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Original article

Characterization of plant growth-promoting traits of bacteria isolated from the rhizosphere of grapevine grown in alkaline and acidic soils

Kenan Karagöz [a], Fadime Ateş [b], Halit Karagöz [c], Recep Kotan [a], Ramazan Çakmakçı [c,*]

[a] Atatürk University, Faculty of Agriculture, Department of Plant Protection, 25240 Erzurum, Turkey
[b] Manisa Viticulture Research Institute, 45040 Manisa, Turkey
[c] Atatürk University, Faculty of Agriculture, Department of Agronomy, 25240 Erzurum, Turkey

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ABSTRACT

The purpose of this study was to investigate the diversity of cultivable N_2-fixing, P-solubilising and siderophores-producing bacteria originated from acidic and alkaline rhizospheric soil of native grapevine grown at three locations. Ninety–five dominant, morphologically distinct rhizobacteria were purified, which belonged to 27 genera and 44 species. Gram-negative bacteria were dominating in the grapevine environment. *Bacillus* spp. and *Pseudomonas* spp. were common at both the acidic and alkaline soils. Among different groups, *Gammaproteobacteria*, *Firmicutes* and *Actinobacteria* comprised the largest groups contributing to about 42.1, 33.7 and 9.5% of the total N_2-fixing isolates, respectively. The cultivated bacterial community from rhizosphere of native grapevine represented 27 different known bacterial genera represented by *Pseudomonas*, *Bacillus*, *Brevibacillus*, *Stenotrophomonas,* and *Paenibacillus* as as the predominant genera. Of the 95 nitrogen fixing isolates, 12 were able to fixe nitrogen and solubilize phosphates, 12 were able to fixe nitrogen and produce siderophores, only five isolates were able to fix nitrogen, produce siderophores and solubilize phosphates at the same time. In addition, the majority of the isolates were able to grow under high NaCl concentration. Our result showed that different geographical locations, soil pH, and vegetation type in the investigated sites resulted in the different bacterial population and bacterial type.

1. Introduction

Microorganisms colonizing the rhizosphere can affect plant growth both positively and negatively, the term plant growth promoting rhizobacteria (PGPR) often describes beneficial rhizobacteria that stimulate plant growth. PGPR in turn having a great impact on root biology, influence plant growth, nutrition and development are important for long-term sustainability. Plant-associated N_2-fixing and P-solubilizing bacteria are regarded as a possible alternative for inorganic nitrogen fertilizers, and PGPR strains have previously been attracted the attention of agriculturists as soil inoculums to improve the plant growth and yield [1,10,13,23,35]. Apart from fixing N_2, PGPR can influence plant growth directly by the synthesis of phytohormones, antibiotics, vitamins, enzymes and/or fungicidal compounds, inhibition of plant ethylene synthesis, improvement of nutrient uptake, enhanced stress resistance, production of siderophores, solubilization of inorganic phosphate, and mineralization of organic phosphate [19,27].

The thin layer of soil surrounding crop roots and the volume of soil occupied by roots is known as the rhizosphere. The rhizosphere is well known to host a variety of PGPR and the majority of plant-associated bacteria derive from the soil environment [17]. One strategy that may contribute to the establishment of pre-selected beneficial organisms in the root zone of soils is through early establishment of selected communities of bacteria in the rhizosphere. In the last few years, the number of PGPR that have been identified has seen a great increase, mainly because the role of the rhizosphere as an ecosystem has gained importance in the functioning of the biosphere and also because mechanisms of action of PGPR have been deeply studied. Rhizosphere soil is influenced by plant roots which select for specifically adapted microbial communities [2,4]. Several studies have demonstrated that the bacterial diversity in rhizospheres can be influenced by a number of different factors, i.e., the plant species, varietal differences within a species, plant age, plant genotype, agricultural management, or soil properties [7,12,18,30,39]. An important factor to be considered when screening new isolates is their activity in the range of

* Corresponding author. Tel.: +90 442 231 2403; fax: +90 442 231 1469.
E-mail addresses: rcakmak@atauni.edu.tr, ramazan_cakmakci@yahoo.com (R. Çakmakçı).

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environments in which they would be expected to be used; in particular different plants and soil types. Previous isolations of N_2-fixing bacteria have revealed a broad diversity of diazotrophs to inhabit the crop rhizosphere [7,11]. Nevertheless, many rhizospheric, N_2-fixing and P-solubilizing bacterial species remain unknown and more studies are needed to reveal the high biodiversity of these bacteria.

Isolating of native strains adapted to the environment and their study may contribute to the formulation of inoculants to be used in region crops. Native isolates may be preferred in the selection of bacteria for inoculation, as they are adapted in the environment and can be more competitive than the foreign bacteria. The advantage of using natural soil isolates is the easier adaptation and succession when inoculated into the plant rhizosphere [13]. On the other hand, characterization and identification of these bacteria are necessary for wide ecological studies of the plant rhizosphere. Some endophytic bacteria colonize upper grapevine organs, especially berries, flowers, fruits and seeds on grapevine [16] and inoculation of *Vitis vinifera* explants with PGPR strain increased grapevine growth and physiological activity at low temperature [1]. Though much information is available on the activity of soil microorganisms and nitrogen fixation and phosphate solubilization for annual crops, information about the characteristics of composition and diversity of the bacterial community in perennial crops soil ecosystems is scarce. The diversity of PGPR species in grapevine soils remains unknown, especially of those strains that can fix nitrogen and solubilise phosphate besides having several plant growth promoting and biocontrol properties. Also, there is not sufficient knowledge on rhizosphere microbiology of the wild or cultured grape regions. For this reason it is important to study native strains isolated in the wild or cultured grape-growing regions where they may be used as berries and/or grapevine inoculants. The study was focused on rhizosphere bacteria as these represent an important group of soil microorganisms interacting with plant nutrition and health. The purpose of the present work was to isolate and identify PGPR of rhizosphere soils of native wild grapevines at three different region locations in Turkey and evaluation of their N_2-fixing, P-solubilising, siderophores-producing status and other plant growth traits.

2. Materials and methods

2.1. Soil samples and isolation of bacteria

We conducted a survey of PGPR, naturally colonizing a mild and continental climate and mostly acidic and alkaline rhizospheric soil of native grape; in the mild climate with high precipitation and acidic environmental area of the Storm (Fırtına) Valley in the Black Sea Region, a hard, grey and limestone alkaline soil of Akdamar Island in the Eastern Anatolia and Mediterranean climate of the Kemalpaşa in the Aegean Region have never been studied before. These regions have very widespread and common with a great diversity of wild and cultured grape species and/or ecotypes. Some properties of the soils in the three region used for isolation of rhizobacteria were given in Table 1. Storm Valley (300–700 m above sea level) is like a natural botanic garden which is ancient natural forest and rich flora and fauna. Soils samples of the valley were generally clay loam and sandy clay loam texture, strong and medium acid reactions (pH 4.6–6.6). Akdamar Island is a small island westward in Lake Van (alkaline-saline lake, pH 9.8) in the Eastern Anatolia region of Turkey, about 0.7 km^2 in size, situated about 3 km from the shoreline. In this island, the soils surveyed were alkaline and calcareous with low organic matter content. Kemalpaşa with characteristic quite typical of a Mediterranean climate, the summer are hot and dry, and winters cool and rainy. The soils in this region were neutral or slightly alkaline reactions (Table 1).

Table 1
Characteristics of the soils used for isolation of rhizobacteria.

Soil properties	Storm valley	Akdamar Island	Kemalpaşa
pH (1:2.5 W/V H_2O)	4.6–6.6	7.1–8.7	7.1–7.6
Organic matter (%)	2.1–7.4	0.8–1.9	1.2–2.3
Salt (%)	0.04–0.34	0.06–0.78	0.04–0.65
CaCO3 (%)	0.5–2.4	1.8–36.2	1.7–34.5
Total N (%)	0.13–0.45	0.05–0.098	0.08–0.15
Available P (ppm)	2.1–18.2	3.3–19.7	1.4–16.9
K (ppm)	50–768	74–1215	48–486
Ca (ppm)	462–3480	840–6256	580–5678
Mg (ppm)	101–473	85–729	56–635
Na (ppm)	41–281	16–639	22–884
Fe (ppm)	8–196	3–98	2–58
Zn (ppm)	0.6–8.8	0.5–3.2	0.4–5.9
Cu (ppm)	0.9–14.7	0.8–9.7	0.6–32,4
Mn (ppm)	2–104	6–43	3–109
B (ppm)	0.6–6,9	0.5–3.1	0.3–5.7
N_2-fixing bacteria (CFU g^{-1} dry soils)	1.1×10^5–4.2×10^7	4.0×10^5–6.9×10^6	5.5×10^5 –5.1×10^6

Rhizosphere soil samples were collected from wild grape. Ten grams the soil for each individual grape plant adhering to the roots, considered as the rhizospheric soil, was mixed and used for the bacterial isolation procedures. The soil adhering strongly to the root was referred to as rhizosphere soil. Rhizosphere soil samples were collected carefully by uprooting the root system and placed in a cool box for transport and stored at 4 °C. Ten grams of soil from each sample was aseptically weighed and transferred to an Erlenmeyer flask with 100 ml sterile water, and was shaken for 30 min at 150 rpm. Immediately after shaking, a series of ten-fold dilutions of the suspension was made for each sample by pipetting 1 ml aliquots into 9 ml sterile water. The final dilution was 10^5- fold; 0.1 ml of each dilution of the series was placed onto a Petri dish. Three replicate dishes were made for each dilution. Dishes were placed in an incubator at 28 °C for seven days (aerobically). Rhizobacteria isolates were selected from N-free solid malate–sucrose medium (NFMM) modified from Döbereiner [20]. Modified NFMM medium per liter distilled water (sucrose, 10.0 g; L-malic acid, 5.0 g; $MgSO_4 \cdot H_2O$, 0.2 g; $FeCl_3$, 0.01 g; NaCl, 0.1 g; $CaCl_2 \cdot 2H_2O$, 0.02 g; K_2HPO_4, 0.1 g; KH_2PO_4, 0.4 g; $Na_2MoO_4 \cdot H_2O$, 0.002 g) with 18 g agar for solid medium was used for isolation. The medium adjusted to pH 7.2 with 1 N NaOH prior to agar addition and was then sterilized at 121 °C for 20 min in an autoclave [47]. N-free medium was used in order to obtain nitrogen fixing PGPR [38].

2.2. Extraction of cellular fatty acids, FAME profiling and characterization of bacterial strains

Cells were streaked in a quadrant pattern and grown overnight on TSBA. Approximately 50 mg of bacterial cells, harvested from the third and fourth quadrant streak of growth, were used for the extraction using standard extraction techniques [43]. FAME profiles were obtained by running samples on a Hewlett Packard Agilent GC 6890 GC fitted with a microprocessor containing the Sherlock Microbial Identification System (MIDI) Software (V.A. 06. 03). The FAME profiles were compared with the TSBA40 aerobe library. FAME profiles were routinely used to identify genera, species, and strains of bacteria [9]. Only strains with the similarity index (SIM) $\geq$0.3 were considered a good match [34]. The bacterial strains were characterized by morphological, biochemical and physiological tests including pigment production on nutrient agar medium, the Gram reaction, catalase, oxidase, sucrose,starch hydrolysis, salt tolerance, nitrate reduction activities and growth at 36 °C on N-free

basal medium [21]. Nitrate reduction was determined in the nitrate broth supplemented with KNO_3. A small inverted Durham tube was added to each tube in order to detect gas formation. The cultures were incubated for 7 days at 24 °C. The presence of nitrate and nitrite in tubes after incubation was evaluated using standard nitrate reagent solution A (sulfanilic acid), reagent B (alpha-naphthylamine) and powdered zinc to reduce the remaining nitrate to nitrite [21]. Subsequently, salt and extreme pH tolerance were evaluated by observing the growth on Tryptic Soy Broth supplemented with different concentrations of NaCl (3, 5, and 7%, w/v), and varying initial pH (5, 8, and 9) conditions.

2.3. Phosphate solubilisation

Phosphate solubilization activity of the bacterial isolates was detected on Pikovskaya (PVK) and National Botanical Research Institute's phosphate growth medium (NBRIP-BPB). NBRIP-BPB contained (per liter): glucose, 20 g; $Ca_3(PO_4)_2$, 10 g; $MgCl_2$ $6H_2O$, 5 g; $MgSO_4$ $7H_2O$, 0.25 g; KCl, 0.2 g; $(NH_4)_2SO_4$, 0.1 g, and bromophenol blue (BPB), 0.025 g. The pH of the media was adjusted to 7.0 before autoclaving, as described earlier [31]. 5 ml of NBRIP-BPB medium was transferred to a sterile test tube and autoclaved. Autoclaved, uninoculated broth medium served as controls. The sterile liquid medium was inoculated with 500 μl suspension of the tested bacterial strains. The test tubes were incubated for 14 days at room temperature. At the end of the incubation period, change in pH of the culture broth was recorded. All the pure isolates were also tested in triplicate for their phosphate solubilizing capacity in sucrose-tricalcium phosphate agar media [37]. Pikovskaya's medium contained per litre: glucose, 10 g; $Ca_3(PO_4)_2$, 5 g; $(NH_4)SO_4$, 0.5 g; NaCl, 0.2 g; $MgSO_4.7H_2O$, 0.1 g; KCl, 0.2 g; NaCl, 0.2 g; $MnSO_4.7H_2O$, 0.002 g; $FeSO_4.7H_2O$, 0.002 g; yeast extract, 0.5 g. After incubation for 6 days, water soluble P was determined colorimetrically by the vanadomolybdophosphoric acid colorimetric method [25].

2.4. Acetylene reduction assay (ARA)

Nitrogen fixation of the isolates was determined in a nitrogen-free medium by the acetylene reduction assay [22]. Cultures for the acetylene reduction assay were prepared according to Holguin and Bashan [24] and incubated at 30 °C for 24 and 48 h without agitation. Ethylene production was measured using a Hewlett Packard gas chromatograph (Model 6890, USA).

2.5. Siderophore production ability

Ability of the rhizobacterial isolates to synthesize siderophore under iron limiting conditions was assayed using the "universal" Chrome Azurol S (CAS) assay [44]. Siderophore activity was detected after 3 days in terms of the intensity of colour change (blue to orange-yellow).

3. Results

The number of cultivable N_2-fixing bacteria expressed as colony-forming units (CFU) ranged between $4.0 \pm 0.4 \times 10^5$ and $4.2 \pm 0.9 \times 10^7$ CFU g^{-1} dry soils in the sampled the rhizosphere of wild grape-growing zones of various agro climatic regions in Turkey. Morphological, biochemical and physiological tests showed that 95 rhizobacterial isolates obtained from the wild grape rhizosphere have several common characteristics (Table 2). For example, 44.2 and 27.4% of them were oxidase and sucrose positive, 30.5 and 61.1% showed positive starch hydrolysis and nitrate reduction activity, respectively. Most of the isolates were also able to grow in N-free basal medium and high NaCl concentration, and solubilise phosphate. Isolated strains were capable of nitrogenase activity, but the amounts of C_2H_4 varied with bacterial species and nitrogenase activity ranging from 0.22 to 0.98 nmol C_2H_4 10^7 cfu/h. These isolates showed significant differences in their phosphate solubilizing potential, theirs extend of solubilization ranged between 55.6 and 168.3 mg L^{-1} liquid medium.

In our study, a total of 95 nitrogen fixing bacteria were isolated from the rhizospheres of wild grape and identified using FAME profile analysis. The MIDI system identified (SIM gt; 0.3) 74.2% (95 out of 128) of the bacteria isolated from the rhizosphere of grapevine and 12.6% (12 out of 95) of the bacteria solubilized P from insoluble calcium phosphate on PVK and NBRIP medium (Table 2). Also about 11.7% of the isolates could not be identified by the MIDI system since there were no matches or the analysis was of unacceptable quality. Of the 95 nitrogen fixing isolates, 12 were able to fixe nitrogen and solubilize phosphates, 12 were able to fixe nitrogen and produce siderophores, and five isolates were able to fix nitrogen, produce siderophores and solubilize phosphates at the same time. The rhizospheric soils from Akdamar Island had the highest number of nitrogen fixer and phosphate solubilizer strains in relation to the other sites (Table 2). This site presented the highest number of rhizospheric soil isolates that produce siderophores. About 44 diazotrophic species were found to be in common in the acidic and alkaline soils tested. The distribution of nitrogen fixing bacterial species varies significantly across the two rhizospheric soils of native grape. A total of 17 and 11 Gram-negative and positive N_2-fixing bacterial species were identified as exclusive to alkaline soils (not in acidic soils), and 4 and 1 were exclusive to acidic soils, respectively (Table 2).

The analysis of FAME profiles for the totally isolated nitrogen fixing bacteria facilitated their classification under four bacterial divisions: *Bacteroidetes* (5.3%), γ, β and α-subdivisions of *Proteobacteria* (42.1%, 5.3% and 4.1%, respectively), *Firmicutes* (33.7%), and *Actinobacteria* (9.5%). The nitrogen fixing bacterial community of native grape rhizosphere was composed of Gram-negative (56.8%) and Gram-positive bacteria (43.2%). Out of a total of 95 isolates, 54 belonged to Gram-negative, which included 40 γ-*proteobacteria* and 9 α- and β-*proteobacteria*; 5 isolate belonged to the *Bacteroidetes* group. Major β-proteobacterial genera recovered from grape rhizospheres included *Variovorax, Acidovorax* and *Ralstonia,* while *Pseudomonas*, *Stenotrophomonas*, *Citrobacter* and *Vibrio* dominated the γ-proteobacterial genera. The 41 Gram-positive isolates included 32 *Firmicutes* and 9 *Actinobacteria* (Table 2).

Of the N_2-fxing 95 (39 were from Akdamar Island and 56 from other sites), in which 27 differently known bacterial genera were represented by *Bacillus* (24.2%), *Pseudomonas* (17.9%), *Stenotrophomonas* (7.4%), *Brevibacillus* (5.3%), and *Paenibacillus* (4.2%) as the predominant genera. The genus *Pseudomonas* was the 31.5% of the Gram-negative population, with a prevalence of *Pseudomonas putida* (8 strains), followed by *Pseudomonas fluorescens* (6 strains); while *Bacillus* was the 56.1% of the Gram-positive population, with a prevalence of *Bacillus cereus* (26.8%), followed by *Bacillus megaterium* and *Brevibacillus choshinensis*. Other N_2-fxing *Firmicutes* identified included three isolate each of *Bacillus atrophaeus*, *Bacillus mycoides* and *Paenibacillus macquariensis,* two strains of others. Among the other proteobacterial species, 5 isolates of *Stenotrophomonas maltophilia*, 3 isolates each of *Variovorax paradoxus* and *Vibrio alginolyticus*, 2 isolates each of *Rhizobium radiobacter*, *Stenotrophomonas acidaminiphila*, *Pseudomonas syringae*, *Aeromonas ichthiosmia* and *Citrobacter amalonaticus* and 15 isolates of other species were confirmed as nitrogen fixing bacteria (Table 2).

The results obtained indicated that two isolates of *Brevibacterium epidermidis*, and one isolate each of *Arthrobacter aurescens*,

Table 2
Diversity, biotechnological potential and characteristics of culturable bacteria from the land of grapevine.

Taxonomic identification/Order	Bacterial strain FAME identification	Total number of isolates[b]	SIM value	Number of N_2-fixing isolates[a]			Biochemical characteristics					
				Storm valley	Akdamar Island	Kemalpaşa	Oxidase	Catalase	Sucrose	Starch hydrolysis	Nitrate reduction	Growth in 3, 5, and 7% NaCl[c]
Alphaproteobacteria												
Rhizobiales	*Rhizobium radiobacter*	2	0.702–0.910	1(0/1)	1		+	+	−	−	+	5
	Rhizobium rubi	1	0.713			1	+	+	−	−	−	ND
Rhodobacterales	*Paracoccus denitrificans*	1	0.512			1	+	+	+	−	+	5
Betaproteobacteria												
Burkholderiales	*Ralstonia eutropha*	1	0.727	1			+	+	−	−	+	ND
	Acidovorax delafieldii	1	0.709		1		+	−	−	−	+	5
	Variovorax paradoxus	3	0.526–0.851	1	2		+	+	−	−	D	5–7
Gammaproteobacteria												
Xanthomonadales	*Xanthomonas* sp.	1	0.648		1		−	+	−	+	−	5
	S. acidaminiphila	2	0.501–0.787	1		1	+	+	−	−	+	3
	S. maltophilia	5	0.487–0.818	4 (1/0)	1 (1/0)		−	D	−	−	+	3–5
Pseudomonadales	*Pseudomonas fluorescens*	6	0.735–0.899	3	2 (1/2)	1	+	+	D	−	+	3–5
	Pseudomonas mendocina	1	0.906		1		+	+	−	−	+	7
	Pseudomonas putida biotype A	3	0.729–0.803	2 (1/1)	1(1/1)		+	+	D	−	−	3–5
	Pseudomonas putida biotype B	5	0.596–0.875	2 (1/2)	3 (1/3)		+	+	D	−	D	3–5
	Pseudomonas syringae	2	0.795–0.819			2 (0/1)	−	D	D	−	−	5
Aeromonadales	*Aeromonas ichthiosmia*	2	0.596–0.600		2		+	+	+	+	+	−
Vibrionales	*Vibrio alginolyticus*	3	0.599–0.624		2 (1/0)	1	+	+	−	+	+	7
	Vibrio fluvialis	1	0.536	1 (1/0)			+	+	+	+	+	7
Enterobacteriales	*Citrobacter freundii*	1	0.836			1	−	+	+	−	+	5
	Citrobacter amalonaticus	2	0.709–0.812	2			−	+	−	−	+	5
	Hafnia alvei	1	0.445		1		−	+	−	−	+	3
	Enterobacter hormaechei	1	0.773	1			−	+	+	−	+	3
	Raoultella terrigena	1	0.702		1		−	+	+	−	+	ND
	Pantoea agglomerans	1	0.719			1(1/0)	−	−	+	−	+	5
	Serratia marcescens	1	0.685			1 (1/0)	−	+	+	−	+	7
	Serratia odorifera	1	0.830			1	−	+	+	+	+	7
Firmicutes												
Bacillales	*Bacillus atrophaeus*	3	0.632–0.669	1	1 (0/1)	1	−	+	+	+	+	5–7
	Bacillus cereus	11	0.540–765	7	2	2	−	D	D	+	D	5–7
	Bacillus megaterium	5	0.558–0.698	1	3 (1/0)	1	D	+	−	−	D	5–7
	Bacillus mycoides	3	0.344–0.541	2		1	−	+	−	D	D	5–7
	Bacillus sp.	1	0.444			1	−	+	−	−	+	5
	Paenibacillus alginolyticus	1	0.552		1		−	+	−	−	−	5
	Paenibacillus macquariensis	3	0.578–0.638		2	1	−	+	−	−	−	3
	Brevibacillus choshinensis	5	0.578–0.698		2	3	D	+	−	−	−	ND
Actinobacteria												
Actinomycetales	*Arthrobacter aurescens*	1	0.595		1		+	+	−	−	+	5
	Arthrobacter globiformis	1	0.738	1			−	+	−	−	+	5
	Arthrobacter oxydans	1	0.816			1	−	+	−	−	+	7
	Kocuria rosea	1	0.780			1	−	+	−	−	+	7
	Kocuria kristinae	1	0.553		1		+	+	−	−	−	7
	Brevibacterium epidermidis	2	0.536–0.663			2	−	+	+	+	D	7
	Curtobacterium flaccumfaciens	1	0.483		1		−	+	+	+	−	7
	Microbacterium barkeri	1	0.657		1		−	−	+	+	+	5
Bacteroidetes												
Flavobacteriales	*Flavobacterium johnsoniae*	1	0.417		1		+	+	+	+	+	2
	Chryseobacterium balustinum	2	0.572–0.639	1	1		+	+	−	−	+	5
	Chryseobacterium indoltheticum	1	0.530		1		+	+	−	−	−	5
Sphingobacteriales	*Sphingobacterium spiritivorum*	1	0.438		1		+	+	+	+	−	3
Total		95		31 (4/4)	39 (6/7)	25 (2/1)						

D: Different reaction given by different isolate; ND: Not Determined
[a] Numbers in parentheses indicate the number of P-solubilizing/siderophores-producing strains where bacterial genera were detected.
[b] Isolates number with a similarity index < 0.3.
[c] The highest salt concentration (3, 5, and 7%) which did not inhibit growth.

Arthrobacter globiformis, *Arthrobacter oxydans*, *Kocuria rosea*, *Kocuria kristinae*, *Curtobacterium flaccumfaciens* and *Microbacterium barkeri* were the most prominent N_2-fixing Actinobacterial species in the rhizosphere of grapevine. Five isolate belonged to the *Bacteroidetes* group, two isolates of *Chryseobacterium balustinum* and one isolate each of *Flavobacterium johnsoniae*, *Chryseobacterium indoltheticum* and *Sphingobacterium spiritivorum* were able to fix nitrogen.

A total 12 izolates were identified as N_2-fixing and siderophore producing isolates, 7 were *P. putida* (4 from Akdamar Island and 3 from Storm Valley), 2 isolates of *P. fluorescens* (1 each from Akdamar and Storm Valley) and one isolates each of *R. radiobacter*, *P. syringae* and *B. atrophaeus* (Table 2). Out of these 4 isolates of *P. putida* and 1 isolates of *P. fluorescens* were able to fix nitrogen, produce siderophores and solubilize phosphates at the same time. Also, two *S. maltophilia* strains, *B. megaterium*, *V. alginolyticus*, *Vibrio fluvialis*, *Pantoea agglomerans* and *Serratia marcescens* were the most frequent P-solubilizing and N_2-fixing species in the native grape rhizosphere soils.

4. Discussion

The taxonomic identities of 27 genera and 44 species from approximately 95 rhizospheric root-associated bacteria isolated from rhizospheric soil samples of grape, grown at 3 sites were determined. Identification of the bacterial isolates was more successful in the grapevine rhizosphere samples expressing an overall identification of about 74.2% of the total isolates. Characterization of the isolates on the basis of their FAME profiles revealed the presence of both Gram-positive and Gram-negative bacteria within the grape rhizosphere soils although larger number was that of Gram-negative. Among the Gram positives N_2-fixing bacteria, the dominant ones were *Bacillus* spp., followed by *Brevibacillus* spp. and *Paenibacillus* spp. Among the Gram-negative *Pseudomonas* spp. dominated and were followed by *Stenotrophomonas* sp. The data obtained show a greater abundance of Gram-negative bacteria in the grape rhizosphere, in agreement with previous studies [15,39] that show a higher level of Gram-negative species in the rhizosphere relative to Gram-positive species. In contrast, other studies show a higher level of Gram-positive species in the rhizosphere relative to Gram-negative species [11,41,42].

The soils of Akdamar and Kemalpaşa sites sampled in the present study had pH values that ranged from 7.1 to 8.7. Cultivated nitrogen-fixing bacterial community from native grape rhizosphere samples and represented members of the genera *Pseudomonas, Bacillus*, *Paenibacillus*, *Brevibacillus*, *Serratia*, *Kocuria* and *Variovorax* were commonly found in alkaline soils. The results obtained indicated that *Bacillus*, *Stenotrophomonas* and *Citrobacter* genera were the most prominent N_2-fixing groups in the acidic grape rhizosphere (pH, 4.6–6.6) and soil populations of the Storm Valley. These bacteria are often identified as dominant taxa of cultivable microbial populations from rhizosphere of various crop plants [3,9,10,26,35,42]. The widely studied *Pseudomonas, Bacillus* and *Paenibacillus* genus represents one of the most diverse genera in the plant rhizosphere and soil populations [7,11,33] and these species can be characterized with the ability to tolerate unfavourable conditions [8].

In addition, the majority of the isolates were salt tolerant up to 3% NaCl and pH-tolerant. Salt- and pH-tolerant traits of these strains might be of some significance for its survival in high salt accumulated and alkaline soils. The pH tolerance investigations have highlighted the fact that our strains possess wide ecological tolerance values. Our study indicated that habitat had a strong influence on the diversity of N_2-fixing species that were the most abundant among the isolates in plant rhizosphere. PGPR strains isolated from the rhizosphere of different crops have been developed in different acidic soils [36,45], and acidic and alkaline grapevine environments have never been studied before.

The distribution patterns of the bacterial species differed among the three different site (Table 2). For example, Among the 95 strains obtained in grape, N_2-fixing strains of *Acidovorax delafieldii*, *Pseudomonas mendocina*, *A. ichthiosmia*, *Hafnia alvei*, *Raoultella terrigena*, *Paenibacillus alginolyticus*, *A. aurescens*, *K. kristinae*, *C. flaccumfaciens*, *F. johnsoniae* and *S. spiritivorum* were isolated only from alkaline Akdamar Island, but not from others sites. In contrast, *Ralstonia eutropha*, *C. amalonaticus*, *Enterobacter hormaechei* and *A. globiformis* were isolated only from acidic Storm Valley and *Rhizobium rubi*, *Paracoccus denitrificans*, *P. syringae*, *Citrobacter freundii*, *Serratia odorifera*, *A. oxydans*, *K. rosea* and *B. epidermidis* were from Kemalpaşa. It was also notable that a number of N_2-fixing and P-solubilizing *P. agglomerans* and *S. marcescens* were isolated from rhizosphere of grape at Kemalpaşa, *V. fluvialis* was from Storm Valley. N_2-fixing and P-solubilizing and/or siderophores-producing strains of *P. putida* and *S. maltophilia* were isolated more frequently from Akdamar and Storm Valley, while the *B. atrophaeus*, *B. megaterium* and *P. fluorescens* were isolated from all sites the rhizosphere of grape.

Members belonging to *Gammaproteobacteria* were the most abundant among the isolates in most cases, with many of these isolates assigned to *Pseudomonas* in the rhizosphere soils of the grapevine, whereas the composition of species was different according to the environment. *Bacillus* was the next most common genus, and the species composition was again significantly different between samples. These results indicated that the pH of the soil and habitat had a strong influence on the distribution of N_2-fixing and P-solubilizing species that were frequently isolated in grape rhizosphere. However, PGPR strains isolated from the wild grapevine in alkaline and acidic environments have never been studied before. The composition of the rhizobacterial community associated with plant roots is influenced by a variety of sites, soil pH and type, and environmental factors.

Several strains of bacilli, mainly species of the genera *Bacillus* and *Paenibacillus*, displaying important PGP characteristics were isolated from twenty wild grape rhizosphere soils. Among the strains isolated in the present study *B. megaterium*, *B. atrophaeus*, *B. cereus*, *B. mycoides*, *P. macquariensi, P. alginolyticus*, and *B. choshinensis* have also been isolated from the rhizosphere as N_2-fixers [11,39,41,47]. Among the N_2-fixing and P-solubilizing actinomycetes isolated in the present study, *K. rosea*, *B. epidermidis*, *C. flaccumfaciens*, and *M. barkeri* were previously reported as N_2-fixers or P-solubilizers [11,40,41].

In the present study, several pseudomomads were identified to have N_2-fixing and P-solubilizing and/or siderophores-producing properties from the rhizosphere of wild grape. Most of the species had the ability to fix nitrogen and the majority of the strains were the most powerful phosphate solubilizers and/or siderophores producer such as *P. fluorescens*, *P. putida*, *P. mendocina* and *P. syringae* [11,23,32,33,35,39,47], *S. maltophilia* [35] and *A. ichthiosmia* [39] and nitrogen-fixing *S. acidaminiphila* [11] have also been frequently isolated from the rhizosphere of a variety of other plants.

Acidic and alkaline wild grapevine rhizosphere soils for the predominant N_2-fixing and P-solubilizing *Enterobacteriales* and *Vibrionales* species, from which some are known as N_2-fixing and P-solubilizing strains *C. freundii*, *C. amalonaticus*, *H. alvei*, *R. terrigena*, *P. agglomerans, S. marcescens*, *S. odorifera* and *V. alginolyticus* and the newly found species, are also important for plant growth as a result of their great capacity to fix nitrogen and/or solubilize phosphate [6,11,14,41,46,47]. Among the Gram-negative N_2-fixing and/or P-

solubilizing α- and β-proteobacterial strains isolated in the present study *R. radiobacter* and *V. paradoxus* were previously reported as dinitrogen fixers [5,39]. Five Gram-negative N_2-fixing *Bacteroidetes*, four of them were isolated from rhizosphere of grape in alkaline and calcareous of Akdamar Island. Nitrogen fixing *S. spiritivorum* [39], plant growth promoting *C. balustinum* and *F. johnsoniae* [28,29], and *C. indoltheticum* were able to fix nitrogen.

Although it is well known that many species of *Bacillus* and *Paenibacillus* can contribute to plant growth and health in many ways, there are only a few studies concerning the acid tolerant strain of P-solubilizing and N_2-fixing bacteria. In this study, several alkaline and/or acid tolerant strains displaying important PGP characteristics (nitrogen-fixer and phosphate-solubilizers and/or siderophore-producers) were isolated, and some strains (e.g., 5 isolates of *P. putida* biotype B, 2 isolates each of *P. putida* biotype A, *P. fluorescens* and *S. maltophilia*, a single isolate each of *B. atrophaeus*, *B. megaterium*, *V. paradoxus* and *S. marcescens*) proved to be very efficient in promoting the growth of grapevine. Some of the above tested isolates could exhibit more than two or three PGP traits, which may promote plant growth directly or indirectly or synergistically. This strain could be useful in the formulation of new inoculants, improving the cropping systems into which it can be most profitably applied. The identification and the isolation of PGP bacteria from acidic and alkaline soils, which combine the ability to solubilize phosphate and to produce siderophore with the fixation nitrogen capable of promoting plant growth, could also significantly increase the productivity of crops in acidic and alkaline soil.

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