

DIVERSITY AND METABOLIC POTENTIAL OF CULTURABLE N₂-FIXING AND P-SOLUBILISING BACTERIA FROM RHIZOSPHERE OF WILD CROPS IN VAN LAKE BASIN -TURKEY

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Abstract

The diversity of phosphate solubilizing and nitrogen-fixing bacteria from rhizospheric soil samples of different plant species (native wild cereals, beet, stonecrops, onion, rose, raspberry, and 10 other plant genera) grown at four different locations in the Van Lake Basin, Turkey were investigated. A total of 169 rhizospheric soil samples were gathered from these plant species, and over 777 rhizoplane bacteria were haphazardly selected from agar-solidified trypticase soy broth and examined for fatty acid methyl ester (FAME) reports. As a result, 57 bacterial genera were recognised, which 64.8% gone to five genera viz., *Bacillus* (29.6%), *Pseudomonas* (9.8%), *Stenotrophomonas* (9.7%), *Paenibacillus* (5.7%), *Micrococcus* (5.1%), and *Arthrobacter* (4.9%). Approximately 56.8% of bacteria were found to be gram-positive (GPB), while 43.2% as gram-negative bacteria (GNB). In the of total GPB, 56.3% were N₂-fixing bacteria and 46.6% were P-solubilising bacteria, while in the case of GNB, 43.7% were N₂-fixing bacteria and 53.4% were P-solubilising bacteria. A total of 651 bacteria were isolated from the rhizospheric samples of the 20 plant genera, and subjected to further analysis. Of the 651 isolates, 542 were able to fix nitrogen, 279 were able to solubilize phosphates, and 247 isolates could fix both nitrogen and solubilize phosphates all at once. *Bacillus*, *Pseudomonas*, *Stenotrophomonas*, *Paenibacillus*, *Micrococcus*, *Serratia*, and *Pantoea* genera were the most prominent N₂-fixing and P-solubilising groups. Additionally, *B. megaterium*, *B. atrophaeus*, *B. cereus*, *B. pumilus*, *B. licheniformis*, *B. subtilis*, *Pb. polymyxa*, *Pb. macerans*, *Pb. macquariensis*, *Bb. choshinensis*, *Ste. maltophilia*, *Ps. fluorescens*, *Ps. putida*, *Ps. stutzeri*, *Pn. agglomerans*, *Mic. luteus*, *Rco. erythropolis*, *Kocuria rosea*, *Csb. Meningosepticum*, and *Serratia fonticola* were the mainly frequent P-solubilising and N₂-fixing species in the native rhizosphere soils of plants.

Key words: Plant growth promoting rhizobacteria, Wild cereals, Beet, Isolation, Biodiversity.

Introduction

The colonization of microorganisms in the rhizosphere influences the plant growth depending upon their nature and function. Plant growth-promoting rhizobacteria (PGPR) are beneficial that live in association with plants, stimulate plant growth and protect plants from environmental stresses through a variety mechanisms (Asghar *et al.*, 2002; Çiğ *et al.*, 2021). Many PGPR are recommended as “soil inoculants” to secure high yields in different crops under adverse conditions. Moreover, the PGPR, for example N₂-fixing bacteria (NFB) and P-solubilising bacteria (PSB), could be regarded as possible alternatives to inorganic fertilizers that are harmful to our environment and the ecosystem. The PGPR might facilitate a healthy environment and ecosystem for future generations. PGPR will receive the research attention of agricultural researchers (Park *et al.*, 2005a, b; Chen *et al.*, 2006; Çakmakçı 2006, 2007a, b; Yao *et al.*, 2008; Hariprasad & Niranjana, 2009; Ahmad *et al.*, 2022).

The presence of plant growth-promoting NFB and PSB and the probability of a significant improvement in plant performance and yield under the limitation of nutrient conditions by root-connected bacteria have been

considered for many years. Studies compared microbial communities among soils from different ecosystems (Fischer *et al.*, 2007), fertilizing needs (Poonguzhali *et al.*, 2006), land-use histories (Benizri & Amiaud, 2005), cropping regimes (Larkin, 2003), environmental factors along with different management practices (Park *et al.*, 2005b). Several research looked at the effects of different plant species, soil properties, varietal differences within the plant species, and genetic improvements of plants on rhizosphere microbial population structures (Poonguzhali *et al.*, 2006; Beneduzi *et al.*, 2008; Crump & Koch, 2008; El Sabagh *et al.*, 2020).

Environmental factors have greater influence on the bacterial community residing in the root zone as well as in the rhizosphere (Poonguzhali *et al.*, 2006; Costa *et al.*, 2006); while some researchers (Fierer & Jackson, 2006) suggested that the nature of soil has the greatest effect on PGPR. Previous isolations of NFB have shown a wide-ranging diversity of diazotrophs to inhabit the crop rhizosphere (Vessey, 2003). Bacteria in soil are very varied, numerous, and functionally imperative, and they have historically been an important research topic by microbiologists (Topp, 2003). PGPR are associated with many plant species and are commonly present in several environmental

conditions (Compant *et al.*, 2005; Islam *et al.*, 2021). Screening of new isolates in a wide range of environmental conditions is essential to observe their activity. Besides, native plants have unique biology and endemism. Thus, it is crucial to study the isolation of native strains where they may be used as non-legumes and/or cereal inoculants. It is believed that novel rhizospheric PGPR may be discovered from these plants. Studying rhizospheric bacteria is hard due to the high number of bacteria present in the soil. Conversely, classification and recognition of these bacteria in the rhizosphere are essential for ecological studies (Peix *et al.*, 2003).

The research on the various PGPR species found in Eastern Anatolia soils will continue indefinitely, particularly those PGPR that can fix nitrogen and solubilize phosphate. Therefore, the present study aims to separate and classify the PGPR in the rhizosphere of native plants at four different locations in Van Lake, Turkey, and to evaluate their N₂-fixing and P-solubilizing capabilities.

Materials and Methods

Soil samples, isolation and identification of bacteria:

To characterize N₂-fixation and P-solubilisation, the diazotrophic bacteria from the rhizosphere of different wild plants including (*Triticum* spp., *Aegilops* spp., *Hordeum* spp., *Secale* spp., and *Avena* spp.), wild beet (*Beta* spp., and *Corollinae* spp.), stonecrops (*Sedum* spp.), onion (*Allium* spp.), rose (*Rosa* spp.), raspberry (*Rubus* spp.) and others (*Orchis* spp., *Sempervivum* spp., *Salvia* spp., *Traxacum* spp., *Fragaria* spp., *Gladiolus* spp., *Inula* spp., *Helichrysum* spp., *Peganum* and *Acroptilon* spp.) grown in the Van Lake basin were isolated. Native wild plants grown in various agro-climatic regions in Van Lake were selected to isolate bacterial communities from May to September 2007-2008. Four sites along the geographic gradient of the Van Lake in Turkey were selected. The average annual precipitation of these sites varies between 390 and 470 mm across the study sites. Ulupamir is located in the northern part of Van City, which comes under Turkey's continental climate zone. The Zeylan

valley, elevation of 1850 to 3500 m, is a geothermal area located 30 km north of Erciş and Van Lake. *Tendirek mountain* (2100–3530 m elevation) is an area north of Van Lake in which volcanic rocks are represented by alkali basalts (39°22' 12' N and 43°52' 12' E). The Artos Mountain karstic carbonate rocks (1900-3550 m elevation) are also found on the south coast of Van Lake and cover heavily degraded and overgrazed tragacanth steppe and/or calcareous mountain slopes (38° 15' N and 43° 6' E). The soil samples were collected from the experimental sites and analyzed in the laboratory. The soil was generally sandy, sandy loam, clay loam, and sandy clay loam in texture; the soil pH values ranged from 4.9 to 9.1 (average 7.4); the organic matter content ranged from 0.43 to 4.78%; and the N, P, and K content of soil were determined as 0.01-0.18%, 0.4-21.6 mg kg⁻¹, and 15-221 mg kg⁻¹, respectively. Except for three plants rhizosphere samples (stonecrops, raspberry, and rose), the pH value of rhizosphere soils of wild plants had a slightly alkaline reaction (Table 1). The region has been surveyed and 169 soil samples (61 from wild cereals, 24 wild beta, 16 stonecrops, 12 raspberries, eight onions, eight roses, and 40 from other species) from four locations were collected (Table 1). Five plant samples were taken for rhizosphere soil sampling within each geographic zone, and bacterial isolations were performed on the same day.

For bacterial isolation, ten grams of rhizospheric soil were collected and mixed thoroughly. Sampled plants and non-rhizospheric soil were packed in polythene bags and brought to the laboratory for air drying. Rhizospheric soil of 10 g was measured and moved into an Erlenmeyer flask with 100 mL of sterile water, and placed on a shaker set at 150 rpm for 30 min. To prepare a series of 10-fold dilutions, 1 mL of aliquot was piped into 9 mL of sterile water. The final dilution was 10⁵- fold; 0.1 ml of each dilution (with three replicates) of the series was placed into a Petri-dish followed by placing them in an incubator for seven days at 28°C. With the help of fatty acid methyl ester (FAME) profiles, rhizobacteria were recognized randomly from the agar-solidified trypticase soy broth. The recognised bacterial strains were cultured in nutrient broth (NB) with 30% glycerol at -86°C for further examination at the genus level.

Table 1. Number and average pH values of rhizosphere soil samples, and enumeration of colony forming units (CFU) grown on N-free medium plates.

Sampling plants	Number of soil samples	Average soil pH	Minimum-Maximum	Mean ^b
<i>Triticum</i> spp.	32	7.30	4.6 x 10 ⁵ - 5.4 x 10 ⁸	3.9 x 10 ⁷ e
<i>Aegilops</i> spp.	8	7.90	5.2 x 10 ⁸ - 9.9 x 10 ⁸	8.4 x 10 ⁸ a
<i>Hordeum</i> spp.	8	7.33	1.2 x 10 ⁷ - 1.7 x 10 ⁸	4.3 x 10 ⁷ e
<i>Secale</i> spp.	8	7.12	1.4 x 10 ⁵ - 2.7 x 10 ⁸	6.9 x 10 ⁷ de
<i>Avena</i> spp.	8	7.81	1.6 x 10 ⁸ - 9.7 x 10 ⁸	5.8 x 10 ⁸ b
<i>Beta</i> spp.	24	7.67	2.1 x 10 ⁵ - 2.7 x 10 ⁸	4.3 x 10 ⁷ e
<i>Sedum</i> spp.	16	6.36	2.1 x 10 ⁵ - 6.0 x 10 ⁷	6.8 x 10 ⁶ e
<i>Rubus</i> spp.	12	5.67	3.1 x 10 ⁵ - 1.6 x 10 ⁸	3.3 x 10 ⁷ e
<i>Allium</i> spp.	8	7.85	1.1 x 10 ⁷ - 1.1 x 10 ⁸	4.9 x 10 ⁷ e
<i>Rosa</i> spp.	8	6.83	1.0 x 10 ⁸ - 3.8 x 10 ⁸	2.7 x 10 ⁸ c
Others ^a	40	7.34	4.2 x 10 ⁷ - 8.9 x 10 ⁸	1.6 x 10 ⁸ d

^a*Orchis* spp., *Sempervivum* spp., *Salvia* spp., *Taraxacum* spp., *Fragaria* spp., *Gladiolus* spp., *Inula* spp., *Helichrysum* spp., *Peganum* spp., *Acroptilon* spp.; ^bDifferent lowercase letters indicate significant differences (p<0.01)

Extraction of cellular fatty acids and FAME profiling: TSBA was used to grow the bacterial cells overnight. By using a standard technique, bacterial cells (50 mg) were extracted to obtain the FAME profiles to select and identify bacterial strains, species, and genera (Caesar-TonThat *et al.*, 2007). Moreover, they provide insights into the microbial communities' structural and functional attributes (Larkin, 2003). According to Oka *et al.*, (2000) and Poonguzhali *et al.*, (2006), strains containing ≥ 0.3 similarity index (SIM) value are considered a good match. The physio-morphological and biochemical characterization (pigment production, antioxidant capacity, sucrose, amylase, starch, nitrate reductase) of bacterial strains was performed on an N-free basal medium at 36°C (Forbes *et al.*, 1998).

Phosphate solubilization and nitrogen fixation potential: To detect the activity of the phosphate solubilization bacterial isolates, Pikovskaya (PVK) and phosphate growth medium (NBRIP-BPB) of the National Botanical Research Institute were used. The NBRIP-BPB contained (per liter) 5 g of $MgCl_2 \cdot 6H_2O$, 10 g of $Ca_3(PO_4)_2$, 0.25 g of $MgSO_4 \cdot 7H_2O$, 0.2 g of KCl, 20 g of glucose, 0.025 g of bromophenol blue (BPB), and 0.1 g of $(NH_4)_2SO_4$. Bromophenol blue containing pH 7.0 was used as an indicator to compare the halo formation reproducibility (Mehta & Nautiyal, 2001). NBRIP-BPB medium of 5 mL was autoclaved in a test tube. In contrast, previously autoclaved broth medium was used as a comparison control. The tested bacterial strains of 500 μ l suspension were subjected to the sterile liquid medium. Incubation of test tubes was performed at room temperature for 14 days. According to Pikovskaya (1948), all isolates had phosphorus solubilizing capacity on agr media. Vanadomolybdophosphoric acid colorimetric method was used to determine the soluble P and these bacteria were examined according to Döbereiner (1988), Han *et al.*, (2005), and Rau *et al.*, (2009).

Results

A total of 777 colonies (276 from the cereals and 501 from the other wild plant species) were selected from the rhizosphere in the Van Lake basin, Turkey. The MIDI system identified (SIM > 0.3) 83.0% (229 out of 276) and 84.2% (422 out of 501) rhizospheric bacteria from the cereals and other plant species for a total of 30 and 55 different genera, respectively (Tables 2, 3). The findings of the current investigation stated that the MIDI system was unable to identify the 777 isolates (3.5%), while 12.7% of the isolates were identified with a SIM < 0.3 , which showed uncertainty of results.

Isolated bacteria were classified into four divisions through FAME profiles analysis, viz., i) γ , β and α -subdivisions of *Proteobacteria* (33.2, 4.2, and 0.6%, respectively), ii) *Bacteroidetes* (5.2%), iii) *Actinobacteria* (16.1%), and iv) *Firmicutes* (40.7%). It comprised 43.2% of gram-negative bacteria and 56.8% of gram-positive bacteria. Gram-negative bacteria (281 isolates out of 651: 195 from other plants and 86 from cereals) included 216 γ -proteobacteria, 31 α - and β -

proteobacteria, and 34 separates belonged to the *Bacteroidetes* group. *Achromobacter* and *Alcaligenes* fall within the β -proteobacterial genera, while γ -proteobacterial genera have *Pseudomonas*, *Stenotrophomonas*, *Serratia*, and *Pantoea*.

The 370 gram-positive isolates were from two bacterial divisions, viz., 265 *Firmicutes* (104 isolated from the cereals and 161 from others), and 105 *Actinobacteria* (39 isolated from the cereals and 66 from the other plants). Three orders, i.e., *Actinomycetales*, *Bacillales*, and *Lactobacillales* were included in the gram-positive group. Genus *Enterococcus* was represented by the order *Lactobacillales*. Similarly, five different genera viz., *Bacillus*, *Paenibacillus*, *Brevibacillus*, *Kurthia*, and *Staphylococcus*, fall within the most diverse *Bacillales* order. The order *Actinomycetales* includes 10 genera, viz., *Cellulomonas*, *Kocuria*, *Arthrobacter*, *Micrococcus*, *Rhodococcus*, *Kytococcus*, *Curtobacterium*, *Brevibacterium*, *Microbacterium*, and *Nocardia* (Tables 2 and 3).

The *Enterobacteriales*, *Pseudomonads*, and *Xanthomonads* group were the most dominated, with five species identified (*St. Maltophilia*, *Pseudomonas fluorescens*, *P. putida*, *Pn. agglomerans*, and *Serratia fonticola*) among non-eteric gram negative bacteria. Among gram-negative bacteria, 10 strains of *Pseudomonas agarici*, seven strains of *Lysobacter enzymogenes*, seven strains of *Alcaligenes faecalis*, four strains of *Pseudomonas stutzeri*, and *Rahnella aquatilis* were recognized. The genus *Bacillus* was 52.2% of the gram-positive, with an occurrence of *B. megaterium* (46.1%), followed by *B. cereus* (14.5%), *B. atrophaeus* and *B. subtilis*. The *Bacillus* group, with 10 other species identified, was the most abundant group (*Bacillus* sp., *B. pumilus*, *B. psychrosaccharolyticus*, *B. sphaericus*, *B. laevolacticus*, *B. mycoides*, *B. thuringiensis*, *B. licheniformis*, *B. coagulans*, and *B. oleronius*). The gram-positive species of the genus *Bacillus* (*B. megaterium*, *B. cereus*, *B. atrophaeus*, *B. subtilis*, *B. pumilus*, and others) showed the highest species abundance, representing more than 35.8% of the isolates (68% isolated mainly from wheat) obtained in cereals and almost 26.3% of those (57% from wild beet and stonecrops) obtained in the rhizosphere of other plant species (Tables 2, 3). The Gram-positive *Paenibacillus* genus, with eight species identified (*Pb. polymyxa*, *Pb. validus*, *Pb. macquariensis*, *Pb. macerans*, *Pb. larvae*, *Pb. lentimorbus*, *Pb. azotofixans*, and *Pb. alginolyticus*), was the second most abundant group. Among the other gram-positive bacteria, 28 strains of *Mic. luteus*, ten nine strains of *Bb. choshinensis*, and ten five strains of *Kocuria rosea* and 10 strains of *Arb. globiformis* and *Arb. agilis* were identified.

Rhizobacterial isolates of 651 taken from wild plant rhizosphere had numerous common characteristics as observed from physio-morphological and biochemical analysis. According to the obtained results, oxidase and catalase activity were 47.2 and 86.8%, while sucrose and amylase showed 24.3 and 22.9% activity, respectively. These isolates also presented significant variances in their phosphate solubilizing potential. Their solubilization range varied from 16.7 to 174.6 mg L⁻¹ liquid medium.

Table 2. Diversity of bacterial associated with wild *Triticum*, *Aegilops*, *Hordeum*, *Secale* and *Avena* spp.

Taxonomic identification/ Order	Bacterial strain FAME identification	Number of isolates*				
		<i>Triticum</i> spp.	<i>Aegilops</i> spp.	<i>Hordeum</i> spp.	<i>Secale</i> spp.	<i>Avena</i> spp.
Betaproteobacteria						
<i>Burkholderiales</i>	<i>Acidovorax facilis</i>				1 (1/1)	
	<i>Achb. xylos. denitrificans</i>	2 (2/2)		2 (2/0)		
	<i>Alcaligenes latus</i>				1 (1/0)	
	<i>Alcaligenes faecalis</i>			2 (1/0)		2 (1/1)
	<i>Variovorax paradoxus</i>	1 (1/1)				
Gammaproteobacteri						
<i>Xanthomonadales</i>	<i>Ste. maltophilia</i>	9 (7/2)	1 (1/0)	3 (3/1)	6 (5/2)	5 (5/3)
<i>Pseudomonadales</i>	<i>Pseudomonas aeruginosa</i>	1 (1/0)				
	<i>Pseudomonas agarici</i>	2 (2/1)				1 (1/0)
	<i>Pseudomonas alcaligenes</i>	1 (1/1)				
	<i>Pseudomonas fluorescens</i>	6 (5/4)		1 (1/1)	1 (1/1)	
	<i>Pseudomonas putida</i>	2 (1/1)		1 (1/1)		
	<i>Pseudomonas savastanoi</i>	2 (2/1)				
	<i>Acinetobacter calcoaceticus</i>				1 (1/0)	
<i>Vibrionales</i>	<i>Photobacterium angustum</i>	2 (2/1)				
<i>Enterobacteriales</i>	<i>Hafnia alvei</i>		2 (2/2)			
	<i>Salmonella typhimurium</i>	1 (1/1)	1 (1/1)	1 (1/1)		
	<i>Serratia fonticola</i>	1 (1/0)	2 (2/1)		1 (1/0)	3(2/2)
	<i>Serratia plymuthica</i>	1 (1/1)		1 (1/1)		
	<i>Yersinia bercovieri</i>			1 (1/1)		
	<i>Rahnella aquatilis</i>	1 (1/0)	1 (1/1)			
	<i>Pantoea agglomerans</i>	4 (4/2)			1 (1/1)	
Firmicutes						
<i>Bacillales</i>	<i>Bacillus atrophaeus</i>	9 (7/6)		1 (1/0)		
	<i>Bacillus cereus</i>	7 (5/3)				3 (2/1)
	<i>Bacillus coagulans</i>	2 (2/0)				
	<i>Bacillus laevolacticus</i>	2 (2/0)				
	<i>Bacillus megaterium</i>	28 (26/12)		2 (1/1)	8 (7/4)	6 (3/2)
	<i>Bacillus mycoides</i>				1 (1/0)	
	<i>Bacillus pumilus</i>	1 (1/1)	1 (1/1)		1 (1/0)	
	<i>Bacillus</i> sp.	5 (3/0)				
	<i>Bacillus subtilis</i>			1 (1/0)	2 (1/0)	
	<i>Bacillus thuringiensis</i>	2 (2/0)				
	<i>Paenibacillus alginolyticus</i>	1 (1/0)				
	<i>Paenibacillus macquariensis</i>	2 (2/0)				
	<i>Paenibacillus polymyxa</i>	5 (4/1)		3 (3/3)	1 (1/0)	
	<i>Paenibacillus validus</i>	1 (1/0)		1 (1/1)		
	<i>Brevibacillus choshinensis</i>	3 (3/0)				1 (1/1)
<i>Staphylococcus cohnii</i>	2 (1/1)					
<i>Kurthia sibirica</i>	2 (1/0)					
Actinobacteria						
<i>Actinomycetales</i>	<i>Micrococcus luteus</i>	8 (7/2)		3 (3/0)		4 (3/1)
	<i>Arthrobacter agilis</i>	6 (5/3)				
	<i>Arb. histidinolorans</i>	1 (1/1)				
	<i>Arthrobacter viscosus</i>	2 (2/0)				
	<i>Kocuria rosea</i>	6 (5/2)				
	<i>Kocuria kristinae</i>	1 (1/1)				
	<i>Brevibacterium epidermidis</i>	2 (2/0)		1 (1/0)		
	<i>Cellulomonas fimi</i>	2 (2/0)				
	<i>Cellulomonas turbata</i>	1 (1/0)				
	<i>Microbacterium barkeri</i>					1 (1/0)
	<i>Rhodococcus erythropolis</i>	1 (1/1)				
Bacteroidetes						
<i>Flavobacteriales</i>	<i>Bergeyella zoohelcum</i>	3 (2/2)			1 (1/1)	1 (1/0)
	<i>Weeksella virosa</i>			2 (2/2)		
<i>Sphingobacteriales</i>	<i>Sphingobacterium faecium</i>	1 (1/0)				
No library match		5	1	3	4	1
Unidentified**		15	3	5	4	6
Total		162 (123/54)	12 (8/6)	33(24/13)	34 (23/10)	34(20/11)

*Numbers in parentheses indicate the number of N₂-fixing/P-solubilizing strains where bacterial genera were detected;

**Isolates number with a similarity index < 0.3

Table 3. Diversity of bacterial associated with wild *Beta*, *Sedum*, *Rubus*, *Allium*, *Rosa* and other plants.

Taxonomic identification/ Order	Bacterial strain FAME identification	Number of isolates ^a					
		<i>Beta</i> spp.	<i>Sedum</i> spp.	<i>Rubus</i> spp.	<i>Allium</i> spp	<i>Rosa</i> spp.	Others plants ^b
<i>Betaproteobacteria</i>							
<i>Burkholderiales</i>	<i>Achb. Xylos. denitrificans</i>			1 (1/1)	2 (2/1)	1 (1/1)	2 (2/1)
	<i>Alcaligenes faecalis</i>		1 (1/1)		2 (2/2)		
	<i>Delftia acidovorans</i>	1 (1/1)					
	<i>Duganella zoogloeoides</i>	1 (0/1)					
<i>Gammaproteobacteria</i>							
<i>Xanthomonadales</i>	<i>Lysobacter antibioticus</i>						1 (1/0)
	<i>Lysobacter enzymogenes</i>		1 (1/0)				
	<i>Ste. maltophilia</i>	11 (9/6)	5 (4/1)	2 (2/1)	3 (3/1)	6 (5/1)	12 (12/6)
<i>Pseudomonadales</i>	<i>Pseudomonas aeruginosa</i>	2 (1/1)					
	<i>Pseudomonas agarici</i>		2 (2/1)	2 (2/1)			3 (2/1)
	<i>Pseudomonas alcaligenes</i>	1 (1/1)		1 (1/0)			
	<i>Pseudomonas fluorescens</i>	3 (3/2)			3 (2/1)		5 (4/2)
	<i>Pseudomonas putida</i>	4 (3/3)	4 (3/0)	2 (1/0)	2(2/0)	1 (1/1)	3 (3/1)
	<i>Pseudomonas stutzeri</i>	2 (2/2)					2 (2/1)
	<i>Pseudomonas mendocina</i>						2 (2/1)
	<i>Pseudomonas syringae</i>						2 (1/1)
	<i>Acinetobacter baumannii</i>	1 (1/0)					
	<i>Acn. calcoaceticus</i>		1 (0/1)				
	<i>Acn. haemolyticus</i>			1 (1/0)			
	<i>Aeromonadales</i>	<i>Aeromonas ichthiosmia</i>			1 (1/1)		
<i>Aeromonas hydrophilia</i>							1 (1/0)
<i>Enterobacteriales</i>	<i>Citrobacter freundii</i>		1 (1/1)				1 (1/1)
	<i>Enterobacter asburiae</i>						1 (1/1)
	<i>Kluyvera cryocrescens</i>				2 (2/2)		
	<i>Proteus vulgaris</i>			1 (1/1)	1 (1/1)		
	<i>Serratia fonticola</i>	1 (1/1)	2 (2/1)			1 (1/1)	3 (2/2)
	<i>Serratia grimesii</i>	2 (2/2)					
	<i>Serratia odorifera</i>			1 (1/0)			
	<i>Rahnella aquatilis</i>	2 (2/2)					
	<i>Pantoea agglomerans</i>	1 (1/1)	2 (2/2)		2 (2/1)	1 (1/1)	4 (3/3)
	<i>Firmicutes</i>						
<i>Bacillales</i>	<i>Bacillus atrophaeus</i>	6 (5/ 2)	3 (2/1)			1 (0/0)	1 (1/1)
	<i>Bacillus cereus</i>	7 (6/3)	4 (2/1)	3 (3/1)		1 (1/0)	3 (2/1)
	<i>Bacillus laevolacticus</i>	2 (2/1)					
	<i>Bacillus licheniformis</i>			1 (1/1)			2 (2/2)
	<i>Bacillus megaterium</i>	18 (15/8)	7 (6/2)	4 (3/1)	1 (1/1)	3 (3/2)	12 (10/3)
	<i>Bacillus mycoides</i>	1 (1/1)		1 (1/1)			
	<i>Bacillus oleronius</i>	1 (1/0)					
	<i>B. psychrosaccharolyticus</i>	1 (1/0)		4 (3/1)	1 (1/1)	1 (1/0)	
	<i>Bacillus pumilus</i>	1 (1/0)		5 (3/2)			

Table 3. (Cont'd.).

Taxonomic identification/ Order	Bacterial strain FAME identification	Number of isolates ^a					
		<i>Beta</i> spp.	<i>Sedum</i> spp.	<i>Rubus</i> spp.	<i>Allium</i> spp	<i>Rosa</i> spp.	Others plants ^b
	<i>Bacillus</i> sp	1 (1/0)		1 (0/0)			1 (1/0)
	<i>Bacillus sphaericus</i>	2 (2/0)	1 (0/0)		1 (0/1)		
	<i>Bacillus subtilis</i>	1 (1/0)		3 (2/2)		2 (2/0)	2 (2/0)
	<i>Bacillus thuringiensis</i>		1 (1/1)				
	<i>Paenibacillus azotofixans</i>	1 (1/0)			1 (1/0)		
	<i>Paenibacillus larvae</i>	2 (2/1)					
	<i>Paenibacillus lentimorbus</i>	1 (1/1)					
	<i>Pb. macquariensis</i>		1 (1/1)				2 (2/0)
	<i>Paenibacillus macerans</i>	1 (1/1)	1 (1/1)		1 (1/1)	1 (1/0)	1 (1/1)
	<i>Paenibacillus polymyxa</i>	2 (2/2)		3 (2/0)		1 (1/0)	2 (1/1)
	<i>Paenibacillus validus</i>			1 (1/0)			1 (1/1)
	<i>Brevibacillus choshinensis</i>	6 (4/0)	2 (2/1)			1 (1/0)	6 (5/2)
	<i>Brevibacillus centrosporus</i>						4 (3/0)
	<i>Brevibacillus reuszeri</i>						1 (0/1)
	<i>Kurthia sibirica</i>	1 (0/1)					1 (1/0)
Actinobacteria							
<i>Actinomycetales</i>	<i>Micrococcus luteus</i>	4 (3/1)	1 (1/0)	3 (1/1)			5 (5/4)
	<i>Micrococcus lylae</i>		4 (1/0)			1 (1/0)	
	<i>Arthrobacter agilis</i>	1 (1/1)		1 (1/0)			2 (2/0)
	<i>Arthrobacter aurescens</i>	1 (1/1)	2 (2/0)				
	<i>Arb. crystallopoietes</i>						1(1/0)
	<i>Arb. histidinolorans</i>	1 (1/1)	1 (1/0)	1 (1/0)			
	<i>Arthrobacter globiformis</i>	2 (2/0)	1 (1/1)	1 (1/0)			6 (4/1)
	<i>Arthrobacter ramosus</i>					1 (1/1)	
	<i>Arthrobacter viscosus</i>					1 (1/1)	
	<i>Kocuria rosea</i>		1 (1/0)	2 (1/1)		4 (3/2)	2 (2/1)
	<i>Kocuria kristinae</i>	1 (1/0)					
	<i>Brb. epidermidis</i>	1 (1/0)					
	<i>Brb. liquefaciens</i>		2 (2/1)		1 (1/0)		
	<i>Brevibacterium lyticum</i>						1 (1/0)
	<i>Cub. flaccumfaciens</i>	1 (1/1)					1 (1/0)
	<i>Rhodococcus erythropolis</i>	1 (1/0)					1 (1/1)
Bacteroidetes							
<i>Flavobacteriales</i>	<i>Csb. balustinum</i>			3 (3/2)		1 (1/0)	
	<i>Csb. meningosepticum</i>	2 (1/2)	6 (4/4)	2 (2/1)	3 (2/0)		2 (2/0)
<i>Sphingobacteriales</i>	<i>Sph. spiritivorum</i>		2 (2/2)	1 (1/1)			
	<i>Sph. multivorum</i>		2 (2/2)				
Others ^c		14 (10/6)	8 (4/3)	4 (3/2)	5 (3/3)	7 (4/3)	11 (6/5)
No library match		6	2	1	2		2
Unidentified ^d		16	11	8	4	3	24
Total		138 (97/57)	82 (52/29)	65(44/22)	37(26/16)	39 (30/14)	140(95/47)

Tables 1 and 2 showed that 279 out of the 651 (42.9%) tested isolates were able to solubilize phosphate, and 542 isolates (83.3%) could fix nitrogen. Among the 651 bacterial strains, 247 were efficient at N₂-fixation and P-solubilization at the same time. Nitrogen fixation and phosphate solubilisation were detected in 93 and 73% of isolates of *Pantoea*, followed by *Paenibacillus* (92 and 43%), *Arthrobacter* (91 and 34%), *Stenotrophomonas* (89 and 38%), *Serratia* (89 and 63%), *Pseudomonas* (84 and 48%), *Bacillus* (82 and 37%), and *Micrococcus* (76 and 27%), respectively. Of the P-solubilizing isolates (249; 94 from rhizospheric soil of cereals and 185 from other plant species) a total of 46 different recognised bacterial genera were represented as follows: *Bacillus* (25.8%), *Pseudomonas* (11.8%), *Stenotrophomonas* (8.6%), *Paenibacillus* (5.7%), *Serratia* (4.3%), *Pantoea* (3.9%), and *Arthrobacter* (3.9%). The highest P-solubilizing isolates, i.e., 37.6, 34.8, and 12.5% were belonged to *Gammaproteobacteria*, *Firmicutes*, and *Actinobacteria*, respectively.

Among the P-solubilising *Bacillus*, the dominant one were the following ones: *B. megaterium* (36 strains), followed by 10 strains of *B. atrophaeus*, *B. cereus*, *B. pumilus*, *B. licheniformis*, *B. subtilis*, *B. mycoides*, *B. psychrosaccharolyticus*, *B. sphaericus*, *B. laevolacticus* and *B. thuringiensis* (Tables 2, 3). Among the *Paenibacillus* and *Brevibacillus* strains, seven isolates of *Pb. polymyxa*, four isolates each of *Pb. macerans* and *Bb. choshinensis*, two separates of *Pb. validus* and a single isolate of *Pb. macquariensis*, *Pb. lentimorbus*, *Pb. larvae* and *Bb. reuszeri* were confirmed as PSB. Other identified P-solubilizing isolates included one isolate each of *Kurthia sibirica* and *Staphylococcus cohnii*. Thirty-five isolates belonged to genus *Arthrobacter*, nine to *Mic. luteus*, six strains to *Kocuria rosea*, two strains to *Rhodococcus erythropolis* and 17 strains to other actinobacterial genera. In addition, 21 strains namely, *Csb. balustinum*, *Csb. meningosepticum*, *Bergeyella zoohelcum*, *Weeksella virosa*, *Sph. spiritivorum*, *Sph. multivorum*, *Flavobacterium* sp. and *Bergeyella* sp.

Out of 279 P-solubilising isolates, 149 fitted to gram-negative, which involved 110 γ -proteobacteria, 19 α - and β -proteobacteria, and 20 isolates belonging to the Bacteroidetes group. Among the gram-negative P-solubilising *Pseudomonas*, the dominant ones were *Pseudomonas fluorescens* (11 strains), followed by *Pseudomonas putida* (eight strains), *P. agarici*, *P. stutzeri*, *P. alcaligenes*, *P. aeruginosa*, *P. mendocina*, *P. savastanoi* and *P. syringae*. It is found that *Ste.* In the soil of the Van region, *Maltophilia* was the most dominant gram-negative phosphate-solubilizing strain. Among the other γ -proteobacteria, 11 isolates of *Pantoea agglomerans*, eight isolates of *Serratia fonticola*, three isolates each of *Rahnella aquatilis* and *Salmonella typhimurium*, two isolates each of *Aeromonas ichthiosmia*, *Hafnia alvei*, *Citrobacter freundii*, *Kluyvera cryocrescens*, *Proteus vulgaris*, *Serratia grimesii* and *Serratia plymuthica* and single isolate each of *Photobacterium angustum*, *Enterobacter asburiae* and *Yersinia bercovieri* were confirmed as PSB. A total of 19 following P-solubilising β -proteobacterial strains were isolated from the rhizosphere of cereals and other plant species: six isolates of *Achromobacter xylosoxidans denitrificans*, four isolates of *Alcaligenes faecalis* and nine strains to other genera.

Out of 542 nitrogen-fixing bacterial isolates, 237 (77 from cereals and 160 from other plants) were to gram-negative, which involved 184 γ -proteobacteria and 25 α - and β -proteobacteria, and 28 isolates belonged to the Bacteroidetes. Among gram-negative N₂-fixing proteobacteria, *Ste. Maltophilia* (56 strains), *Pseudomonas fluorescens* (16 strains), *P. putida* (15 strains), *Pn. agglomerans* (14 strains), *Serratia fonticola* (12 strains), *Pseudomonas agarici* (nine strains), *Achromobacter xylosoxidans* subsp. *denitrificans* (eight strains), *Alcaligenes faecalis* (five strains), and *Rahnella aquatilis* (four strains) were found to be the most prominent root-associated culturable diazotrophs. *Csb. meningosepticum* and *Csb. balustinum* was the most dominant member of the N₂-fixing Bacteroidetes group.

Among the 305 gram-positive N₂-fixing isolates were 216 firmicutes (86 from cereals and 130 from other sources) and 89 actinobacteria (35 from cereals and 54 from other sources). Among the N₂-fixing *Bacillus*, the dominant ones were *B. megaterium* (75 strains), followed by *B. cereus* (21 strains), *B. atrophaeus* (16 strains), *B. subtilis* (nine strains), *B. pumilus* (seven strains), *B. psychrosaccharolyticus* (six strains), *Bacillus* sp. (five strains), *B. laevolacticus* (four strains), and three isolates each of *B. licheniformis*, *B. thuringiensis* and *B. mycoides*, respectively (Tables 2, 3). 16 isolates of *Bb. choshinensis*, 14 isolates of *Pb. polymyxa*, five isolates of each of *Pb. macerans* and *Pb. macquariensis*, four isolates of *Pb. validus*, three isolates of *Bb. centrosporus*, two isolates of each of *Pb. azotofixans* and *Pb. larvae* and a single isolate of *Pb. lentimorbus* and *Pb. alginolyticus* was able to fix nitrogen. The results indicated that *Mic. luteus* (23 strains), *Kocuria rosea* (12 strains), *Arb. agilis* (nine strains), and *Arb. globiformis* (eight strains) was the most noticeable N₂-fixing in the study. Other N₂-fixing isolates identified included four isolates each of *Arb. histidinolovorans* and *Brb. epidermidis*, three isolates each of *Arb. viscosus*, *Arb. aurescens*, *Rco. erythropolis* and *Brb. liquefaciens* and 17 strains of other species.

Discussion

Numerous strains of bacteria, mostly species of the 11 genera (*Bacillus*, *Stenotrophomonas*, *Pseudomonas*, *Paenibacillus*, *Arthrobacter*, *Micrococcus*, *Brevibacillus*, *Serratia*, *Chryseobacterium*, *Kocuria*, and *Pantoea*), were separated from the rhizosphere of various plant species such as wild wheat, aegilops, barley, rye, oats, beet, stonecrops, raspberry, onion, rose, orchids, houseleeks, sage, dandelion, strawberry, gladiolus, elecampane, strawflower, peganum, and knapweed grown in the Van Lake Basin. Subsequently, the taxonomic characteristics of 57 genera out of nearly 651 rhizospheric root-associated bacteria isolated from 169 rhizospheric soil samples of 20 plant genera were developed at four different regions. Analyses through FAME resulted in about 84% identification of the bacterial isolates. However, the ratio of identified isolates was high in the current study as compared to results of Germida & Siciliano (2001), and Poonguzhali *et al.*, (2006).

The FAME profiles analysis revealed the presence of both gram negative and positive rhizobacteria, however the gram-positive bacteria was higher in percentage (57%). In contrast, the gram-negative bacteria were dominant in the rhizosphere of wild onion, knapweed, elecampane, and barley. Thus, it appears that the diversity of rhizosphere bacterial populations varied significantly among crop species. Previous literature recorded a higher rate of gram-positive bacteria in the rhizosphere of wild grasses (Garbeva *et al.*, 2003; Rusznyák *et al.*, 2008; Rau *et al.*, 2009). Xue *et al.*, (2008) found that the gram-positive bacterial in tea soils were higher than those of gram-negative bacteria. In contrast, some other reports showed a higher of gram-negative relative to gram-positive species as mentioned in the previous findings (Poonguzhali *et al.*, 2006; Chowdhury *et al.*, 2007; Fischer *et al.*, 2007; Tian *et al.*, 2009).

Cultivated bacterial population from aboriginal plant rhizosphere tastes characterised members of the genera including *Bacillus*, *Pseudomonas*, *Stenotrophomonas*, *Paenibacillus*, *Arthrobacter*, *Micrococcus*, *Brevibacillus*, *Serratia*, *Chryseobacterium*, *Kocuria* and *Pantoea* (Selvakumar *et al.*, 2008; Aravind *et al.*, 2009; Hariprasad & Niranjana, 2009). The species of *Bacillus*, *Paenibacillus*, *Arthrobacter*, *Brevibacillus*, and *Micrococcus* among the gram-positive bacteria are found in barley, wheat, and alfalfa soil (Germida *et al.*, 1998; Caesar-TonThat *et al.*, 2007). The widely studied *Bacillus* genus is characterized by the capability to tolerate negative environmental conditions (Borsodi *et al.*, 2007; Garbeva *et al.*, 2003; Beneduzi *et al.*, 2008).

We surveyed PSB of naturally colonizing, suitable for a continental climate and mainly in the alkaline rhizospheric soil of 20 different native plants, in the geothermal area of the Zeylan valley, volcanic alkali basalt *Tendürek* Mountain and karstic carbonate rocks of the Artos Mountains in the Van region in Eastern Anatolia. A total of 279 isolates were identified as PSB: 54 from wild wheat, 40 from other cereals, 56 from beet, 30 from sedum, 21 from raspberry, 18 from onion, 13 from rose, and 47 from other plants. The obtained results indicated that *Bacillus* (72 strains), *Pseudomonas* (33), *Stenotrophomonas* (24), *Paenibacillus* (16), *Serratia* (12), and *Pantoea* (11) genera were the most prominent P-solubilising groups. Similar results were presented in previous literature (Çakmakçı *et al.*, 2009; Hariprasad & Niranjana, 2009). Actually, *Pseudomonas* and *Bacillus* species have more solubilizing ability in soluble inorganic phosphate (Tambekar *et al.*, 2009).

A total of 279 isolates were able to solubilize phosphate, and 542 isolates had the ability to fix nitrogen. A whole of 42.3% of the isolates showed considerably lower P solubilization ability as compared to the previous studies reported by Hariprasad & Niranjana (2009) and Rau *et al.*, (2009), however was superior to the P solubilization ability as reported by Beneduzi *et al.*, (2008). Nevertheless, the distribution shapes of the bacterial species among the 20 different native plant species differed (Tables 2, 3). For example, among the 229 strains obtained in cereals, N₂-fixing and P-solubilising strains of *Bacillus atrophaeus*, *Achromobacter xylosoxidans denitrificans*, *Variovorax*

paradoxus, *Pseudomonas agarici*, *P. alcaligenes*, *Kocuria rosea*, and *Rco erythropolis* were isolated only from wheat, but not from others. In contrast, *Hafnia alvei* and *Rahnella aquatilis* were isolated only from *Aegilops* and *Acidovorax facilis* was from rye. It was also notable that many N₂-fixing *Alcaligenes latus*, *Acinetobacter calcoaceticus*, and *B. mycoides* were isolated from the rhizosphere of *Secale* sp., but not from other cereals. *Pseudomonas fluorescens*, *P. putida*, *Pn. agglomerans*, *B. cereus*, and *Arb. agilis* was isolated more frequently from the wheat than from others, while the *Ste. maltophilia* and *B. megaterium* were isolated more frequently from the rhizosphere of wheat, rye, and oats. Also, N₂-fixing and P-solubilising strains of *Alcaligenes faecalis* and *Bb. choshinensis* isolated only from oats. N₂-fixing and P-solubilising *Pb. polymyxa*, *Pb. validus* and *Weeksella virosa* were commonly isolated in the rhizosphere of barley. Dominance of *Bacillus* and *Pseudomonas* in the wheat rhizosphere has been reported previously by Mittal & Johri (2007). Also, *Paenibacillus polymyxa* is one of the best-established species in wild barley rhizosphere (Timmusk *et al.*, 2009).

Members belonging to Firmicutes were predominant in major cases, with several of these isolates allocated to *Bacillus* in the rhizosphere of the wild beet, stonecrops, and raspberry, while the composition of species varied due to variation of the environment. Our data showed that the dominant rhizospheric N₂-fixing and P-solubilising strains were *Bacillus* (*B. megaterium*, *B. licheniformis*, *B. cereus*, *B. atrophaeus*, *B. cereus*, and *B. pumilus*) and *Pseudomonas* (*Pseudomonas fluorescens*, *P. agarici*, *P. putida*, *P. stutzeri*, *P. mendocina* and *P. syringae*) which were equally dominantly distributed in the rhizosphere of plant species such as orchids, houseleeks, sage, dandelion, strawberry, gladiolus, elecampane, strawflower, peganum, and knapweed. Several P-solubilising and N₂-fixing strains were found to be bacilli in this study. Strains of *B. megaterium*, *B. atrophaeus*, *B. cereus*, *B. pumilus*, *B. licheniformis*, *B. subtilis*, *B. mycoides*, *B. psychrosaccharolyticus*, *B. sphaericus*, *B. laevolacticus*, *B. thuringiensis*, and *B. coagulans* have been isolated from the rhizosphere as N₂ fixers (Vazquez *et al.*, 2000; Egamberdiyeva, 2005; Hafeez *et al.*, 2006; Gray *et al.*, 2006; Poonguzhali *et al.*, 2006; Hariprasad and Niranjana, 2009; Rau *et al.*, 2009; Rajeshkumar *et al.*, 2009). Besides, some strains of PGPR was isolated in our study; similarly, Xie *et al.*, (2003) isolated many species of nitrogen-fixing *B. megaterium*, *B. cereus*, *B. subtilis*, *B. pumilus*, *B. Licheniformis*, *Aeromonas hydrophila*, *Citrobacter freundii* and *Ps. mendocina* from the rhizosphere of rice. Numerous strains of bacilli, mostly species of the genera *Bacillus* and *Paenibacillus*, displaying imperative PGP properties were separated from 20 plant species. In the current study, *Pb. polymyxa*, *Pb. validus*, *Pb. macquariensis*, *Pb. macerans*, *Pb. larvae*, *Pb. lentimorbus*, *Pb. azotofixans*, and *Pb. alginolyticus* identified which were earlier described as dinitrogen fixers, P-solubilisers or PGP (Vazquez *et al.*, 2000; DasGupta *et al.*, 2006; Medeiros *et al.*, 2009; Rau *et al.*, 2009). *Pb. lentimorbus* and *Pb. polymyxa* are beneficial to plants and can show the strongest antifungal and nematicidal activities (DasGupta *et al.*, 2006; Son *et al.*, 2006). *Bb. choshinensis*, *Bb. reuszeri* and *Kurthia sibirica* were able to solubilize phosphate and three bacterial isolates namely, *Bb. choshinensis*, *Bb. centrosporus* and *Kurthia sibirica*, are being reported as a nitrogen fixer.

Several pseudomonades were also recognized to have N₂-fixing and P-solubilising characters from the rhizosphere of wild native plants in the present study. Most of the species included efficient phosphate solubilizers such as *Pseudomonas fluorescens*, *P. putida*, *P. agarici*, *P. stutzeri*, *P. alcaligenes*, *P. aeruginosa*, *P. mendocina*, *P. savastanoi* and *P. syringae* (Yao *et al.*, 2008; Hariprasad & Niranjana, 2009; Jha *et al.*, 2009; Rau *et al.*, 2009), *Ste. maltophilia* (Park *et al.*, 2005b; Liba *et al.*, 2006), *Aeromonas hydrophilia* (Xie *et al.*, 2003), *Aeromonas ichthiosmia* (Poonguzhali *et al.*, 2006) and gibberellins producing, nitrogen-fixing and phosphate solubilizing *Acinetobacter calcoaceticus* (Liba *et al.*, 2006; Kang *et al.*, 2009) stated also been frequently separated from the rhizosphere of a difference of additional crops. *Ste. maltophilia* and *P. fluorescens* also have been isolated previously from the rhizosphere of soils of wheat, maize, and rice crops verifying their nitrogen-fixing capacity (Park *et al.*, 2005b).

The soil in the Van Lake region predominates N₂-fixing and P-solubilising *Enterobacteriales* and *Aeromonadales* species, from which some are recognised as N₂-fixing and P-solubilising strains: *Pantoea agglomerans*, *Serratia fonticola*, *Rahnella aquatilis*, *Aeromonas ichthiosmia*, *Citrobacter freundii*, *Kluyvera cryocrescens*, *Serratia plymuthica*, *Enterobacter asburiae*, *Aeromonas hydrophilia*, and *Aeromonas ichthiosmia*. The recently discovered species are also crucial for plant growth due to their good capacity to fix nitrogen and solubilize phosphate (Pérez *et al.*, 2007; Ben Farhat *et al.*, 2009; Hariprasad & Niranjana, 2009; Kumar *et al.*, 2009). It is revealed that the plant growth-promoting *Serratia odorifera* (Kai & Piechulla, 2009), auxin-producing and growth-promoting *Proteus vulgaris* (Karadeniz *et al.*, 2006; Rani *et al.*, 2008), antagonistic plant-associated bacteria *Serratia grimesii* (Lottmann *et al.*, 2000), and *Hafnia alvei* isolated from the rhizospheric soil of *Aegilops* sp., were able to solubilize phosphate and fix nitrogen. Interestingly, *Hafnia alvei* isolated from volcanic alkali basalt rocks in the northwest *Tendürek Mountains* at an altitude of 2850 m above mean sea level.

Among the N₂-fixing and P-solubilising actinomycetes isolated in the current research *Mic. luteus*, *Arb. mysorens*, *Rco. erythropolis*, *Cub. flaccumfaciens*, and *Mbm. barkeri* were previously reported as N₂-fixers or P-solubilisers (Belimov *et al.*, 1995; Elo *et al.*, 2000; Purnomo *et al.*, 2005; Chen *et al.*, 2006; Chowdhury *et al.*, 2007; Rau *et al.*, 2009). Biological control agents *Cellulomonas turbata* (Byrne *et al.*, 2005), atrazine-degrading *Arthrobacter histidinovorans* (Sajjaphan *et al.*, 2010), polysaccharides producing *Arthrobacter viscosus* (Lopez *et al.*, 2003), and other actinomycetes isolated in the present study also have N₂-fixing or P-solubilising properties.

Nitrogen-fixing Firmicutes constituted 39.9% and were the most dominant lineage followed by gamma-proteobacteria (33.9%), Actinobacteria (16.4%), Bacteroidetes (5.2%), beta-proteobacteria (4.1%), and alpha-proteobacteria (0.5%). Among the nitrogen-fixing and/or P-solubilising β -proteobacterial strains isolated in current research *Acidovorax facilis*, *Achromobacter xylosoxidans denitrificans*, *Alcaligenes faecalis*, *Alcaligenes latus*, and

Variovorax paradoxus were earlier stated as dinitrogen fixers (Vermeiren *et al.*, 1999; Joo *et al.*, 2005; Belimov *et al.*, 2009; Bergmann *et al.*, 2009; Jha & Kumar, 2009). *Achromobacter xylosoxidans* and *Delftia acidovorans* are reported to have the ability to produce siderophores (Tian *et al.*, 2009). Several gram-negative N₂-fixing and P-solubilising *Bacteroidetes*, most of them were isolated from the rhizosphere of stonecrops and raspberry in volcanic alkali basalt and karstic carbonate rocks of the *Tendürek* and *Artos mountain* at an altitude of 2250-2740 m above mean sea level. Nitrogen fixation was previously reported for *Csb. meningosepticum* and *Sph. spiritivorum* (Poonguzhali *et al.*, 2006), and auxin production for plant growth-promoting *Csb. balustinum* (Lukas García *et al.*, 2004). The ability of *Chryseobacterium* sp. and *Delftia* sp. to solubilize phosphate has been reported earlier by Chen *et al.*, (2006).

Except for cereal species, a total of 422 strains were isolated from the rhizospheric soil of the other plants, among which 344 exhibited N₂-fixing activity and 185 were efficient in P-solubilisation; 157 strains were efficient in N₂-fixation and P-solubilisation obtained in the four localities. There were obvious differences among the geographical locations, altitudes, rhizosphere soil bacterial communities, and plant species. The nature of plants and soils influenced the Rhizobacterial diversity. Zhang *et al.*, (2006) revealed that various environmental factors like the content of soil organic carbon and nitrogen, and altitude could influence the diversity of soil bacteria, including nitrogen-fixing bacteria. The composition of the rhizobacterial community associated with plant roots is influenced by various plants, sites, environmental factors, and management practices (Park *et al.*, 2005a).

N₂-fixing and P-solubilising bacteria such as *Ste. maltophilia* and *B. megaterium* was found in all the tested rhizospheric soil. N₂-fixing and P-solubilising *Pantoea agglomerans* was found in almost all soil samples except in the soil of raspberry, while both *Ps. putida* and *Serratia fonticola* have already been identified from beet, stonecrops, rose, and houseleeks. *Ste. maltophilia*, *Ps. putida*, *B. atrophaeus*, *B. cereus*, *B. megaterium* and *Paenibacillus polymyxa* were isolated more frequently from the wild beet than from others, while the *Csb. meningosepticum* and both *Sphingobacterium* species were isolated more frequently from the rhizosphere of stonecrops. The gram-positive *Kocuria rosea* was the most dominant nitrogen-fixing and P-solubiliser in the rhizospheres of rose. *Delftia acidovorans*, *Ps. aeruginosa*, *Serratia grimesii*, *Rahnella aquatilis*, *B. laevolacticus*, *Pb. larvae*, *Pb. lentimorbus* and *Cub. flaccumfaciens* was exclusively found in the beet rhizosphere, whereas *B. licheniformis* was found only at raspberry and strawberry. N₂-fixing and P-solubilising *B. pumilus*, *B. subtilis* and *Csb. balustinum* were common in the rhizosphere of raspberry but not found in other plants, whereas *Kluyvera cryocrescens* was common in the wild onion but not found in the other plants rhizosphere. *Arb. ramosus* and *Arb. viscosus* were exclusively found in rose rhizosphere, whereas *Proteus vulgaris* was found only at raspberry and onion. Similarly, *Alcaligenes faecalis* was found in the rhizosphere of stonecrops and onions, and *Ps. stutzeri* and *Rco. erythropolis* were found at both beet and dandelion.

This study is the first report on the diversity of culturable bacteria associated with the 20 major plant species, wild cereals, and beta (*Triticum* spp., *Aegilops* spp., *Hordeum* spp., *Secale* spp., *Avena* spp. *Beta* spp. and *Corollinae* spp.) and other 14 native plants, which are found in the alkali basalts, volcanic and karstic carbonate rocks and geothermal areas of the Van Lake region. Overall, there is less information on the rhizosphere microbiology of the wild plants, and the distribution patterns of the major bacterial taxa differed among the tested plant species. Thus, it seems that the diversity of rhizobacterial communities differs significantly among crop species, suggesting that plants play a dominating function in determining the composition of the rhizobacterial community. Suppose the plants play a dominating role in controlling the ecology of the root-associated microbial community. In that case, this means that the appropriate plant growth-promoting bacterial inoculants may work in various environments (Germida *et al.*, 1998). Several bacilli strains, mainly species of the genera *Bacillus*, *Pseudomonas*, and *Paenibacillus*, displaying important PGP properties were isolated from four distinct regions in the rhizosphere of different native plants.

We assessed the rhizospheric isolates relative to their potential use in plant growth advancement by selection for nitrogen fixation and phosphate solubilization. Many species of PGPR can provide for plant growth and productivity in several methods, only a few reports considering the alkaline and acid-tolerant native strains of N₂-fixing and P-solubilising bacteria adapted to the geothermal, volcanic, and karstic carbonate environments have been made up to now (Chen *et al.*, 2006).

Conclusions

Our results showed that different plant species, altitudes, geographical locations and vegetation types in the investigated sites resulted in the different bacterial populations and bacterial types. Consequently, the strains identified in this study could help to formulate new inoculants, improving the cropping systems into which they can be most profitably applied.

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