Cilt 10, Sayı 2, Sayfa 182-190, 2021 Volume 10, Issue 2, Page 182-190, 2021

Araştırma Makalesi https://doi.org/10.46810/tdfd.937841 Research Article

Türk Doğa ve Fen Dergisi
Turkish Journal of Nature and Science
www.dergipark.gov.tr/tdfd

Rhizospheric PGPR Strains of Wheat, Barley and Trefoil Grown in Ağrı Province

Burak ALAYLAR[1*], Medine GULLUCE[2], Mehmet KARADAYI[2], Taha Yasin KOC[3], Gökce KARADAYI[4]

[1]*Agri Ibrahim Cecen University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, Agri, Turkey
[2] Ataturk University, Faculty of Science, Department of Biology, Erzurum, Turkey
[3] Ataturk University, Institute of Natural and Applied Sciences, Erzurum, Turkey
[4] Ataturk University, Faculty of Science, Department of Molecular Biology and Genetics, Erzurum, Turkey

Burak ALAYLAR ORCID No: 0000-0001-6737-3440
Medine GULLUCE ORCID No: 0000-0002-5957-8259
Mehmet KARADAYI ORCID No: 0000-0002-2473-0409
Taha Yasin KOC ORCID No: 0000-0002-7786-5462
Gökce KARADAYI ORCID No: 0000-0003-2044-9609

Corresponding author: balaylar@agri.edu.tr

(Alınış: 16.05.2021, Kabul: 04.11.2021, Online Yayınlanma: 31.12.2021)

Keywords
PGPR, Nitrogen fixation, Phosphate-solubilization, Siderophore production, 16S rRNA

Abstract: The present study aimed to isolate potential plant growth rhizobacteria (PGPR) from various crops in agricultural areas in Ağrı-Turkey and their characterization. For this goal, rhizospheric soil samples of wheat, barley and trefoil were collected from 12 different locations in Ağrı province. Bacteria isolation studies were carried out with these rhizospheric samples. Then, to determine the PGPR properties of each isolate; nitrogen fixation, phosphate dissolution, siderophore, ammonia and HCN production tests were performed. Molecular identification of active isolates determined as suitable for development of biofertilizers, biostimulants and/or bioprotectants was done by PCR and sequencing applications performed with universal 16S rRNA primers. According to the results, 29 potential PGPR isolates were determined and their molecular characterization was done. These isolates were distributed in *Arthrobacter*, *Bacillus*, *Brevibacterium*, *Enterobacter*, *Pantoea*, *Pseudomonas*, *Microbacterium* and *Stenotrophomonas* according to their 16S rRNA sequence similarities.

Ağrı İlinde Yetiştirilen Buğday, Arpa ve Yoncanın Rhizosferik PGPR Suşları

Anahtar Kelimeler
PGPR, Azot fiksasyonu, Fosfat çözünmesi, Siderofor üretimi, 16S rRNA

Öz: Mevcut çalışmamızda; Ağrı ili ve çevresinde yer alan tarım alanlarında yetişmekte olan buğday, arpa ve yonca gibi zirai mahsullerden potansiyel bitki büyümesini teşvik eden rizobakterilerin (PGPR) izolasyonu ve karakterizasyonu amaçlanmıştır. Bu amaçla, Ağrı ilinin 12 farklı noktasından buğday, arpa ve yonca rizosferik toprak örnekleri toplanmıştır. Bakteri izolasyonu çalışmaları bu rizosferik örneklerle gerçekleştirilmiştir. Daha sonra her bir izolatın PGPR özelliklerini belirlemeye yönelik; azot bağlama, fosfat çözme, siderofor, amonyak ve HCN üretimi testleri yapılmıştır. Biyogübre, biyostimülan ve/veya biyo-koruyucuların geliştirilmesine uygun olduğu belirlenen aktif izolatların moleküler tanımlaması, evrensel 16S rRNA primerlerinin kullanıldığı PCR ve dizileme uygulamaları ile yapılmıştır. Elde edilen Sonuçlara göre 29 potansiyel PGPR izolatı tanılanmış ve moleküler karakterizasyonu yapılmıştır. Bu izolatlar, 16S rRNA sekans benzerliklerine göre *Arthrobacter*, *Bacillus*, *Brevibacterium*, *Enterobacter*, *Pantoea*, *Pseudomonas*, *Microbacterium* ve *Stenotrophomonas* cinsleri içerisinde dağılım göstermiştir.

1. INTRODUCTION

The world population is increasing rapidly in recent years and the food is one of the basic demands for human life. This situation has revealed the necessity of determining various alternative strategies to feed the growing population [1, 2]. With the start of the green revolution, there has been a significant increase in the

use of pesticides and chemical fertilizers in the strategies developed in this field and an alternative solution has been produced to meet the demands in the short term [3, 4]. However, the production costs of these chemical fertilizers have increased and their improper use have caused both economic and environmental destruction such as; high costs, decreasing of microbial diversity and soil productivity, arising of phytopathogens and insect resistance and pollution of environment and agricultural lands [3-5]. As an alternative solution to chemical fertilizers, microbial fertilizers are widely utilized all over the world recently. Among microbial fertilizers, bacteria based fertilizers play crucial role in plant growth and health under different climate conditions [6]. Beneficial effects of soil bacteria have been discovered in crop production for decades.

Many soil bacteria colonize the roots of plants or free-living organisms that have profitable effects on the growth and health of the plant are called plant growth promoting rhizobacteria (PGPR) [7-11]. It has been reported plenty of rizospheric bacteria genera which have beneficial roles for enhancing crop productivity from various agricultural products [12-15]. *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Chromobacterium*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Micrococcus*, *Pantoea*, *Pseudomonas* and *Serratia* are a group of PGPR microorganisms, with the ability to stimulate and plant growth via different mechanisms [16-18].

These mechanisms are divided into three groups as biofertilizers, biostimulants and bioprotectants. For instance; enhancing to nitrogen fixation and phosphate-solubilization which help to nutrients proper to plants can be good example of PGPR's biofertilizer properties [19]. It is also known that their biostimulant effects on plant via production of phytohormones such as indole-3-acetic acid (IAA) [16]. Furthermore, antibiotics, siderophores and lytic enzyme production have caused suppression of soil borne pathogens as bioprotectant [17].

In the current study, we aimed to isolate and characterize of potential PGPR strains from agricultural areas of Ağrı Province-Turkey. It is the promising step for identification of novel local potential PGPR strains gain to improve usage of new microbial based fertilizers in agricultural areas. Hence, 29 potential PGPR strains were characterized from 12 different locations from the agricultural regions in order to assessment of their potential ability to promote plant growth.

2. MATERIALS AND METHODS

2.1. Soil Sampling

Rhizospheric soil samples were collected in May 2019 from agricultural areas in Ağrı-Turkey. Sampling was taken from 12 different sites, at depts of 0-7 cm in the region of directly surrounding the roots of various agricultural crops such as wheat, barley and trefoil. Each soil samples were labeled with codes according to their localities. Then, the samples were brought to the Central Research and Application Laboratory, Agri Ibrahim Cecen University aseptically.

2.2. Isolation of bacterial isolates

The serial dilution method was utilized to isolate bacterial isolates. According to this method, soil samples were transferred into 10 ml of sterile isotonic saline water, and homogenized. Then, dilution series were prepared between 10^{-1} and 10^{-6}. These dilutions were spread on nutrient agar (NA) plates, incubated for 2 days at 28 ºC. After the incubation period, distinct bacterial colonies were streaked on NA media to obtain pure single colonies.

2.3. Plant growth promoting activity tests

2.3.1. N_2 Fixation Assay

Nitrogen fixation assay was performed to determine the nitrogen fixation ability of bacterial strains. The assay was used according to the Jensen's modified method [20] into Nitrogen free medium (NFM). Isolates were inoculated onto this medium, incubated, and those that could grow there were considered positive for nitrogen fixation.

2.3.2. Phosphate-Solubilization Assay

Phosphate solubilization potential of the bacterial strains was determined by using modified Pikovskaya's (PKV) agar medium. Isolates were inoculated onto this medium. Plates were incubated at 28±1 ^{0}C for 2–7 days and phosphate solubilization was observed for halo zones around the colonies [21].

2.3.3. Siderophore Production Assay

The siderophore production assay of the bacterial strains performed to Louden et al. procedure [22]. According to this procedure; all glassware wash with 6M HCI to detract from any trace elements and it should rinse with ddH_2O. After Chrome azurol S (CAS) agar need to prepare respectively as three following main solution as; blue dye solution, mixture solution and CAS agar solution. After inoculation of bacterial cultures on CAS agar plates, the plates were incubated at 28 °C for 2-7 days. Siderophore-producing bacteria demonstrated an alteration in color, from blue to orange around the colonies. The bacteria with orange halo around itself was determined as siderophore-producing bacteria.

2.3.4. Determination of Ammonia Potential Assay

The ammonia production property was determined by using Marques et al. procedure [23]. The bacterial strains were transferred to peptone water (Peptone 20.0 g/L and NaCl 30.0 g/L) with constant shaking at 140 rpm for 5 days at 30 °C. After incubation, 0.2 mL of the culture supernatant was mixed with 1 mL Nessler's reagent. The OD of the mixture was measured at 450 nm using a

spectrophotometer, and an end point of a brown to yellow color was evaluated as ammonia production.

2.3.5. HCN Production Assay

HCN production assay was performed by modified method of Bakker and Schippers's method [24]. Bacterial strains were streaked on solid agar plates added with or without 4.4 g glycine l^{-1}. Then, filter paper soaked in 0.5% picric acid in 1% Na_2CO_3 in the plates. The plates were covered with parafilm and incubated at 28± 1 ◦C. At the final stage; from yellow to light brown, moderate brown or strong brown was determined as HCN production.

2.4. Identification of Bacterial Strains

2.4.1. Biochemical, Morphological and Physiological Characterization of Bacterial Strains

Staining procedures, microscopic examinations and biochemical tests were carried out to the Harley and Prescott method (Cell morphologies, Gram property, catalase and oxidase activities *etc*.) [25].

2.4.2. Molecular Characterization of Bacterial Strains

DNA extraction studies of the potential PGPR bacterial strains were utilized with the method described by Sezen [26]. Extracted DNA samples of the potential PGPR strains were used as a template for 16S rRNA gene analysis. The 16S rRNA genes were amplified with PCR using the following forward and reverse primers: 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-CGGTTACCTTGTTACGACTT-3'). Each reaction was carried out in a 30 µl reaction mixture containing; 3 µl 10 x PCR buffer, 0.6 µl dNTP, 3 µl primer 16SF, 3 µl primer 16SR, 1.2 µl DMSO, 0.6 µl $MgCl_2$ (50 µM), 0.3 µM / ml Taq DNA polymerase, 15.3 µl sdH_2O and 3 µl DNA. PCR program was as follows; The reaction was performed with an initial step at 95 ºC for 2 min, and 36 cycles of 1 min at 94 ºC, 1 min at 53 ºC, 2 min at 72 ºC, followed by a final 5 min extension step at 72 ºC, then brought down to 4 ºC. Then, in the electrophoresis stage, 7 µl of the PCR products was mixed with 3 µl of 6× gel loading buffer and loaded onto an agarose gel (1.5% w/v) supplemented with ethidium bromide. Electrophoresis was done in 0.5× TBE (Tris-Borate-EDTA) buffer at 90 V for 120 min. The DNA product was detected by using the Bio Doc Image Analysis System with Uvisoft analysis package (Cambridge, UK). The amplified gene products were sequenced by Macrogen Inc. (Netherlands). The nucleotide BLAST (Basic Local Alignment Search Tool) search program of NCBI was used to determine the nucleotide sequence homology. The gene sequences were assigned to GenBank®.

3. RESULTS

Twelve sites were selected from different regions in Ağrı province. Location information and sampling data of the regions are given in the Table 1. According to the field studies, 32 rhizospheric soil samples from 12 sites were brought to the laboratory for bacterial isolations. Totally, isolation of 178 bacteria has been done by considering colony characteristics of bacterial strains. Single colonies were chosen for further studies and colony morphology different strains were distinguished.

Among these 178 bacteria were prepared for the detection of PGPR properties. Then, nitrogen fixation, phosphate-solubilization, siderophore, HCN, and ammonia production assays were done respectively. According to the results the best capability of plant growth properties of bacterial strains was chosen for potential local PGPR strains. Inside of these bacterial strains; 6 of the bacterial strains were nitrogen-fixing, 11 phosphate-solubilization, 21 siderophore production, 22 bacteria ammonia production. 5 of the bacterial strains were showed to phosphate-solubilization, siderophore and ammonia production. Also, 2 of the bacteria both phosphate and nitrogen-fixing. The detailed information about PGPR properties were given in Table 2. All the potential PGPR strains were selected for the molecular identification studies.

Twenty-nine species of potential PGPR isolated and identified by using 16S rRNA gene analysis. Data of the 16S rRNA gene sequencing showed that the active strains grouped in *Arthrobacter*, *Bacillus*, *Brevibacterium Enterobacter*, *Pantoea*, *Pseudomonas*, *Microbacterium* and *Stenotrophomonas* genera. According to the results, 1 isolate was assigned to *Brevibacterium frigoritolerans*, 1 isolate to *Bacillus safensis*, 1 isolate to *Bacilllus simplex*, 1 isolate to *Bacillus thuringiensis*, 3 isolates to *Bacillus* sp., 4 isolates to *Bacillus pumilus*, 1 isolate to *Microbacterium hydrocarbonoxydans*, 2 isolates to *Microbacterium* sp., 3 isolate to *Pseudomonas* sp., 1 isolate to *Pseudomonas brassicacearum*, 1 isolate to *Pseudomonas kilonensis*, 1 isolate to *Pseudarthrobacter oxydans*, 3 isolate to *Arthrobacter* sp., 2 isolates to *Enterobacter ludwigii*, 2 isolates to *Pantoea* sp., 1 isolate to *Stenotrophomonas* sp., and 1 isolate to *Brevibacterium frigoritolerans*. The strains were on 100% identical to the closest relatives registered in GenBank. The length of the identified nucleotides sequences of 16S rRNA genes of the strains recognized as adequate for reliable identification based on 16S rRNA genes analysis using BLAST tool. The accession number was obtained from the NCBI-GenBank® and detailed information about these strains were given in Table 3. The phylogenetic tree was constructed from the partial 16S rRNA region sequencing data via the neighbor-joining method using the software package MEGA 4.0 [27] and demonstrated in Figure 1.

Table 1. Bacterial strains isolation locations and sampling data informations from agricultural areas in Ağrı

No	Sampling regions	Locations	Sampling number	Isolation source
1	Doğubeyazıt Road 12. Km/Yoncalı village - Ağrı	39°42'41"N 43°7'59"E	3	Agricultural areas
2	Doğubeyazıt Road - Kazlı village - Ağrı	39°41'30"N 43°12'49"E	3	Trefoil
3	Doğubeyazıt Road Kazlı village - Ağrı	39°41'30"N 43°12' 51"E	3	Barley
4	Taşlıçay-Ağrı	39°39'13"N 43°21' 16"E	2	Wheat
5	Taşlıçay-Ağrı	39°39'28"N 43°20' 0"E	4	Agricultural areas
6	Doğubeyazıt Road Murat Bucağı village - Ağrı	40°31'21" N 41°32'4" E	2	Agricultural areas
7	Hamur-Yoğun hisar village – Ağrı	39°37'27.2893"N 43°0' 12.204"E	3	Agricultural areas
8	Hamur–Ağrı	39°36'25.6464"N 42°58' 21.414"E	2	Agricultural areas
9	Eleşkirt-Müftüselim District/Ağrı	39°48'18.5256 N 42°42' 52.5492 E	3	Trefoil
10	Sadaklı village - Eleşkirt/Ağrı	39°48'49.4352" N 42°45' 54.126"E	2	Wheat
11	Eleşkirt/Ağrı	39°48'53.0424"N 42°45' 53.802"E	3	Barley
12	Eleşkirt/Ağrı	39°48'53.0424"N 42°45' 53.802"E	2	Trefoil

Table 2. Investigation of PGPR properties of bacterial strains using conventional methods

Codes	NCBI codes	N_2 fixation	Phosphate solubilization	Siderophore production	Ammonia production	HCN production
BP9	Agri-1	-	+	+	+	-
BP11	Agri-2	-	+	+	+	-
BP14	Agri-3	-	+	+	+	-
BP15	Agri-4	-	-	+	+	-
BP18	Agri-5	-	-	+	+	-
BP48	Agri-6	+	-	+	+	-
BP51	Agri-7	+	-	+	slight	-
BP52	Agri-8	-	+	-	+	-
BP69	Agri-9	-	-	+	+	-
BP90	Agri-10	-	+	-	+	-
BP2	Agri-11	-	-	+	+	-
BP4	Agri-12	-	-	+	slight	-
BP5	Agri-13	+	+	-	+	-
BP7	Agri-14	-	-	+	+	-
BP8	Agri-15	-	+	+	+	-
BP10	Agri-16	-	-	+	+	-
BP26	Agri-17	+	-	-	slight	-
BP27	Agri-18	-	-	+	slight	-
BP29	Agri-19	-	-	+	+	-
BP31	Agri-20	-	-	+	+	-
BP33	Agri-21	-	-	+	+	-
BP45	Agri-22	-	+	-	slight	-
BP46	Agri-23	-	+	+	+	-
BP55	Agri-24	+	+	-	+	-
BP64	Agri-25	-	-	+	slight	-
BP74	Agri-26	-	+	-	+	-
BP83	Agri-27	+	-	-	+	-
BP85	Agri-28	-	-	+	+	-
BP98	Agri-29	-	-	+	+	-

Table 3. Taxonomic affiliation of the bacterial isolates and their GenBank® accession numbers

Strain Code	NCBI Code	Strain Name	Length (bp)	Accession number	Percentage identity
BP9	Agri-1	*Bacillus safensis*	1424	MN900701	100
BP11	Agri-2	*Pseudomonas* sp.	1400	MN900702	100
BP14	Agri-3	*Bacillus pumilus*	1416	MN900703	100
BP15	Agri-4	*Bacillus* sp.	1416	MN900704	100
BP18	Agri-5	*Microbacterium* sp.	1397	MN900705	100
BP48	Agri-6	*Stenotrophomonas* sp.	1414	MN900706	100
BP51	Agri-7	*Bacillus* sp.	1429	MN900707	100
BP52	Agri-8	*Pseudomonasbrassicacearum*	1410	MN900708	100
BP69	Agri-9	*Microbacterium sp.*	1377	MN900709	100
BP90	Agri-10	*Pseudomonas* sp.	1410	MN900710	100
BP2	Agri-11	*Glutamicibacter* sp.	1394	MT102719	100
BP4	Agri-12	*Bacillus simplex*	1423	MT102720	100
BP5	Agri-13	*Bacillus pumilus*	1407	MT102721	100
BP7	Agri-14	*Bacillus pumilus*	1420	MT102722	100
BP8	Agri-15	*Bacillus pumilus*	1415	MT102723	100
BP10	Agri-16	*Microbacterium hydrocarbonoxydans*	1373	MT102724	100
BP26	Agri-17	*Bacillus* sp.	1429	MT102725	100
BP27	Agri-18	*Pseudarthrobacter oxydans*	1360	MT102726	100
BP29	Agri-19	*Pantoea agglomerans*	1071	MT102727	100
BP31	Agri-20	*Pantoea sp.*	1046	MT102728	100
BP33	Agri-21	*Arthrobacter sp.*	1359	MT102729	100
BP45	Agri-22	*Arthrobacter sp.*	1102	MT102730	100
BP46	Agri-23	*Enterobacter ludwigii*	913	MT102731	100
BP55	Agri-24	*Pseudomonas kilonensis*	1399	MT102732	100
BP64	Agri-25	*Brevibacterium frigoritolerans*	1422	MT102733	100
BP74	Agri-26	*Pseudomonas sp.*	1054	MT102734	100
BP83	Agri-27	*Enterobacter ludwigii*	1384	MT102735	100
BP85	Agri-28	*Arthrobacter* sp.	1108	MT102736	100
BP98	Agri-29	*Bacillus thuringiensis*	1416	MT102737	100

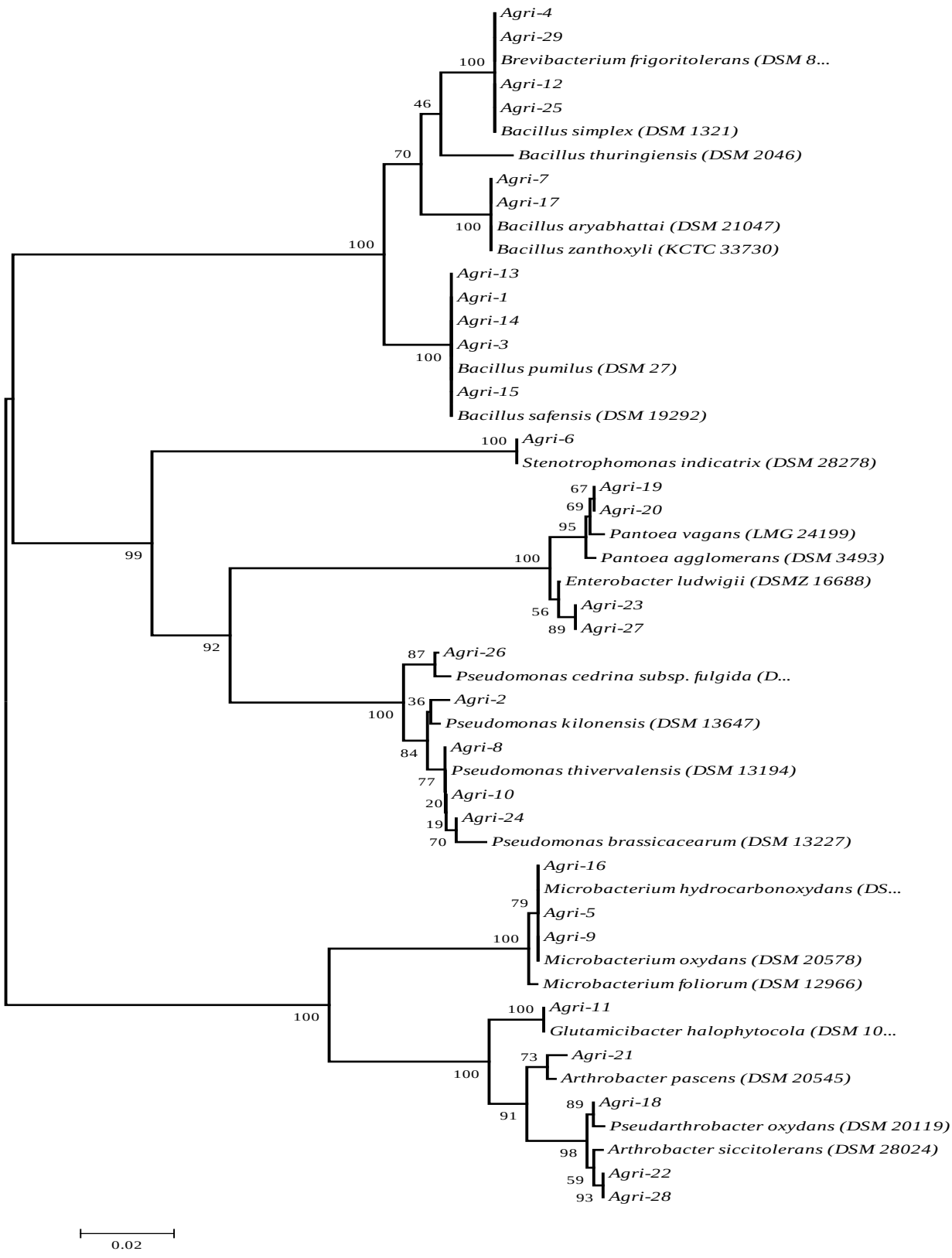

Figure 1. Neighbor joining phylogenetic tree on the basis of 16S rRNA gene sequence data of potential PGPR strains from agricultural areas Ağrı in Turkey. The scale bar represented 2% divergence.

4. DISCUSSION

Recent researches showed that the detrimental effects of various chemical fertilizers utilized in agricultural areas have caused to researchers to find alternative solutions and methods [4]. PGPR is the one of the most promising alternative application in agricultural areas since past decades because of their beneficial effects on agricultural lands, product yield, soil and plant health [18]. Furthermore, PGPR can support plant growth and development with various mechanisms. Among these mechanisms, nitrogen fixation, phosphate-solubilization, siderophores producing, ammonium production, HCN production etc. are the well-studied PGPR mechanisms according to the literature [15, 16, 28]. Each of these mechanisms have critical role in not only plant growth and health but also soil health. For instance, Nitrogen-fixation is played crucial role in conversion of atmospheric dinitrogen to ammonia. Because nitrogen (N_2) is a unique element for all living organism and plants cannot utilize it straightly. Therefore, nitrogen-fixation have significant role in plant growth and development [4]. The other nitrogen source for plants is ammonia because of its fruitful nitrogen containing molecules [29]. After the N_2, phosphorus (P) is a fundamental and pivotal macronutrient for plant development and growth.

Therefore, phosphate solubilization is one of the most crucial properties of PGPR [2, 30-31]. Also, siderophore production is well-known PGPR mechanism which have pivotal role in improve the bioavailability of iron and synthesis auxins, cytokinins, gibberellins *etc*. These phytohormones have key roles in different stage of plant growth processes [29].

In the current study; we isolated 29 potential PGPR strain from agricultural areas in Ağrı-Turkey. Profiling of isolates showed that these contained 29 strains belonging to (10) *Bacillus* species as [*B. safensis* (Agri-1), *B. pumilus* (Agri-3), *Bacillus* sp. (Agri-4), *Bacillus* sp. (Agri-7), *B. simplex* (Agri-12), *B. pumilus* (Agri-13), *B. pumilus* (Agri-14), *B. pumilus* (Agri-15), *Bacillus* sp. (Agri-17), *B. thuringiensis* (Agri-29)] and (5) *Pseudomonas* species as; [*Pseudomonas* sp. (Agri-2), *Pseudomonas brassicacearum* (Agri-8), *Pseudomonas* sp. (Agri-10), *Pseudomonas kilonensis* (Agri-24), *Pseudomonas* sp. (Agri-26)] and (4) *Arthobacter* species as; *Pseudarthrobacter oxydans* (Agri-18), *Arthrobacter* sp. (Agri-21), *Arthrobacter* sp. (Agri-22), *Arthrobacter* sp. (Agri-28),] and (3) *Microbacterium* species as; [*Microbacterium* sp. (Agri-5), *Microbacterium* sp. (Agri-9), *Microbacterium hydrocarbonoxydans* (Agri-16)] and (2) *Enterobacter* species as ; [*Enterobacter ludwigii* (Agri-23), *Enterobacter ludwigii* (Agri-27)] and (2) *Pantoea* species as; [*Pantoea agglomerans* (Agri-19), *Pantoea* sp. (Agri-20). Also (1) *Stenotrophomonas* sp. and *Brevibacterium frigoritolerans* were identified. As it cited previous studies showed that *Arthrobacter*, *Bacillus*, *Brevibacterium*, *Enterobacter*, *Pseudomonas*, *Microbacterium*, *Pantoea*, and *Stenotrophomonas* are known as isolated potential PGPR strains from soil rhizosphere. Among these genera, *Bacillus* and *Pseudomonas* play a critical role in host root colonization at very high productivity. Therefore, these potential PGPR strains are contributed to production of growth metabolites for enhanced to strategic crops yield [32].

According to a report by Banerjee et al. [12] *Arthrobacter* sp. and *Bacillus* sp. isolated tomato rhizosphere from Kalyani, West Bengal in India showed phosphate solubilization. Kumar et al. [13] reported that isolation and characterization of rhizobacteria associated with coastal agricultural ecosystem of rhizosphere soils of cultivated vegetable crops which have capability of siderophore production, inorganic phosphate solubilization and IAA production and these strains and their planth growth properties (PGP) were reported as respectively; belonging to [*Bacillus subtilis*, *Bacillus licheniformis*, *Pseudomonas synxantha* (siderophore production)], [*Staphylococcus sciuri* sp. *sciuri*, *Staphylococcus intermedius*, *Bacillus pumilus* (phosphate solubilization)] and [*Alcaligenes faecalis* sp. *faecalis*, *Enterobacter cloacae* sp. *dissolvens* (IAA production). Kumar et al. [33] reported that *Bacillus* isolates identified from rhizosphere of common bean growing at Uttarakhand Himalaya in India demonstrated PGP activities such as IAA, HCN and siderophore production and phosphate-solubilization potentials. In other study, *P. agglomerans* Ima2 demonstrated significantly most of the plant growth properties [34]. Kesaulya et al. [35] isolated numerous potential PGPR strains from potato plant rhizosphere which have capability of siderophore production, inorganic phosphate solubilization and (IAA) production. A study by Tara and Saharan [36] revealed that *Brevibacterium frigrotolerans* SMA23 isolated from *Aloe vera* rhizosphere have multiple plant growth promotion properties such as phosphate solubilization, IAA production and siderophore production. A study by Wang et al. [37] revealed that *Stenotrophomonas maltophilia* W-6 have nitrogen-fixing capability. In the study by Habibi et al. [15] were found to strains of *Enterobacter ludwigii* have IAA production, phosphate solubilizing and siderophore production capabilities. *Microbacterium maritypicum* strain DSM 12512 was found to have IAA and siderophore production by Nadiéline et al. [38]. The result obtained in the present paper revealed that the most of the bacterial isolates studied had at least one of the PGP properties among the nitrogen fixation, phosphate-solubilization, siderophore, ammonia and HCN production. The PGP parameters of the 29 bacterial strains were identified. Moreover, the results of the present study indicated that predominantly of the isolated bacterial strains have substantial PGP parameters thought to play a pivotal role in control several soil borne diseases and ameliorate the health of soil and agricultural crops according to the previous literature knowledge. To the best of our knowledge, this is the first study investigating of potential PGPR strains from agricultural areas in Ağrı-Turkey. Our findings showed that soil rhizosphere has high potential of cultivable bacteria exhibiting multiple PGP activities. Because it is well known that PGPR having PGP activities can increase plant growth, soil fertility and

crop productivity. In this context; these 29 strains are potential bacterial strains with promise in crop growth and soil fertility in agricultural fields in Ağrı province.

Moreover, these potential PGPR strains can be utilized in similar agricultural products, climate and soil conditions. The strains isolated in this study, containing diverse species were found to beneficial effects on various crops such as; wheat, barley and trefoil *etc*.

5. CONCLUSION

The effective usage of potential PGPR strains can be used for reduce the hazardous effects of chemical fertilizers on the environment for sustainable agriculture. Therefore, local strains in this study can be used agricultural areas in Ağrı-Turkey for sustainable crop production as a local biofertilizers, biostimulants and bioprotectants.

Acknowledgement
This work was supported by Agri Ibrahim Cecen University Scientific Research Project (Project number: FEF.18.006-2018).

Conflict of interests
The author declares that they have no conflict of interest.

REFERENCES

[1] Wezel A, Casagrande M, Celette F, Vian JF, Ferrer A, et al. Agroecological practices for sustainable agriculture. A review. Agronomy for Sustainable Development. 2014;34: 1–20.
[2] Alaylar B, Güllüce M, Karadayı G, Karadayı M. Isolation of pgpr strains with phosphate solubilizing activity from Erzurum and their molecular evaluation by using newly designed specific primer for *pqqB* Gene. International Journal of Scientific and Engineering Research. 2018;9(5);103-106.
[3] Aeron A, Khare E, Jha CK, Meena VS, Abdel Aziz SM, Islam MT, et al. Revisiting the plant growth-promoting rhizobacteria: lessons from the past and objectives for the future. Archives of Microbiology. 2020;202:665-676.
[4] Alaylar B, Güllüce M, Karadayı M. Detection of the *nifH* gene in nitrogen-fixing bacteria from agricultural areas in Erzurum. Fresenius Environmental Bulletin. 2020;29 (2): 809–814.
[5] Ibal JC, Jung BK, Park CE, Shin JH. Plant growth-promoting rhizobacteria used in South Korea. Applied Biological Chemistry. 2018; 61(6):709-716.
[6] Egamberdieva D, Davranov K, Wirth S, Hashec A, Abd_Allah EF. Impact of soil salinity on the plant-growth-promoting and biological control abilities of root associated bacteria. Saudi Journal of Biological Sciences. 2017;24(7):1601-1608.
[7] Abbas-Zadeh P, Saleh-Rastin N, Asadi-Rahmani H, Khavazi K, Soltani A, Shoary-Nejati AR, et al. Plant growth-promoting activities of *fluorescent pseudomonads*, isolated from the Iranian soils. Acta Physiologiae Plantarum.2010;32:281-288.
[8] Rasool L, Asgha M, Jamil A, Rehman SU. Identification of *Azospirillum* species from wheat rhizosphere. The Journal of Animal & Plant Sciences. 2015;25 (4):1081-1086.
[9] Shaheen T, Khan AA, Rahman M, Qamar MTU, Rahman M. Estimation of genetic diversity of plant growth promoting rhizobacteria (pgpr) strains found in different areas of Pakistan using RAPD and 16S rRNA analysis. The Journal of Animal & Plant Sciences.2015;25(5):1457-1465.
[10] Karadayı M, Güllüce M, Doğan S, Güllüce E. Isolation and molecular characterization of bacteria with plant growth-promoting characteristics from magnesite mining fields in Kütahya-Turkey. Journal of Applied Biological Sciences. 2016;10(3): 27-32.
[11] Rehman B, Hassan TUI, Bano A. Potential of indole-3-acetic acid-producing rhizobacteria to resist Pb toxicity in polluted soil. Soil and Sediment Contamination. 2019;28(1): 101-121.
[12] Banerjee S, Palit R, Sengupta C, Standing D. Stress induced phosphate solubilization by Arthrobacter sp. and *Bacillus* sp. isolated from tomato rhizosphere. Australian Journal of Crop Science. 2010;4(6):378-383.
[13] Kumar K, Amaresan N, Bhagat S, Madhuri K, Srivastava RC. Isolation and characterization of rhizobacteria associated with coastal agricultural ecosystem of rhizosphere soils of cultivated vegetable crops. World Journal of Microbiology and Biotechnology. 2011;27: 1625-1632.
[14] Pérez-Montano F, Alías-Villegas C, Bellogín RA, Del Cerro P, Espuny MR, Jiménez-Guerrero I, et al. Plant growth promotion in cereal and leguminous agricultural important plants: From microorganism capacities to crop production. Microbiological Research. 2014;169(5-6):325-336.
[15] Habibi S, Djedidi S, Ohkama-Ohtsu N, Sarhadi WA, Kojima K, Rallos RV, et al. Isolation and screening of indigenous plant growth-promoting rhizobacteria from different rice cultivars in Afghanistan soils. Microbes and Environments. 2019;34(4): 347-355.
[16] Sharan BS, Nehra V. Plant growth promoting rhizobacteria: A critical review. Life Sciences and Medicine Research. 2011;2011:1-30.
[17] Chuks Kenneth O. Plant growth promoting rhizobacteria (pgpr): A bioprotectant bioinoculant for sustainable agrobiology. A review. International Journal of Advanced Research in Biological Sciences. 2017;4(5):123-142.
[18] Oleńska E, Małek W, Wójcik M, Swiecicka I, Thijs S, Vangronsveld J, et al. Beneficial features of plant growth-promoting rhizobacteria for improving plant growth and health in challenging conditions: A methodical review. Science of the Total Environment. 2020;743: 1-21.
[19] Vessey JK. Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil. 2003; 255:571-586.
[20] Jensen HL. Pro Line Soc. NSW; 1942.

[21] Pikovskaya RI. Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. Mikrobiologiya. 1948;17:362–370.
[22] Louden BC, Haarmann, Lynne AM. Use of blue agar CAS assay for siderophore detection. Journal of Molecular Biology and Biology Education. 2011;12(1):51-53.
[23] Marques APGC, Pires C, Moreira H, Rangel AOSS, Castro PML. Assessment of the plant growth promotion abilities of sixbacterial isolates using *Zea mays* as indicator plant. Soil Biology and Biochemistry. 2010;42:1229–1235
[24] Bakker AW, Schippers B. Microbial cyanides production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp. mediated plant growth stimulation. Soil Biology and Biochemistry. 1987;19(4):451–457.
[25] Harley JP, Prescott LM. Laboratory Exercises in Microbiology. New York; USA. McGraw-Hill Press; 2002.
[26] Sezen S. Isolation and molecular characterization of microorganisms with usage potential for lignite enrichment technology from Afşin-Elbistan lignite mine [dissertation]. Erzurum: University of Ataturk; 2015.
[27] Baltaci MO, Genc B, Arslan S, Adiguzel G, Adiguzel A. Isolation and characterization of thermophilic bacteria from geothermal areas in Turkey and preliminary research on biotechnologically important enzyme production. Geomicrobiology Journal. 2017; 34(1):53–62.
[28] Ferchichi N, Toukabri W, Boularess M, Smaoui A, Mhamdi R, Trabelsi D. Isolation, identification and plant growth promotion ability of endophytic bacteria associated with lupine root nodule grown in Tunisian soil. Archives of Microbiology. 2019;201(3): 1333-1349.
[29] Orhan F. Alleviation of salt stress by halotolerant and halophilic plant growth-promoting bacteria in wheat (*Triticum aestivum*). Brazilian Journal of Microbiology. 2016;47 (3):621-627.
[30] Alaylar B, Güllüce M, Karadayı M, Isaoglu M. Rapid detection of phosphate-solubilizing bacteria from agricultural areas in Erzurum. Current Microbiology. 2019; 76(7):804-809.
[31] Alaylar B, Egamberdieva D, Gulluce M, Karadayi M, Arora NK. Integration of molecular tools in microbial phosphate solubilization research in agriculture perspective. World Journal of Microbiology and Biotechnology. 2020;36(93):1-12.
[32] Bhattacharyya PN, Jha DK. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World Journal of Microbiology and Biotechnology. 2012; 28:1327-1350.
[33] Kumar P, Dubey RC, Maheshwari DK. Bacillus strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. Microbiological Research. 2012;167: 493-499.
[34] Silini-Chérif H, Silini A, Ghoul M, Yadav S. Isolation and characterization of plant growth promoting traits of a rhizobacteria: *Pantoea agglomerans* Ima2. Pakistan Journal of Biological Sciences. 2012;15 (6): 267-276.
[35] Kesaulya H, Baharuddin B, Zakaria B, and Syaiful SA. Isolation and physiological characterization of PGPR from potato plant rhizosphere in medium land of Buru Island. First International Symposium on Food and Agro-biodiversity, ISFA 2014. Semerang: Elsevier; 2014. p. 190-199.
[36] Tara N, Sharan BS. Plant growth promoting traits shown by bacteria *Brevibacterium frigrotolerans* SMA23 isolated from Aloe vera rhizosphere. Agricultural Science Digest. 2017;37 (3): 226-231.
[37] Wang S, Xu Y, Li Z. Nitrogen utilization and transformation of *Stenotrophomonas maltophilia* W-6 with nitrogen-fixing ability. The Preprint Server for Biology. 2018;1-32.
[38] Nadiéline CV, Ndiaye MF, Fall S, Krasova T, Le Quéré A, Diouf D. Isolation and characterization of potential phosphate solubilizing bacteria in two regions of Senegal. African Journal of Microbiology Research. 2019;13(28):609-618.