An Acad Bras Cienc (2022) 94(1): e20191460 DOI 10.1590/0001-3765202220191460
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

CELLULAR AND MOLECULAR BIOLOGY

Isolation and characterization of the most abundant rhizobacterial species associated with *Vuralia turcica* (Fabaceae: Papilionoideae)

DİLEK TEKDAL, CEM ÇİFTÇİ, BURÇİN ÇINGAY & SELİM ÇETİNER

Abstract: *Vurali turcica* is naturally grown in a limited area in Central Anatolia in Turkey and was categorized as a critically endangered plant in the Red Data Book of Turkish Plants. This study aimed to analyze whether the symbiotic and mutualistic relation between *V. turcica* rhizomes and present microflora in the habitat can be active on its distribution. Plant growth-promoting rhizobacteria (PGPRs) colonize the rhizosphere and promote plant growth and physiology. In this paper, the diversity of PGPRs of rhizomes of *V. turcica* was analyzed. Rhizome samples were obtained from the natural habitats of *V. turcica* by the workers of Nezahat Gökyiğit Botanical Garden, and bacterial isolation was conducted on the collected samples. MIS analysis, 16S rRNA, and 16S-23S rRNA ITS region sequencing were implemented, and as a result, *Bacillus megaterium* was found to be one of the most abundant bacterial species of the rhizomes of *V. turcica* based on nucleotide homology. This study is the first report on the identification of rhizobacterial species in *V. turcica*.

Key words: MIS, Nezahat Gökyiğit Botanical Garden, 16S rRNA, 16S-23S rRNA ITS region, *V. turcica*.

INTRODUCTION

Plant growth-promoting rhizobacteria (PGPR) affect plant growth positively through plant growth regulator production, nutrient mobilization in the soil, plant growth regulator production, inhibition, and toxic compound degradation, and plant-pathogen control (Ahemad et al. 2009, Ahemad & Kibret 2014). Plant roots facilitate nutrient and water uptake and provide mechanical support to the plant. Besides, plant roots synthesize and secrete compounds that contribute to metabolizing microbial populations (Walker et al. 2003). Secondary metabolites produced by PGPRs may have a role in facilitating nutrient uptake of plants as those metabolites convert into available forms for plant roots to absorb (Glick et al. 2007).

Vuralia turcica (Tan et al. 1983) Uysal et al. 2014 belongs to the Fabaceae family and is naturally distributed in Konya province in Turkey. This species is a rare and endemic plant species. The habitat of *V. turcica* is limited to an area in Central Anatolia in Turkey (Vural 2009). The symbiotic and mutualistic relation between *V. turcica* rhizomes and microflora may be a reason for the distribution of the plant in a very narrow area. To date, microbial activity at the rhizomes of *V. turcica* has not been studied. To close this gap in the literature, PGPRs living on the rhizomes of *V. turcica* were isolated and identified.

MATERIALS AND METHODS

Collection of rhizome samples from various habitats of *V. turcica*

Rhizome samples were collected from 6 different locations of the habitats of *V. turcica* in April 2017 by the workers of Nezahat Gökyiğit Botanical Garden (NGBG) (Figure S1 – Supplementary Material). The coordinates of each sample collected were identified with a Global Positioning System (GPS; Magellan eXplorist 310) (Table I). Each rhizome sample was taken into sterile cups containing sterile distilled water. Samples were labeled according to the collected location and kept at +4°C until further analysis.

Nodule sampling and the isolation of plant growth-promoting rhizobacteria

The collected rhizome samples were used for bacterial isolation. Nodules were removed from the rhizomes of each plant and grinded in liquid Yeast Malt (YM) medium described by Vincent (1970). The diluted rhizome-liquid YM medium mix was taken on a YM agar medium and incubated at 28°C until bacterial colonies were obtained. For the pre-selection of PGPRs, YM agar medium containing either Bromothymol blue or Congo red was used. Gram staining of the isolates was also conducted. To obtain single colonies, suspensions of bacteria were diluted in liquid medium as 1 in 6 serial dilutions and reseeded on solid medium.

Molecular analysis of the isolates

After bacteria were isolated according to their color and morphology, the isolates were characterized by amplifying 16S rRNA and 16 S-23S rRNA Internal Transcribed Spacer (ITS) region. 16S rDNA genes were amplified using D1F (5’ AGAGTTTGATCCTGGCTCAG -3’) and D1R (5’ AAGGAGGTGATCCAGCC -3’) primers and 16S-23S rDNA ITS region was identified using FGPS1490-72 (5’ TGCGGCTGGATCCCCTCCTT -3’) and FGPL132-38 (5’ CCGGGTTTCCCCATTCGG -3’) primers. Polymerase Chain Reaction (PCR) was implemented in a 25-µL reaction volume by mixing the final concentration of 5 ng DNA extract with the polymerase reaction buffer (1X), Taq DNA polymerase (0.125 U/µL), dNTPs (0.2 mM), and each primer (0.8 µM). PCR amplification was performed in a C100 Touch (Bio-Rad Laboratories, Segrate, Milan, Italy) thermal cycler adjusted to the following PCR conditions: initial denaturation at 95 °C for 7 min, 35 amplification cycles (denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 min),

Table I. The coordinates of each rhizome sample collected from six locations of the Central Anatolia Region of Turkey.

Location	Samples	X (North)	Y (East)	Height (m)	Field name	Depth (cm)
1	Rhizome 1	38° 46´ 987´´	31° 34´ 559´	980	Gölçayır	0-30
2	Rhizome 2	38° 28´ 10.5´´	31° 21´ 04.4´´	996	Gölçayır	0-30
3	Rhizome 3	38° 28´ 17.328´´	31° 20´ 52.468´´	976	Akşehir	0-30
4	Rhizome 4	38° 30´ 36.702´´	31° 17´ 56.702´´	966	Dereçine	0-30
5	Rhizome 5	38° 36.42´3864´´	31° 08´ 55.968´´	960	Eber	0-30
6	Rhizome 6	38° 32´43.2168´´	31° 16´ 54.4728´´	956	Sultandağı	0-30

and final extension at 72°C for 5 min. Negative control (H_2O; containing no template) was also used to check for experimental contamination. PCR products were checked by agarose (1%, w/v) gel electrophoresis. Isolated and purified PCR products were directly sequenced. The sequencing service was commercially provided by BM Laboratory Systems, Ankara, Turkey (https://www.bmlabosis.com/).

Sequence analysis and phylogenetic analysis

The consensus sequences of 16S rRNA and 16S-23S rRNA ITS region were used to search for similar sequences in GenBank at the National Center for Biotechnology Information (NCBI), Bethesda, USA, using the BLAST search program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul et al. 1990). Phylogenetic analysis of the isolated bacteria was carried out by analyzing consensus sequences obtained from the amplification of 16S rRNA and 16 S-23 S rRNA ITS regions. Phylogenetic analysis was performed by the Molecular Evolutionary Genetics Analysis (MEGA) program version 7.0 (https://www.megasoftware.net/). Multiple sequence alignments were developed for both 16S and 16S-23S ITS sequences, separately. Phylogenetic trees were generated from these nucleotide sequences in the dataset using the Neighbor-Joining method based on the Kimura 2 parameter (Kimura 1980). Aligned 16S rDNA and 16S-23S rDNA ITS sequences were evaluated with bootstrap analysis (1000 replicates) (Felsenstein 1985). 2 outer sequences of *Bacillus megaterium* and *Agrobacterium tumefaciens* obtained from NCBI GenBank were included sequence dataset for sequence comparison and were aligned with both 16S rRNA gene sequence and 16S-23S ITS region sequence of isolates.

Microbial identification system (MIS) analysis

MIS analysis of the bacterial isolates was implemented using gas chromatography (6890N GC, Agilent Technologies INC., USA) and MIS software (Sherlock 6.0 MIDI, Inc., Newark, DE, 2005). The resulting data were compared with the commercial database (RTSBA6). Fatty acid methyl ester (FAME) groups were identified by MIS software. The unit with the highest score between the diagnosis results was considered as the ideal result.

The 16S-23S sequences of the isolates Vt1N1, Vt1N2, Vt1N3, Vt1N4, Vt3N1, Vt3N2, Vt6N1, and Vt6N2 were submitted to GenBank under accession numbers MN565946, MN565947, MN565948, MN565949, MN565950, MN565951, MN565952, and MN565953, respectively. Also, the GenBank accession numbers of 16S sequences of the isolates Vt1N1, Vt1N2, Vt1N3, Vt1N4, Vt3N1, Vt3N2, Vt6N1, and Vt6N2 are MN561035, MN561036, MN565037, MN565038, MN565039, MN565040, MN565041, and MN565042, respectively.

RESULTS

Pre-selection of the bacteria isolated from *V. turcica* rhizomes

Bacterial colonies were detected at dilutions until 10^{-5} from the rhizome samples taken from locations 1, 3, and 6 of *V. turcica*. Pre-selective studies resulted in 4 isolates from location 1, and 2 isolates from locations 3, and 6.

The reaction results of isolates against Congo red, Bromothymol blue, and gram-staining were given in Table II. Images of isolates in mediums containing Congo red and Bromothymol blue were presented in Figure S2, and gram-staining results can be seen in Figure S3. The results of the Gram staining experiment of the isolates showed that all isolates were Gram-positive.

Table II. **Morphological and physiological characteristics of isolated bacterial species from rhizomes of *V. turcica*.**

Location	No	Strain Code	Gram-staining	Congo red	Bromothymol blue
1	1	Vt1N1	Positive-Bacillus	Red-Black	White-Basic
	2	Vt1N2	Positive -Bacillus	Red-Black	White-Basic
	3	Vt1N3	Positive -Bacillus	Red	Yellow-Acidic
	4	Vt1N4	Positive -Bacillus	Red-Black	Yellow-Acidic
3	1	Vt3N1	Positive -Bacillus	Red-Black	Yellow-Basic
	2	Vt3N2	Positive -Bacillus	Red-Black	Yellow-Basic
6	1	Vt6N1	Positive -Bacillus	Red-Black	Yellow-Basic
	2	Vt6N2	Positive -Bacillus	Red-Black	Yellow-Basic

Phylogenetic analysis of rhizome-isolated bacteria

After pre-selection of 8 bacteria, MIS analysis, 16S-23S rDNA ITS region, and 16S rRNA gene sequence analysis were implemented on these isolates. gDNAs were extracted from the bacterial isolates for molecular analysis. 16S rRNA and 16S-23S rDNA ITS regions of each isolate gDNAs were amplified and sequenced using the primers mentioned above. Consensus sequences (Tables SI and SII – Supplementary Material) were used for the identification of the isolates using the BLAST search program. According to the BLAST result, the 16S rDNA sequences of isolates, high sequence similarities with *Bacillus aryabhattai* (≥99 coverage and identity) was found in all isolates (Table III). The BLAST result also showed that isolate Vt6N1 has a 100% sequence similarity with *B. aryabhattai* and *B. megaterium* (Table III). On the other hand, BLAST results based on the 16S-23S rDNA ITS region sequences of isolates showed high sequence similarities with *Bacillus megaterium* (≥99 coverage and identity) were found in all isolates.

The phylogenetic trees generated using 16S rRNA and 16S-23S ITS analysis results of the isolates were shown in Figure 1 and Figure 2, respectively. The 8 isolates were clustered into three branches by comparison of the sequences identified from the 16S rDNA PCR profiles (Figure 1). Isolates Vt1N1, Vt1N2, Vt1N3, Vt1N4, Vt3N1, and Vt3N2 were pooled together into the same branch, but Vt6N1 and Vt6N2 were grouped into the different branches (Figure 1). According to the phylogenetic tree constructed based on the 16S-23S rDNA ITS region sequence, Vt1N3 was clustered into different branches, and other isolates were clustered with GenBank sequences (FJ969767.1) of *B. megaterium* (Figure 2). In both phylogenetic constructs based on the sequences of 16S rDNA and 16S-23S rDNA ITS region of isolates with two out-groups, *Agrobacterium tumefaciens* was clustered on different sub-branches.

MIS results

The bacterial identity of the isolates was tried to be revealed by analyzing their FAME groups with MIS. As a result of MIS analysis, *B. megaterium* was the most dominantly colonized bacteria on the rhizomes of *V. turcica* (Table IV).

Table III. Sequence similarities according to the BLAST search against NCBI database based on identified 16S rRNA and 16S-23S rDNA ITS sequences.

gDNA	Species	Accession No	Similarity (%)
The BLAST search based on identified 16S rRNA sequences			
Vt1N1	*Bacillus aryabhattai* B8W22	NR_115953.1	99.90
Vt1N2	*Bacillus aryabhattai* B8W22	NR_112636.1	99.78
Vt1N3	*Bacillus aryabhattai* B8W22	NR_112636.1	99.85
Vt1N4	*Bacillus aryabhattai* B8W22	NR_112636.1	100
Vt3N1	*Bacillus aryabhattai* B8W22	NR_112636.1	99.93
Vt3N2	*Bacillus aryabhattai* B8W22	NR_112636.1	99.93
Vt6N1	*Bacillus aryabhattai* B8W22 *Bacillus megaterium* NBRC 15308 = ATCC 14581	NR_112636.1 NR_112636.1	100 100
Vt6N2	*Bacillus aryabhattai* B8W22	NR_112636.1	99.81
The BLAST search based on identified 16S-23S rDNA ITS sequences			
Vt1N1	*Bacillus megaterium* strain KCCM 11934	FJ969785.1	99.03
Vt1N2	*Bacillus megaterium* strain KCCM 11934	FJ969785.1	99.03
Vt1N3	*Bacillus megaterium* strain KCCM 11934	FJ969785.1	99.03
Vt1N4	*Bacillus megaterium* strain KCCM 11776	FJ969784.1	99.61
Vt3N1	*Bacillus megaterium* strain KCCM 11934	FJ969785.1	99.33
Vt3N2	*Bacillus megaterium* strain KCCM 11934	FJ969785.1	99.35
Vt6N1	*Bacillus megaterium* strain KCCM 11776	FJ969784.1	99.03
Vt6N2	*Bacillus megaterium* strain KCCM 11934	FJ969785.1	99.33

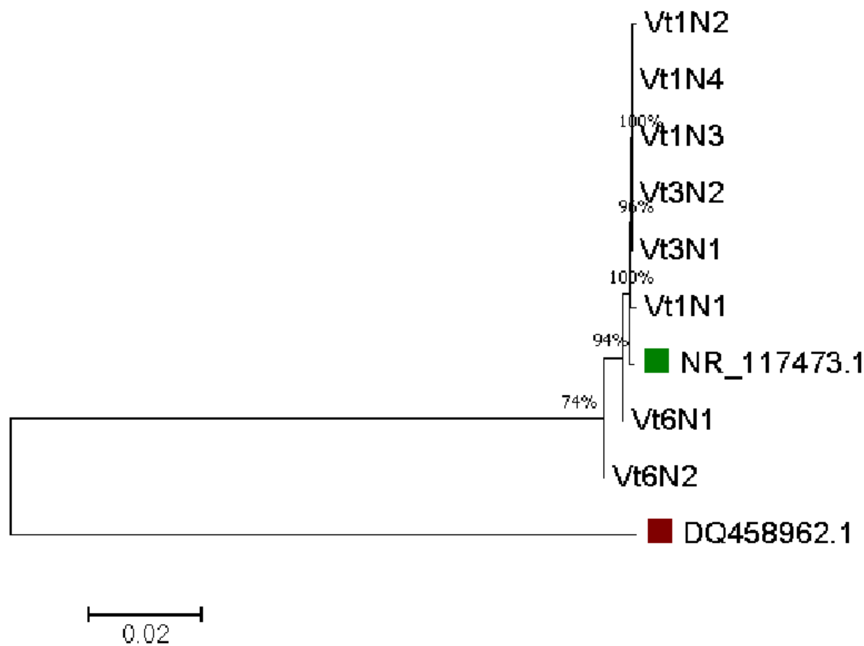

Figure 1. Neighbor-joining tree based on partial sequences of 16S rDNA region of the isolates with each other for phylogenetic inference. The evolutionary history was inferred using the N-J method (Saitou & Nei 1987) with the Kimura-2 parameter method (Kimura 1980). NR_117473.1 (*Bacillus megaterium*) and DQ458962.1 (*Agrobacterium tumefaciens*) were used as an out-group.

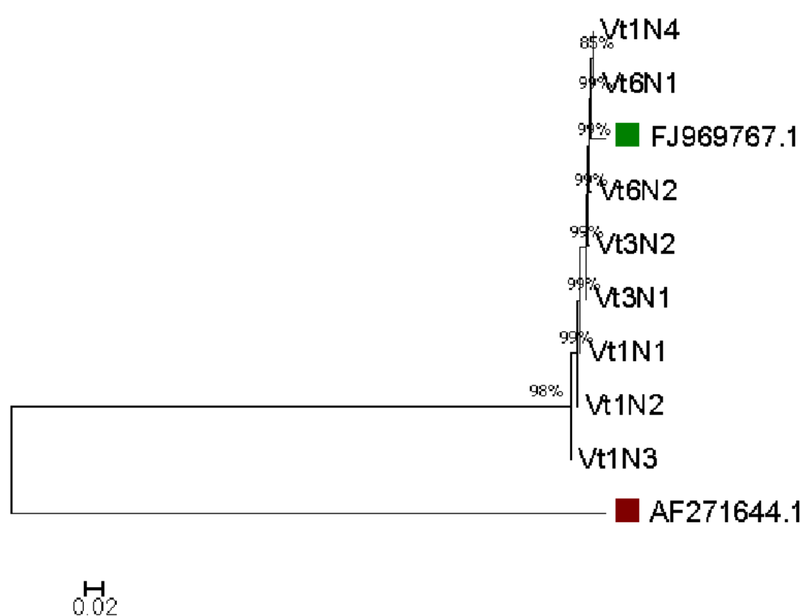

Figure 2. Neighbor-joining tree based on partial sequences of 16S-23S rDNA ITS region of the isolates with each other for phylogenetic inference. The evolutionary history was inferred using the N-J method (Saitou & Nei 1987) with the Kimura-2 parameter method (Kimura 1980). FJ969767.1 (*Bacillus megaterium*) and AF271644.1 (*Agrobacterium tumefaciens*) were used as an out-group.

Table IV. MIS results of the bacteria isolated from rhizomes.

Location	Strain Code	MIS result
1	Vt1N1	*Bacillus megaterium*-GC subgroup B (0.469)
	Vt1N2	*Bacillus megaterium*-GC subgroup A (0.855)
	Vt1N3	*Bacillus megaterium*-GC subgroup A (0.851)
	Vt1N4	*Bacillus megaterium*-GC subgroup A (0.755)
3	Vt3N1	*Bacillus megaterium*-GC subgroup A (0.729)
	Vt3N2	*Bacillus megaterium*-GC subgroup A (0.598)
6	Vt6N1	*Bacillus megaterium*-GC subgroup A (0.729)
	Vt6N2	*Bacillus megaterium*-GC subgroup A (0.834)

DISCUSSION

Microorganism identification is essential for various applications, such as industrial and environmental studies. In this study, to identify which bacterial species are useful for the growth of *V. turcica*, rhizomes of the plant were obtained from the plants grown in 6 different natural habitats given in Table I. YM medium is a selective medium for the reproduction of *Rhizobium* sp. and *Agrobacterium* sp. Since *Agrobacterium* sp. can absorb Congo red dye, the color of the colonies of this bacterial genus is red and shades when grown on YM medium, including Congo red dye, whereas *Rhizobium* sp. colonies are white and orange on that of medium (Kado & Heskett 1970). Also, yellow color formation in a medium containing Bromothymol blue is indicative of acid formation in a medium; thus, this medium acts as a pH indicator, and isolates can be classified as alkalizers or acid producers. For nitrogen fixation and nodulation, acid producers are essential (Boakye et al. 2016). Since to identify various types of rhizobacteria which are active in the growth and physiology

of *V. turcica* was aimed in this study, 8 isolates that differed from each other in terms of their color, shape, and morphology were chosen from many colonies (~100 colonies) according to their reactions in selective media mentioned above. No laboratory contaminants were detected on the bacterial strains. Bacterial colonies from the samples belonging to locations 2, 4, and 5 were not obtained. It could be possible due to chemical usage during surface sterilization. Morphological and physiological analysis indicated that four isolates (Vt1N1, Vt1N2, Vt3N1, and Vt3N2) were acid producers, and all isolates were Gram-positive.

In order to identify a bacterial species, the rRNA sequence-based analysis is mostly preferred. 16S rRNA gene of bacterial species contains highly conserved regions; therefore, 16S rRNA analysis is used for the characterization of new strains. On the other hand, since both the sequence and the length of the 16S-23S rDNA ITS region can vary considerably between bacterial species, this region is also essential for the identification of microorganisms (Hoffmann et al. 2010).

The BLAST result of the 16S-23S rDNA ITS region sequence showed that the isolates have ≥99% sequence similarity (Table III). Also, 16S-23S rDNA ITS region sequence and 16S rDNA gene sequence-based phylogenetic trees were generated, which show the relationship between the isolates and selected outer groups (Figures 1 and 2). As a result of MIS analysis and 16S-23S rRNA ITS region sequence to identify the bacterial isolates from rhizomes of *V. turcica*, *Bacillus megaterium* was the dominant bacterial species (Tables III and IV). On the other hand, according to BLAST analysis of the 16S rDNA sequences of the isolates, *B. aryabhattai* was the dominant bacterial species. It is known that the colony color of *B. megaterium* is white/cream, and *B. aryabhattai* is peach-colored from the previous study (Shivaji et al. 2009). In this study, the isolates were identified as *B. megaterium* as a result of the colony colors of the isolates and molecular studies (Figure S4).

The ability of *B. megaterium* to metabolize different root exudates as a carbon source might have put this species one step forward than other bacteria while competing for colonization. More chances to find nutrition can result in better colonization. As this bacteria enhances the photosynthetic activity by accumulating the N in the plant, higher amounts of photosynthates would be produced and utilized by the bacteria as a carbon source. This mutualistic loop might be one of the reasons why the microflora on the *V. turcica* rhizomes is dominated by *B. megaterium*. Plants have a significant role in controlling the bacterial community and the diversity of the root-associated bacteria (Germida et al. 1998). *V. turcica* has rhizome and nitrogen fixation (acetylene reduction) associated with rhizomes. Various plants with *B. megaterium* root-inoculation showed significant growth with increased total plant N and N-fixing activities compared to control plants (Elkoca et al. 2007). *B. megaterium* has been found to contain nitrogenase iron protein (NifH) genes, which encode essential enzymes taking a role in N-fixing mechanisms, and those genes are widely used as marker genes to identify N-fixing bacteria (Ding et al. 2005, Gaby & Buckley 2012). In light of this information, it is assumed that *B. megaterium* has growth-promoting effects on *V. turcica* plants by fixing or helping to fixate N.

Plant growth-promoting activity between *B. megaterium* and host plants was reported many times in the literature (López-Bucio et al. 2007, Liang et al. 2011, Chakraborty et al. 2012). It was known from the previous study that *B. megaterium* was endophytic bacterial species in legume plants and promotes plant growth (Khalifa & Almalki 2015). It has been shown that

B. megaterium application on tea plants leads to the accumulation of phenolic compounds in the leaves, a compound reported to be related to plant resistance against various stresses (Chakraborty et al. 2012). *Curcuma longa* has a rhizomic root structure like *V. turcica,* and turmeric plants had shown an increase in compounds related to induced systemic resistance (ISR) and prevention from fungal infections when *B. megaterium* were present at their rhizomes (Uthandi & Sivakumaar 2013). Expression levels of superoxide dismutase and catalase, together with the previously mentioned ISR-related enzymes, were observed to be higher in *B. megaterium* inoculated cucumber plants in means of defense against fungal infection (Liang et al. 2011). This endophytic presence of this bacterium might have protective effects on the rhizomes against invasive pathogens. *B. megaterium* is also found in the natural habitat of *V. turcica*, but *V. turcica* is only found in a restricted area in Central Anatolia (Tekdal et al. 2018). This bacterium probably plays a crucial role in the survival of this plant. The indirect growth-promoting ability of *B. megaterium* consists of antibiotic production. It has been previously shown that this bacterium is capable of producing antibiotics such as bacimethrin, cytidines, oxetanocin, iturin, bacillomycin, zwittermycin A, surfactin and other fungitoxins (Malanicheva et al. 2012, Uthandi & Sivakumaar 2013). *B. megaterium* might contribute to *V. turcica* growth indirectly with this antimicrobial activity, and this might explain the colonial dominance of this bacterium at *V. turcica* rhizomes.

CONCLUSION

Identification of beneficial rhizobacterial species promoting *V. turcica* growth in this study has shown that there was a strong dominance of *B. megaterium* at the rhizomes of *V. turcica*. The finding of this study might be a clue for further explanations on the endemism of *V. turcica*.

Further investigations on testing the direct PGPR effect on *V. turcica* and on secondary metabolite production ability, which is essential to convert soil minerals into available forms for plant roots to absorb, should be carried out.

Acknowledgments

The authors are grateful to Ali Nihat Gökyiğit and Prof. Dr. Adil Güner (Nezahat Gökyiğit Botanical Garden - NGBG) and would like to thank Ömer Demir (Nezahat Gökyiğit Botanical Garden - NGBG) for helping with the collecting samples. This work was supported by the Ali Nihat Gökyiğit Foundation of Turkey.

REFERENCES

AHEMAD M, KHAN S, ZAIDI A & WANI PA. 2009. Remediation of herbicides contaminated soil using microbes in sustainable agriculture. In: Khan MS, Zaidi A & Musarrat J (Eds), Microbes in Sustainable Agriculture, Nova Science Publishers, New York, USA.

AHEMAD M & KIBRET M. 2014. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. J King Saud Univ – Sci 26: 1-20.

ALTSCHUL SF, GISH W, MILLER W, MYERS EW & LIPMAN D. 1990. Basic local alignment search tool. J Mol Biol 215: 403-410.

BOAKYE EY, LAWSON IYD, DANSO SKA & OFFEI SK. 2016. Morphological, physiological, cultural characteristics, and IAA production of PGPR bacterial strains isolated from the wheat rhizosphere. Symbiosis 69: 89-99.

CHAKRABORTY U, CHAKRABORTY BN & CHAKRABORTY AP. 2012. Induction of plant growth Promotion in *Camellia sinensis* by *Bacillus megaterium* and its bioformulations. World J Agric Sci 8: 104-112.

DING Y, WANG J, LIU Y & CHEN S. 2005. Isolation and identification of nitrogen-fixing *Bacilli* from plant rhizospheres in Beijing Region. J Appl Microbiol 99: 1271-1281.

ELKOCA E, KANTAR F & SAHIN F. 2007. Influence of nitrogen fixing and phosphorus solubilizing bacteria on the nodulation, plant growth, and yield of chickpea. J Plant Nutr 31: 157-171.

FELSENSTEIN J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evol 39: 783-791.

GABY JC & BUCKLEY DH. 2012. A comprehensive evaluation of PCR primers to amplify the nifH gene of nitrogenase. PLoS ONE 7: e42149. doi: 10.1371/journal.pone.0042149.

GERMIDA JJ, SICILIANO SD, RENATO DE FREITAS J & SEIB AM. 1998. Diversity of root-associated bacteria associated with fieldgrown canola (*Brassica napus* L.) and wheat (*Triticum aestivum* L.). FEMS Microbiol Ecol 26: 43-50.

GLICK BR, CHENG Z, CZARNY J & DUAN J. 2007. Promotion of plant growth by ACC deaminase-producing soil bacteria. Eur J Plant Pathol 119: 329-339.

HOFFMANN ET AL. 2010. PCR-based method for targeting 16S-23S rRNA intergenic spacer regions among *Vibrio* species. BMC Microbiol 10: 90.

KADO CI & HESKETT MG. 1970. Selective media for isolation of *Agrobacterium, Corynebacterium*, *Erwinia*, *Pseudomonas* and *Xanthomonas*. Phytopathology 60: 969-976.

KHALIFA AYZ & ALMALKI MA. 2015. Isolation and characterization of an endophytic bacterium, *Bacillus megaterium* BMN1, associated with root-nodules of *Medicago sativa* L. growing in Al-Ahsaa Region, Saudi Arabia. Ann Microbiol 65: 1017-1026.

KIMURA M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16: 111-120.

LIANG JG, TAO RX, HAO Z, WANG LP & ZHANG X. 2011. Induction of resistance in cucumber against seedling damping-off by plant growth-promoting rhizobacteria (PGPR) *Bacillus megaterium* Strain L8. Afr J Biotechnol 10: 6920-6927.

LÓPEZ-BUCIO ET AL. 2007. *Bacillus megaterium* rhizobacteria promote growth and alter root-system architecture through an auxin-and ethylene-independent signaling mechanism in *Arabidopsis thaliana*. Mol Plant Microbe In 20: 207-217.

MALANICHEVA IA, KOZLOV DG, SUMARUKOVA IG, EFREMENKOVA OV & ZENKOVA VA. 2012. Antimicrobial activity of *Bacillus megaterium* strains. Microbiol 81: 178-185.

SAITOU N & NEI M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4: 406-425.

SHIVAJI S, CHATURVEDI P, BEGUM Z, PINDI PK & MANORAMA R. 2009. *Janibacter hoylei* sp. nov., *Bacillus isronensis* sp. nov. and *Bacillus aryabhattai* sp. nov., isolated from cryotubes used for collecting air from the upper atmosphere. Int J Sys Evol Microbiol 59: 2977-2986.

TAN K, VURAL M & KUCUKODUK M. 1983. An unusual new *Thermopsis* from Turkey. Notes Roy Bot Gard, Edinburgh.

TEKDAL D, CINGAY B & CETINER MS. 2018. Determination of soil nutrient status in *Vuralia turcica* populations growing at different locations in the Central Anatolia Region of Turkey. Turk J Bot 42: 317-326.

UTHANDI B & SIVAKUMAAR PK. 2013. *Bacillus megaterium* (AUM72)-mediated induction of defense related enzymes to enhance the resistance of turmeric (*Curcuma longa* L.) to *Pythium aphanidermatum* causing rhizome rot. Agr Sci J 10: 1-8.

UYSAL T, ERTUĞRUL K & BOZKURT M. 2014. A new genus segregated from *Thermopsis* (Fabaceae: Papilionoideae): *Vuralia*. Plant Syst Evol 300: 1627-1637.

VINCENT JM. 1970. A manual for practical study of root nodule bacteria. IBP Handbook No. 15, Blackwell Scientific Publishers, Oxford, 164 p.

VURAL M. 2009. Piyan (*Thermopsis turcica*). Bağbahçe 25: 14-16.

WALKER TS, BAIS HP, GROTEWOLD E & VIVANCO JM. 2003. Root exudation and rhizosphere biology. Plant Physiol 132: 44-51.

SUPPLEMENTARY MATERIAL

Figure S1. **Images of the rhizomes extracted from the determined coordinates (black numbers indicate the rhizome number, and the detail is given in Table I).**
Figure S2. **Status of isolated bacteria from the rhizomes collected from various habitats of *V. turcica* in YMA medium containing Congo red (left) or Bromothymol blue (right); Location 1 (1: Vt1N1, 2: Vt1N2, 3: Vt1N3, 4: Vt1N4), Location 3 (1: Vt3N1, 2: Vt3N2), Location 6 (1: Vt6N1, 2: Vt6N2).**
Figure S3. **Gram-staining results of rhizome-isolated bacteria; Location 1 (1: Vt1N1, 2: Vt1N2, 3: Vt1N3; 4: Vt1N4), Location 3 (1: Vt3N1, 2: Vt3N2), Location 6 (1: Vt6N1, 2: Vt6N2).**
Figure S4. **General view of colonies of isolates (1: isolate Vt1N1; 2: isolate Vt6N1).**
Table SI. **16S rRNA sequence results of rhizome-isolates.**

Table SII. **16S-23S rDNA ITS region sequence results of rhizome-isolates.**

How to cite
TEKDAL D, ÇİFTÇİ C, ÇINGAY B & ÇETİNER S. 2022. Isolation and characterization of the most abundant rhizobacterial species associated with *Vuralia turcica* (Fabaceae: Papilionoideae). An Acad Bras Cienc 94: e20191460. DOI 10.1590/0001-3765202220191460.

Manuscript received on 26 November, 2019;
accepted for publication on 29 January, 2020

DİLEK TEKDAL[1,2]
https://orcid.org/0000-0002-4545-9005

CEM ÇİFTÇİ[2,3]
https://orcid.org/0000-0001-8101-7602

BURÇİN ÇINGAY[4]
https://orcid.org/0000-0003-1001-1937

SELİM ÇETİNER[2]
https://orcid.org/0000-0002-9679-2748

[1] Department of Biotechnology, Faculty of Science and Letters, Mersin University, Yenişehir, 33343, Mersin, Turkey

[2] Biological Sciences and Bioengineering Program, Faculty of Engineering and Natural Sciences, Sabanci University, Orhanli-Tuzla, 34956, Istanbul, Turkey

[3] Department of Genetics and Bioengineering, Faculty of Engineering, Yeditepe University, Kayisdagi, 34755, Istanbul, Turkey

[4] Nezahat Gökyiğit Botanical Garden, 34774, Istanbul, Turkey

Correspondence to: **Dilek Tekdal**
E-mail: dilektekdal@mersin.edu.tr

Author contributions

Experiments were designed and conceived by [Dilek Tekdal]. Experiments were performed by [Dilek Tekdal and Cem Ciftci]. Rhizomes were collected by [Burçin Çıngay]. Data collection and analysis were performed by [Dilek Tekdal]. The sequences were deposited into the GenBank database by [Dilek Tekdal]. The first draft of the manuscript was written by [Dilek Tekdal and Cem Ciftci], and all authors commented on previous versions of the manuscript. Revisions were made by [Dilek Tekdal]. [Selim Çetiner] supervised the study. All authors read and approved the final manuscript.