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

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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.

OBJECTIVES

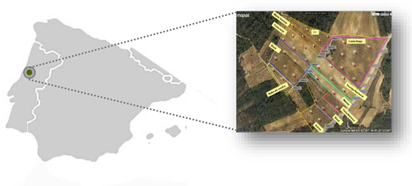
Deep study of the natural microbial populations of grapevine

- Characterize phytopathogens and beneficial microorganisms
- Understand their dynamics, microbial-plant interaction

Looking for phytoprotectors in/for grapevine protection

(Understand their ecology, dynamics and impact)

METHODOLOGY



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

Grapevine samples

- **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

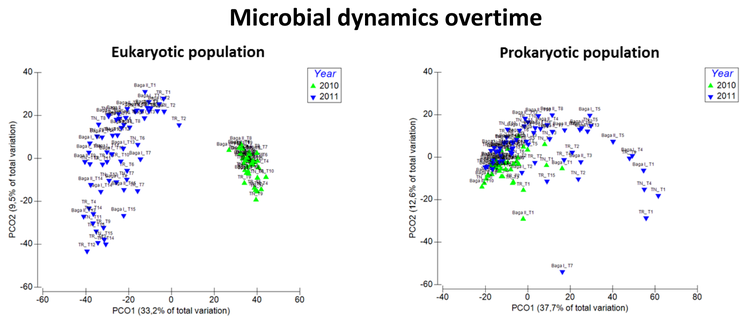
Grave varieties: TR – Tinta Roriz; TN – Touriga Nacional; Baga

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

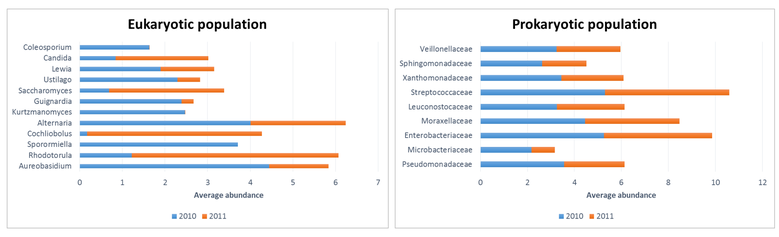


PCO ordinations of Bray-Curtis similarities calculated based on abundances of sequence reads (genus and families, respectively)

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

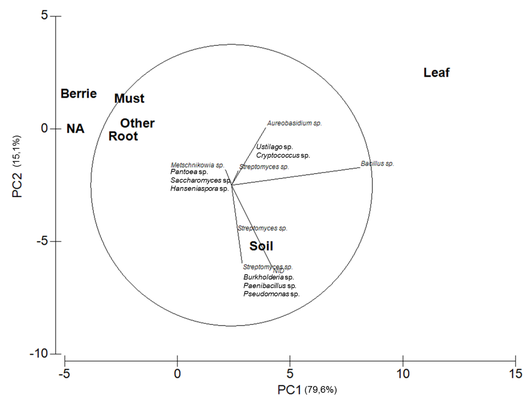


Average dissimilarity: 84.06 **Average dissimilarity: 42.34**

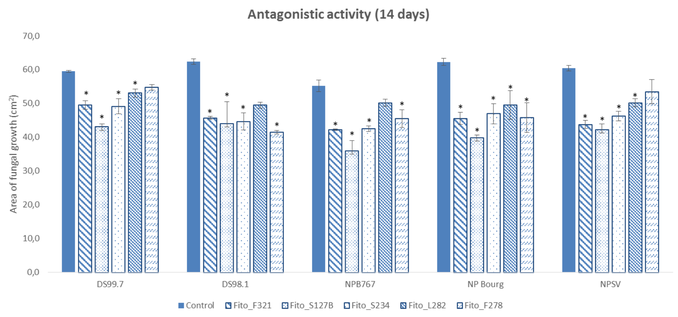
*Average abundance calculated based on sequence reads, after a logx transformation and Bray-Curtis similarity.

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.

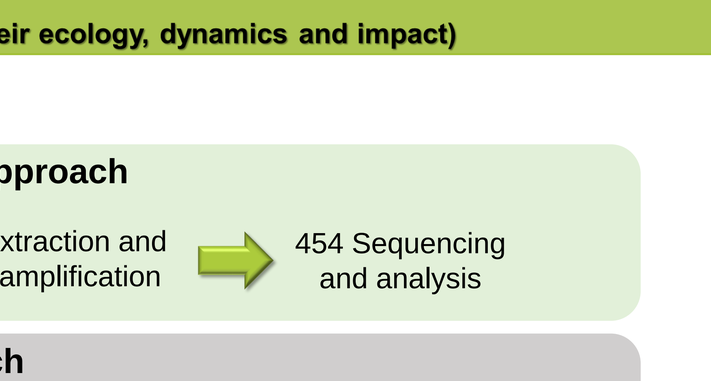


*Ds99.7/ Ds98.1 – *Diplodia seriata*; NPB767/ NP Bourg/ NPSV - *Neofusicoccum parvum*

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.*

Aknowledgments

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