Journal of Tekirdag Agricultural Faculty
Tekirdağ Ziraat Fakültesi Dergisi

Aralık/December 2023, 20(4)
Başvuru/Received: 07/02/23
Kabul/Accepted: 06/06/23
DOI: 10.33462/jotaf.1244111

http://dergipark.gov.tr/jotaf
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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Determination of Bioremediation Potentials and Plant Growth-Promoting Properties of *Bacillus* Species Isolated from The Rhizosphere of *Dactylorhiza urvilleana**

Dactylorhiza urvilleana Rizosferinden İzole Edilen *Bacillus* Türlerinin Biyoremediasyon Potansiyellerinin ve Bitki Büyümesini Destekleyici Özelliklerinin Belirlenmesi*

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Abstract

Industrial activities have been one of the biggest factors of environmental destruction by affecting natural resources for decades. Heavy metals, which are one of the greatest dangers especially for the biosphere, can be found in industrial waste. Heavy metals that enter agricultural areas through industrial wastewater cause heavy metals to accumulate in the soil after a certain period. These accumulated heavy metals become an important environmental problem, threatening the life of living beings due to their toxic properties. In soils contaminated with wastewater containing heavy metals, microorganism populations are severely damaged in terms of both number and diversity. This heavy metal accumulation in water and soil has become a global health threat. Alternative processes are needed in the fight against heavy metal pollution. Bioremediation activity, defined as the removal process of environmental pollutants through microorganisms and plants, has gained significant importance in recent years. In our study, the tolerance potentials of *Bacillus* species isolated from the rhizosphere of *Dactylorhiza urvilleana* (Steudel) Bauman in the Ovit plateau of Rize province to metals (such as copper, lead, zinc, iron and silver) were investigated. In addition, plant growth promoting Indole Acetic Acid (IAA) production, phosphate dissolution, and ACC (1-Aminocyclopropane-1-Carboxylic acid) deaminase production were determined. It was determined that the isolated *Bacillus* species had a wide pH growth range and some *Bacillus* species were salt tolerant. The results showed that *Bacillus* species have bioremediation potential and plant growth promoting properties. It is thought that the bacteria isolated from the study can be used to make areas with heavy metal pollution suitable for plant cultivation and act as plant growth promoters in these areas. These bacteria strains are planned to be used as cheaper and more effective methods in studies in agriculture or areas with heavy metal pollution.

Keywords: *Bacillus*, Bioremediation, Metal tolerance, Salt tolerance, Indole Acetic Acid (IAA)

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Atıf/Citation: Üreyen Esertaş, Ü. Z., Bozdeveci, A., Uzunalioğlu, E., Alpay Karaoğlu, Ş. (2023). Determination of bioremediation potentials and plant growth-promoting properties of *Bacillus* species isolated from the rhizosphere of *Dactylorhiza urvilleana*. *Journal of Tekirdag Agricultural Faculty*, 20(4): 948-958.

*This study cited from the Master thesis of Ülkü Zeynep Üreyen ESERTAŞ under Şengul Alpay Karaoğlu supervision titled "Determination of bioremidant characteristic of soil born bacillus and its effect on the development of *Zea mays* in the presence of copper" at the Institute of Natural and Applied Sciences, Recep Tayyip Erdoğan University.

Öz

Sanayi faaliyetleri onlarca yıldır doğal kaynakları etkileyerek çevresel tahribatın en büyük etkenlerinin başında gelmektedir. Özellikle biyosfer için en büyük tehlikelerden biri olan ağır metaller sanayi atığının içeriğinde bulunabilmektedir. Endüstriyel atık sular yoluyla tarım alanlarına giren ağır metaller, belirli bir süre sonra ağır metallerin toprakta birikmesine neden olmaktadır. Biriken bu ağır metaller, toksik özellikleri nedeniyle canlıların yaşamını tehdit eden önemli bir çevre sorununa dönüşmektedir. Ağır metaller içeren atık sularla kirlenmiş topraklarda mikroorganizma popülasyonları hem sayı hem de çeşitlilik açısından ciddi zarar görmektedir. Suda ve toprakta meydana gelen bu ağır metal birikimi evrensel boyutlara ulaşan bir sağlık tehdidi haline gelmiştir. Ağır metal kirliliği ile mücadelede alternatif süreçlere ihtiyaç duyulmaktadır. Mikroorganizmalar ve bitkiler aracılığı ile çevresel kirleticilerin giderim süreci olarak tanımlanan biyoremidasyon faaliyeti son yıllarda büyük önem kazanmıştır. Çalışmamızda Rize ili Ovit yaylasındaki *Dactylorhiza urvilleana* (Steudel) Bauman rizosferinden izole edilen *Bacillus* türlerinin bakır, kurşun, çinko, demir, gümüş gibi metallere olan tolerans potansiyelleri araştırıldı. Ayrıca bitki gelişimini teşvik edici İndol Asetik Asit (IAA) üretimi, fosfat çözündürme, ACC (1-Aminosiklopropan-1-Karboksilat) deminaz üretimi gibi özellikleri belirlendi. İzole edilen tüm *Bacillus* türlerinin geniş bir pH üreme aralığına sahip olduğu ve bazı *Bacillus* türlerinin tuz toleransına sahip olduğu belirlendi. Sonuçlar, *Bacillus* türlerinin biyoremediasyon potansiyeline ve bitki büyümesini teşvik edici özelliklere sahip olduğunu göstermiştir. Çalışmadan izole edilen bakterilerin ağır metal kirliliği olan alanların bitki yetiştiriciliğine uygun hale getirilmesinde kullanılabileceği ve bu alanlarda bitki gelişimini destekleyici olarak görev yapabileceği düşünülmektedir. Bu bakteri suşlarının tarımda veya ağır metal kirliliği olan bölgelerde yapılacak çalışmalarda daha ucuz ve daha etkili yöntemler olarak kullanılması planlanmaktadır.

Anahtar Kelimeler: *Bacillus*, Biyoremediasyon, Metal toleransı, Tuz toleransı, İndol asetik asit (IAA)

1. Introduction

Efforts are being made to develop more environmentally friendly alternatives and to provide biological control in the fight against environmental pollutants and plant pathogens (Ongena and Jacques, 2008). Microorganisms in the soil have an important role in maintaining and protecting natural ecosystems. Microorganisms play a direct or indirect role in physical, chemical, and biological changes in plants and soil with specialized molecules and signals. In the soil ecosystem, there are microorganisms (bacteria and fungi) that have a positive effect on plant health, as well as microorganisms that are harmful to plant health. Beneficial bacteria in the plant root system promote plant growth by producing phytohormones, dissolving inorganic phosphorus, producing iron-chelate siderophores and increasing iron intake, producing plant hormones (such as auxin, cytokinin, and gibberellin), or facilitating the uptake of minerals in the environment. The plant growth-promoting properties of beneficial microorganisms are important for their use in agricultural production (Karapire and Özgönen, 2013). Some rhizosphere-resident bacteria, with their ability to produce aminocyclopropane carboxylate deaminase (ACC), reduce the amount of ethylene in plant roots and promote root elongation and development (Penrose and Glick, 2001). *Bacillus* species, which are frequently found in the soil and plant rhizosphere, attract attention with their stimulating properties on plant growth and with their inhibitory properties on pathogens (Güldoğan et al., 2022; Soylu et al., 2022). The characteristics that make *Bacillus* species biotechnologically attractive are their diverse secondary metabolisms and their ability to produce structurally different antagonistic substances (Stein, 2005). Therefore, *Bacillus* species are becoming a group of microorganisms with the potential for biotechnological applications.

Heavy metals that mix with and accumulate in soils affect soil fertility, microbial activity, and biological diversity (Yaldız and Şekeroğlu, 2013). Excessive amounts of heavy metals in the environment slow down the metabolic reactions of living organisms and may have toxic effects. In the world, the spread of heavy metals and environmental pollution with dyestuffs is increasing due to industrial development. Since industrial waste water contains a large amount of heavy metals, their uncontrolled discharge into the environment without treatment causes toxic and mutagenic effects on the living things in that environment. Although various metals are used in very little amounts to maintain life in some organisms, their high concentrations cause harmful effects on the cell. Although toxic metals such as Ag, Al, Au, Cd, Pb, and Hg have no biological significance, their presence in the cell, even in low concentrations, is dangerous (Bruins et al., 2000; Nies, 2004). Living organisms require certain heavy metals in trace amounts. However, negative results are observed due to high heavy metal intake due to toxic effects in plants, animals and humans. They can cause serious physiological and neurological damage, especially for the human body.

Our study includes the purification and biochemical characterization of *Bacillus* species isolated from the rhizosphere soil of *Dactylorhiza urvillenana* species grown on the Ovit plateau of Rize. In addition, it aimed to determine the salt tolerance, heavy metal tolerance, and plant growth-promoting properties of isolated bacteria.

2. Materials and Methods

2.1. Bacterial isolation from orchid specimens

Orchid samples were taken from the Ovit plateau in Rize province in 2012-2013. The tuber of the orchid plant and the rhizosphere soil at a depth of 20 cm were taken into sterile plastic bags. After the orchid samples were identified, bacteria were isolated from the root and rhizosphere soil. Orchid specimens were soaked in 70% ethanol for 3 minutes after the surfaces were washed in sterile tap water. The washing process was repeated three times with sterile distilled water to remove the alcohol. With the help of a sterile scalpel, 1 cm^2 pieces of orchid roots and tubers were cut and placed in Mueller Hinton agar petri dishes and incubated at 37 °C for 2 days. Soil samples were weighed equivalent to 10 g dry weight of wet soil and mixed in 90 mL sterile distilled water. Using the macrodilution method, 100 µL of dilution liquid was spread on MHA medium and incubated at 37 ºC for 2 days. Different colonies grown in the MHA media were examined microscopically by Gram staining. Gram-positive colonies grown in the medium were inoculated on MHA medium for pure culture with the single colony technique and incubated for 1-2 days at 37 ºC.

2.2. Biochemical identification of isolates

Bacteria purified as single colonies were incubated overnight at 36 °C in MHA medium. The bacteria's morphology was observed at 1000x magnification after Gram staining under a light microscope (BX63; Olympus, Tokyo, Japan). The isolated bacterial strains were identified by biochemical tests such as motility test, indole test, methyl red test, gelatin test, citrate test, nitrate reduction test, lecithinase, catalase test, Voges-Proskauer (VP) test, urease test, starch hydrolysis test and sugar fermentation test(Cappuccino and Sherman, 1996; Kandler and Weiss, 1986; Holt et al., 1994).

2.3. Determination of the effect of temperature, pH and NaCl on bacterial growth

Mueller Hinton Broth (MHB) containing different pH (4.5, 5.5, and 8.5) and salt (10%, 15%) concentrations were used to determine pH and salt tolerance of bacterial isolates. A single colony was taken from the fresh cultures of the isolates and inoculated into MHB and incubated at 36 °C for 24 hours. Bacterial suspension McFarland 0.5 turbidity or $1x10^8$ CFU/mL was prepared from overnight culture. The Eliza plate wells were dispensed with 190 µL of MHB medium that had different salt and pH concentrations, and 10 µL of bacterial suspension was put into each well. The isolates were incubated for 48 h at different temperatures (10 °C and 45 °C), pH levels, and salt concentrations. The temperature, pH, and salt concentrations at which bacterial isolates could grow were determined. The experiments were carried out with 3 repetitions.

2.4. Detection of plant growth-promoting traits (PGPR) of the selected isolates

Phosphate solubilization activity was performed according to the methods of Fürnkranz et al. (2009) and Aydoğan et al. (2013). Bacterial isolates phosphate growth agar (NBRIP) (10 g glucose, 5 g $Ca_3(PO_4)_2$, 5 g $MgCl_2x6\ H_2O$, 0.25 g $MgSO_4x7H_2O$, 0.2 g KCl, 0.1 g $(NH4)_2SO_4$, and 15 g agar) was inoculated into the medium and incubated for 5 days. For testing of ACC(1-Aminocyclopropane-1-Carboxylate) deaminase activity, bacterial culture was seeded on ACC deaminase agar medium and incubated at 36±2°C for 2-7 days until colony formation. Colony growth was recorded as ACC-deaminase positive after incubation (Dworken and Foster, 1958; Brígido et al., 2015). All experiments were performed in triplicate. Chrome A qualitative test of siderophore production was performed on the agar medium of Chrome Azurole (CAS) (Schwyn and Neilands 1987). CAS agar plates were prepared and spot inoculated with bacterial isolates. Inoculated CAS agar plates were incubated at 36±2°C for 3-5 days. The development of a yellow-orange halo around the bacterial colony was considered a positive result (Schwyn and Neilands, 1987; Alexander and Zuberer, 1991). Indole acetic acid (IAA) production in bacterial isolates was tested by inoculating the bacterial suspension (10% v/v) in Mueller-Hinton broth containing 50 µg/mL L-tryptophan. Cultures were incubated at 36±2°C for 48 hours and then centrifuged at 10,000 g for 10 minutes. The concentration of IAA in the culture supernatant was tested using Salkowski's reagent (0.5 M $FeCl_3$ and 35% perchloric acid in a mixture 1:49). 1 mL of supernatant was placed on the spectrum plates, 4 mL of Salkowski reagent was added, and it was incubated at 37°C for 30 minutes after incubation in the dark, measurements were made at 535 nm in the spectrophotometer. Each sample was performed in triplicate and the mean value of the measured value was taken (Gordon and Weber, 1951; Fürnkranz et al., 2009).

2.5. Determination of Metal Tolerance of Isolates

It was aimed to determine the tolerance of the isolated bacteria to silver (Ag), copper (Cu^{+2}), iron (Fe^{+2}), zinc (Zn^{+2}) and lead (Pb^{+2}) metals. MHA medium containing metal salts ($AgNO_3$, $CuSO_4.5H_2O$, $FeCl_3.6H_2O$, $ZnCl_2$, and $Pb\ (NO_3)_2$) at different concentrations (1.0, 2.5, 5.0, and 10.0 mM/L) was used to determine metal tolerance (Velásquez and Dussan, 2009; Üreyen Esertas et al., 2020). The overnight cultures of bacterial isolates were incubated at 36±2°C for at least 5 days by spot seeding in MHA medium containing different concentrations of metal salts. Colony growth was recorded as positive by following the bacterial growth.

3. Results and Discussion

3.1. Identification of bacteria by traditional methods

It was observed that oxidase and catalase reactions were positive in the majority of bacteria, some showed weak positive reactions, most of them were R-type colonies and spore localization in the cell was mostly subterminal (*Table 1*).

Table 1. Biochemical characteristics of the Bacillus isolates.

NO	Species	Spore	O_2	Oxi	Cat.	Mot	Nit	Ami	Üre	Les	Gel	İn	MR	Cit	D/Y	H_2S	G
5O7	*B. macerans*	Subterminal	FA	-	+	-	-	-	-	4+	+	+	+	-	A/Al	-	-
5O6	*B. mycoides*	Central	AE	+	+	-	+	2,8/3,4	-	4+	+	-	+	-	Al/Al	-	-
1412	*B. insolitus*	Subterminal	FA	+	+	-	+	-	-	nd	+	-	+	-	Al/Al	-	-
5O11	*Bacillus* sp.	Subterminal	AE	-/+	+	-	-	-	-	-	-	-	-/-	-	Al/Al	-	-
146	*Bacillus* sp.	Subterminal	A	-	+	-	-	-	±	nd	+	-	-	±	Al/Al	-	-
5O5Y12	*Bacillus* sp.	Subterminal	A	+	+	-	-	-	-	nd	+	-	+	±	A/A	-	-
112O3	*Bacillus* sp.	Subterminal	FA	+	+	-	+	-	++	nd	+	-	-	±	A/A	-	-
141	*B. insolitus*	Subterminal	AE	+	+	-	+	-	-	4+	-	-	+	-	A/A	-	-
5O5Y10	*Bacillus* sp.	Central	FA	-	+	-	+	-	-	nd	+	-	-	++	A/A	-	-
142	*B. insolitus*	Central	AE	+	+	-	+	-	++	4+	-	-	+	-	A/A	-	-
5O5Y2	*Bacillus* sp.	Subterminal	AE	+	+	-	-	-	±	nd	+	-	-	±	A/A	-	-
144	*B. mycoides*	Subterminal	FA	+	+	-	+	1,8/2,8	-	4+	-	+	+	-	Al/Al	+	-
5O3	*B. laterasporus*	Subterminal	FA	-	+	-	-	-	-	4+	+	-	+	-	A/A	-	-
5O5Y13	*B. globisporus*	Central	FA	-/+	+	-	+	2,9/3,4	-	4+	+	-	+	-	A/A	-	-

In; İndole, Mot; Motility, Oxi; Oxidase, Cat; Catalase, MR; Methyl-Red, Cit; Citrate, KIA: Kligler iron agar, D: Deep, Y; Surface, G; Gase, Nit; Nitrate, Ami: Amilase, Üre; Ürease Lest: Lesitinase, Gel; Gelatinase, nd; Not detected. -/-; Not Growth, -; Reproduction positive but negative test result, ±; weak positive, +; Positive, 4+; very good,

3.2. Determination of the effect of temperature, pH and NaCl on bacterial growth

Since temperature, pH and salt (NaCl) concentration have roles in enzymatic function as well as overall metabolic efficiency, these factors do have an effect on survivability. The temperature, pH profile and salt (NaCl) concentration of the strain indicate that the strain has the ability to survive in an adverse condition (Samanta et al., 2012). It was aimed to determine the growth potential of the isolated *Bacillus* strains at different temperatures and pHs. In our study, it was observed that *Bacillus* species can grow well in especially 10°C conditions with a wide temperature range (10-45°C). A similar result has been obtained in the study of two isolates *Bacillus sonorensis* and *Bacillus subtilis* that showed growth between 10-40°C (Jadhav et al., 2010).

It was observed that *Bacillus* species can grow well in both acidic and basic conditions with a wide pH range (4.5-8.5). In our study was consistent with previous studies, which reported a wide pH range (5.0–10.0) where Bacillus can grow. The study highest growth was observed at pH 7.0, 8.5, and 10.0, respectively. However, the results also indicated that isolates *Bacillus* could grow at an acidic pH of 5.0 (Wekesa et al., 2022).

In determining the salinity tolerance, it was observed that the growth changed depending on the species. It was determined that growth was better when the salinity rate was 10%, but some *Bacillus* species could not reproduce when the salinity rate was 15%. Determining the growth of bacteria at different temperatures, pH and salinity rates is important because of adaptation to seasonally changing soil conditions. Because when the microorganism is applied to the plant, it should be able to adapt easily to the root rhizosphere and be able to benefit the plant by maintaining its vitality. It was observed that 85% of the isolates (except three) grew well in the presence of salt (10%), and the 5O5Y10 strain was very good in the presence of 15% salt. It was determined that only 5O5Y12 grew better in the presence of 10% salt (*Table 2*). In the study has been reported that the soil-based *Bacillus subtilis* strain AS-4 was able to tolerate high salt concentration in a growing medium that contained both 10% and 15% salt. Study according the growth was observed solely in the initial hours up to 25 hours of incubation and later on it decreased (Satapute et al., 2012). In general, enzymes/enzyme manufacturers that are resistant to harsh conditions such as high temperature, pH or salt are preferred in the industry (Duman et al., 2016). It is thought that the temperature, pH and salt tolerance abilities observed in the isolates of 5O7, 1412, 5O11, 112O3, 5O5Y10, 142 which have been identified as species, can also be evaluated industrially.

Table 2. Effect of Salinity, pH and temperature on bacterial growth.

Code	Species	10% NaCI	15% NaCI	10 °C	45 °C	pH 4.5	pH 5.5	pH 8.5
5O7	*B. macerans*	±	±	++	+	++	++	++
5O6	*B. mycoides*	-	-	+	+	+	++	++
1412	*B. insolitus*	±	+	+	+	++	++	++
5O11	*Bacillus* sp.	+	+	++	+	++	+	++
146	*B. macerans*	+	-	++	++	++	+	++
5O5Y12	*B. mycoides*	++	-	+	+	++	++	++
112O3	*B. insolitus*	+	+	+	+	+	+	+
141	*Bacillus* sp.	+	±	-	+	+	+	++
5O5Y10	*B. macerans*	+	++	++	+	++	+	++
142	*B. mycoides*	±	+	+	+	++	++	++
5O5Y2	*B. insolitus*	-	-	+	-	+	++	++
144	*Bacillus* sp.	+	-	+	-	-	+	+
5O3	*B. macerans*	+	-	±	+	++	++	±
5O5Y13	*B. mycoides*	-	-	+	-	++	++	+++

3.3. Detection of plant growth-promoting traits (PGPR) of the selected isolates

Plant-associated bacteria play an important role in the adaptation of the host to the changing environment by altering plant cell metabolism or promoting plant growth (Ma et al., 2011). The most well-known of features that plant growth promoting bacteria is promote plant growth under heavy metal stress conditions, including indole acetic acid (IAA) production, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, siderophore production, phosphate solubility, ammonia production and nitrogen fixation (Ullah et al., 2015).

Bacterial endophytes either directly by producing phytohormones, including indole-3-acetic acid (IAA), gibberellins, cytokinins, phosphate solubility, nitrogen fixation, or indirectly support the growth of host plants through the production of antibiotics, siderophores. Soil microorganisms can affect trace metal mobility and availability to plants (Idris et al., 2004). In addition, IAA, siderophores, ACC deaminase and phosphate solubilizing bacteria can stimulate plant growth (Rajkumar et al., 2012). In previous studies, metal-resistant bacterial isolates from different genera (such as *Pseudomonas* and *Bacillus*) were reported to produce IAA and enhance plant growth (Rajkumar et al., 2012). A number of plant growth promoting factors (Siderophore production, Phosphate solubility, ACC deaminase activity and Ammonium production) of the identified bacteria were tested in liquid or solid media (on agar plate). Strong siderophore production (≥20) ability was detected in 4 of the isolates, but moderate in most of the isolates. In a study to draw attention to the importance of siderophores, pyoverdine (Pvd), a bacterial siderophore produced by *Pseudomonas aeruginosa*, was applied. In metal-contaminated soil (Cd and Cu), the mobility, phytoavailability, and uptake of Cu by tomatoes and barley increased, while cadmium fate did not change (Cornu et al., 2014). In our study, low levels of siderophore were detected only in isolates 5O5Y2 and 144, while siderophore production was not detected in the strain 1412. Other *Bacillus* strains in the study showed a strong ability to produce siderophores by forming yellow zones on agar plates. Phosphate solubility activity was detected in 6 of them, 112O3 and 144 better. It was observed that all isolates had the ability to produce ammonium, but some isolates were better than others (*Figure 1*, *Table 3*). In addition, ACC deaminase positivity was determined in 6 of the isolates, one of which was low. In this study, the ability of the strains to produce indole acetic acid was investigated by spectrophotometric method. Good level of IAA activity was detected in most of the samples, very good level of IAA activity in 146 and 5O5Y12 strains, while no activity was detected in 6 of them. The plant growth promoting properties of the strains in the study promise potential to be local PGPB strains due to concerns about the potential ecological risks of introducing non-native PGPR to the field, especially in recent years (Ambrosini et al., 2016).

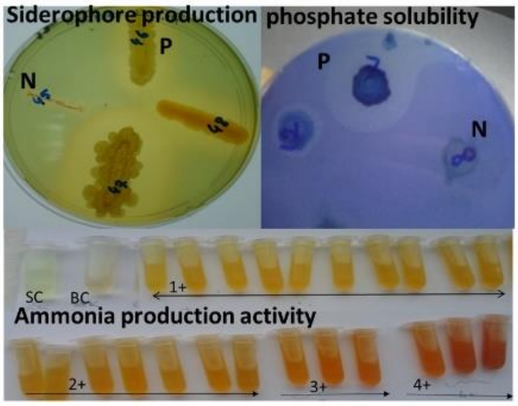

Figure 1. Siderophore production, phosphate solubility and ammonium production activity of bacterial isolates. P: Positive, N: Negative, SC: Nessler solution control, BK: Bacteri control. 1+;weak, 2+; moderate activity, 3+;good activity, 4+; strong activity

Table 3. Determination of plant growth-promoting some properties.

Code	Sid(mm)	Pho(mm)	ACC	Am	IAA activity
5O7	10/12	6/-	-	+++	7,54±0,26
5O6	18/48	5/-	-	++	-5,15±0,10
1412	-	-	-	+	-2,80±0,36
5O1	8/15	3/-	-	+++	10,92±2,45
146	17/20	6/-	+	+	15,29±0,26
5O5Y12	8/10	5/-	-	+++	11,10±0,12
112O3	8/10	6/14	±	++	18,14±0,63*
5O3	19/24	5/-	+	++	5,81±1,35
5O5Y10	9/14	7/9	-	++	3,76±0,65
5O5Y13	18/45	5/-	-	++	14,68±0,62
142	7/18	5/8	+	++	-5,56±0,17
5O5Y2	6/9	4/-	-	++	5,94±7,32
144	4/7	9/12	-	++	-4,18±0,95
141	6/10	7/9	-	++	17,00±0,51*

U; Growth, Z; Zone diameter(mm), -; negative activity, +; positive activity, ACC; ACC deaminase, Am; Ammonium production, Sid.; Siderophore production, Pho: phosphate solubility, 1-9 mm; positive activity, 10-19 mm; good activity, ≥20; very good activity

3.4. Determination of metal tolerances of bacteria

Studies show that a large number of plants are used to remove heavy metals from contaminated soils (Ullah et al., 2015). Accordingly, PGPB are widely used to inoculate plants as a biologically defined approach to increase the phytoremediation efficiency of toxic metals (Chen et al., 2017; Zornoza et al., 2017). The growth characteristics five different heavy metals salts (copper(II), lead(II), iron(II), silver and zinc(II)) of the Ovit strains tested in the study were examined on agar medium with 1, 2.5, 5 and 10 mM concentrations (*Table 3, Figure 2*). All strains in the study were found to grow easily even in the presence of 10 mM iron. Five of the strains were able to grow in the presence of 10 mM silver, nine in the presence of 10 mM copper, and eleven in the presence of 10 mM lead. It was determined that all strains in the study did not grow in the medium in the presence of 10 mM zinc. In the presence of silver, obvious color change and transparent zone formation were observed in some strains. In the presence of lead, this event was detected as darkening of the colonies. In the presence of copper in the medium, no color change was observed in the petri dish, but a zone of transparency was observed around the bacterial colony. In the presence of zinc and iron metals, no shape changes were observed in the petri dishes. Colonies grown in the presence of copper showed a clear color change in the medium when kept at room temperature (*Figure 2*). It is thought that the metal salts will probably be reduced by accumulating on the surface of the bacteria or adsorbing the metals to the surface of the bacteria as they wait. Similar to our study, Njoku et al., (2020) reported in their study that the uptake of Pb, Cd and Ni from the media increased with the increase of the incubation period of *Bacillus megaterium* and *Rhizopus stolonifer*.

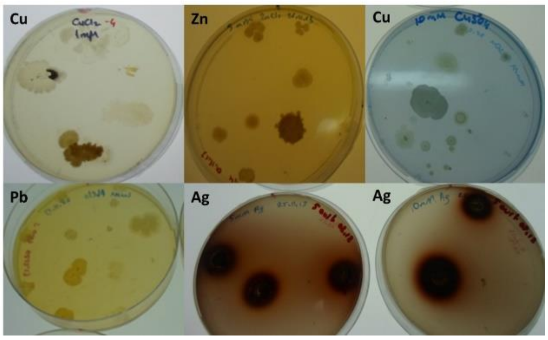

Figure 2. Petri image of bacterial isolates in an agar medium containing different metal salts

Table 4. Determination of the tolerance of bacteria to different metal concentrations

Code	Ag	Fe	Cu	Pb	Zn
5O7	nd	≤10 /+	nd	TE	TE
5O6	-	≤10 / +	≤10 / +	≤10 / +	≤5 / +
1412	nd	≤10 /+	nd	nd	Nd
5O1	1 / +	≤10 / +	≤10 / +	≤10 / +	≤2,5 / +
146	≤10 / +	≤10 / +	≤2,5 / +	≤5 / +	≤1 / +
5O5Y12	-	≤10 / +	≤10 / +	≤10 / +	≤5 / +
112O3	1 / +	≤10 / +	≤5 / +	≤10/ +	-/-
5O3	≤10 / +	≤10 / ++	≤5 / +++	≤1 / +	≤5 / +
5O5Y10	-	≤10 / ++	≤5 /+++	≤10 / ++	≤5 / +
5O5Y13	≤10 / +	≤10 / +	≤5 / +	1 (-/+)	≤5 / +
142	-	≤10 / +	≤10 / +	≤10 / +	≤2,5 / +
5O5Y2	≤2,5 / +	≤10 / +	≤10 / +	≤10 / +	≤5 / +
144	-	≤10 / ++	≤10 / ++	≤10 / +	≤2,5 / +
141	-	≤10 / +	≤10 / +++	≤10 / +	≤5 / +

≤0; negative activity, 0-9, positive activity, 10-15; good activity, ≥16; very good acitivity, Ability to grow in the presence of 1, 2.5, 5 and 10 mM metal, nd: not detected, (≤); 1-10 mM growth, (-); not growth, (+): growth, (2+); good growth, (3+); very growth.

Biomass that is used or turned into waste should be disposed of safely without harming the environment. Since the discharge or burning of spent adsorbents pollutes the soil, air and groundwater, suitable alternative techniques such as thermal desorption and maximum possible recycling of biosorbents are needed for sustainable use (Priyadarshanee and Das, 2021). Bacteria are characterized by their high adaptability to different environments, even under extreme conditions. Studies suggest many mechanisms to explain the rapid adaptation and tolerance of bacteria to different environmental conditions (Casacuberta and González, 2013). Utilizing plant-beneficial rhizobacteria not only improves biocontrol and reduces the use of environmentally harmful pesticides, but also promotes plant growth and increases crop uptake. It is recognized that PGPB play a key role in agricultural practices and enable more sustainable farming opportunities (Zhang et al., 2022). PGPB can help growth by increasing the resistance of plants to different environmental stresses (Gururani et al., 2013). Studies show that these rhizobacteria, which are beneficial for plants, have high potential in applications for sustainable agriculture and this is of great importance for agricultural activities (Chen et al., 2022). As we stated in our study, it is seen that PGPB with high metal tolerance are of great importance for environmental and agricultural applications. Liu et al., (2014) stated in their study that the plant growth rate was more than 300% after the treatment of the soil with copper-tolerant bacteria. Bioremediation is the removal of waste with microorganisms and is one of the most effective strategies. For effective bioremediation, it is of great importance to identify metal-resistant bacteria that can promote plant growth and minimize the exposure of plants to metals in the soil. Different studies show that the application of heavy metal resistant PGPB in soils exposed to artificial heavy metal application prevents plants from being exposed to these heavy metals due to their microbial activities in the rhizosphere (Tak et al., 2013). It shows that plants should be treated with PGPR if heavy metal accumulation is increased. However, in parallel with the bacterial metal dissolving activity, other factors such as nutrient level, pH, metal species and plant variety in the soil highly affect the metal solubility in the soil, which in turn changes the metal uptake (Rajkumar et al., 2013). *Bacillus* genus can be grown in many culture media. In addition, it is mostly in the first place in studies carried out in heavy metal-contaminated areas, along with those based on enrichment techniques for the detection of metal-resistant microorganisms. In a field study, it was reported that 31.51% of the bacterial population belonged to the genus *Bacillus* in the microbiota examination of soil samples known to contain cadmium (0.5 to 1.6 mmol L^{-1}), zinc (138.2 to 900.7 mmol L^{-1}), and lead (6.1 to 442.1 mmol L^{-1}) (Lenart and Wolny-Koladka, 2013). Jing et al. (2012) reported that three of the four species isolated from heavy metal-contaminated soils belonged to the genus *Bacillus*. New approaches based on using contaminated biomass aim to produce catalysts for the synthesis of organic molecules (Grison, 2015). Among these studies, *Bacillus* genus is of great importance as it is the most isolated bacterium. Clavé et al. (2016) show that copper "eco-catalysts" prepared from the roots of *Eichhornia crassipes* are highly effective in the azide-alkyne cycloaddition reaction. Sevim and Sevim (2015) reported that *Bacillus* strains isolated from soil samples taken from different locations in Rize were resistant to copper, chromium, zinc, iron, and nickel. Another study revealed that inoculation with metal-resistant endophytic bacteria can effectively increase growth parameters in plants under Cd and Ni stress (Jan et al., 2019). Similar to these studies, the results of our study are considered to have potential for bioremediation and growth regulating properties for plants. *In vitro* and *ex vivo* studies on different conditions and plants should be continued in the future to fully demonstrate this potential.

4. Conclusions

Plant growth-promoting bacteria (PGPB) may be one of the most viable alternatives in the future to reduce negative stress threats with their natural, environmentally friendly and sustainable approach. In addition to improving plant health, using PGPB can optimize conditions for plants in the soil and the rhizosphere by maintaining nutrient availability, synthesizing phytohormones, and dissolving phosphate. Since the metal-bacteria interaction is initiated at the metal uptake level, the uptake mechanism is thought to be closely related to the metal resistance mechanism. Biodegradation abilities in bacteria may differ between species, even within species within the same genus. However, data on the bioremediation of heavy metals is limited, and further research is required to identify candidate species that can provide bio-recovery of heavy metal-contaminated soils.

Acknowledgment

This work supported supported by the Recep Tayyip Erdogan University Scientific Research Project (Project No: RTEU-2015.53002.102.03.01), Rize, Turkey.

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