

Screening of a novel Biological Control Agent targeting the phytopathogen *Xylella fastidiosa*

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Introduction

In autumn 2013, a disease outbreak affecting olive trees occurred in Gallipoli, a restricted area in the southern of Italy. It was the occurrence of the Olive Quick Decline Syndrome (OQDS) caused by a devastating xylem bacterium, the so called *Xylella fastidiosa* subsp. pauca De Donno strain. In spite of the pertinent research effort devoted to control the disease, so far there are no control measure available to eliminate the bacteria from a diseased host plant. Therefore, the aim of this study is the screening of potential antagonistic bacteria on leaves of 8 different host plants species.

Collection of samples and isolation

Sampling of symptomatic and asymptomatic leaves from 8 different hosts of (xf) was carried out in various *X. fastidiosa* outbreaks in Apulia region. The hosts sampled were: *Olea europaea*, *Polygala myrtifolia*, *Rosmarinus officinalis*, *Nerium oleander*, *Laurus nobilis*, *Myrtus communis*, *Prunus Dulcis* and *Prunus avium*. For epiphytic bacterial isolation(Fig 1), the steps are:

- Ten leaves/sampling from each species were washed separately in flasks containing 50ml of sterile distilled water
- shaking for 2 hours at 150rpm
- Dillution series was made of each washing solution and (0,1ml) were spread onto king B and NA.
- After 3 days of growth at 25C°, the single colonies were picked up and purified by repeated streaking on the same medium.

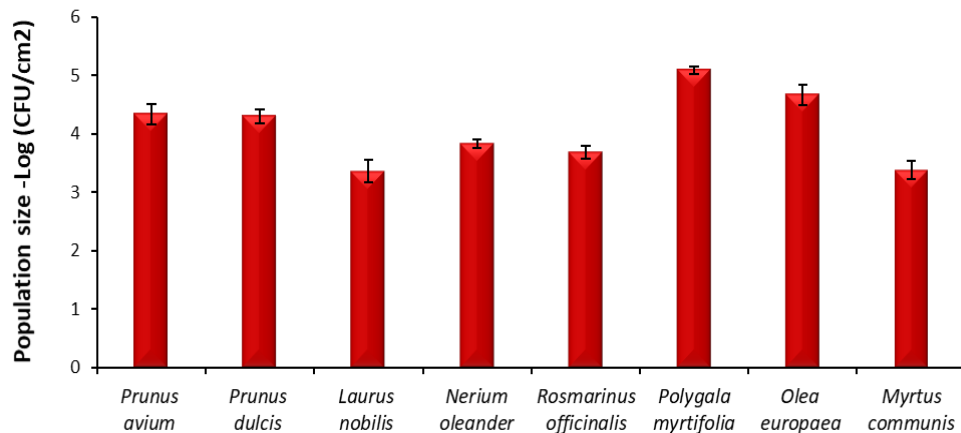


Figure 1: Total bacterial population on leaves of different host plant species of *Xylella fastidiosa*, Errors bars indicate the standard error (89 isolates in total).

Screening of antagonistic activity in vitro

The antagonistic activity against (*Xf*) was studied on BCYE medium by the dual culture method. Three drops of (*Xf*) suspension (10^6 CFU/ml), each containing 20 μ l, were placed at the top of each Petri dish, 1 cm apart, and allowed to slowly flow down to the opposite side of the plate, resulting in three parallel rows. After 24 h of incubation at 28 °C, an aliquot of 5 μ l of a suspension of the potential BCA (10^8 CFU/ml) was placed on the top of each row of (*Xf*) cultures. After 7–12 days of incubation at 28 °C, the antagonistic activity was detected as an area of (*Xf*) growth inhibition, that was measured and the test were performed in triplicate. (Fig. 2 and 3).

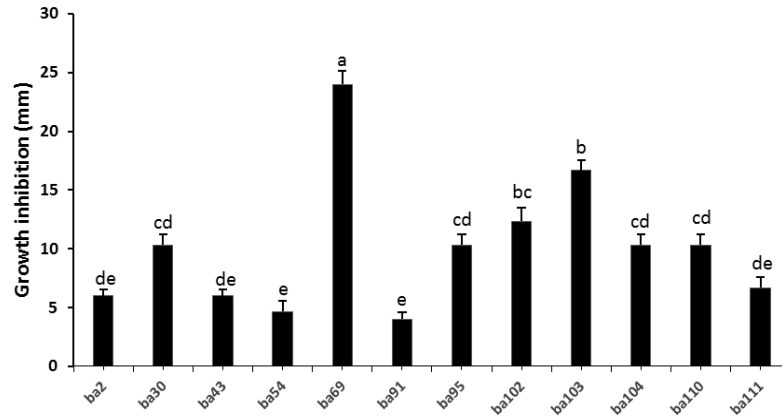


Figure 2 : Growth inhibition of (*Xf*) (ST53) by antagonistic bacteria. Different letters indicate statistically different antagonistic activities with $P < 0.05$ as determined by one-way analysis of variance (ANOVA) followed by the Tukey test.

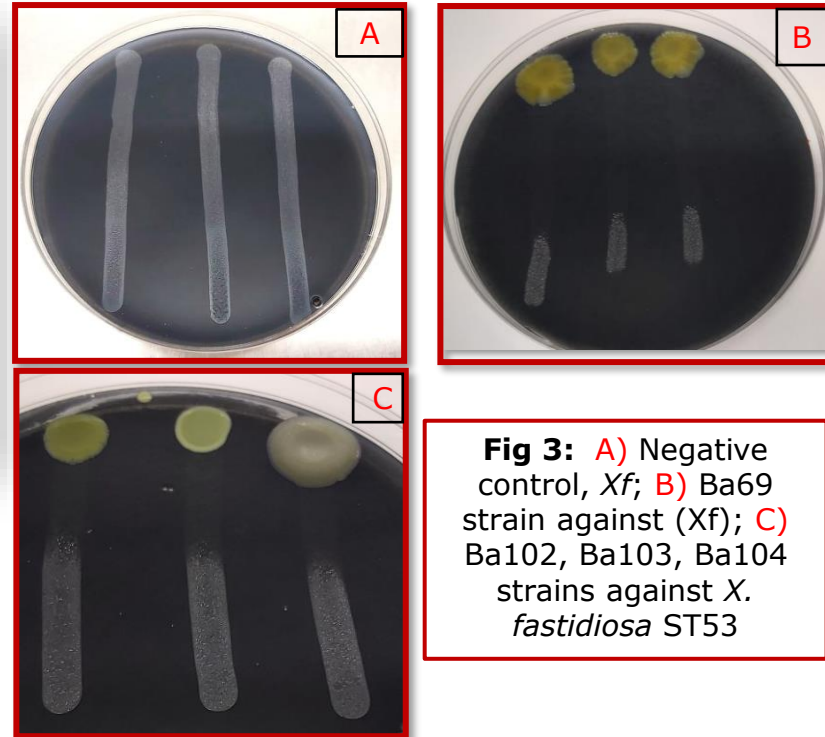


Fig 3: A) Negative control, *Xf*; B) Ba69 strain against (*Xf*); C) Ba102, Ba103, Ba104 strains against *X. fastidiosa* ST53

Characterization and identification of potential antagonistic bacteria

The 12 antagonistic bacteria were submitted to biochemical tests (e.g. fluorescence production, levan production, KOH assay and oxidase test) and were identified by analysis of DNA sequence of 16S rDNA (Fig 4)

Fig 4: Main characteristic of antagonistic bacteria OX (oxidase test) ; LE (levan production); FL (fluorescence pigment production on King B); GR (Gram Stain), - = negative, + = positive.

BCAs Isolates Name	Collected from: Host plant	GR	Ox	FL	LE	Belonging to	% of 16S rRNA gene identity
Ba2	<i>Rosmarinus officinalis</i>	-	-	-	-	<i>Pseudomonas graminis</i>	98,28%
Ba30	<i>Rosmarinus officinalis</i>	-	+	-	-	<i>Delftia acidovorans</i>	100%
Ba43	<i>Olea europaea</i>	-	+	-	-	<i>Delftia acidovorans</i>	100%
Ba54	<i>Olea europaea</i>	+	+	-	-	<i>Curtobacterium flaccumfaciens</i>	100%
Ba69	<i>Neirum oleander</i>	-	-	-	-	<i>Pantoea agglomerans</i>	99,91%
Ba91	<i>Olea europaea</i>	+	-	-	-	<i>Microbacterium oleivorans</i>	99,03%
Ba95	<i>Myrtus Communis</i>	+	+	-	-	<i>Sphingomonas molluscorum</i>	99,91%
Ba102	<i>Polygala myrtifolia</i>	-	+	-	-	<i>Stenotrophomonas rhizophila</i>	100%
Ba103	<i>Polygala myrtifolia</i>	+	-	-	-	<i>Microbacterium spp.</i>	99,91%
Ba104	<i>Polygala myrtifolia</i>	+	-	-	-	<i>Microbacterium oxydans</i>	99,82%
Ba110	<i>Olea europaea</i>	+	-	-	-	<i>Microbacterium spp.</i>	100%
Ba111	<i>Polygala myrtifolia</i>	+	-	-	-	<i>Microbacterium phyllosphaerae</i>	99,91%

Conclusion and Future perspectives

Our screening of a novel Biological Control Agent targeting the phytopathogen *Xylella fastidiosa* ST53 strain pointed out a twelve epiphytic bacterial isolates belonging to different bacterial species that were able to reduce the growth of the bacterium. Several tests are ongoing to understand their characteristics and their mode of action. As future work, the antagonistic efficacy of the most efficient isolates *in vitro* needed to be tested *in vivo* and depending on these results, it could be a precious help to cure and protect the European olive orchards.



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