

Screening of epiphytic rhizosphere-associated bacteria in Argentinian Malbec and Cabernet-Sauvignon vineyards for potential use as biological fertilisers and pathogen-control agents

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ABSTRACT

The rhizosphere-associated microbiome has diverse functions that support plant growth and health, varying among plant species, vegetation growth stages and environmental habitats. This microbiome includes a group of bacteria denominated plant growth-promoting rhizobacteria (PGPR) which can colonize plant roots. Certain PGPR isolates improve the ability of plants to adapt to a stressful environment. In this study, we collected and characterised the rhizosphere-associated bacteria, or epiphytic rhizobacteria, from Malbec and Cabernet-Sauvignon vineyards from the main wine-producing provinces of Argentina to analyse their potential use as biologic fertilisers and/or as pathogen-control agents. A total of 170 bacterial isolates were obtained, distributed into eleven different genera and classified into three phyla, Proteobacteria, Actinobacteria and Firmicutes. The *in vitro* analysis for plant-growth-promoting (PGP) activities demonstrated that a significant number of bacterial isolates had one or more of these traits. The *Pseudomonas* was the genus with the highest number of isolates and PGP activities, followed by the *Arthrobacter*, *Serratia*, *Bacillus* and *Pantoea*. We observed that bacterial isolates identified as *Bacillus* exhibited a remarkable production of hydrolytic enzymes related to biocontrol activities. Biocontrol trials from the *Bacillus* collection revealed that at least five isolates were able to inhibit the fungal growth of *Botrytis cinerea* and *Alternaria alternata*. The results obtained suggest the biological potential of each isolate and the relevance of proceeding to greenhouse and field assays to obtain long-term environmentally compatible bio-products for vineyard management.

KEYWORDS

plant-growth-promoting rhizobacteria (PGPR), rhizosphere, grapevine, biocontrol, biofertilisation

Supplementary data can be downloaded through: <https://oenone.eu/article/view/4655>

INTRODUCTION

Associations of microorganisms with plants range from mutually beneficial to commensalistic or pathogenic (Kogel *et al.*, 2006; Newton *et al.*, 2010). The soil zone adhered directly to the plant root system has a high diversity of these microorganisms also known as rhizospheric microorganisms. These plant-microorganism associations play a key role in soil quality, productivity and plant health, through direct or indirect mechanisms, such as mineralisation of soil organic matter, activation of plant defence mechanisms and production of antibiotics against phytopathogens (Bacon and White, 2016; Compant *et al.*, 2005; Lugtenberg and Kamilova, 2009). The rhizosphere, defined as the area of the soil immediately surrounding the plant root, is considered a hotspot of microbial activity and represents one of the most complex ecosystems. Root exudates include a range of organic acids, amino acids, sugars and other small molecules that act as strong chemo-attractants of the soil microbiota (Compant *et al.*, 2019). The rhizosphere microbiome of grapevines (as other plants) is impacted by hierarchically structured relationships between geographic location, plant genotype and edaphic factors including land-use history (Burns *et al.*, 2016; Griggs *et al.*, 2021; Zarraonaindia *et al.*, 2015). Plant growth-promoting rhizobacteria (PGPR) include a wide variety of rhizospheric and endophytic bacteria which not only benefit from the nutrients secreted by the plant root but also beneficially influence the plant directly or indirectly, resulting in a stimulation of its growth (Bloemberg and Lugtenberg, 2001; Compant *et al.*, 2005). Rhizobacteria isolated from grapevines have been demonstrated to possess different beneficial effects. These effects have been attributed to nutrient acquisition (Baldan *et al.*, 2015), secondary metabolites (Ait Barka *et al.*, 2006; Rolli *et al.*, 2015) and the ability to induce and/or increase the plant tolerance against different abiotic and biotic stresses (Calvo-Garrido *et al.*, 2018). An investigation undertaken in Argentina by Salomon *et al.* (2016) demonstrated that plant bacterisation with rhizobacteria isolated from grapevine cv. Malbec shows a delayed water loss by inducing abscisic acid (ABA) synthesis and triggers the accumulation of terpenes that protect cells against reactive oxygen species. In addition, strains with multiple *in vitro* plant growth-promoting (PGP) activities, such as indole-3-acetic acid (IAA) production, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, exopolysaccharide production, phosphate solubilisation, siderophore

production, potential nitrogen fixation, protease and ammonia production, were able to improve grapevine growth in the field, even if they originate from different grapevine cultivars (Rolli *et al.*, 2016; Sabir *et al.*, 2012). In concordance with this, a stimulating effect of the nitrogen-fixing strain *Azospirillum brasilense* Sp245 on nursery propagation of rootstocks and during the vegetative development of young grapevine plants was observed in the field (Bartolini *et al.*, 2017).

Biological control including the use of PGPR, or their natural compounds has received remarkable attention as a promising method that can contribute to overcoming the ecological issue associated with pesticides overuse in agriculture. They reinforce plant resistance against pathogens and consequently reduce the significant loss of plant productivity through diverse mechanisms. The mechanisms include root colonisation and competition with other microbes present in the environment (Santoyo *et al.*, 2021), the production of antimicrobial metabolites (Cawoy *et al.*, 2014) and the induction of plant-mediated resistance responses (Aziz *et al.*, 2015; Calvo-Garrido *et al.*, 2018). Among the main grapevine diseases, grey mould and bunch rot have been reported to affect grapevine cultivation in Argentina and some regions around the world. *Botrytis cinerea* is responsible for the first disease and is also one of the most important causal agents of the second one (together with *Alternaria alternata*, *Aspergillus* spp. and *Penicillium* spp.) (Fillinger and Elad, 2015; Muñoz and Moret, 2010). These pathogens cause severe losses in the quality and volume of grapes harvested for wine production or fruit market. Different strains belonging to *Pseudomonas*, *Pantoea*, *Bacillus* and *Burkholderia* genera were used in biocontrol studies against necrotrophic fungus *Botrytis cinerea* (Aziz *et al.*, 2015; Calvo-Garrido *et al.*, 2018).

Today, the wine industry faces challenges that include good grapevine productivity and sustainability through the application of processes that help to obtain quality products, safe for the consumer and workers in the sector. On a global scale, the effects of continuous non-sustainable agricultural practices, such as chemical fertilisation, can cause serious damage to the environment (Mateo-Sagasta *et al.*, 2018). Microbial inoculations are a major and preferred sustainable practice in agriculture because that approach encompasses an alternative use to toxic chemicals, where living microorganisms establish associations with plants and promote their growth

using multiple beneficial factors. The combination of different sustainable agricultural methods for the production of crops has involved researching and applying new technologies, which study the adequate use of bacterial isolates and identifying their PGP traits, relying on their efficiency to survive and thrive under laboratory conditions as well as in field cultivation experiments (Souza *et al.*, 2015). The favourable response to soil or plant inoculation with PGPR varies considerably depending on the bacteria employed, the plant and soil type and the environmental conditions (Dries *et al.*, 2021; Fedele *et al.*, 2020; Santoyo *et al.*, 2021). These aspects imply that the biogeographical origin and environmental conditions constitute relevant considerations in the search for new bacterial isolates. Therefore, this study aimed to obtain a collection of cultured rhizobacteria isolated from the epiphytic microbial communities of Malbec and Cabernet-Sauvignon cultivars, located in the four main wine-producing provinces of Argentina and to characterise their PGP potential *in vitro*. Some of these isolates are proposed as potential PGPR for reaching a more sustainable development of pesticide-reduced viticulture.

MATERIALS AND METHODS

1. Sampling of grapevine roots and rhizospheric soil

Grapevine roots samples were collected from eleven vineyards located along four wine-producing provinces of Argentina during 2016,

one week prior to the harvest period (Table 1). Information of each site (vineyard), age of grapevine plants, type of cultivation (organic, conventional), inter-row-management (bare ground or presence of other plants) are described in Table S1. Rhizosphere soil and roots samples were taken at 30 cm depth and 20–30 cm distance from the vine trunk into the vineyard rows, following the scheme implemented by Oyuela Aguilar *et al.* (2020). The study included nine vines sampled per plot, covering a 49 m² area, placed at least 7 m away from the plot edge. Samples were later stored in sterile bags at 4 °C until processed. The nine samples were pooled in one for bacterial isolation.

2. Determination of soil physicochemical characteristics

Rhizospheric soil samples were sent to the Centro Nacional de Investigaciones Agropecuarias (CNIA-INTA) (Buenos Aires, Argentina) for physicochemical analysis. The soil treatment and analysis were the same as mentioned in Oyuela Aguilar *et al.* (2020), where the evaluated soil chemical composition included the water pH, organic material, carbon and nitrogen, C/N relationship, absorbable phosphorus content, while the physical components included lime, clay and sand content. Soil physico-chemical analysis is described in Table S2. The Principal Component Analysis (PCA) of soil physicochemical characteristics as well as Redundancy Analysis (RDA) were analysed with PAST v.3 (Hammer *et al.*, 2001).

TABLE 1. Sampling sites of rhizosphere-associated bacterial isolates.

Province	Site	Varietal	Location	masl*	Name	No. of isolates
Río Negro	Mainqué	Malbec	39° 02.190' S 67° 19.757' W	600	NMr	16
Mendoza	Agrelo	Malbec	33° 09.437' S 68° 53.700' W	930	Tms	19
Mendoza	Agrelo	Cabernet-Sauvignon	33° 09.416' S 68° 53.657' W	920	Tct	10
San Juan	Finca Norte	Malbec	31° 27.114' S 68° 42.523' W	780	Nmp	7
San Juan	Finca Norte	Cabernet-Sauvignon	31° 27.002' S 68° 42.109' W	770	Ncqc	19
San Juan	Finca Arriba	Malbec	31° 28.407' S 68° 45.486' W	800	Fam	14
San Juan	Finca Arriba	Cabernet-Sauvignon	31° 28.407' S 68° 45.347' W	800	FACn	22
Salta	Cafayate	Malbec	26° 04.846' S 66° 00.022' W	1700	Fau	21
Salta	Cafayate	Cabernet-Sauvignon	26° 04.815' S 66° 00.206' W	1700	AMCv	13
Salta	Molinos	Malbec	25° 30.495' S 66° 23.360' W	2200	Cmw	16
Salta	Finca Arenal	Malbec	25° 02.669' S 66° 04.460' W	2600	Aex	13

*masl: meters above sea level.

3. Isolation of epiphytic bacteria from the grapevine rhizosphere

For the isolation of epiphytic rhizobacteria, the grapevine roots were suspended in 50 ml of sterile saline solution (NaCl 0.9 % [w/v]) and mixed 3 times for 60 seconds in a vortex to recover firmly adhered soil. Then, the solution was diluted in serials of 1:10, 1:20 and 1:50 before plating on Luria-Bertani (LB) agar (Miller, 1971) containing cycloheximide (100 µg/ml) to prevent fungal growth. Plates were incubated at 28 °C for 72 hours.

From plates containing isolated colonies, 15 to 20 clearly different in some phenotypic characteristics (such as morphology, colour, mobility and growth rate), were chosen. They were then subjected to subsequent purification steps to ensure that pure colonies were obtained. Isolates were stored in LB broth plus 50 % (v/v) glycerol at –20 °C until further analyses. Initially, the bacterial collection was represented by approximately 20 isolates from each sampled site. However, only 170 isolates could be later recovered for their characterisation since not all survived in subcultures or after glycerol storage.

4. Bacterial identification by MALDI-TOF mass spectrometry

The identification of cultivable bacteria was carried out by whole-cell matrix-assisted–laser–desorption–ionisation–time-of-flight mass spectrometry (UV-MALDI-TOF MS) with an Ultraflex III UV-MALDI-TOF/TOF mass spectrometer through the use of the MALDI Biotyper 3.1 software (Bruker Daltonics, Bremen, Germany) (Maier *et al.*, 2006), as described in López *et al.* (2018). Previously to this study, the commercial database was expanded with the corresponding MS spectra of different bacterial isolates from seeds, plants and nodules which were incorporated into an in-house library (López *et al.*, 2018; Toniutti *et al.*, 2017).

Analysis by MALDI-TOF MS was performed in the Center of Chemical and Biological Studies of the Buenos Aires University (Centro de Estudios Químicos y Biológicos por Espectrometría de Masa (CEQUIBIEM), Mass Spectrometry Facility, FCEN-UBA, IQUBICEN-CONICET). MALDI-TOF MS identifications were classified using the following score values: ≥ 2 species identification, between 1.7 and 1.9 genus identification and < 1.7 no identification.

5. *In vitro* plant-growth-promoting (PGP) assays

Bacterial isolates were screened by an agar-plate assay for phosphate solubilisation, siderophore production and synthesis of lytic enzymes through cellulase, protease, amylase and pectinase activities. Assays were repeated three times for each isolate. The ability to solubilise mineral phosphate was tested on the solid phosphate-growth medium of the National Botanical Research Institute (NBRIP) (Nautiyal, 1999). Siderophore production was assayed using chromium azurol S (CAS) agar medium (Schwyn and Neilands, 1987) by the bilayer method (Pérez-Miranda *et al.*, 2007), in which the formation of orange halos around the bacterial colonies is indicative of a positive result. Enzymatic activity was evaluated in specific media previously described for amylase López *et al.* (2018), protease Brown and Foster (1970), cellulase Hankin and Anagnostakis (1977) and pectinase Soares *et al.* (1999) activities.

6. Antagonistic activity against phytopathogenic fungi

Bacterial isolates identified as *Bacillus* spp. were tested against the phytopathogenic fungi *Botrytis cinerea*, *Alternaria alternata*, *Fusarium graminearum*, *Fusarium oxysporum*, *Penicillium expansum* and *Penicillium oxalicum* by a dual-culture assay on potato-dextrose-agar medium at 23 °C, according to a modified protocol based on Whipps (1987) and described by Príncipe *et al.* (2007). Briefly, agar disks containing the fungal mycelium were applied on the Petri dish 3 cm apart from each bacterial culture spot. A negative control consisting of fungal agar disks in the absence of bacterial culture spots was also conducted. The Petri dishes were incubated at 23 °C for 4 days. Then, the radius of mycelial growth was measured in cm. Three replicates were performed.

RESULTS AND DISCUSSION

1. Taxonomic identification of rhizobacteria isolates

A final collection of 170 grapevine rhizosphere-associated bacteria were obtained from Malbec and Cabernet-Sauvignon cultivars located in eleven vineyards of the four-leading wine provinces of Argentina. The largest percentage of bacterial isolates was associated with Malbec cultivar (88 %), which was present at all sampled sites. In accordance with the number of sampled sites, most of the isolates were recovered from vineyards located in Salta and San Juan provinces

(37 %, respectively), and smaller percentages from Mendoza (17 %) and Río Negro (9 %) (Table 1).

By MALDI-TOF mass spectrometry, it was possible to identify 151 isolates that were grouped into 11 genera belonging to Proteobacteria (α and γ), Actinobacteria and Firmicutes phyla. A summary of the identified genera and their geographical origin is shown in Figure 1. The additional data, shown in Table S1, indicates the genus of each isolate, their associated vineyard and the grapevine cultivar of origin. Only 5 genera clustered 95 % of the identified isolates; they were *Pseudomonas* (39 %), *Serratia* (22 %), *Pantoea* (15 %), *Arthrobacter* (13 %) and *Bacillus* (11 %). *Pseudomonas* spp. were found in the four provinces, predominantly in Salta and San Juan. Members of the *Serratia* genus were predominant in Mendoza and San Juan. Rhizobacteria belonging to the *Pantoea* genus were collected at Río Negro, San Juan and Mendoza but not in Salta. With respect to Gram-positive isolates, which were less frequent than Gram-negative isolates, these represented 53 %, 27 %, 18 % and 10 % of the total bacteria obtained from Río Negro (*Arthrobacter* and *Microbacterium*), Salta (*Bacillus* and *Arthrobacter*), San Juan

(*Bacillus* and *Arthrobacter*) and Mendoza (only *Bacillus*), respectively.

The *Pseudomonas*, *Serratia*, *Arthrobacter* and *Bacillus* genera were found associated with the rhizosphere of both cultivars, while the *Pantoea* genus was only isolated from Malbec grapevines (Figure 2). The remaining genera (5 %) included a few isolates belonging to *Rhizobium* genera (2 %), recovered from both grapevine cultivars and unique isolates of different genera. Among these, *Microbacterium* and *Aeromonas* genera were associated with Malbec grapevines while *Paenibacillus*, *Acinetobacter* and *Stenotrophomonas* genera were isolated from Cabernet-Sauvignon cultivar (Figure 2).

MALDI-TOF biotyping enables species assignment; the assurance of such assignment depends on the number of spectra stored in the database. To increase the identification power of the MALDI-TOF MS on genus and species level in case of environmental bacterial strains, we used an in-house library. It is noteworthy that most of the isolates (58 %) were classified to the species level as high-confidence identification (score value > 2) from which some species were identified with a very high score (> 2.3),

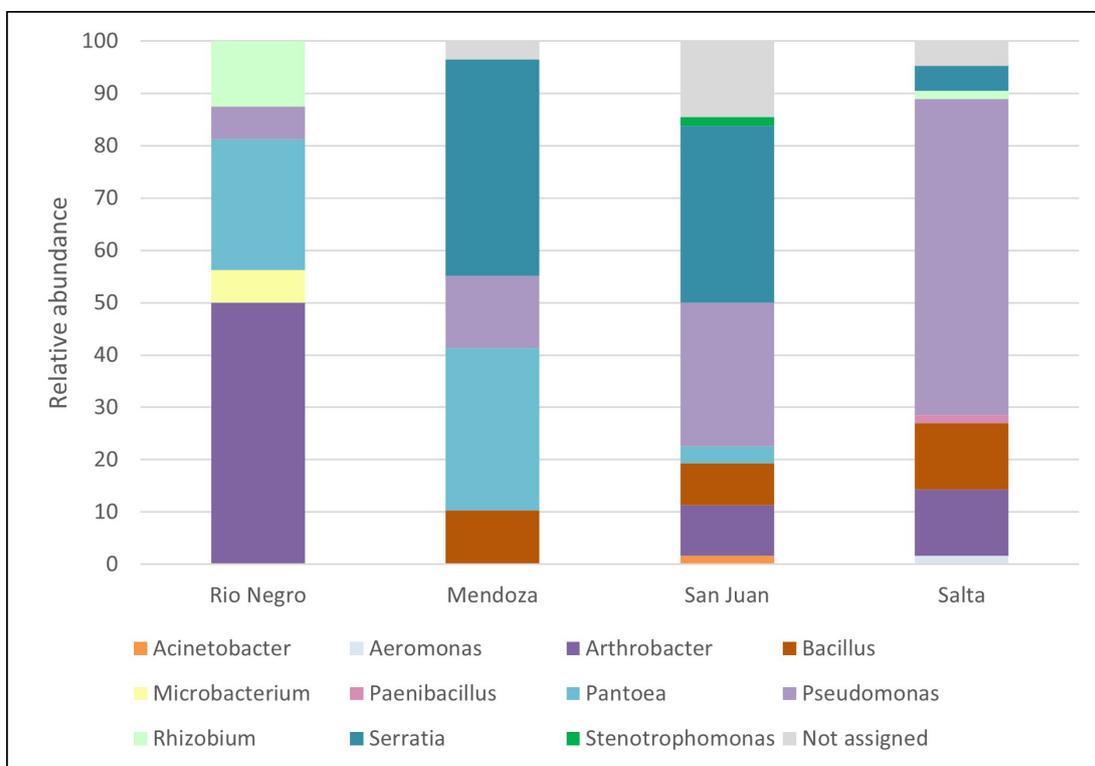


FIGURE 1. Distribution of the bacterial genera identified by MALDI-TOF mass spectrometry according to the province of origin.

The total number of isolates analysed for each province was: Río Negro, 16; Mendoza 29; San Juan, 61; Salta 63.

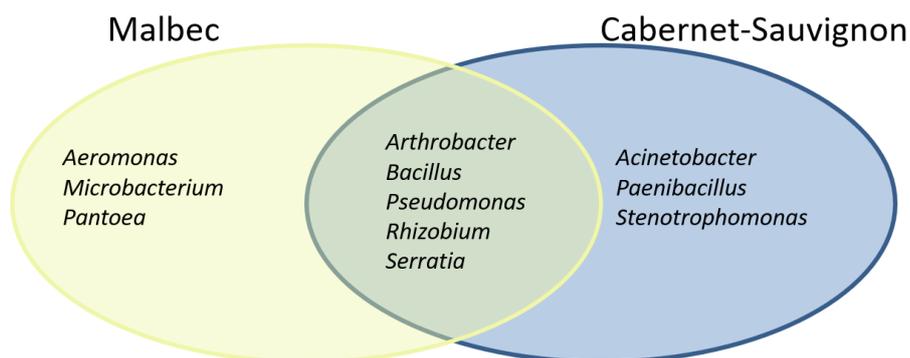


FIGURE 2. Venn diagram representing the single and shared bacterial genera identified from each grapevine cultivar.

The genera isolated from the Malbec cultivar samples are represented in yellow, while those from the Cabernet-Sauvignon samples are indicated in blue, the light-green intersection represents the genera common to both cultivars. The total number of Malbec isolates were 106 and Cabernet-Sauvignon were 64.

such as *Pseudomonas jessenii*, *Pseudomonas chlororaphis* subsp. *chlororaphis*, *Pseudomonas chlororaphis* subsp. *aurantiaca*, *Pseudomonas brassicacearum*, *Pseudomonas agglomerans*, *Serratia ficaria*, *Serratia marcescens*, *Rhizobium radiobacter* and *Bacillus megaterium*, which has been recently transferred into the genera *Priestia* (Gupta *et al.*, 2020). In addition, some isolates belong to *Bacillus subtilis*, *Bacillus cereus* and *B. simplex*/*B. muralis* groups (Table S1). Many of these species have been characterised as PGPR in different crops (Alvarez *et al.*, 2012; Batista *et al.*, 2021; Pusey *et al.*, 2011; Robles Montoya *et al.*, 2019; Singh and Jha, 2016). As not all bacteria could be assigned to species level the comparisons were limited to genus level.

A principal component (PCA) analysis of the soil physicochemical characteristics showed that samples were distributed according to their origin (Figure 3). The first two principal components accounted for 93.9 % of the total variance, 59.3 % and 34.6 %, respectively. The soils samples from Salta were mainly influenced by the sand proportion and the low organic carbon and organic nitrogen. On the other hand, the soils from Mendoza, San Juan and Rio Negro provinces presented more clay and lime content. These last three provinces could be divided into two groups according to the content of organic carbon and organic nitrogen: one group includes samples from San Juan and a second one samples of Rio Negro and Mendoza.

To better explore the microbial differences among sites, a redundancy analysis (RDA) was performed to assess the effects of soil physicochemical properties variation of genera diversity (Figure 4) with isolates identified in more than one site.

It could be observed that the amount of *Pseudomonas* in the sites is correlated to the proportion of sand in the soils. On the contrary, members of *Pantoea* could be isolated in soils with lower sand content, while *Arthrobacter* in soils with an abundance of assimilable phosphorus. Several DNA studies supported that the grape microbiome is related to vineyard location, climatic conditions and other vineyard-related factors (Mezzasalma *et al.*, 2018; Oyuela Aguilar *et al.*, 2020; Zarraonaindia *et al.*, 2015).

2. Analysis of phenotypic traits related to PGP activities

The collection of 170 epiphytic rhizobacteria isolates was screened by *in vitro* assays for six biochemical activities related to PGP and/or biological control. The traits assayed were the enzymatic activities amylase, protease, cellulase and pectinase along with siderophore production and ability to solubilise phosphate (Table S3). Certain bacterial isolates exhibited several positive traits, suggesting the use of those strains in future plant assays to analyse the corresponding effects. The results, summarised in Table 2, indicated that the most prevalent traits were protease activity (59 %), siderophore production (56 %) and phosphate solubilisation (47 %).

The most remarkable ability to solubilise inorganic phosphate was detected in some isolates belonging to *Pantoea*, *Pseudomonas*, *Microbacterium* and *Serratia* genera (Table 2, Table S3). Second to nitrogen, phosphorus is the most required inorganic nutrient by plants and microorganisms (Gyaneshwar *et al.*, 2002). Therefore, the ability to solubilise phosphate constitutes a fundamental characteristic in

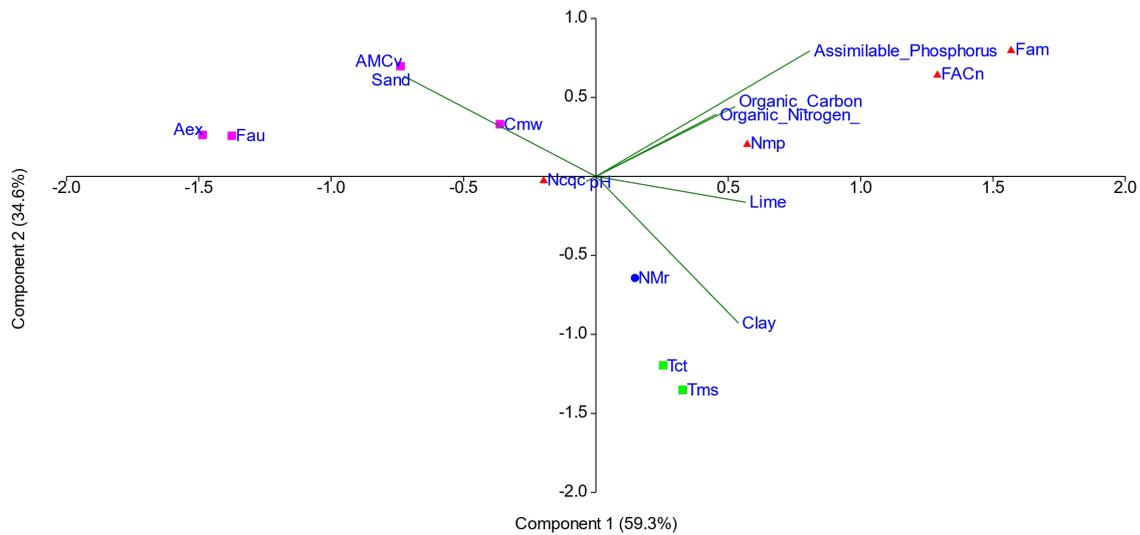


FIGURE 3. PCA of soil physicochemical characteristics of sampled vineyards.

Samples from Rio Negro (blue circles), San Juan (red triangles), Mendoza (green squares) and Salta (purple squares) are shown in two first dimensions PCA.

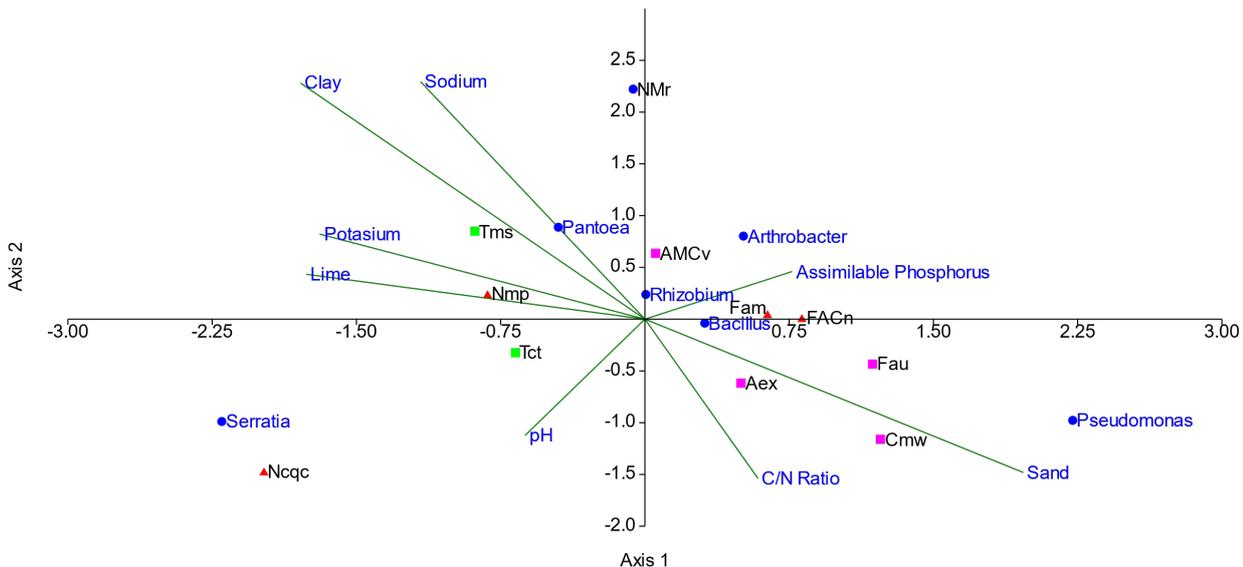


FIGURE 4. Redundancy Analysis (RDA) of bacterial isolates.

The two first dimensions are shown, axis 1 represented 61.75 % and axis 2 20.43 %. Samples from Rio Negro (blue circles), San Juan (red triangles), Mendoza (green squares) and Salta (purple squares) are shown. ($R^2:0.9369$, $R^2 \text{ adj}:0.6846$, $F:3.714$, $\text{Perm p. (n = 999):}0.023$).

rhizosphere-dwelling microorganisms. Phosphate solubilising bacteria secrete organic acids which chelate divalent cations (such as Ca^{+2}) from complex mineral phosphorous (P) and release free P that can be taken up by the plant (Goldstein, 1995). Many Gram-negative bacteria employ periplasmic glucose oxidation through pyrroloquinoline quinone (PQQ)-dependent glucose dehydrogenase enzyme to produce gluconate, resulting in the acidification of the microbial cells and their surroundings. This mechanism was well-documented in *Pantoea* (Son *et al.*, 2006;

Tahir *et al.*, 2020), *Pseudomonas* (Suleman *et al.*, 2018) and *Serratia* (Ludueña *et al.*, 2017) strains.

Siderophore production by microorganisms has received great attention due to their application in different branches of the agriculture sector such as soil science, plant pathology and environmental sciences (Albelda-Berenguer *et al.*, 2019; Johnstone and Nolan, 2015). Siderophores are secondary metabolites with a high specific affinity to chelate iron. Iron is a scarce nutrient in neutral or basic soils due to the low solubility of the Fe^{+3} ion, which is substantially necessary

for plant health. Furthermore, iron chelation by beneficial bacteria can prevent the colonisation of the rhizosphere by pathogenic microorganisms (Ghazy and El-Nahrawy, 2021; Siddiqui, 2006). Chrome azurol S (CAS)-based assays were widely used for the screening of siderophore-producing bacteria, even those chemically different (catecholates, hydroxamates, carboxylates and mixed types). We found that 71 % of the isolates belonging to the *Pseudomonas* genus were substantially active in siderophores production (Table 2, Table S3). As in our results, the well-suited for iron scavenging pseudomonads were the main siderophore-producing rhizobacteria from Concord grape vineyards, suggesting a potential role in iron turnover in vineyard systems (Lewis *et al.*, 2019). Other important siderophore-producing genera were *Pantoea* (67 %), *Bacillus* (50 %) and, particularly, *Serratia* genus (45 %) (Table 2, Table S3). In these genera, more than one type of siderophore have been described: pyochelin (Youard *et al.*, 2011), enterobactin (Soutar and Stavrinides, 2018), bacillibactin (Dertz *et al.*, 2006) and serratiochelin (Khilyas *et al.*, 2019), which are most commonly produced by *Pseudomonas*, *Pantoea*, *Bacillus* and *Serratia*, respectively. In addition, a significant proportion of isolates belonging to *Arthrobacter* genera (63 %), especially those from Rio Negro and

Cafayate (Salta) locations, showed siderophore production. Although siderophores produced by *Arthrobacter* spp., are not well-characterised, some interesting strains have shown resistance to toxic heavy metals and stressful conditions (Binnewies *et al.*, 2006) in which siderophores could be involved (Johnstone and Nolan, 2015).

Regarding synthesis of lytic enzymes, bacterial isolates belonging to *Arthrobacter* and *Bacillus* genera exhibited positive results for protease, amylase and/or pectinase activity, while members of *Pseudomonas* and *Serratia* genera displayed mainly protease activity. In a general way, *Pantoea* spp. isolates exhibited a very weak hydrolase activity, except *P. agglomerans* NMr9 that showed protease and pectinase activity (Table S3). The production of hydrolytic enzymes contributes to the recycling of organic matter in the soil and, therefore, to soil biodiversity. The bulk of plant biomass comprises cell wall material, which is deconstructed into glucose by multiple synergistic enzyme activities such as cellulases and pectinases. In addition, several lytic enzymes are involved in biocontrol mechanisms (Compant *et al.*, 2019). It is worth mentioning that *Arthrobacter* sp. FACn18, *Paenibacillus* AMCV14, *Serratia* sp. Ncq3b, *Bacillus* sp. Fau8 and *Pseudomonas jessenii* Fau9 were grouped among isolates with a better hydrolytic profile.

TABLE 2. *In vitro* assays to detect functional traits of grapevine rhizosphere bacterial isolates, grouped by genus.

Genus	Isolates	Phosphate solubilisation	Siderophore production	Protease activity	Amylase activity	Cellulase activity	Pectinase activity
Acinetobacter	1	0	0	1	0	0	0
Aeromonas	1	0	0	0	0	0	0
Arthrobacter	19	1	12	10	16	1	10
Bacillus	16	4	8	10	11	0	12
Microbacterium	1	1	1	0	0	0	0
Paenibacillus	1	0	0	1	1	0	1
Pantoea	15	13	10	1	1	0	1
Pseudomonas	59	32	42	39	2	4	11
Rhizobium	3	0	3	0	0	0	0
Serratia	33	22	15	30	0	10	8
Stenotrophomonas	1	1	0	1	0	0	0
Unassigned	19	6	4	7	8	0	13
Total	169	80	95	100	39	15	56
Average (percentage)		47.3	56.2	59.2	23.1	8.9	33.1

3. Growth-inhibition for phytopathogenic fungi control

Plant fungal pathogens cause a large number of soil-borne diseases, many of which lead to serious annual agricultural loss (Agrios, 2009; Koike *et al.*, 2003). Therefore, to avoid environmental deterioration, biological control is being considered as an alternative or a complementary way to reduce the use of synthetic chemicals for disease control (Beneduzi *et al.*, 2012).

Certain *Bacillus* species are suitable candidates for use as biocontrol agents against fungal phytopathogens (Calvo-Garrido *et al.*, 2019; Chen *et al.*, 2019). Among the main attributes of members from this genus is the production of several antimicrobial compounds, which include cyclic lipopeptides (Medeot *et al.*, 2019). In addition, their ability to sporulate is advantageous for time-stable inoculant production.

Based on the above-mentioned, the ability to inhibit the growth of soil-borne fungal phytopathogens was tested in *Bacillus* isolates present in the rhizobacteria collection. Selected necrotrophic fungi include *Botrytis cinerea*, the causal agent of grey mould and botrytis bunch rot, two important vineyard diseases of great concern to the wine industry. Moreover, antagonistic activity toward *A. alternata*, the causal agent of grape bunch rot (Meena and Samal, 2019), *Fusarium* spp. and *Penicillium* spp. was also tested to know the inhibitory capability of *Bacillus* isolates.

According to our results, five isolates belonging to *B. subtilis* and *B. cereus* groups were able to significantly antagonise *Botrytis cinerea*. In addition, isolates belonging to the *B. cereus* group strongly inhibited *Alternaria alternata* (Figure 5, Table S4). Interestingly, isolates Fau18 and Fam11b were able to inhibit at least one species

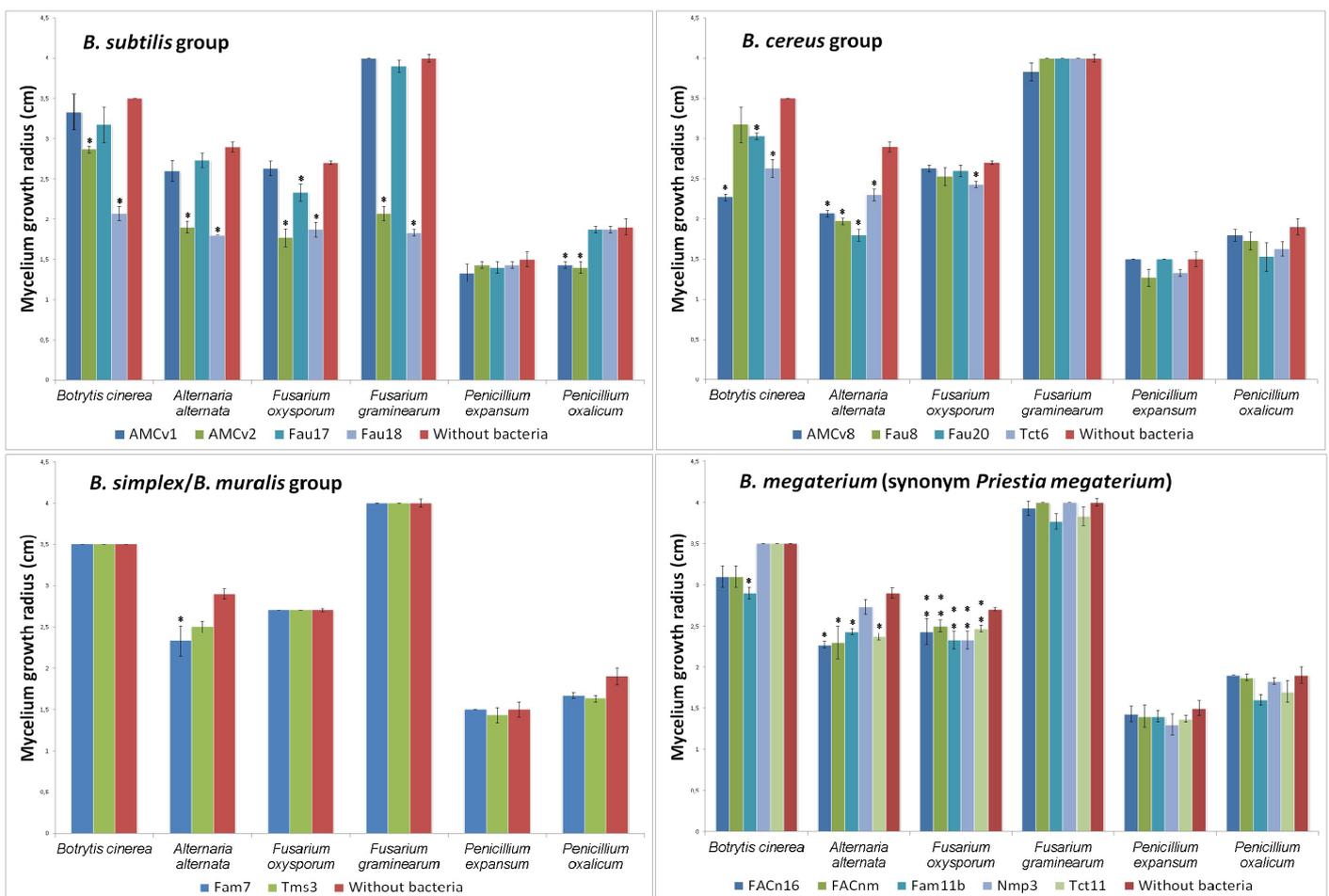


FIGURE 5. Inhibition of phytopathogenic fungi by bacterial isolates belonging to the genus *Bacillus*.

The values of inhibition of mycelium growth by antagonistic activity were calculated by measuring the fungal colony radius (cm) in PDA plates without any bacteria in comparison with dual culture bacteria-fungi antagonistic assays. Data are the means of three replicates with SEM. Star (*) indicates a statistically significant difference (P < 0.001), double stars (**) indicates a statistically significant difference with P < 0.05 after one way ANOVA and multiple comparisons versus Control Group (without bacteria) by Holm-Sidak method.

of *Fusarium* or *Penicillium*, respectively, while ACMv2 showed the broadest spectrum antifungal activity. Complementary studies will be carried out to characterise bioactive metabolites produced by these isolates.

CONCLUSIONS

In this study, we obtained, identified and screened a collection of rhizosphere-associated bacteria from vineyards located in four provinces of Argentina, to investigate the potential of those isolates as biofertilisers and/or biological control agents. The most identified genera were *Pseudomonas*, *Serratia*, *Arthrobacter*, *Bacillus* and *Pantoea*, which were differentially found according to the type of soil. Most rhizospheric isolates exhibited *in vitro* one or more PGP properties such as phosphate solubilisation, siderophore production, hydrolytic activity and fungal antagonism. Based on these results, certain isolates representatives of each site will be selected and evaluated in terms of their potential PGPR effects on grapevine plants. A few isolates showed highly positive results for all tests; therefore, combined isolates could be considered to attain a synergistic effect. The obtained results expose those potentially beneficial traits, considered relevant to develop biological inoculants, that constitute a safer alternative to synthetic chemical fertilisers and pesticides in vineyards.

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