Towards microbiota-based disease management: analysis of grapevine microbiota in plots with contrasted levels of downy mildew infection

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1. Context and hypotheses

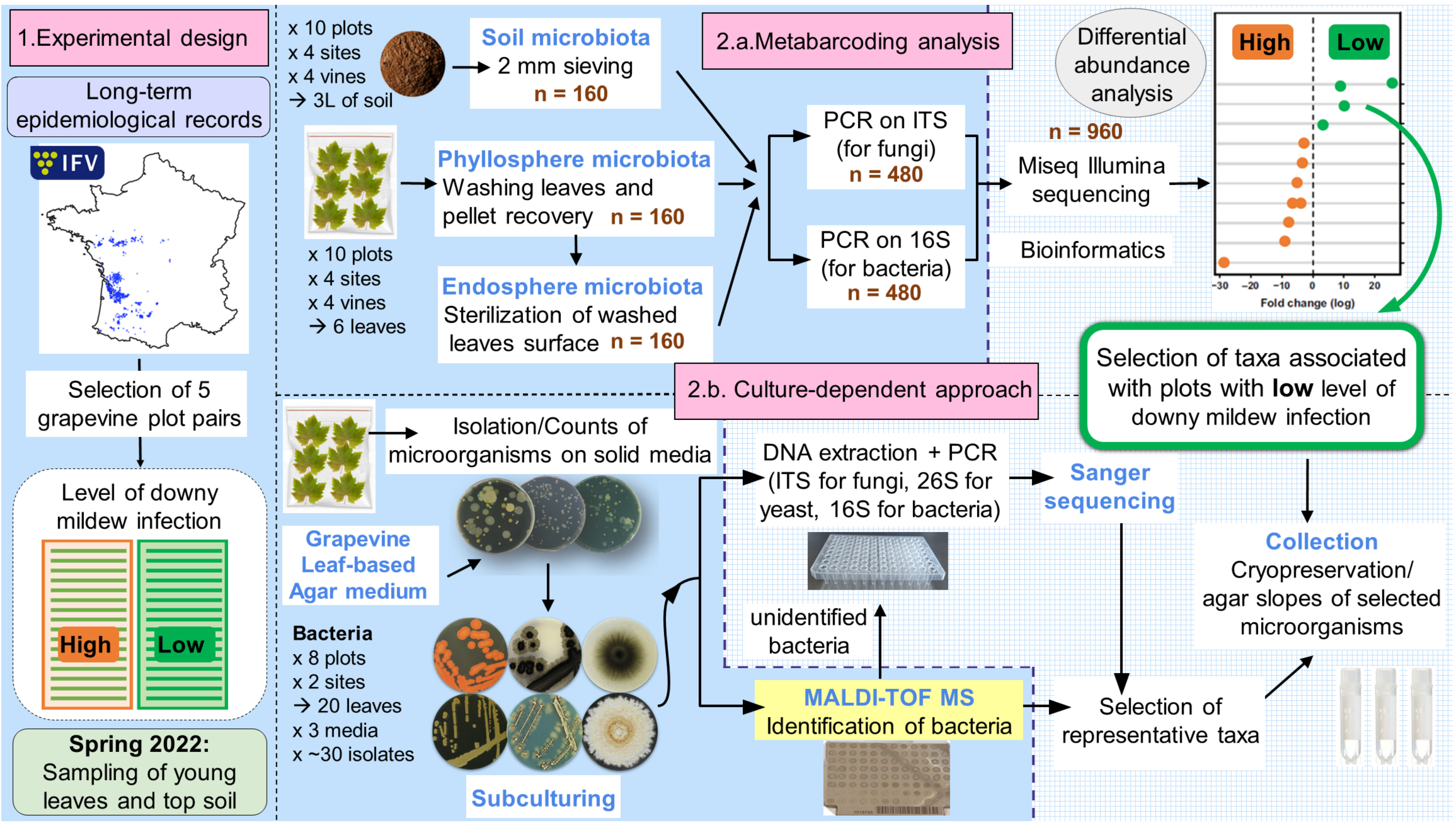
Downy mildew
Plasmopara viticola
Oomycete

Vineyards are colonized by a myriad of microorganisms that interact with each other and with grapevine plants[1,2]. Some microorganisms are plant pathogens, such as the oomycete *Plasmopara viticola* that causes grapevine downy mildew. Others have a positive influence on vine health, such as plant growth promoting bacteria and disease biocontrol agents. These beneficial interactions have gained more attention in recent years because they could represent an alternative to the use of fungicides in viticulture[3].

To develop microbiota-based disease management, we aim to cultivate the myriad of microorganisms that colonize grapevine leaves and then assemble multi-strain microbial consortia that could protect vines against *P. viticola*. To increase the probability of isolating microorganisms of interest, we developed a plant-based culture medium. We tested the hypothesis that it increases the cultivable microbial diversity in comparison to standard culture media.

2. Materials and methods

We combined metabarcoding (Fig.1(2.a)) and culture-dependent (Fig.1(2.b)) approaches to analyze the microbiota of soil and leaf samples collected in plots with contrasted levels of downy mildew infection.



1.Experimental design

Long-term epidemiological records

IFV

Selection of 5 grapevine plot pairs

Level of downy mildew infection

High

Low

Spring 2022: Sampling of young leaves and top soil

x 10 plots
x 4 sites
x 4 vines
→ 3L of soil

Soil microbiota
2 mm sieving
n = 160

Phyllosphere microbiota
Washing leaves and pellet recovery n = 160

x 10 plots
x 4 sites
x 4 vines
→ 6 leaves

Endosphere microbiota
Sterilization of washed leaves surface n = 160

2.a.Metabarcoding analysis

PCR on ITS (for fungi) n = 480

PCR on 16S (for bacteria) n = 480

n = 960

Miseq Illumina sequencing

Bioinformatics

Differential abundance analysis

Selection of taxa associated with plots with **low** level of downy mildew infection

2.b. Culture-dependent approach

Isolation/Counts of microorganisms on solid media

Grapevine Leaf-based Agar medium

Bacteria
x 8 plots
x 2 sites
→ 20 leaves
x 3 media
x ~30 isolates

Subculturing

DNA extraction + PCR (ITS for fungi, 26S for yeast, 16S for bacteria)

Sanger sequencing

unidentified bacteria

MALDI-TOF MS
Identification of bacteria

Selection of representative taxa

Collection
Cryopreservation/ agar slopes of selected microorganisms

Fig.1: Workflow to characterize and isolate bacterial and fungal communities naturally colonizing vineyard soil and leaves in plots with contrasted levels of downy mildew infection. Media used for bacteria: Tryptic Soy Agar (TSA), Luria Bertani Broth Agar (LBA) and a Grapevine Leaf-based Agar medium (GLA).

Color legend

- 🟦 The task has been completed
- ⬜ The task is in progress
- 🟨 The results of this task are presented here

Bibliography
1. Bettenfeld *et al.*, 2021. *J. Adv. Res.*
2. Fournier, Pellan *et al.*, 2022. *Adv. Ecol. Res.*
3. Busby *et al.*, 2017. *PLOS Biol.*

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3. Results and discussion

MALDI-TOF MS
Identification of bacteria

- A total of 1262 bacterial isolates were analyzed by MALDI-TOF, of which 60% could be identified at least to genus level (Fig.2).
- Strains isolated from GLA and LBA were less well assigned than those isolated from TSA (higher % of non-identification and lower % of assignment to the species level) (Fig.2).
- A total of 32 bacterial genera were identified on the three culture media. Eight genera were isolated on all three media (Fig.3).
- The genera *Gordonia* and *Rahnella* were isolated only on GLA (Fig.3).

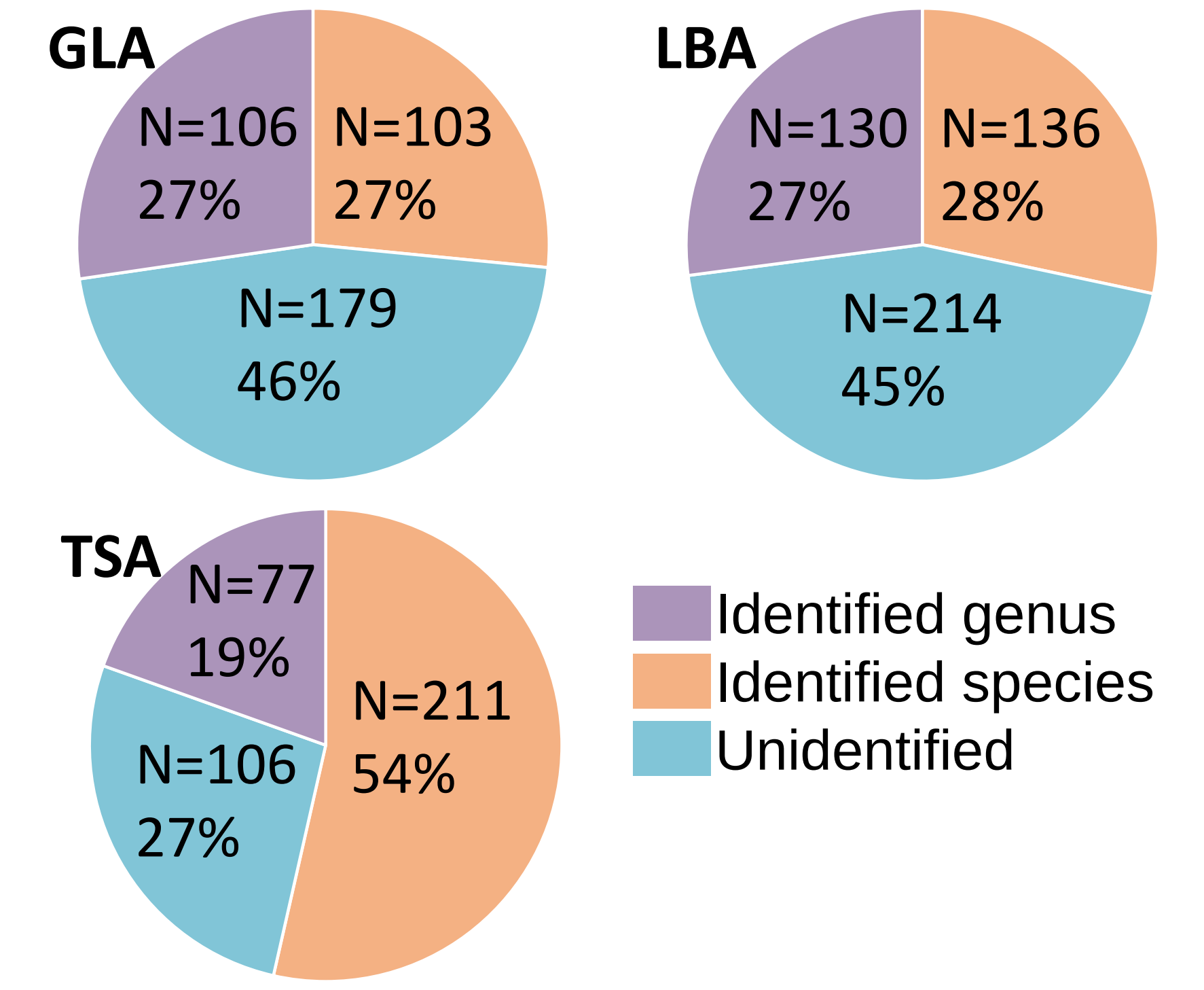

Fig.2: Number of bacterial strains isolated on each culture media and percentage of taxonomic assignments by MALDI-TOF MS.

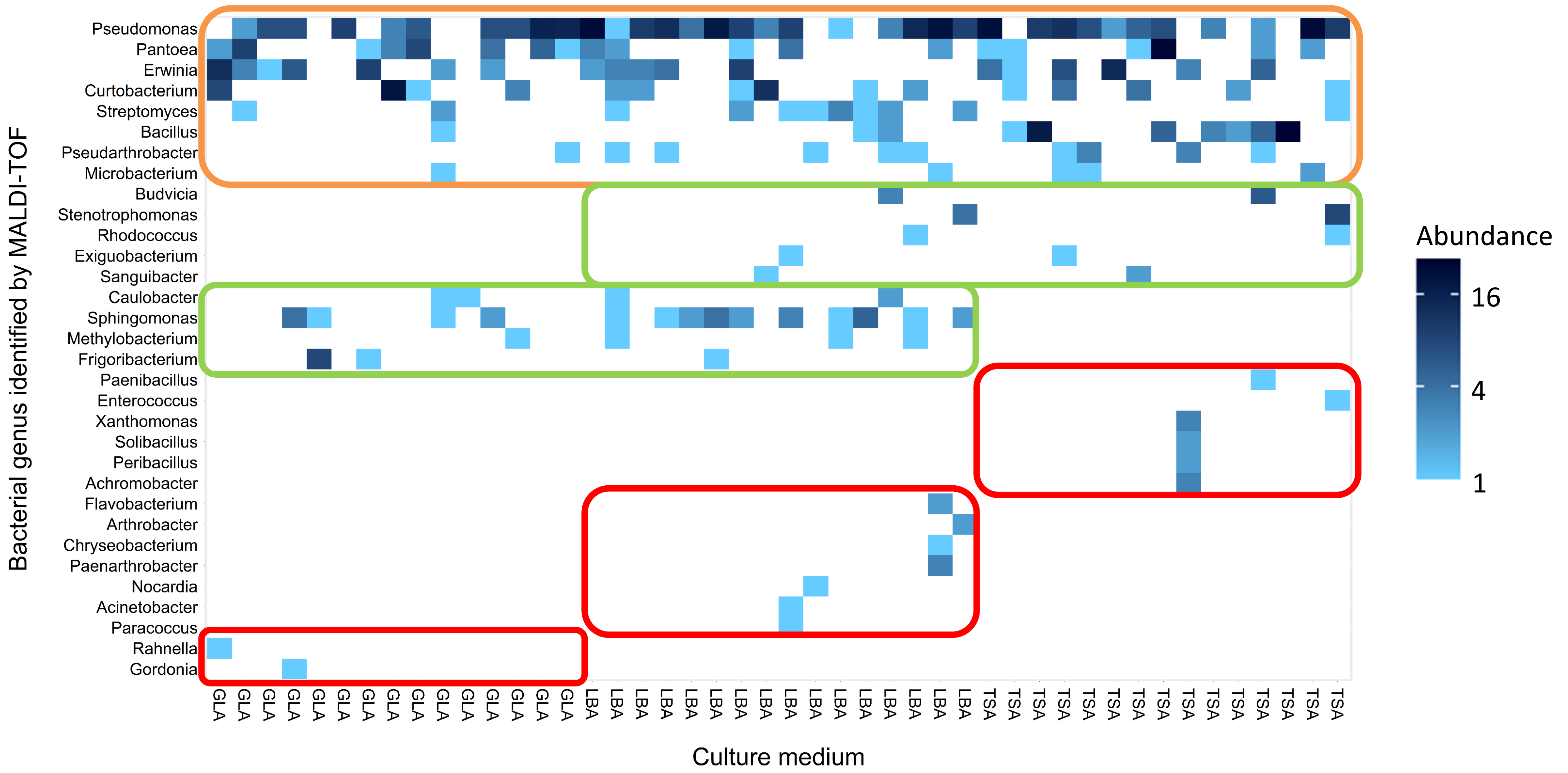

Fig.3: Bacterial genera identified using MALDI-TOF MS in grapevine leaf samples, depending on the culture media (GLA, LBA and TSA). Abundance is the number of bacterial isolates belonging to each genus. Bacterial genera identified in only one, two or three culture media are circled in red, green and orange, respectively.

4. Conclusion and perspectives

We were able to cultivate and isolate >1000 bacterial strains from grapevine leaves. Diversifying the culture media allowed us to isolate more strains. After completing the Sanger sequencing of all microbial isolates (including yeasts and filamentous fungi; Fig. 1 (2.b)), we will analyze the complementarities between the grapevine leaf-based culture medium and standard culture media. We will test the hypothesis that the vine-based medium selects for microbial taxa that are different from those selected on standard media and/or poorly referenced in the current version of the MALDI-TOF MS database. Statistical analysis of metabarcoding data (Fig. 1 (2.a)) will provide an estimate of the % of cultivable taxa and will allow us to identify microbial taxa that are significantly more abundant in vineyard plots with low levels of downy mildew infection. Those microorganisms will be retrieved from our microbial collection. Their effect on *P. viticola* will be tested in vitro and in planta, as single strains and as consortia. Modes of action will be elucidated for the most promising microbial consortia (those that thrive on grapevine leaves, are resilient to disturbances and have a biocontrol activity against *P. viticola*).