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Scientific Article

Prospecting plant growth-promoting rhizobacteria in grapevines in the São Francisco Valley

Prospecção de rizobactérias promotoras de crescimento de plantas em videiras no Vale do São Francisco

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ABSTRACT - Viticulture is the main agricultural production in the São Francisco Valley, Brazil; however, farm soil management systems require large volumes of fertilizer that could contribute to climate change. Therefore, using plant growth-promoting rhizobacteria (PGPR) has been reported to reduce or replace plant input. This study aimed to evaluate the bacteria in the rhizosphere of *Vitis vinifera* cultivated in the São Francisco Valley in Pernambuco, Brazil, and to characterize the mechanisms that promote plant growth. The PGPR with the greatest biotechnological potential was identified using sequencing. The bacteria were isolated from tryptic soy agar (TSA) culture medium inoculated with 100 µL of a serial dilution. The isolates obtained were characterized phenotypically and tested for their ability to solubilize phosphate, promote biological nitrogen fixation (BNF), and produce indole-3 acetic acid (IAA), biofilms, and antibiotic factors against *Xanthomonas campestris* pv. *viticola*. A total of 423 bacteria were obtained, of which 99 presented positive results for at least one of the growth-promotion mechanisms, representing 6.85% for phosphate solubilizers, 0.74% for BNF, 5.7% for IAA synthesizers, 11.27% for biofilm producers, and 4.01% for promoting antibiosis against *X. campestris* pv. *viticola*. Isolates 3.19 and 31.14; 3.17 and 17.04; 5.35 and 5.42; and 5.37 identified as *Stenotrophomonas*, *Bacillus*, *Pseudomonas,* and *Clostridium*, respectively, presented a biotechnological potential for future experiments to promote vine growth.

Keywords: Viticulture. Semiarid. Auxin. Phosphate solubilization. Antibiosis.

RESUMO - A viticultura é a principal produção agrícola do Vale do São Francisco, Brasil; no entanto, os sistemas agrícolas de manejo dos solos requerem grandes volumes de fertilizantes que podem contribuir para as alterações climáticas. Portanto, foi relatado que o uso de rizobactérias promotoras de crescimento de plantas (RPCP) reduz ou substitui insumos vegetais. Este estudo teve como objetivo avaliar as bactérias da rizosfera de *Vitis vinifera* cultivada no Vale do São Francisco, em Pernambuco, Brasil, e caracterizar os mecanismos que promovem o crescimento das plantas. As RPCP com maior potencial biotecnológico foram identificadas por meio de sequenciamento. As bactérias foram isoladas em meio de cultura Agar Triptona de Soja (TSA) inoculado com 100 µL de uma diluição seriada. Os isolados obtidos foram caracterizados fenotipicamente e testados quanto à capacidade de solubilizar fosfato, promover fixação biológica de nitrogênio (FBN) e produzir ácido indol-3 acético (AIA), biofilmes e fatores antibióticos contra *Xanthomonas campestris* pv. *vitícola*. Um total de 423 bactérias foi obtido, das quais 99 apresentaram resultados positivos para pelo menos um dos mecanismos de promoção de crescimento, representando 6,85% para solubilizadores de fosfato, 0,74% para FBN, 5,7% para sintetizadores de AIA, 11,27% para produtores de biofilme e 4,01% para promoção de antibiose contra *X. campestris* pv. *vitícola*. Os isolados 3.19 e 31.14; 3.17 e 17.04; 5.35 e 5.42; e 5.37 identificados como *Stenotrophomonas*, *Bacillus*, *Pseudomonas* e *Clostridium*, respectivamente, apresentaram potencial biotecnológico para futuros experimentos para promover o crescimento de videiras.

Palavras-chave: Viticultura. Semiárido. Auxinas. Solubilização de fosfato. Antibiose.

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INTRODUCTION

Viticulture exports high-quality table grapes from the semiarid São Francisco Valley of Brazil. (ARATA; HAUSCHILD; SCKOKAI, 2017). The climatic conditions of this region allow 2.5 crop cycles per year, ensuring a grape production of roughly 1,500 tons, which is equivalent to more than 30% of the national grape production (IBGE, 2019). Agricultural management on some farms is based on a non-conservationist system, which results in the possibility of carbon loss and greenhouse gas emissions owing to fertilizers applied to the soil (MAIA et al., 2022). In addition, an irrigation system necessary for sandy soils in this region can threaten water bodies through leaching and eutrophication caused by the accumulation of phosphate (PRESTON et al., 2017).

Using plant growth-promoting rhizobacteria (PGPR) is a sustainable alternative to ensure high agricultural production in tropical semiarid regions. This microbial cultivation method is an efficient strategy for isolating bacteria that promote vine development by producing phytohormones, enzymes, antibiotics, and solubilizing nutrients. It has shown promising results in various land use management practices worldwide. (MATES; PONTES; HALFELD-VIEIRA, 2019; SHARMA; SINDHU; SINDHU, 2018; TEWARI; ARORA, 2014). However, the diversity of these organisms can be influenced by differences in

plant variety, management farming systems, and soil properties, which need to be carefully evaluated. Nevertheless, it is necessary to investigate this important soil microbiome to generate biofertilizers and reduce agricultural costs for farmers (MANICI et al., 2017).

Despite the national economic importance of viticulture, there is a lack of studies on developing inoculants based on microorganisms isolated from the soil and vine roots. This study hypothesized that the rhizosphere of grapevines cultivated in the São Francisco Valley harbors a high diversity of bacteria with plant growth-promoting mechanisms. Therefore, this study aimed to investigate PGPR from the rhizosphere of *Vitis vinifera* and characterize their growth promotion mechanisms, such as biological nitrogen fixation (BNF), phosphate solubilization, indole-acetic acid (IAA) production, biofilm formation, and antibiosis against *Xanthomonas campestris* pv. *viticola*. The PGPR with the greatest biotechnological potential was identified using sequencing.

MATERIAL AND METHODS

Site description and sampling strategy

Grape production sets were obtained from the Senador Nilo Coelho Irrigated Perimeter in the São Francisco Valley, Petrolina, Pernambuco, Brazil. The climate is semiarid and is classified as BSwh' by Koppen, with an average rainfall of 578 mm year^{-1} between November and April. The average annual temperature is 26.5 ºC and the relative humidity is 61%. Soils from six areas were classified as Typic Quartzipsamment, with textures ranging from loam to sandy loam in the surface layer (0–20 cm depth). More information regarding the site description can be found in Fracetto et al. (2023).

Rhizospheric soil samples were collected from six grape-producing areas. In each area, three plants were selected from the initial, middle, and final parts of the plots. Three rhizospheric points were collected from each plant to form a composite biological sample, totaling 18 samples (Table 1). Descriptions of the chemical and physical characteristics of the soils are listed in Table 2.

Table 1. Geographical coordinates of the grape production areas, variety of grape, and years of cultivation in São Francisco Valley, Pernambuco, Brazil.

Grape Production Areas	Geographical coordinates	Cultivated grape variety	Years of cultivation
1	9°18'52.25"S; 40°26'38.58"O	Arra-15	11
2	9°19'22.58"S; 40°35'29.76"O	Itália Moscat	14
3	9°20'1.70"S; 40°32'0.93"O	Vitória	13
4	9°22'42.14"S; 40°37'21.61"O	Crimson	16
5	9°15'5.07"S; 40°25'4.31"O	Iris	15
6	9°26'56.19"S; 40°36'59.25"O	Arra-15	14

Table 2. Physical and chemical characteristics of soils used for the prospection of plant growth-promoting rhizobacteria in six locations in the São Francisco Valley, Pernambuco, Brazil.

Physicochemical characteristics	Grape Production Areas					
	1	2	3	4	5	6
pH (1:2,5 W/V H_2O)	6.93	6.53	6.63	7.07	6.83	6.91
P (mg dm^{-3})	428.40	584.30	610.50	521.70	1026.80	792.50
K^+ (cmol$_c$ dm^{-3})	0.48	0.55	1.01	0.84	0.73	1.58
Na^+ (cmol$_c$ dm^{-3})	0.22	0.16	0.63	0.17	0.30	0.24
Ca^{2+} (cmol$_c$ dm^{-3})	7.70	6.20	5.64	4.64	7.39	8.29
Mg^{2+} (cmol$_c$ dm^{-3})	1.54	1.92	2.32	0.65	3.91	1.68
$H^+ + Al^{3+}$ (cmol$_c$ dm^{-3})	1.05	1.38	1.16	0.99	1.57	1.63
OC (g kg^{-1})	18.23	15.08	15.23	12.98	14.24	14.18
OM (g kg^{-1})	31.42	25.99	26.26	22.37	24.54	24.45
SB (cmol$_c$ dm^{-3})	9.94	8.81	9.61	6.30	12.32	11.78
CEC (cmol$_c$ dm^{-3})	10.98	10.18	10.76	7.29	13.89	13.52
V (%)	90.54	85.71	89.02	86.42	88.89	87.22
PES (%)	2.05	1.68	5.98	2.43	2.17	1.77
Sand (g kg^{-1})	872.00	808.00	828.00	880.00	863.00	785.00
Silt (g kg^{-1})	49.00	73.00	55.00	10.00	54.00	62.00
Clay (g kg^{-1})	79.00	119.00	117.00	110.00	83.00	153.00

OC: Organic Carbon; OM: Organic Matter; SB: Sum of bases; CEC: Cations exchange capacity; V: Base saturation; PES: Percentual of exchange sodium.

Bacterial isolates

Each 10 g soil sample was weighed and added to 90 mL of sterile 0.85% NaCl solution. The product was then agitated on a horizontal shaking table for 30 min at 150 rpm. Subsequently, serial 10-fold dilutions of each sample suspension were prepared by pipetting 1 mL of the suspension into 9 mL of a previously sterilized 0.85% NaCl solution; the final dilution was 10^{-5}. The 0.1 mL aliquot of each dilution was placed in TSA culture medium (15 g tryptone, 5 g soy extract, 5 g sodium chloride, and 15 g agar), with three replicates per dilution and homogenized on the surface (GOULD et al., 1985). The plates were incubated in a bacteriological growth incubator at 28 ºC for 72 hours and checked every 24 hours to monitor the growth time of the colonies. The dilution presenting between 20 and 200 colonies per plate was considered the most viable dilution for a population estimate.

Phenotypic characterization

Colonies were isolated from the plates used for CFU counting and diluted in the TSA culture medium. After the incubation period, the colonies were characterized for morpho -physiological descriptors of the culture: growth speed (24 h, fast; 48h, intermediate, between 48 and 72h, fastidious; and >72h, slow), size (<1 mm, between 2 and 1 mm, and >2 mm), shape (circular, irregular, rhizoid, filamentous, punctiform, and fusiform), appearance (homogeneous or heterogeneous), color (white, yellow, cream, and pink), transparency (transparent, translucent and opaque), surface (smooth or rough), colony elevation (flat, concave-convex, crested, papillated, and crateriform), mucus production (dry, sparse, moderate, and abundant), and mucus consistency (dry, flocculent, butyrous, and viscous). Gram group determination was performed using the KOH technique (BUCK, 1982).

Phosphate solubilization

The capacity to solubilize calcium and aluminum phosphates was evaluated by inoculation in GEL medium (10 g glucose, 2 g yeast extract, 5 mL bromothymol blue, and 15 g agar) enriched with $CaHPO_4$. The process of enrichment of the medium with $CaHPO_4$ was performed by reacting the 0.57 M K_2HPO_4 solution with 0.90 M $CaCl_2$ with the GEL medium supplemented with the sources of each precipitated phosphate, as proposed by Silva Filho and Vidor (2000). Subsequently, solubilization indices were calculated as the ratio of solubilization halos to the colony diameter. The solubilization capabilities of the isolates were compared as described by Hara and Oliveira (2004).

Asymbiotic N_2 fixation

The capacity for non-symbiotic BNF was verified using the Dobereiner method in a nitrogen-free culture medium (DÖBEREINER; BALDANI; BALDANI, 1995). Subsequently, isolates with preliminary positive results for BNF were authenticated by amplifying the *nifH* gene, which encodes the nitrogenase enzyme (POLY; MONROZIER; BALLY, 2001). Only isolates that tested positive in all BNF tests were characterized as diazotrophs.

IAA synthesis

The ability to synthesize IAA was verified using a colorimetric method in a medium enriched with L-tryptophan (5 mM). The presence of orange coloration confirmed IAA production. The isolates were tested in triplicate, and their production was estimated using a photocolorimeter at 530 nm (BRIC; BOSTOCK; SILVERSTONE, 1991).

Biofilm formation

Biofilm formation was analyzed using a colorimetric method. Liquid YM medium (0.5 g K_2HPO_4, 0.2 g $MgSO_4.7H_2O$, 0.1 g NaCl, 5 g Mannitol, and 0.4 g yeast extract) was inoculated with 5 µL of the cell suspension with an optical density of 0.1 (540 nm). The bacteria isolates were incubated for 168 hours (7 days) at 30 °C without shaking. Subsequently, the contents of the plates were discarded. The plates were washed three times with distilled water and dried for 20 minutes. After drying, 100 µL of gentian violet (0.25%) was added for five minutes. Subsequently, the plates were washed again three times, and then 200 µL of an alcohol-acetone solution (80:20) was added. Biofilm formation was marked as positive for isolates with purple-blue coloration (MERINO et al., 2009).

Direct antibiosis against *Xanthomonas campestris* pv. *viticola*

Isolates that positively responded to at least one of the previously tested mechanisms were inoculated onto TSA medium next to *X. campestris* pv. *viticola*. Subsequently, the plates were incubated at a controlled temperature of 30 °C for 72 hours. Then, the formation of a growth inhibition halo was verified, and the percent growth inhibition (GIP) was calculated using the Equation:

$$GIP\ (\%) = [(KR - R1)]/KR\ X\ 100$$

where KR is the colony diameter (mm) from the inoculation point on the control plate to the margin of the plate, and R1 is the diameter of the pathogen growth from the inoculation point to the margin of the colony on the plate in the direction of the antagonist (TABLI et al., 2018).

DNA extraction of the PGPR and sequencing of the 16S rRNA gene

Bacteria that tested positive *in vitro* for growth-promoting mechanisms were classified as PGPR. DNA of PGPR was extracted as described by Vingataramin and Frost (2015). Subsequently, 15 PGPR were selected according to their mechanisms and subjected to 16S rRNA sequencing. The bacterial 16S rRNA gene was amplified using universal oligonucleotide primers. 27F (5′-AGRGTTTGATYMTGGCTCAG-3′) and 1492R (5'-GGYTACCTTGTTACGACTT-3') (LANE, 1991). PCR products were sent to Macrogen Laboratory (Macrogen, Inc. South Korea) for sequencing.

The 16S rRNA sequences identified were compared with the NCBI database and the List of Prokaryotic names with Standing in Nomenclature (LPSA). The sequences were compared using local alignment with BLAST and multiple

alignments with ClustalW in MEGA11. The phylogenetic tree was based on the neighbor-joining method using the MEGA version 11 software package (KUMAR et al., 2008). Bootstrap analysis was performed using 500 bootstrap replicates.

Statistical analysis

The phenotypic description of the isolates was performed in the form of a binary matrix. Subsequently, the isolates were grouped into a dendrogram by Jaccard's method, and Shannon diversity indices (H') and equivalent species values (SH') were estimated for the sample plots.

The indices of phosphate solubilization, IAA production, and growth inhibition percentage were evaluated using a one-way ANOVA. The data were subjected to comparisons using Scott–Knott's test ($P < 0.05$). The mechanisms underlying biofilm formation and biological nitrogen fixation were described as nominal data.

RESULTS AND DISCUSSION

In total, 423 bacterial morphotypes were identified. The mean CFU was 8.00 x 10^4, with the maximum CFU among the samples, being 3.87 x 10^5 and the minimum 1.12 x 10^4. Area 1 had the lowest CFU count. The mean was 1.64 x 10^4, with a minimum of 1.24 x 10^4 and a maximum of 2.10 x 10^4. The highest CFU values were obtained in area two. The mean was 3.33 x 10^5, with a minimum of 2.34 x 10^5 and a maximum of 3.87 x 10^5. The Shannon index (H') revealed high diversity per area. The results ranged from 4.03 to 4.42. The equivalent species values (SH') were also calculated based on the H' value (Table 3). The SH value represents the expected number of individuals per species under maximum equity conditions.

Table 3. Shannon and HS' diversity index for rhizobacteria cultivated in TSA medium in *Vitis vinifera* cultivated in six locations in the São Francisco Valley, Pernambuco, Brazil.

Indexes	Grape Production Areas					
	1	2	3	4	5	6
H'	4.42	4.03	4.32	4.03	4.20	4.12
SH'	83.05	56.53	75.00	56.45	66.43	61.80

The clustering resulted in the formation of 353 phenotypically distinct clusters. Gram-positive bacteria predominated (92%) in the KOH test. Regarding the growth speed of the isolates, most were classified as fast and intermediate (28% and 59%, respectively), with only 13% being slow-growing. The size of the colonies varied but were mostly larger than 2 mm (45%). Regarding the shape of the colonies, 54% were irregularly shaped, 36% were circular, 8% were punctiform, and 1% were rhizoidal, filamentous, or fusiform. Only 23% of the isolates had heterogeneous colonies. Most isolates were opaque (56%) and cream-colored (60%). 75% had a smooth surface and some form of colony elevation; 50% were flat, 21% were lens-shaped, and 15% were convex. Mucus production was scarce (24–38%), and when present, it was dry (33%) or viscous (52%) (Figure 1).

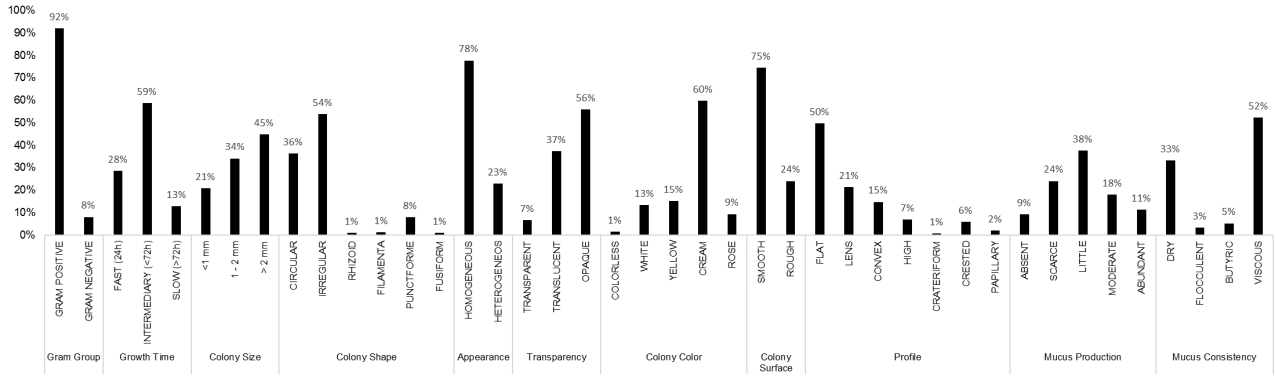

Figure 1. Phenotypic characterization of rhizobacteria in *Vitis vinifera* cultivated in six locations in the São Francisco Valley, Pernambuco, Brazil.

The predominance of gram-positive bacteria in soils from tropical semiarid regions has already been reported and has been related to soil acidity, available P content, and management practices adopted (MUNOZ-ROJAS et al., 2016). The pH values for the rhizospheric samples have a narrow range from 7.07 to 6.53, and the contents of available P were high compared to that of other regions of the São Francisco Valley (FRACETTO et al., 2023; PRESTON et al., 2017). These conditions could favor these bacteria, although most of the P content is leached (XUE et al., 2018). Other possibilities are exposure to heat, low-humidity conditions, and resistance to antibiotics and disinfectant substances used in vine management (BOLO et al., 2021). Aguilar et al. (2020) observed significant differences in the microbial

community composition between vintages and vineyard locations, where the predominant soils showed variances in pH, organic matter, carbon, nitrogen, and absorbable phosphorus content.

Only 29 of the 423 isolates (6.85%) exhibited solubilization halo formation regarding phosphate solubilization. Among the calcium phosphate-solubilizing PGPR, 93% showed a low solubilization index, and only 6.89% showed an intermediate index (Table 4). Among the solubilization indices, 75% of the PGPR showed an IS between 1.15 and 1.55. According to the IS estimation, isolates 31.14, 3.19, 35.18, 17.04, and 3.25 were the five most efficient. According to the classification of Hara and Oliveira (2004), only isolates 31.14 and 3.19 would be medium solubilization (2 < IS < 4). The remaining isolates showed low solubilization (IS < 2). None of the isolates had high solubilization capacity (IS > 4). The isolates showed late solubilization (halo formation after day 7 of inoculation), except for isolate 3.24, which showed early and expressive halo formation on day 4. None of the isolates showed halo formation during aluminum phosphate solubilization.

Table 4. *In vitro* characterization of plant growth promotion mechanisms of rhizobacteria in *Vitis vinifera* cultivated in six locations in the São Francisco Valley, Pernambuco, Brazil.

Isolates	P-Ca (SI)	BNF	IAA (µM)	Biofilm	GIP (%)
1.05	1.235 ± 0.012	-	-	-	50.95
1.06	1.174 ± 0.001	-	-	+	-
1.07	-	-	-	+	-
1.08	-	-	-	+	-
1.11	-	-	-	+	-
1.13	-	-	74.017 ± 0.673	-	-
1.16	-	-	-	+	-
3.01	-	-	-	+	-
3.02	-	-	-	+	-
3.03	1.388 ± 0.013	-	-	+	-
3.05	-	-	-	+	55.41
3.06	1.171 ± 0.001	-	-	-	-
3.07	1.175 ± 0.052	-	-	+	-
3.14	1.651 ± 0.001	-	-	-	-
3.15	1.336 ± 0.006	-	-	+	-
3.17	-	-	-	+	88.12
3.19	2.251 ± 0.521	-	-	-	-
3.20	-	-	-	+	100
3.21	-	-	-	+	54.74
3.22	-	-	-	+	-
3.23	-	-	-	+	-
3.24	1.266 ± 0.103	-	191.701 ± 56.357	+	-
3.25	1.859 ± 0.295	-	-	-	-
3.27	-	-	-	+	-
3.28	1.624 ± 0.353	-	-	+	-
3.29	-	-	335.957 ± 3.751	+	-
5.02	-	-	-	+	-
5.03	-	-	162.581 ± 1.89	+	-
5.04	-	-	-	+	-
5.05	-	-	-	+++	-
5.06	-	-	-	++	-
5.09	-	-	-	++	-
5.11	1.125 ± 0.025	-	-	-	50.86
5.13	-	-	-	+	54.88

P-Ca (SI): Calcium phosphate solubilization index; BNF: Biological Nitrogen Fixation; IAA: Indole-3 acetic acid productions; GIP: Growth Inhibition Percentage.

Table 4. Continuation.

Isolates	P-Ca (SI)	BNF	IAA (µM)	Biofilm	GIP (%)
5.14	-	-	-	+	-
5.18	-	-	-	+	-
5.19	-	-	-	+	-
5.20	-	-	-	+	-
5.21	-	-	-	+	-
5.22	-	-	168.316 ± 3.561	-	-
5.24	-	-	-	+	-
5.25	1.859 ± 0.295	-	-	+	-
5.27	1.165 ± 0.114	-	-	-	-
5.28	-	-	-	+	-
5.31	-	-	-	+	-
5.33	-	-	-	+	-
5.35	-	-	688.786 ± 56.157	-	-
5.36	-	-	-	+	-
5.37	-	-	532.402 ± 9.599	++	-
5.38	-	-	-	+	49.43
5.41	-	-	233.521 ± 15.723	-	-
5.42	-	-	554.496 ± 18.584	-	-
7.05	-	-	-	+	49.38
7.06	-	-	-	+	-
7.07	-	-	-	+	-
7.18	-	+	-	-	-
7.19	-	-	91.838 ± 1.759	-	-
9.04	-	+	-	-	56.07
9.08	-	-	77.915 ± 4.002	-	-
9.17	1.158 ± 0.122	-	-	-	-
11.01	-	-	106.282 ± 1.076	-	-
11.06	-	-	-	-	52.58
11.12	1.203 ± 0.082	-	-	-	-
11.17	-	-	84.983 ± 2.514	-	-
13.12	-	-	-	-	39.21
13.13	-	-	-	-	10.75
13.17	-	-	77.171 ± 1.245	-	-
13.21	-	-	-	-	54.89
13.25	-	-	77.325 ± 3.293	-	-
15.01	1.324 ± 0.115	-	-	-	-
15.02	1.302 ± 0.057	-	-	-	-
15.03	-	-	74.342 ± 1.947	-	-
15.05	-	-	-	-	54.14
15.19	-	-	70.786 ± 1.129	-	-
17.02	1.553 ± 0.154	-	-	-	-
17.04	2.008 ± 0.058	-	-	-	-
17.07	1.146 ± 0.023	-	-	-	-
17.08	1.563 ± 0.151	-	-	-	-
17.23	-	-	-	-	59.43

P-Ca (SI): Calcium phosphate solubilization index; BNF: Biological Nitrogen Fixation; IAA: Indole-3 acetic acid productions; GIP: Growth Inhibition Percentage.

Table 4. Continuation.

Isolates	P-Ca (SI)	BNF	IAA (µM)	Biofilm	GIP (%)
23.03	-	-	119.385 ± 3.118	-	-
23.12	-	-	-	+	-
23.14	-	-	-	+	-
25.02	-	-	-	+	-
25.10	1.194 ± 0.035	-	-	-	-
25.12	-	-	182.256 ± 2.273	-	-
25.15	1.131 ± 0.023	-	-	-	-
25.18	-	-	146.726 ± 14.563	-	49.79
27.09	-	-	234.795 ± 21.475	-	-
27.17	1.059 ± 0.059	-	-	-	-
31.14	2.908 ± 0.211	-	-	-	-
33.02	1.261 ± 0.079	-	-	-	-
33.09	-	-	109.248 ± 0.807	-	-
33.13	-	-	108.248 ± 6.313	-	-
35.17	-	-	80.12 ± 2.001	-	-
35.18	2.051 ± 0.001	-	-	-	-

P-Ca (SI): Calcium phosphate solubilization index; BNF: Biological Nitrogen Fixation; IAA: Indole-3 acetic acid productions; GIP: Growth Inhibition Percentage.

Grape production in the semiarid regions of Brazil depends on high concentrations of phosphate fertilizers. Studies have shown that the available fraction of P in vineyard soils is among the highest recorded in tropical environments (FRACETTO et al., 2023; PRESTON et al., 2017). The low abundance of phosphate-solubilizing bacteria may be due to the high input of inorganic fertilizers in the study area. Bolo et al. (2021) observed that the abundance of phosphate-solubilizing bacteria was strongly increased without P or lime, demonstrating the suppressive effects of increased inorganic P availability on P-solubilizing bacteria. Higher inorganic P levels can block the microbial expression of enzymes associated with P, thereby reducing P abundance and activity (BILLAH et al., 2019).

The ability to perform BNF was observed in 53 of 423 (12.52%) isolates. After the PCR procedure, only two of the 53 isolates retained the *nif*H gene, 7.18 and 9.04 (Table 4). Thus, the percentage of bacteria capable of performing BNF decreased from 12.52% to 0.47%.

Only 5.7% of the isolates synthesized IAA. The average yield among the isolates was 190.96 µM. The PGPR with the lowest IAA synthesis had an estimated production of 70.78 µM. The isolates that produced the highest IAA levels were 5.35 (688.78 µM), 5.42 (554.50 µM), and 5.37 (532.40 µM) (Table 4).

Biological nitrogen fixation and auxin production by PGPR are important mechanisms for promoting plant growth produced by PGPR, and several studies have demonstrated the use of PGPB as a biofertilizer in crops of agronomic importance. However, continuous chemical fertilization can inhibit the growth of beneficial bacteria and the expression of growth-promoting mechanisms. Zhang et al. (2022) demonstrated that the continuous application of chemical fertilizers was strongly associated with a reduced abundance of key ecological clusters within the bacterial co-occurrence network, which contained beneficial bacterial taxa, such as *Lysobacter* sp. and *Haliangium* sp., which are producers of antibiotics and auxins.

The ability to produce exopolysaccharides under biofilm conditions was observed in 11.27% of the 423 isolates evaluated, and only 2.2% showed strong visual expression. The bacteria with the highest biofilm formation were 5.05, 5.06, and 5.37 (Table 4). Only 17 bacterial isolates showed direct antibiosis in the culture medium (Table 4). Some isolates exhibited low GIP. The bacterium 13.13 showed the lowest GIP (10.8%). The second-lowest antibiotic capacity was observed in isolate 13.12 (39.2%). The highest GIP was observed for isolates 3.20 and 3.17, with 100% and 88.1% inhibition of *X. campestris* pv. *viticola* growth. However, isolate 3.20 showed unusual growth behavior. The other isolates showed intermediate antibiotic values between 49.6% and 55.7%.

Antibiotic production by PGPR is essential for promoting plant growth and antimicrobial activity. Various abiotic, biotic, and environmental factors regulate these biochemicals. Zn and Cu concentrations in the São Francisco Valley vineyard soils are high because of the widespread use of Cu fertilizers and fungicides, respectively (PRESTON et al., 2016). These metals can significantly influence the soil bacterial communities. Li et al. (2022) demonstrated that adding Cu significantly inhibited soil respiration and urease activity and that these metals greatly influenced the transfer of antibiotic-resistance genes in bacteria from agricultural soils.

The isolates with the highest number of plant-promoting growth mechanisms were 1.05, 3.17, 3.19, 3.24, 3.29, 5.35, 5.37, 5.38, 5.41, 5.42, 13.12, 17.04, 27.09, 31.14 and 35.18 (Table 4). Owing to the cultivation system adopted by the grape farms, there was a low occurrence of cultivable PGPR. It is known that non-conservation systems (i.e., those that use mineral fertilizers and other inputs) are responsible for reducing microbial diversity by approximately 36% and metabolic activity by 18% (SEMENOV et al., 2020). This is

because both the favoring of copiotrophic groups (r-strategists) and the depletion of prokaryotic groups depend on organic substrates (DAI et al., 2018). This behavior also affects the ability to isolate cultivable microorganisms for industrial use.

Sequencing of the 16S rRNA gene of the selected PGPR revealed genetic diversity among the genera. The predominant genera detected were *Pseudomonas* (20%), *Stenotrophomonas* (13.33%), and *Bacillus* (13.33%). Other genera were also associated with the selected PGPR, such as *Pseudoxanthomonas, Clostridium, Leclercia, Lysinibacilus, Kosakonia, Lueinomonas*, and *Comamonas*. In addition, PGPR 3.19 did not show similarity to any of the genetic sequences available in the BLASTn database (Table 5).

Table 5. Identity of rhizobacteria in *Vitis vinifera* cultivated in six locations in the São Francisco Valley, Pernambuco, Brazil, based on bacterial strains selected from the sequencing of the 16S rRNA.

Isolates	Taxonomical Class	Order	Specie	Query Cover	E Value	Percent Identity	GenBank accession
1.05							
	Gammaproteobacteria	Xanthomonadales	*Stenotrophomonas maltophilia*	41	0.0	96.38	FJ971878.1
	Gammaproteobacteria	Xanthomonadales	*Stenotrophomonas pavanii*	41	0.0	97.69	FJ748683.2
3.17							
	Bacilli	Bacillales	*Bacillus subtilis*	32	0.0	98.14	MG015919.1
	Bacilli	Bacillales	*Bacillus amyloliquefaciens*	45	0.0	97.46	KY047618.1
3.19							
	Gammaproteobacteria	Xanthomonadales	*Stenotrophomonas maltophilia*	44	0.0	75.36	FJ971878.1
	Gammaproteobacteria	Xanthomonadales	*Stenotrophomonas pavanii*	45	0.0	75.10	FJ748683.2
	Gammaproteobacteria	Xanthomonadales	*Stenotrophomonas geniculata*	43	0.0	75.05	AB021404.1
3.24							
	Gammaproteobacteria	Xanthomonadales	*Stenotrophomonas maltophilia*	71	0.0	98.41	FJ971878.1
	Gammaproteobacteria	Xanthomonadales	*Stenotrophomonas pavanii*	81	0.0	97.33	FJ748683.2
3.29							
	Gammaproteobacteria	Xanthomonadales	*Stenotrophomonas geniculata*	70	0.0	89.42	AB021404.1
5.35							
	Gammaproteobacteria	Pseudomonadales	*Pseudomonas putida*	54	0.0	89.79	JN630890.1
	Gammaproteobacteria	Pseudomonadales	*Pseudomonas monteilii*	54	0.0	90.66	KT825516.1
	Gammaproteobacteria	Pseudomonadales	*Pseudomonas taiwanensis*	54	0.0	90.66	EU103629.2
	Gammaproteobacteria	Pseudomonadales	*Pseudomonas plecoglossicida*	52	0.0	91.06	MH021844.1
5.37							
	Clostridia	Clostridiales	*Clostridium hydrogeniformans*	96	0.0	93.91	DQ196623.2
	Clostridia	Clostridiales	*Clostridium frigidicarnis*	96	0.0	91.22	AF069742.1
5.38							
	Gammaproteobacteria	Enterobacteriales	*Enterobacter cloacae*	39	0.0	98.57	AJ417484.1
	Gammaproteobacteria	Enterobacteriales	*Enterobacter ludwigii*	66	0.0	98.13	KF516257.1
	Gammaproteobacteria	Enterobacteriales	*Enterobacter hormaechei*	69	0.0	96.07	MH031693.1
5.41							
	Gammaproteobacteria	Pseudomonadales	*Pseudomonas putida*	72	0.0	95.15	JN630890.1
	Gammaproteobacteria	Pseudomonadales	*Pseudomonas monteilii*	98	0.0	94.83	KT825516.1
	Gammaproteobacteria	Pseudomonadales	*Pseudomonas taiwanensis*	99	0.0	94.84	EU103629.2
	Gammaproteobacteria	Pseudomonadales	*Pseudomonas plecoglossicida*	98	0.0	94.89	MH021844.1
5.42							
	Gammaproteobacteria	Pseudomonadales	*Pseudomonas putida*	72	0.0	94.89	JN630890.1
	Gammaproteobacteria	Pseudomonadales	*Pseudomonas monteilii*	98	0.0	94.33	KT825516.1
	Gammaproteobacteria	Pseudomonadales	*Pseudomonas taiwanensis*	98	0.0	94.33	EU103629.2
	Gammaproteobacteria	Pseudomonadales	*Pseudomonas plecoglossicida*	98	0.0	94.31	MH021844.1

Table 5. Continuation.

Isolates	Taxonomical Class	Order	Specie	Query Cover	E Value	Percent Identity	GenBank accession
13.12							
	Bacilli	Bacillales	*Lysinbacillus macroides*	75	0.0	86.31	MG645263.1
	Bacilli	Bacillales	*Lysinbacillus sphaericus*	98	0.0	84.87	MT760090.1
	Bacilli	Bacillales	*Lysinbacillus fusiformis*	98	0.0	84.99	KY328837.1
17.04							
	Bacilli	Bacillales	*Bacillus paramycoides*	74	0.0	88.90	KJ812444.1
27.09							
	Gammaproteobacteria	Enterobacteriales	*Enterobacter cloacae*	40	0.0	87.00	AJ417484.1
	Gammaproteobacteria	Enterobacteriales	*Enterobacter ludwigii*	94	0.0	85.20	KF516257.1
	Gammaproteobacteria	Enterobacteriales	*Enterobacter homaechei*	78	0.0	86.17	MH031693.1
31.14							
	Gammaproteobacteria	Xanthomonadales	*Stenotrophomonas geniculata*	97	0.0	90.84	AB021404.1
35.18							
	Betaproteobacteria	Burkholderiales	*Delftia tsuruhatensis*	98	0.0	85.98	AB075017.1
	Betaproteobacteria	Burkholderiales	*Delftia lacustris*	98	0.0	86.07	EU888308.1
	Betaproteobacteria	Burkholderiales	*Delftia acidovorans*	94	0.0	85.67	EU024145.1
	Betaproteobacteria	Burkholderiales	*Comamonas phosphati*	98	0.0	83.13	JQ246447.1

In descending order of similarity, 16S rRNA sequences available in the LPSN were used to form five large polyphyletic groups associated with bacterial species through type sequences and BLASTn. (Figure 2).

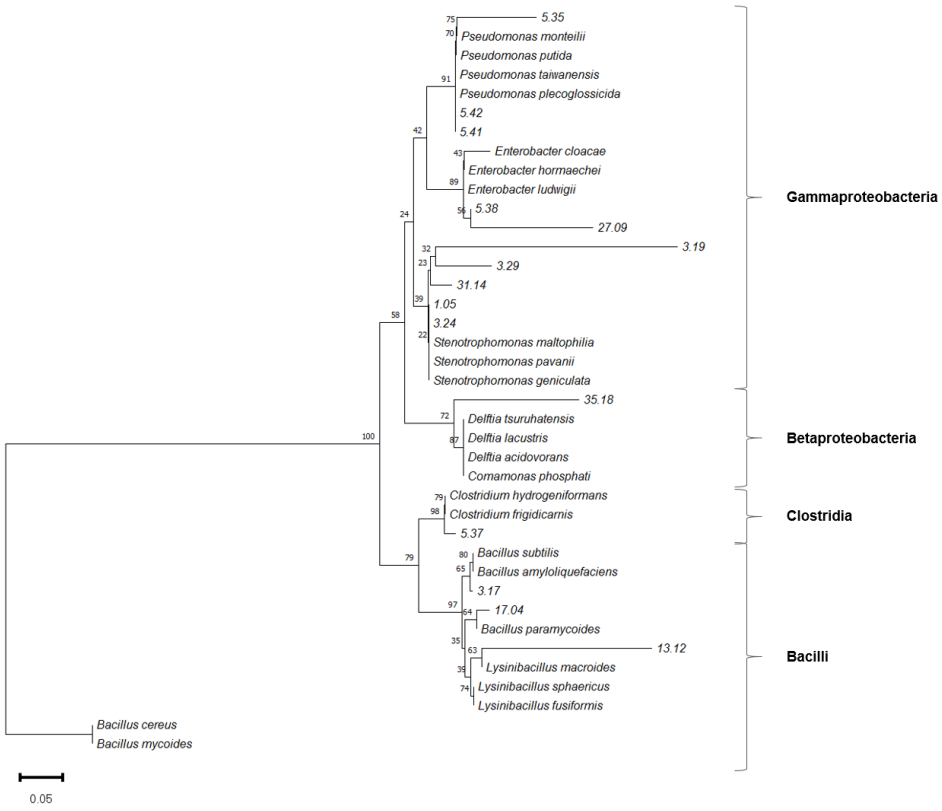

Figure 2. Phylogenetic relationship based on the partial sequence of the 16S rRNA gene of rhizobacteria in *Vitis vinifera* cultivated in six locations in the São Francisco Valley, Pernambuco, Brazil, with the strains obtained from the alignment of the sequences with the NCBI database.

A phylogenetic tree constructed using the 16S rRNA gene sequences of the bacterial isolates revealed that most belonged to Gammaproteobacteria, represented by *Pseudomonas*, *Enterobacter*, and *Stenotrophomonas*. Isolates 3.24, 1.05, 31.14, 3.29, and 3.19 were phylogenetically closest to species belonging to *Stenotrophomonas*. The genera *Enterobacter* was grouped with isolates 5.38 and 27.09, while *Pseudomonas* species were related to isolates 5.41, 5.42, and 5.35.

In particular, *Stenotrophomonas* is well known as a host in many different types of habitats, thus exhibiting environmental adaptability and being characterized as a genus consisting of PGPR with multiple properties (GHOSH; CHATTERJEE; MANDAL, 2020). Similarly, the genus *Pseudomonas* is qualified as ubiquitous, with a great diversity of species that can exert both beneficial and antagonistic relationships with higher plants (PEIX; RAMÍREZ-BAHENA; VELÁZQUEZ, 2018). In particular, they can be easily isolated to guarantee the development of growth factors in vines.

The second most dominant class was Bacilli, with the genera *Bacillus* and *Lysinbacillus*. The sequences of *Bacillus cereus* and *B. mycoides* constituted the root of the phylogenetic tree that gave rise to the clustering of isolated clades 3.17 and 17.04 together with species belonging to the genera *Bacillus* and *Lysinbacillus*. Several strains of *Bacillus* have already been documented as important PGPR in several crops (KUMAR et al., 2021; WEI et al., 2020). The *Bacillus* genus forms endospores that remain in the soil until the environment improves. *Bacillus velezensis* was reported as a PGPR by aiding nutrient assimilation (WEI et al., 2020). The production of exopolysaccharides by *Bacillus* as an extracellular matrix carbon source, providing a natural probiotic for the rhizosphere community, is also an important mechanism linked to the rhizosphere (BANERJEE et al., 2022).

The second clade was composed of *Clostridium,* which resembled isolate ID 5.37. The genera *Delftia* and *Comamonas* exhibited high similarity to the 16S rRNA sequence of isolate 35.18. The bacterial species *Delftia tsuruhatensis* was first described as being pathogenic by Ranc et al. (2018). However, recent studies have linked their presence in soils to high metal concentrations (Li et al., 2018). *D.tsuruhatensis* plays a role in the biomineralization of metals present in the uppermost soil layers. Phylogenetic analysis revealed that PGPR 35.18 is a member of the *Delftia* genus with a query coverage of 99% and 86.08% identity. This same isolate is closely related phylogenetically to the species *Comamonas phospati* (83.13%). *C. phophati* is a bacterium isolated and identified from a phosphate mine and can be solubilized by extruding glycolic acid, rhamnose, and d-melibiose (XIE et al., 2016).

Habitat is the primary factor shaping the structure and function of microbial communities. (BULGARELLI et al., 2013). Our study indicates that long-term agricultural cultivation environments develop PGPR communities with a low isolation capacity. Part of the rhizosphere microbial community of long-term intensive crops becomes auxotrophic (dependent on substances that prevent their growth in other environments), and they cannot grow *in vitro* or be manipulated.

Cropping systems that use organic fertilization benefit microbial biomass, positively affecting taxonomic and functional diversity (MORUGÁN-CORONADO et al., 2022). In contrast, systems that adopt conventional fertilization practices restrict the diversity of bacteria, thereby increasing the occurrence of phyla such as Acidobacteria, Actinobacteria, and Proteobacteria (DAI et al., 2018). The low CFU expression (average among the areas of 8.0 x 10^4) and the reduced number of bacteria capable of performing any of the mechanisms (only 99 out of 423) of growth promotion corroborate the losses in biodiversity of cultivable bacteria due to the adopted cultivation system (NOURI et al., 2021).

CONCLUSION

Only 25% of the bacteria isolated from the vine rhizosphere in São Francisco Valley exhibited *in vitro* plant growth-promoting mechanisms. Isolates 3.19 and 31.14; 3.17 and 17.04; 5.35 and 5.42; and 5.37 that presented biotechnological potential for future experiments to promote vine growth were identified as *Stenotrophomonas, Bacillus, Pseudomonas*, and *Clostridium*, respectively.

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