Phenotypic characterization of two Spanish strains of *Xylella fastidiosa* subsp. *multiplex* ST6 differing in plasmid content

Miguel Román Écija [1]

Blanca B. Landa, Juan A. Navas-Cortés [1]

Laura Gómez, Leonardo De la Fuente [2]

1. Institute for Sustainable Agriculture Córdoba (Spain)

2. Auburn University (Alabama, USA)

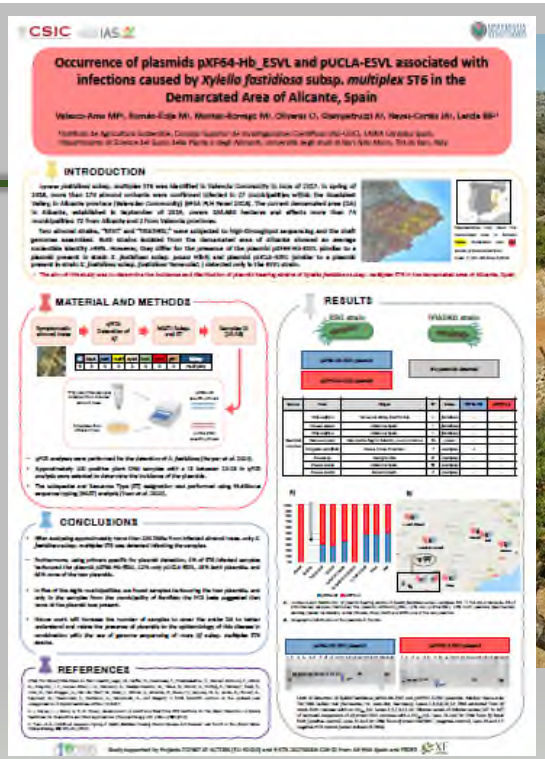
EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

XF ACTORS

Xylella Fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy

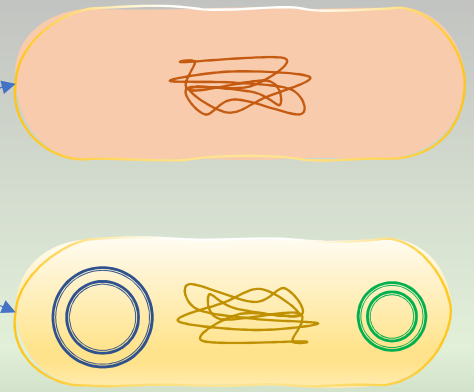
Introduction: isolates

Introduction: isolates



ISOLATE	SUBSPECIES	ST	HOST ORIGIN	GEOGRAPHIC ORIGIN
IVIA5901	*multiplex*	ST6	Almond	Bollulla, Alicante (Spain)
ESVL	multiplex	ST6	Almond	Benimantell, Alicante (Spain)

Giampetruzzi A., et al. 2018. Phytopathology, 109, 219–221



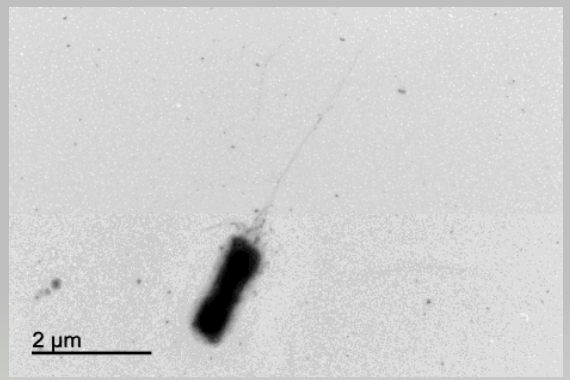
pXF64-Hb_ESVL (59.7 KB)

pUCLA-ESVL (1.28 KB)

- **Both isolates share 99,99 % chromosomal genome (ANI)**
- **Major difference: 2 plasmids in ESVL strain**

Introduction: colonization and disease development

- **Colonization and disease development is related with several factors:**
  - **Celular adhesion**
  - **Cell motility**
  - **Biofilm formation**
  - **Size aggregates**



- **Pili I and IV are related with all of this factors**
  - **I : Biofilm and cell aggregation**
  - **IV : motility**

- **Non fimbrial adhesins**

Main aim

- **To characterize phenotypic traits associated to infection and disease development of these two Spanish isolates of *X. fastidiosa* subsp. *multiplex* ST6 to find out if the differences in plasmid content could be related to differences in phenotypic traits**

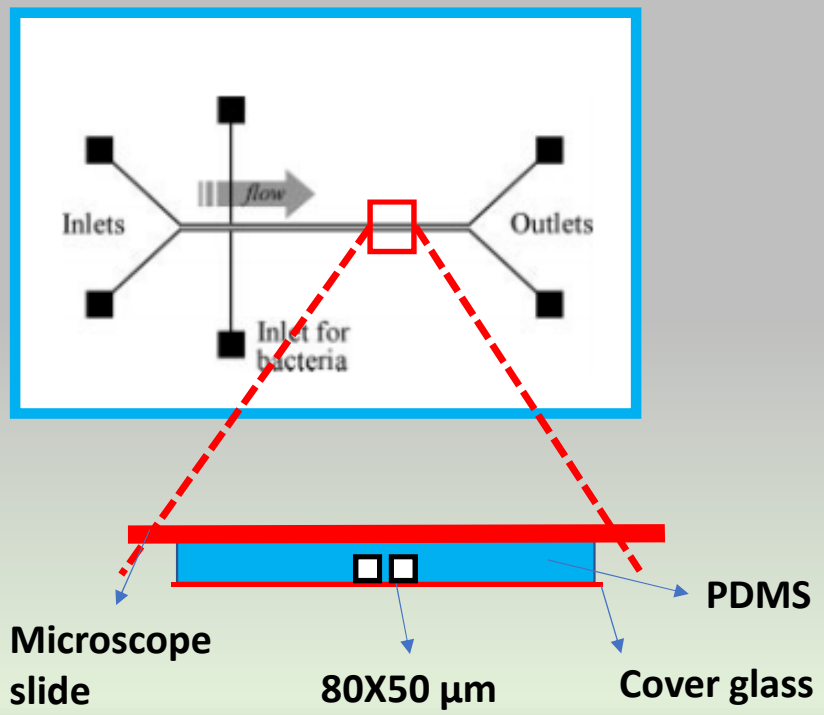
Phenotipic characterizaction

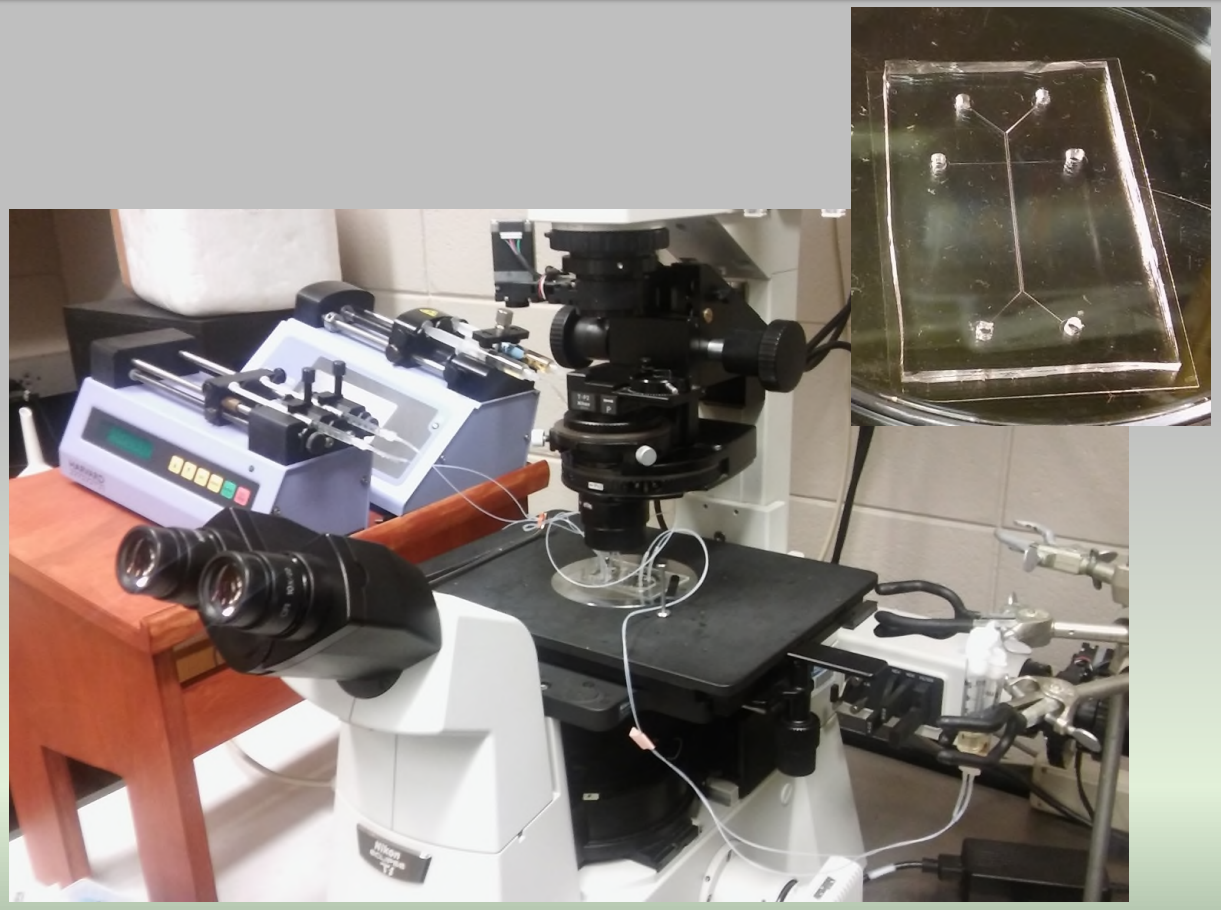
1. **Patterns of bacterial growth**
2. **Cells adhesion**
3. **Biofilm formation**
   - **Quantification in 96-well plates**
   - **Growth observation in crystal tubes**
4. **Assessment of bacterial cell-to-cell aggregation**
5. **Twitching motility**
6. **Pathogenicity experiment**

ISOLATE	SUBSPECIES	ST	HOST ORIGIN	GEOGRAPHIC ORIGIN
Temecula1	*fastidiosa*	ST1	Grape	California (EEUU)
IVIA 5901	*multiplex*	ST6	Almond	Alicante (Spain)
ESVL	*multiplex*	ST6	Almond	Alicante (Spain)
Alma-Em3	*multiplex*	ST42	Blueberry	Georgia (EEUU)
BB08-1	*multiplex*	ST43	Blueberry	Florida (EEUU)

1. Patterns of bacterial growth and cell adhesion experiments

- **Microfluidic Chamber**

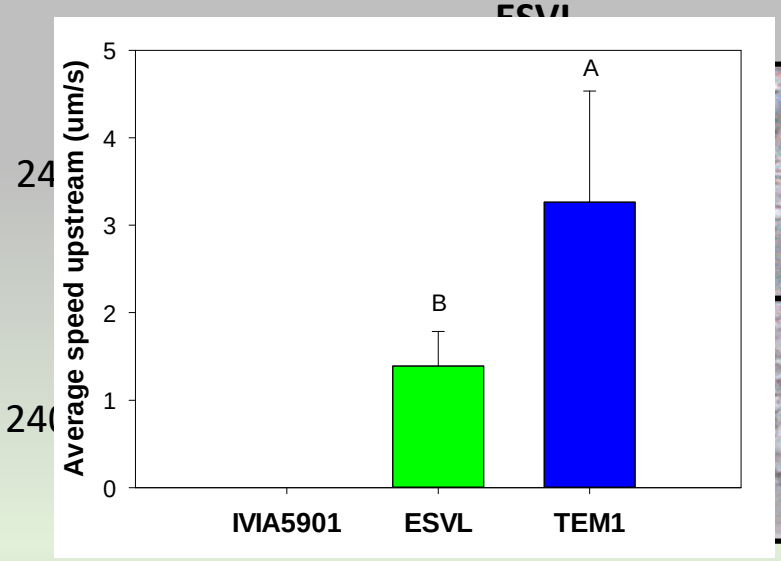


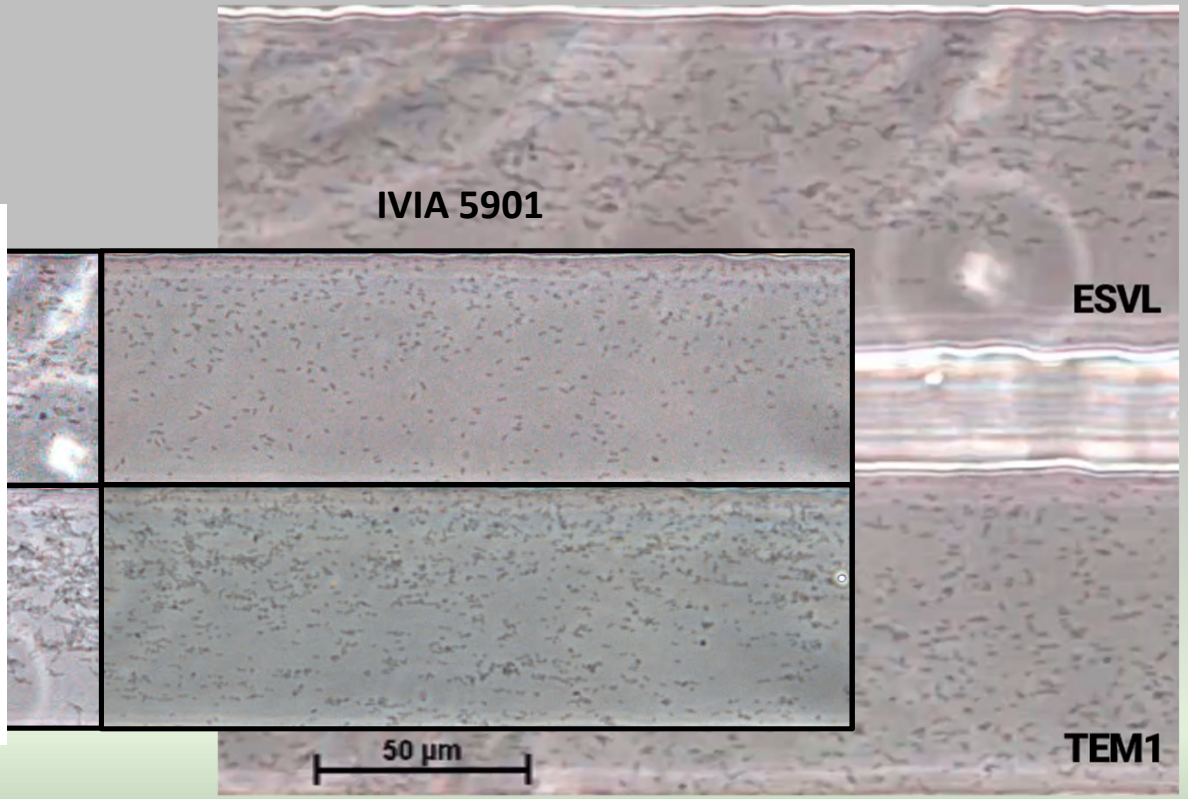


De La Fuente, et al. 2007. Appl. Environ. Microbiol. 73, 2690–2696.

1. Patterns of bacterial growth

- **IVIA5901 seem to have not observable movement**
- **ESVL and TEM1 cells move**
- **TEM 1 cells has higher speed**

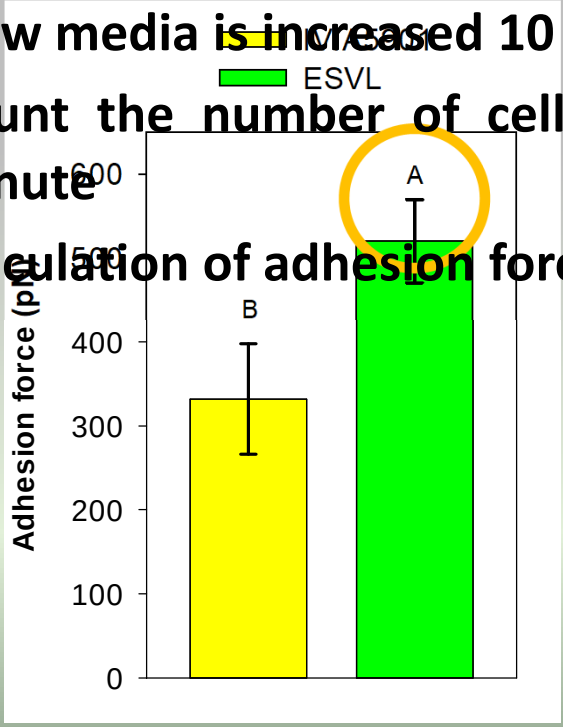


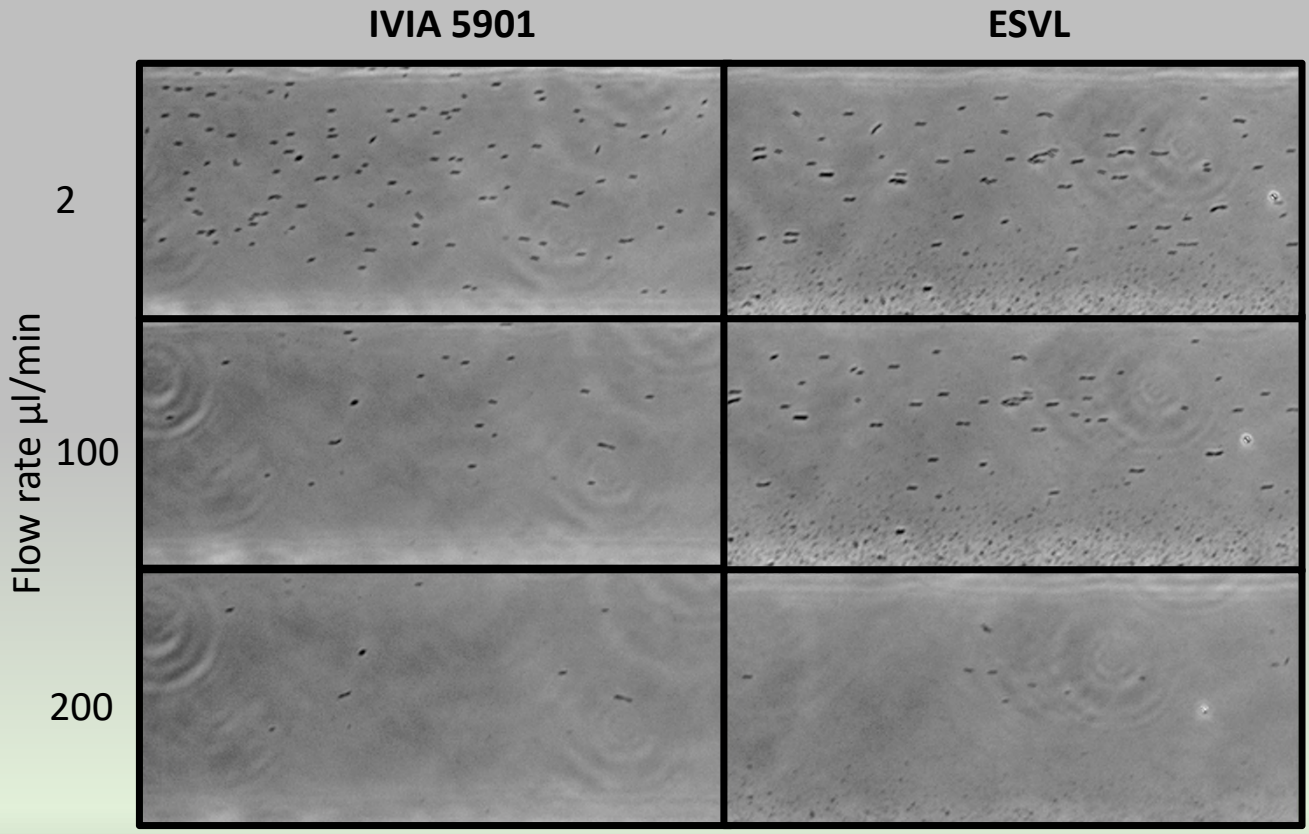


- **Spanish strains form the same type of aggregates**

2. Cells adhesion experiments

- **Flow media is increased 10 ul/min**
- **Count the number of cells each minute**
- **Calculation of adhesion force**

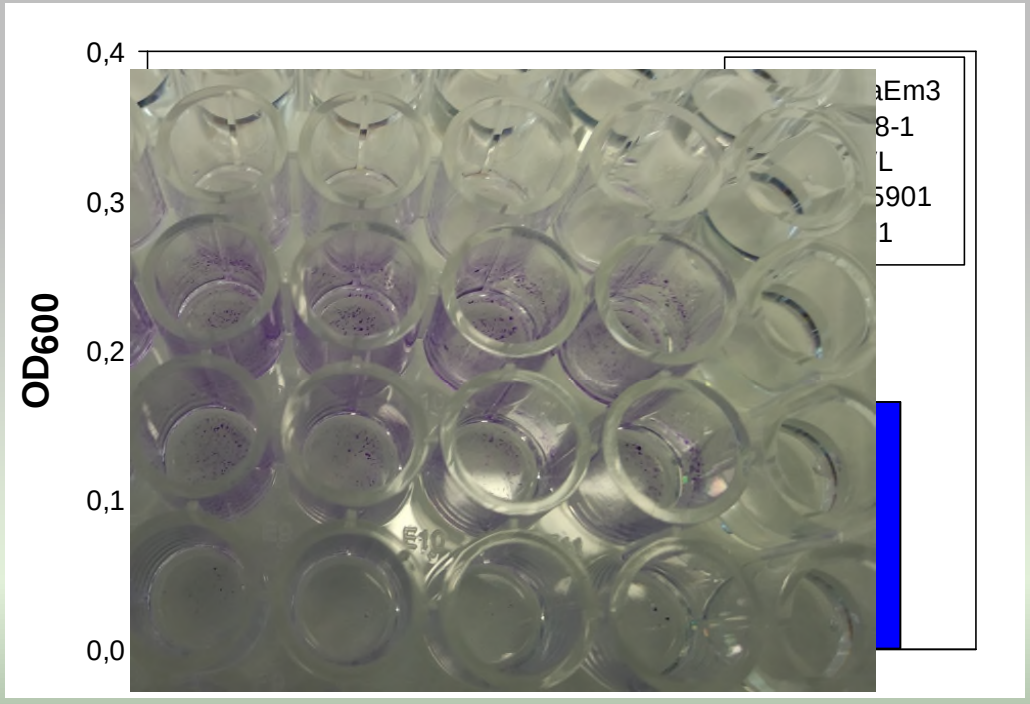


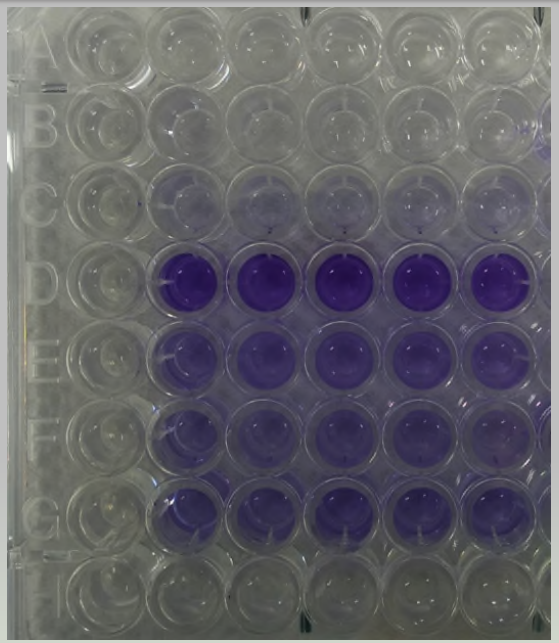


ESVL has a high capacity of attachment to the surface than IVIA5901

3. Biofilm formation

- **Cristal violet protocol**
- **3 independent replicates x 3 repetitions**





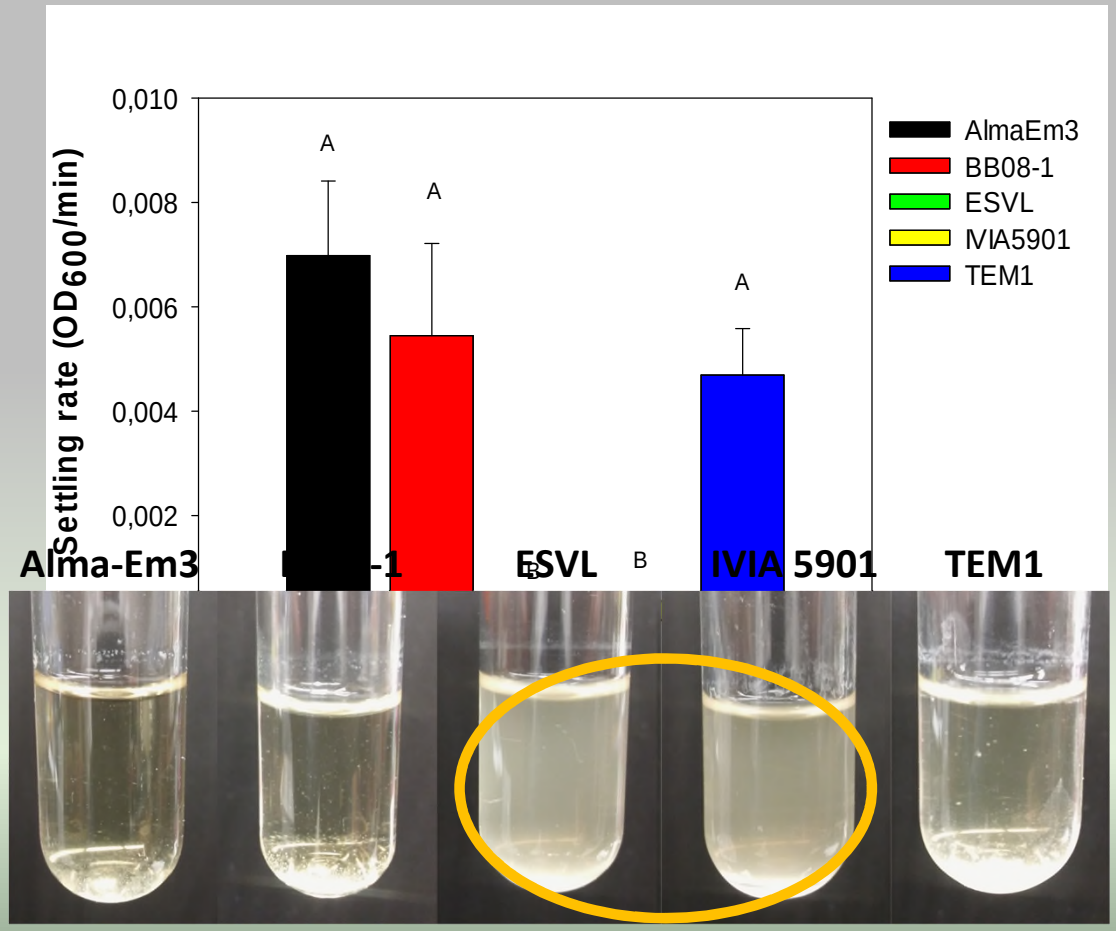
- **Spanish strains has less capacity to form biofilm**
- **There are no differences between Spanish strains**

4. Settling rate

- **Ability to form cell aggregates**
- **Cells aggregates settle at the bottom of the tube**
- **Estimation of the settling rate of the cells:**
  - **measuring OD_{600} in T_0 and T_{60min}**

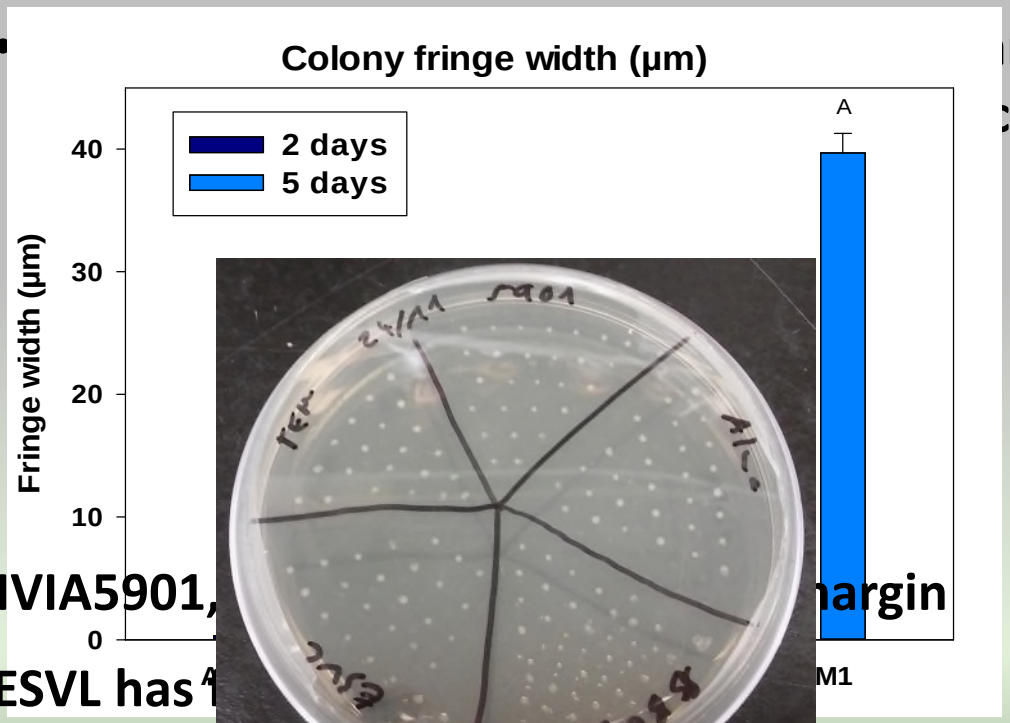
- **Spanish strains have less capacity to aggregate cells**

- **There are no differences between Spanish strains**

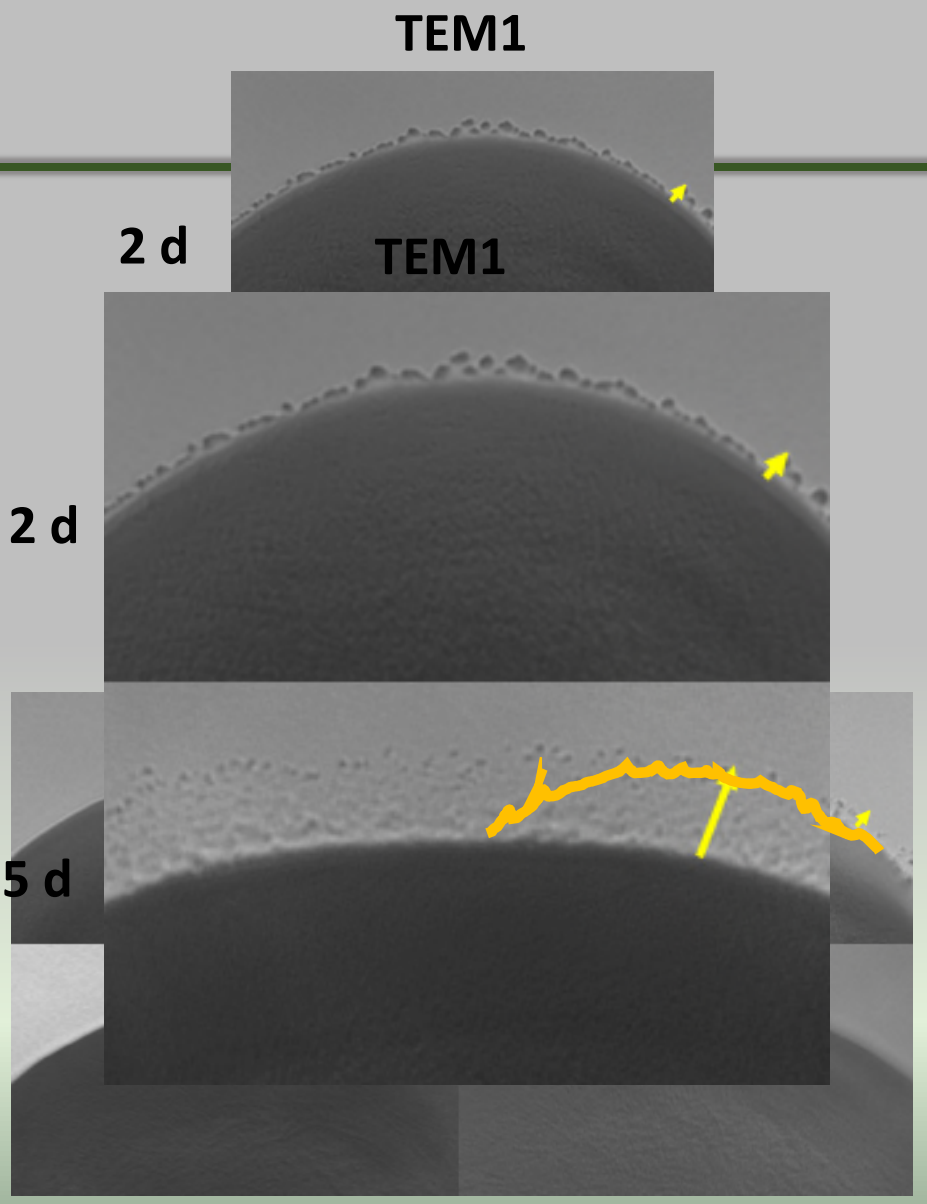


5. Twitching motility

- Morphology of the edge of the colonies
- Measures at 2 and 5 days
- rked
  ching



- IVIA5901, argin
- ESVL has
  - Crenul

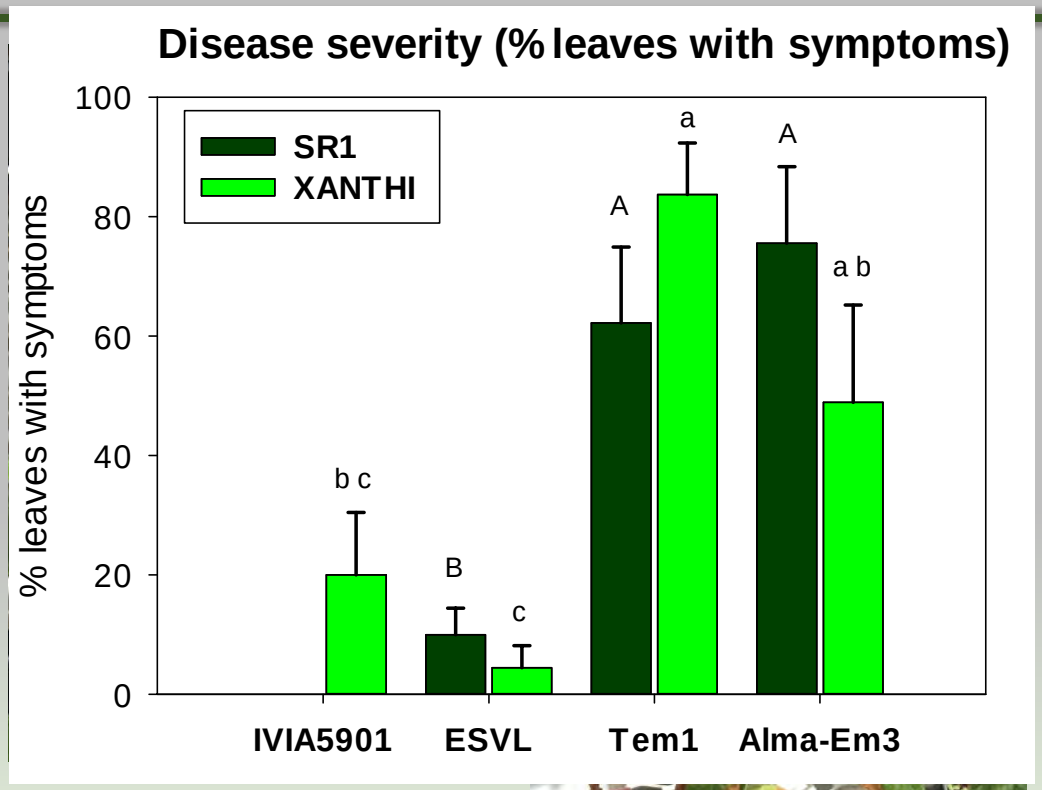


6. Pathogenicity experiment

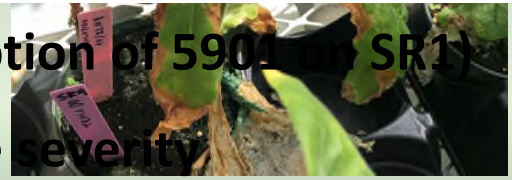
- **2 tobacco cultivars (*Nicotiana tabacum* L)**
  - **Petit Havana SR1**
  - **Xanthi**
  - **9 plants/treatment**
- **4 isolates + Buffer-inoculated control**
  AlmaEm3, IVIA5901, ESVL and Temecula1
- **Pin-prick inoculation method**

6. Pathogenicity experiment

- First symptoms after 3 months PI
- Leaf yellowing and scorch
- Weekly disease severity assessment
- Quantification of leaves showing symptoms



- All isolates were pathogenic in tobacco (exception of 5901 on SR1)
- Spanish strains had significantly lower disease severity



Conclusions

- **Spanish isolates has less motility, ability to form biofilm and cell aggregates and virulence on tobacco compared with other reference strains**
- **Compared with IVIA5901, ESVL has higher motility and adhesion force to surfaces and it is pathogenic in both tobacco cultivars**
- **Our results may indicate that Spanish strains don´t have pili IV or lack of some of the functions related with the formation or functionality of pili IV**
- **This phenotypic difference might be caused by the presence of the two plasmids in the ESVL strain, as both strains are identical at more than 99 % in their genome**
- **Further research is needed to confirm if this phenotypic differences are mainly due to the presence of both plasmid in ESVL strain**

XF ACTORS

Xylella Fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy

CSIC

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

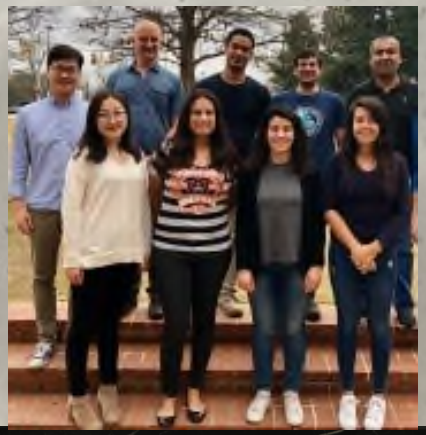
INSTITUTO DE AGRICULTURA SOSTENIBLE IAS

AUBURN UNIVERSITY

COLLEGE OF AGRICULTURE

EuroXanth

COST Action CA16107



AGENCIA ESTATAL DE INVESTIGACIÓN

cost

EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

INIA

Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria