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### ► To cite this version:

C Pinto, V Custódio, A Spagnolo, A Songy, C Clément, et al.. Grapevine microbiome: a challenge to identify beneficial microorganisms for grapevine protection. MICROPE, 2015, VIENNA, Australia. hal-03124141

**HAL Id: hal-03124141**

**<https://hal.univ-reims.fr/hal-03124141v1>**

Submitted on 28 Jan 2021

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# Grapevine microbiome: a challenge to identify beneficial microorganisms for grapevine protection

C. Pinto<sup>1,2</sup>, V. Custódio<sup>1</sup>, A. Spagnolo<sup>2</sup>, A. Songy<sup>2</sup>, C. Clément<sup>2</sup>, F. Fontaine<sup>2</sup>, A. C. Gomes<sup>1</sup>

<sup>1</sup>BIOCANT, Technology Transfer Association, BIOCANT PARK, Núcleo 04, Lote 8, 3060-197 Cantanhede, Portugal

<sup>2</sup>University of Reims Champagne Ardenne, URVVC EA 4707, Laboratory of Stress, Defence and Reproduction of Plantes, BP 1039, 51687 Reims Cedex 2, France

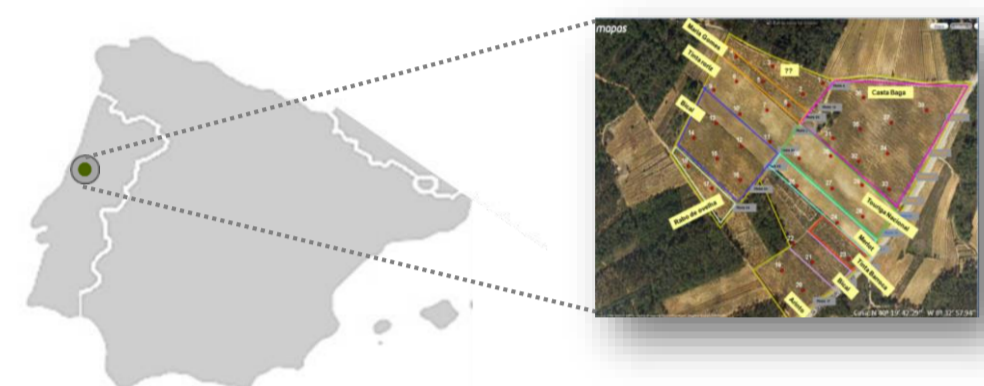
Presenting author: [catia.pinto@biocant.pt](mailto:catia.pinto@biocant.pt); Corresponding author: [acgomes@biocant.pt](mailto:acgomes@biocant.pt)

## INTRODUCTION

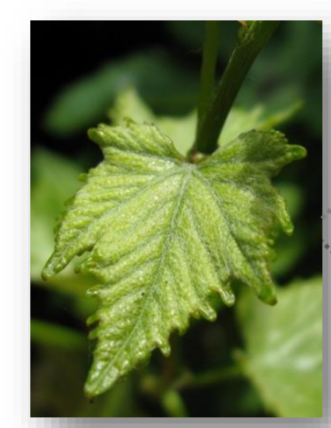
*Vitis vinifera* is in a close interaction with different microbial communities that constitutes the plant microbiome. Such plant-microbial interactions and their balance are essential for the plant growth and health status.

The beneficial microorganisms have the capacity to improve the potential of the plant by reducing the plant disease incidence or by promoting the plant growth. Thus, the deep knowledge of these communities is crucial to develop new sustainable strategies for grapevine protection.

## METHODOLOGY



Vineyard located at Bairrada appellation, Portugal  
Samples collected during 2010 and 2011 vine campaign and across the plant vegetative cycle



Grapevine samples

## OBJECTIVES

Deep study of the natural microbial populations of grapevine

Characterize phytopathogens and beneficial microorganisms

Understand their dynamics, microbial-plant interaction

Looking for phytoprotectors in/for grapevine protection  
(Understand their ecology, dynamics and impact)

### • Cultivation-independent approach

Grapevine leaves of TR, TN and Baga → DNA extraction and rDNA amplification → 454 Sequencing and analysis

### • Cultivation-based approach

Isolation of microorganisms → Molecular and biochemical characterization of potential phytoprotectors → Analysis of grapevine-microbial interactions

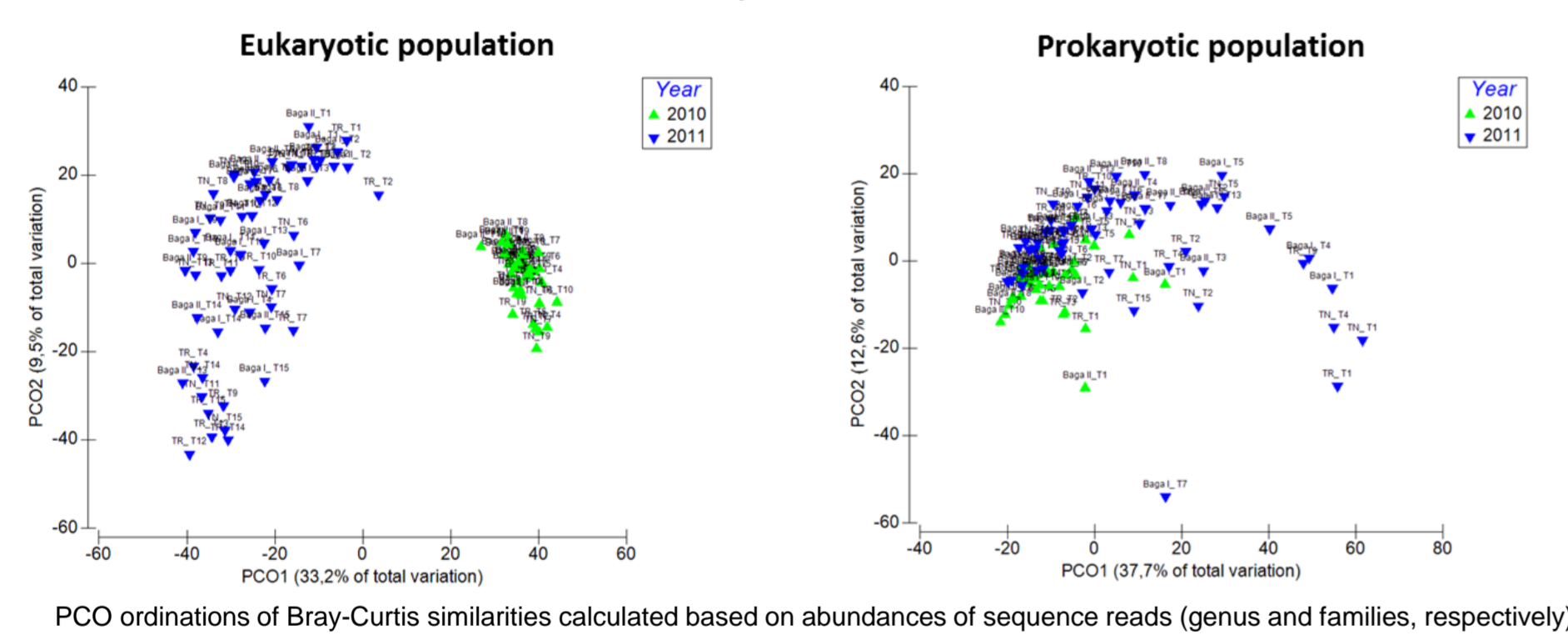
Grape varieties: TR – Tinta Roriz; TN – Touriga Nacional; Baga

## RESULTS

### Grapevine microbiome

Among the eukaryotic population, Ascomycota and Basidiomycota phylum were the most abundant population (average of 34.76% and 5.57%, respectively). For the prokaryotic community, Firmicutes (46.11%), Proteobacteria (44.27%) and Actinobacteria (3.73%) were the most abundant.

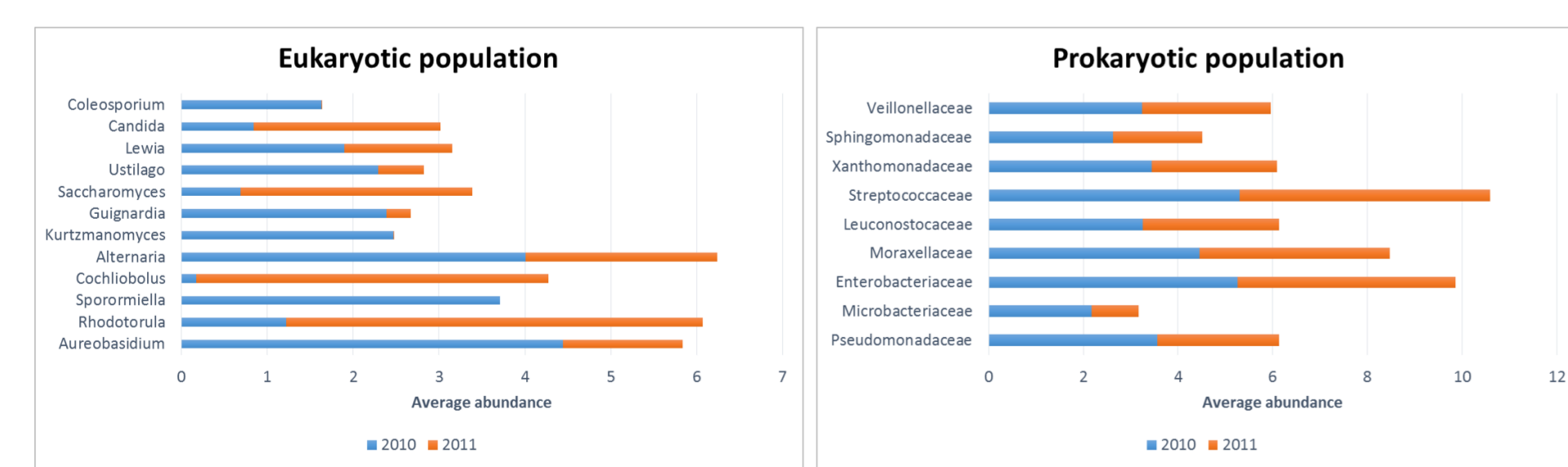
#### Microbial dynamics overtime



Vintage and time exhibited a significant effect on both eukaryotic and prokaryotic population ( $p < 0.002$ ). No differences were observed across grape varieties.

*Aureobasidium* was the most abundant at 2010 (25.91%) and *Rhodotorula* (27.18%) at 2011. Among the prokaryotic population, Enterobacteriaceae (25.89/ 25.08%) and Streptococcaceae (38.48/17.88%) were the most abundant.

#### Microbial dissimilarities between 2010 and 2011



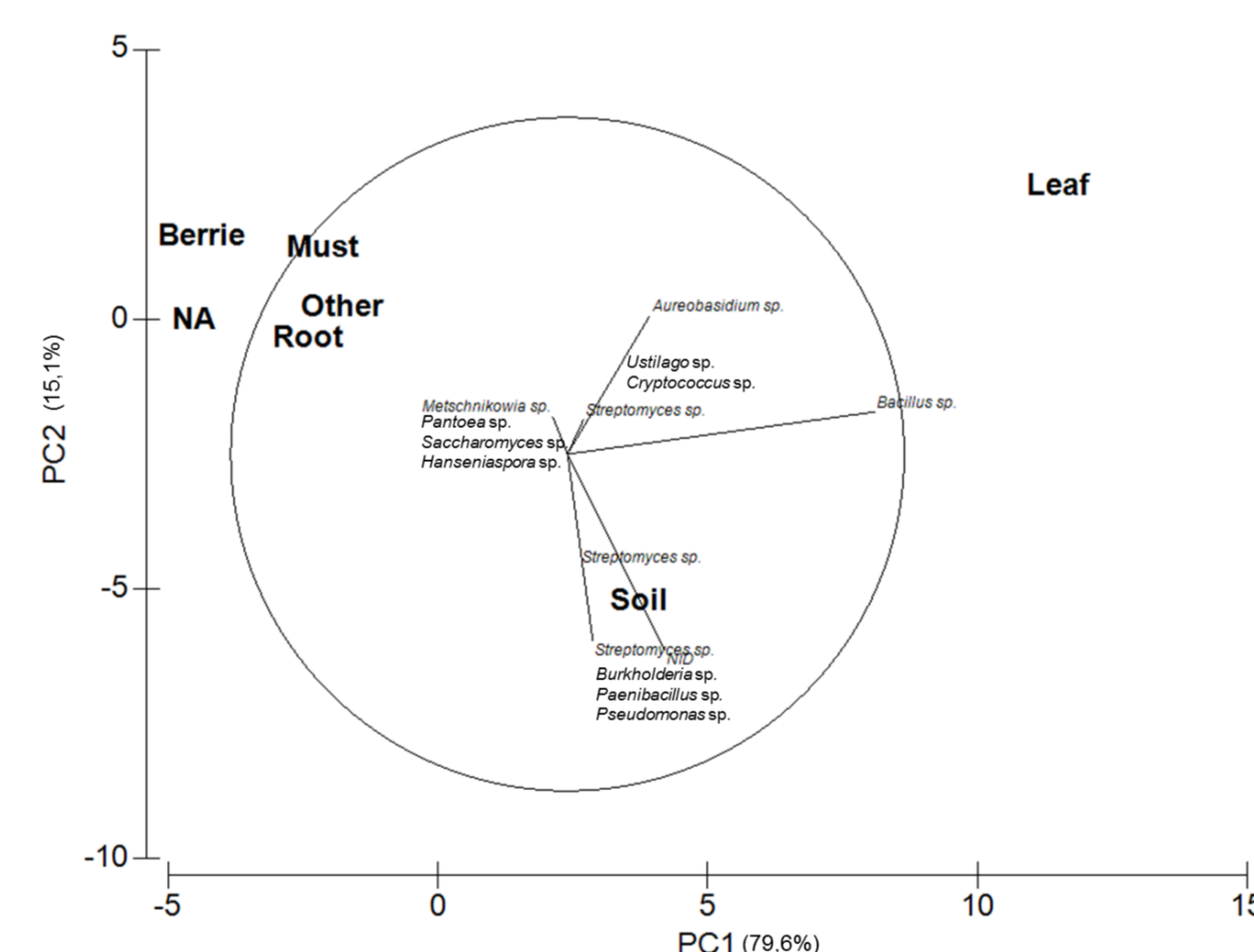
Average dissimilarity: 84.06

Average dissimilarity: 42.34

\*Average abundance calculated based on sequence reads, after a log<sub>2</sub> transformation and Bray-Curtis similarity.

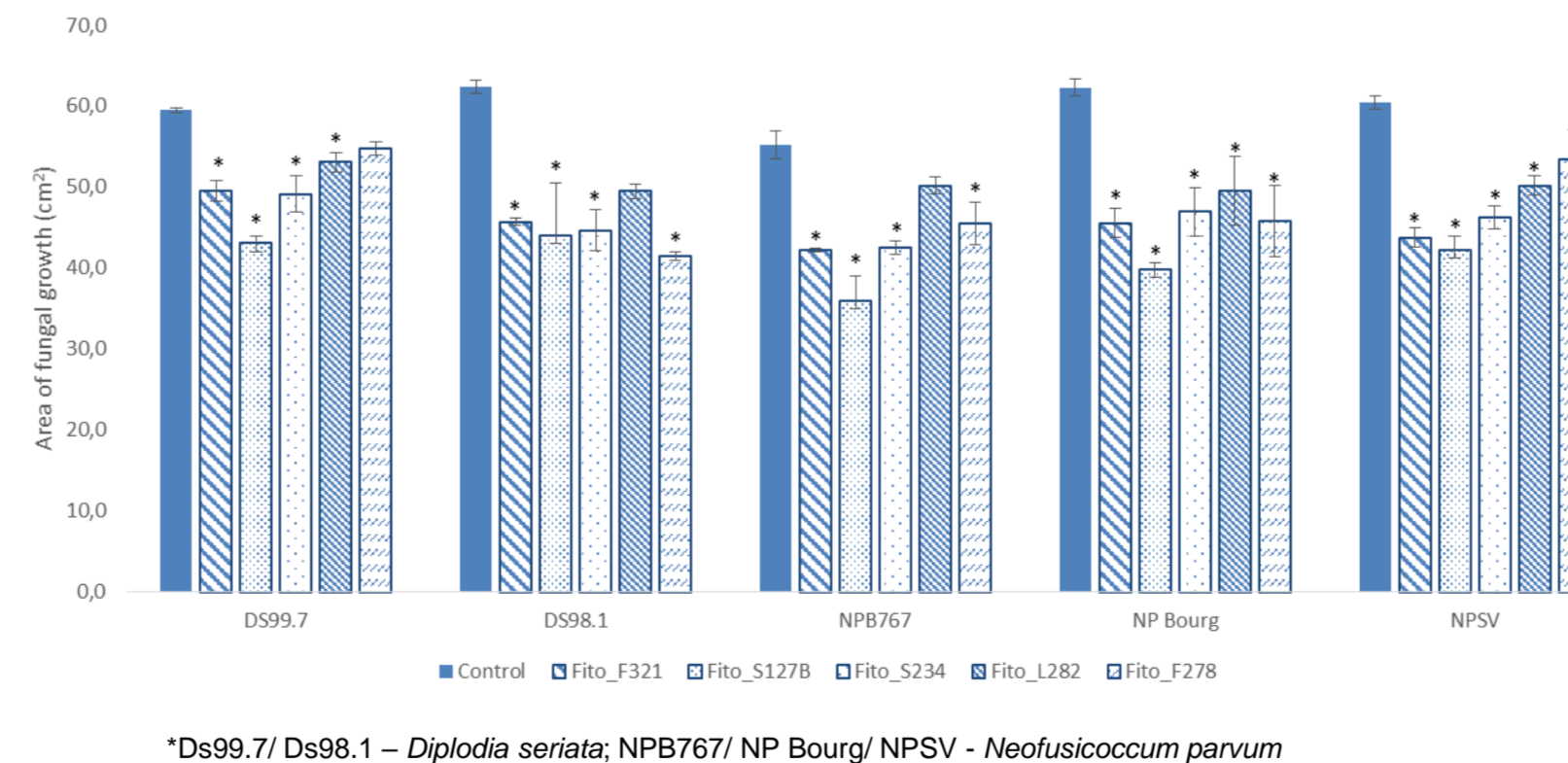
### Cultivation-based approach

A total of 254 isolates were isolated from different grapevine structures (soil, root, leaf, berry, must) and tested for their antagonistic capacity against different grapevine pathogens. From these, 72 positive isolates (34%) were obtained, both bacteria (61%) and yeasts (20%).



Based on these results, the best 5 isolates were chosen and tested against strains responsible of Botryosphaeria dieback, one of the main grapevine trunk diseases.

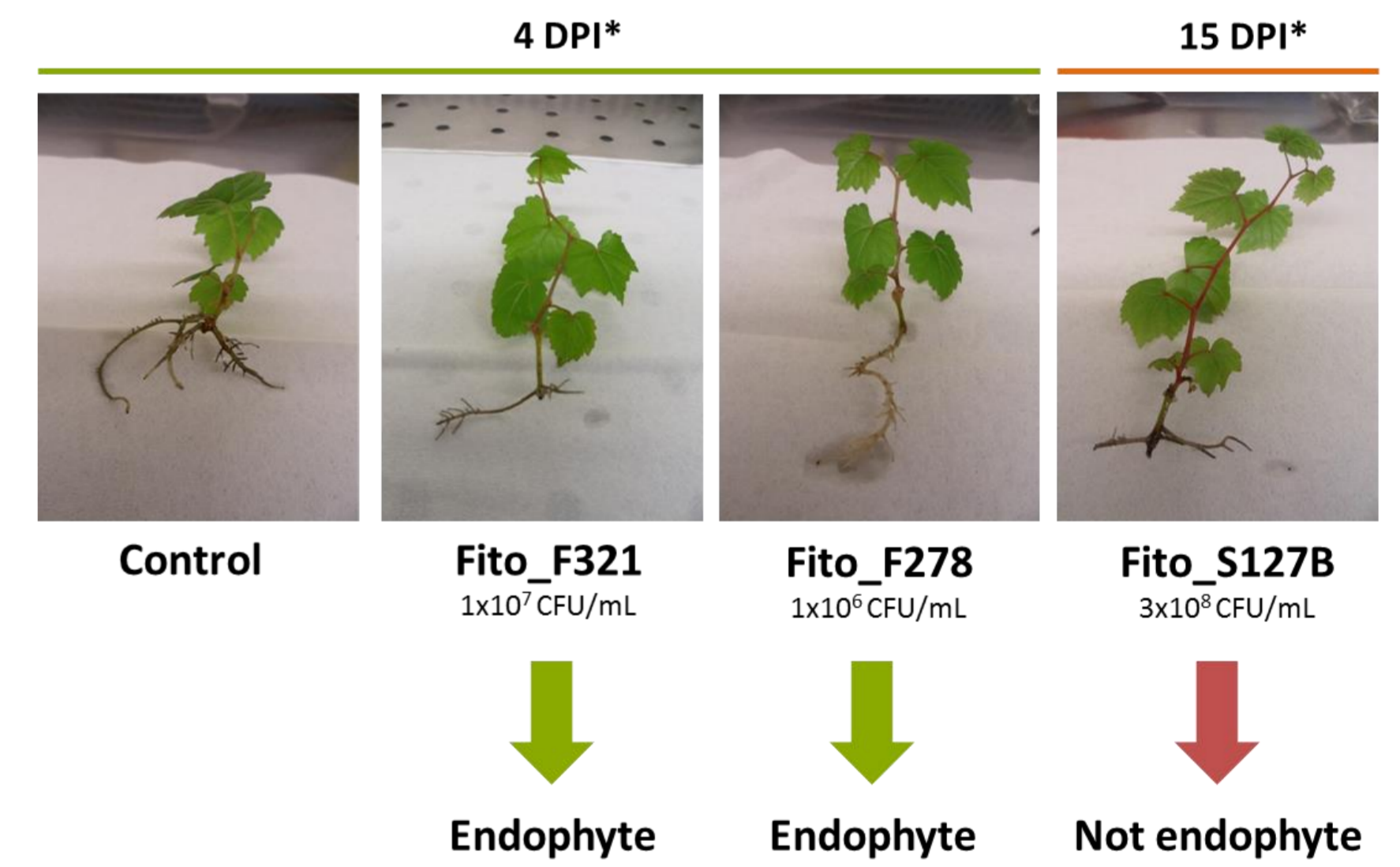
#### Antagonistic activity (14 days)



All 5 strains showed a significant inhibition of the fungal growth

### Plant-microbial interactions

A set of 3 potential phytoprotectors were uncovered and their interactions with *in vitro* plants of *Vitis vinifera* were analysed.



Different concentrations of each potential phytoprotector were tested and the plant health status compared with control plants.

Some potential phytoprotectors showed to be endophytic, with capacity to move across the plant (from roots to leaves) and to growth *in planta* overtime.



Leaves observation after 4DPI\*

\*DPI – Days Post Inoculation

## CONCLUSION

- The plant microbiome can be considered as a plant's second genome.
- A resident microbial communities was uncovered, where *Aureobasidium* and *Rhodotorula*, Enterobacteriaceae and Streptococcaceae were the most abundant population.
- Vintage and temporal distribution represented significant drivers of the microbial community.
- A selection of 3 potential phytoprotectors for grapevine management is investigated, with endophytic capacity and a growth *in planta*.

### Acknowledgments

We are most thankful to the wine producer in the Bairrada appellation for providing the grapevine leaves samples. This work was partially carried out within the project InovWine II (COMPETE/QREN – Ref:FCOMP-01-0202-FEDER-030272, Holiwine (FCT/COMPETE/QREN – Ref: FCOMP-01-0124-FEDER-027411) and by SDRP laboratory. Cátia Pinto is supported by a PhD grant from FCT, with the reference SFRH/BD/84197/2012.