2</sup>	≤10 <sup>2</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>2</sup> -10 <sup>4</sup>	+
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	Eg505	10 <sup>2</sup> -10 <sup>4</sup>	≤10 <sup>2</sup>	≤10 <sup>2</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>2</sup> -10 <sup>4</sup>	+
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	Mo11	10 <sup>2</sup> -10 <sup>4</sup>	≤10 <sup>2</sup>	≤10 <sup>2</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>2</sup> -10 <sup>4</sup>	+
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	P701	10 <sup>2</sup> -10 <sup>4</sup>	≤10 <sup>2</sup>	≤10 <sup>2</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>2</sup> -10 <sup>4</sup>	+
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	P990	10 <sup>2</sup> -10 <sup>4</sup>	≤10 <sup>2</sup>	≤10 <sup>2</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>2</sup> -10 <sup>4</sup>	+
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	Tom50	10 <sup>2</sup> -10 <sup>4</sup>	≤10 <sup>2</sup>	≤10 <sup>2</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>2</sup> -10 <sup>4</sup>	+
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	Tom803	10 <sup>2</sup> -10 <sup>4</sup>	≤10 <sup>2</sup>	≤10 <sup>2</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>2</sup> -10 <sup>4</sup>	+
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	Tom806	10 <sup>2</sup> -10 <sup>4</sup>	≤10 <sup>2</sup>	≤10 <sup>2</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>2</sup> -10 <sup>4</sup>	+
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	Tom930	10 <sup>2</sup> -10 <sup>4</sup>	≤10 <sup>2</sup>	≤10 <sup>2</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>6</sup>	≤10 <sup>2</sup>	+
<i>C. flaccumfaciens</i> pv. <i>flaccumfaciens</i>	Tom999	10 <sup>2</sup> -10 <sup>4</sup>	≤10 <sup>2</sup>	≤10 <sup>2</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>2</sup> -10 <sup>4</sup>	+
<i>C. flaccumfaciens</i> pv. <i>oortii</i>	ICMP 2632 <sup>T</sup>	10 <sup>2</sup> -10 <sup>4</sup>	≤10 <sup>2</sup>	≤10 <sup>2</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>6</sup>	-
<i>C. flaccumfaciens</i>	Cmmeg20	-	-	-	-	-	-	-
<i>C. flaccumfaciens</i>	G105	-	-	-	-	-	-	-
<i>C. flaccumfaciens</i>	Tom827	-	-	-	-	-	-	-
<i>C. flaccumfaciens</i>	Xeu15	-	-	-	-	-	-	-
<i>Curtobacterium</i> spp.	Mo04	-	-	-	-	-	-	-
<i>Clavibacter michiganensis</i> subsp. <i>michiganensis</i>	ICMP 2550 <sup>T</sup>	-	-	-	-	-	-	-
<i>Clavibacter michiganensis</i> subsp. <i>michiganensis</i>	NCPFB 382	-	-	-	-	-	-	-
<i>Clavibacter michiganensis</i> subsp. <i>michiganensis</i>	Tom835	-	-	-	-	-	-	-
<i>Clavibacter</i> spp.	Tom495	-	-	-	-	-	-	-

<sup>a</sup> All strains, which were pathogenic on common bean, were able to grow on different concentrations of both compounds, while nonpathogenic strains were sensitive. *Clavibacter michiganensis* strains were sensitive to both the compounds regardless of their pathogenicity on tomato. S = data statistically significant (ANOVA and Tukey's test,  $P < 0.05$ ).

# Genomic and Phenotypic Metal Resistance Profile

	CHEMICAL ELEMENT	CHEMICAL COMPOUND	TOLERANCE LEVEL		
			SENSITIVE <sup>A</sup>	MODERATELY TOLERANT <sup>B</sup>	RESISTANT <sup>C</sup>
METALS	Al	Aluminum sulfate		x <sup>D</sup>	
	Cd	Cadmium chloride	x		
	Co	Cobalt chloride		x	
	Cr	Potassium chromate			x
		Sodium dichromate			x
		Chromium chloride			x
	Cs	Cesium chloride			x
	Cu	Cupric chloride		x	
	Fe	Ferric chloride		x	
	Li	Lithium chloride		x	
	Mn	Manganese(II) chloride		x	
	Ni	Nickel chloride		x	
	Tl	Thallium(I) acetate		x	
	V	Sodium metavanadate			x
		Sodium orthovanadate			x
	W	Sodium Tungstate		x	
Zn	Zinc chloride		x		
METALLOIDS	As	Sodium m-arsenite		x (D) <sup>E</sup>	
		Sodium arsenate		x (D)	
	B	Boric acid		x	
		Sodium metaborate		x	
	Sb	Antimony(III) chloride		x (D)	
	Si	Sodium metasilicate			x
Te	Potassium tellurite		x (D)		

ICPM 2584

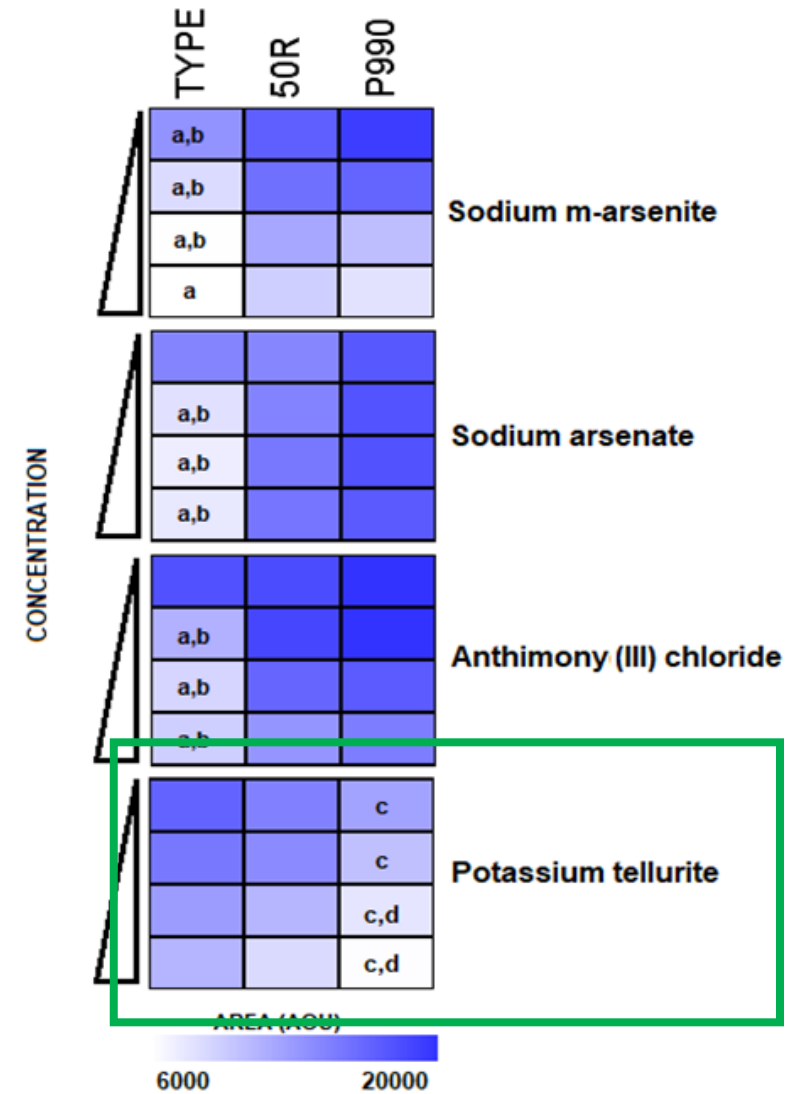
P990

50R

<sup>D</sup>X: the three showed overlapping kinetic curves

<sup>E</sup>X(D): the three strains showed different kinetic curves

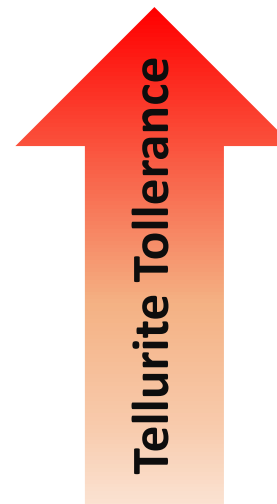
# Potassium Tellurite Tolerance



# Screening for metal and metalloid resistance

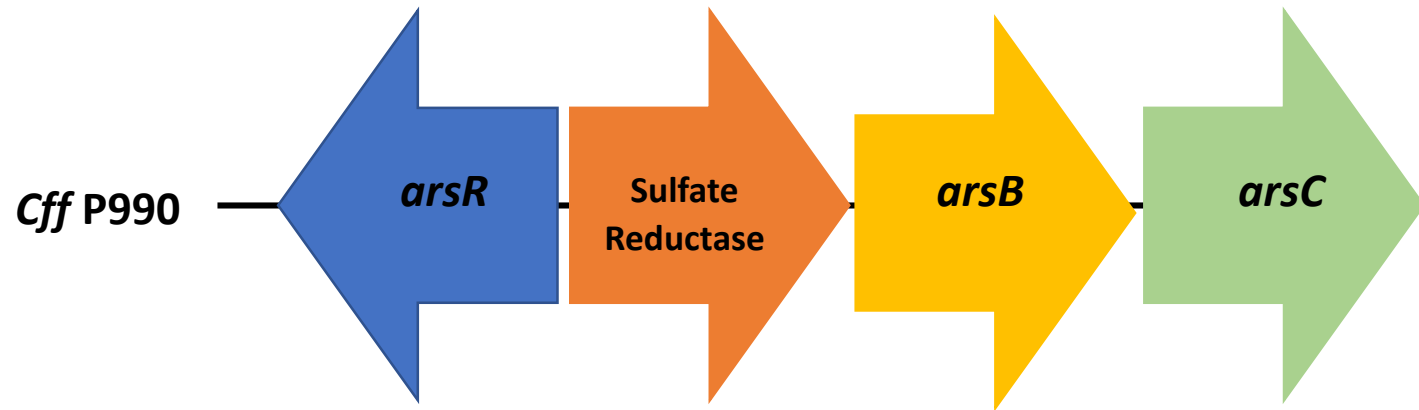
	Resistance to								
	Caesium Chloride			Sodium Metasilicate			Potassium Tellurite		
	75 mM	50 mM	25 mM	8 mM	6.5 mM	5 mM	0.3 mM	0.2 mM	0.1 mM
<b>ICPM 2584</b>	$10^6-10^7$	$10^7-10^8$	$10^7-10^8$	$10^5-10^6$	$10^6-10^7$	$10^6-10^7$	$10^5$	$10^6$	$10^6$
<b>50R</b>	$10^6-10^7$	$10^7-10^8$	$10^7-10^8$	$10^5-10^6$	$10^6-10^7$	$10^6-10^7$	$10^5$	$10^6$	$10^6$
<b>P990</b>	$10^6-10^7$	$10^7-10^8$	$10^7-10^8$	$10^5-10^6$	$10^6-10^7$	$10^6-10^7$	$10^4$	$10^5$	$10^5$
<b>Tom827</b>	$10^6-10^7$	$10^7-10^8$	$10^7-10^8$	$10^5-10^6$	$10^6-10^7$	$10^6-10^7$	$10^4$	$10^5$	$10^5$

Isolated from Solanaceae



Isolated from Fabaceae

## Comparative Gene analysis



### RESEARCH REVIEW

INTERNATIONAL MICROBIOLOGY (2006) 9:207-215  
[www.im.microbios.org](http://www.im.microbios.org)

INTERNATIONAL  
MICROBIOLOGY

Luis M. Mateos  
Efrén Ordóñez  
Michal Letek  
José A. Gil\*

***Corynebacterium  
glutamicum* as a model  
bacterium for the  
bioremediation of arsenic**

Department of Ecology, Genetics  
and Microbiology, Area of

# Comparative Genome Analysis of *Curtobacterium flaccumfaciens*

Letters in Applied Microbiology 2002, 35, 331–337

## PCR-based assay for the detection of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* in bean seeds

S. Tegli, A. Sereni and G. Surico

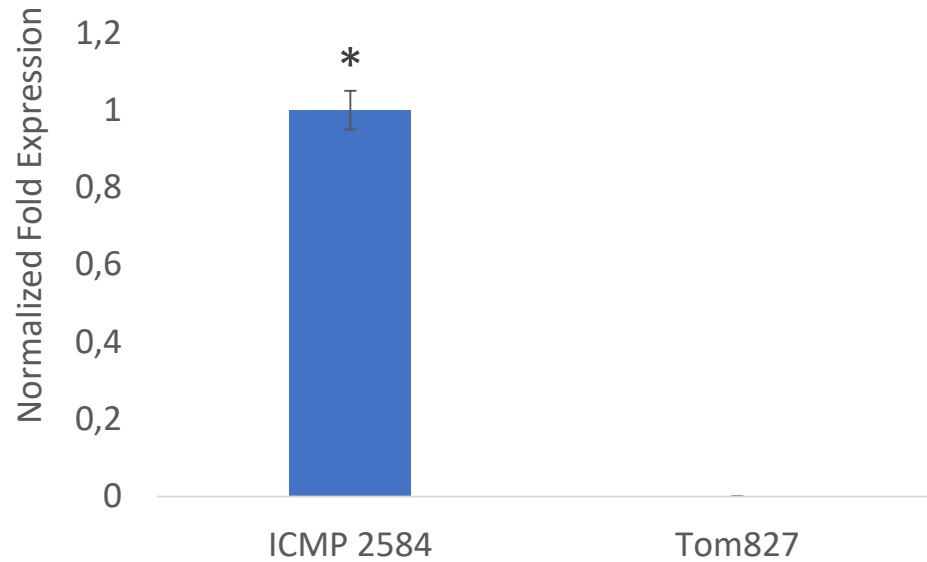
Dipartimento di Biotecnologie Agrarie (DiBA) – Sezione di Patologia Vegetale, Università degli Studi di Firenze, Firenze, Italy

2002/31: received 23 January 2002, revised 1 March 2002 and accepted 25 June 2002

		<i>Cff</i> FOR2 →		← <i>Cff</i> REV4	
		108	129	403	420
<i>Cff</i> - NCPPB	178	GTTATGACTGAACTTCA-CTCC		CTGAACACCGGGAACATC	
<i>Cff</i> - ICMP	5370	GTTATGACTGAACTTCA-CTCC		CTGAACACCGGGAACATC	
<i>Cff</i> - NCPPB	558	GATATGACTGAACTTCA-CTCC		CTGAACACCGGGAACATC	
<i>Cff</i> - NCPPB	1751	GTTATGACTGAACTTCA-CTCC		CTGAACACCGGGAACATC	
<i>Cfp</i> - NCPPB	854	ATTGGAGATCGGCGG-T-CTCC		ATGCGCGGC-----	
<i>Cfb</i> - NCPPB	374	CTTGGGTTAGCTCGGCATCTAC		ATATTGACGTGAAATAGT	
		* * * *		*	



## Gene Expression



## Comparative genome analysis

CDS identifiers P990 strain	CDS identifiers Type strain	Annotation
p990_03611	type.fna_03759	trypsin-like serine protease
p990_03612	type.fna_03758	trypsin-like serine protease
p990_03613	type.fna_03757	serine protease
p990_03614	type.fna_03756	putative Serine/cysteine peptidase protein
p990_03615	type.fna_03755	pectate lyase
p990_03652	type.fna_03718	trypsin-like serine protease
p990_03610	type.fna_03760	transposase
p990_03627	type.fna_03743	transposase
p990_03648	type.fna_03722	transposase
p990_03649	type.fna_03721	integrase
p990_03650	type.fna_03720	integrase

## Comparative genome analysis

P990

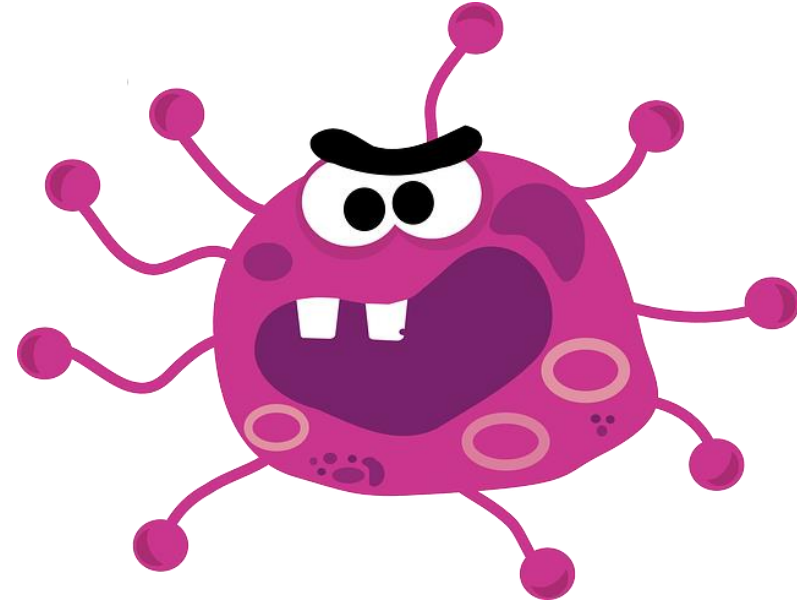
ICPM 2584

~~Tom827~~



SERINE PROTEASE

PECTATE LYASE



## Multiple plasmid-borne virulence genes of *Clavibacter michiganensis* ssp. *capsici* critical for disease development in pepper

In Sun Hwang<sup>1</sup>, Eom-Ji Oh<sup>1</sup>, Donghyuk Kim<sup>2</sup> and Chang-Sik Oh<sup>1</sup>

<sup>1</sup>Department of Horticultural Biotechnology, Kyung Hee University, Yongin 17104, Korea; <sup>2</sup>Graduate School of Biotechnology, Kyung Hee University, Yongin 17104, Korea

MPMI Vol. 22, No. 7, 2009, pp. 809–819. doi:10.1094/MPMI-22-7-0809.

## The Putative Secreted Serine Protease Chp-7 Is Required for Full Virulence and Induction of a Nonhost Hypersensitive Response by *Clavibacter michiganensis* subsp. *sepedonicus*

Riitta Nissinen,<sup>1</sup> Yunjian Xia,<sup>1</sup> Laura M. Susan E. Knudson,<sup>3</sup> Mary Metzler,<sup>4</sup> and

MOLECULAR PLANT PATHOLOGY (2008) 9(5), 599–608

DOI: 10.1111/J.1364-3703.2008.00484.X

## A family of serine proteases of *Clavibacter michiganensis* subsp. *michiganensis*: *chpC* plays a role in colonization of the host plant tomato

INES STORK, KARL-HEINZ GARTEMANN, ANNETTE BURGER AND RUDOLF EICHENLAUB

Fakultät für Biologie, Lehrstuhl für Mikrobiologie/Gentechnologie, Universitaetsstr. 25, D-33615 Bielefeld, Germany

© Wozniak et al., 2010, Nature

# Comparative genome analysis

P990

ICPM 2584

~~Tom827~~



TRASPOSASE

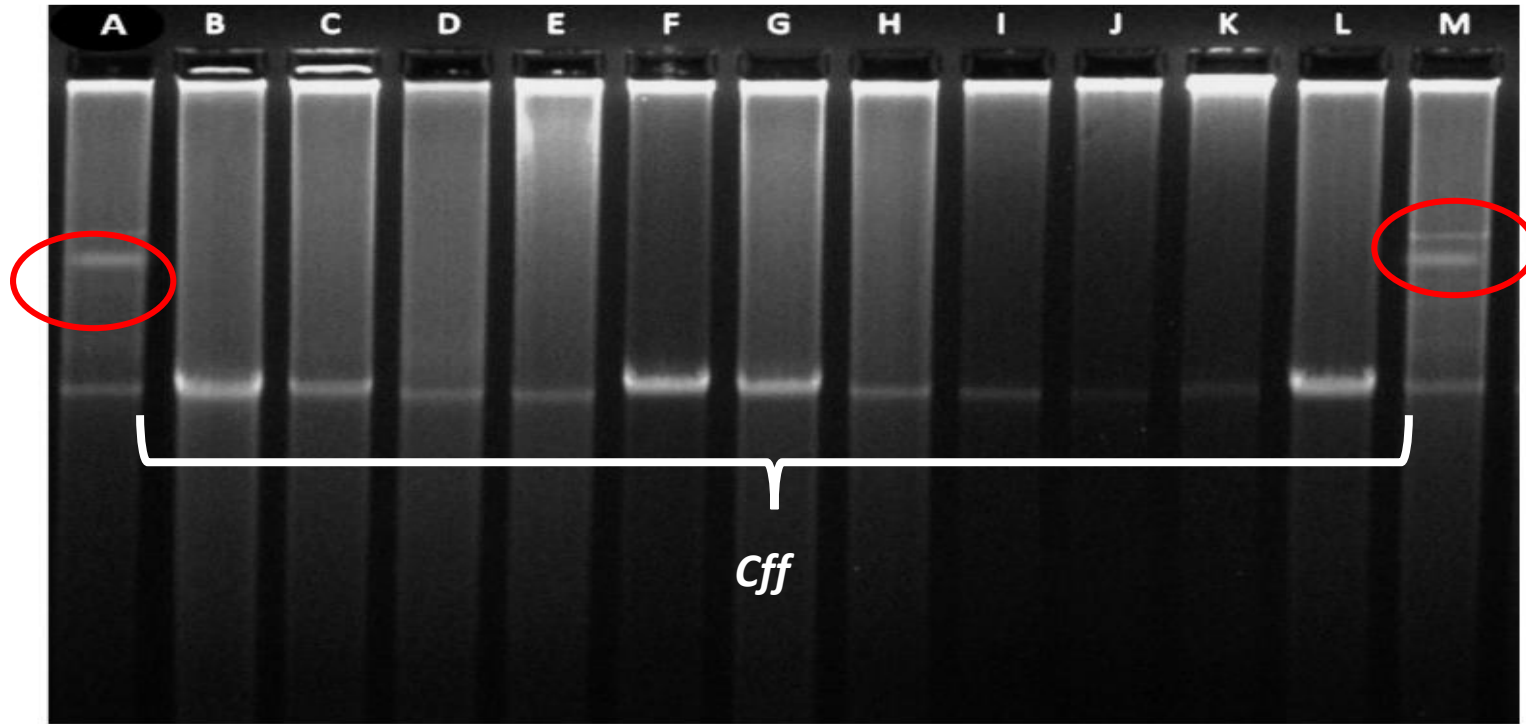
INTEGRASE

CONJUGATIVE GENES

Conjugative Relaxase, Pilus  
assembly protein, VirB4...

ICE ?

## Plasmid Isolation



## Conclusion

✓ Advance in epidemiology → CONTROL STRATEGY

✓ *ArsRBC* operon in P990

✓ Detect virulence determinant → NEW TARGETS

# THANKS!

**Silvia Calamai**  
silvia.calamai@unifi.it