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The Isolation and Identification of Bacteria with the Remediation Potential of Calcerous Soil

Kireçli Toprağı Islah Etme Potansiyeline Sahip Bakterilerin İzolasyonu ve Tanılanması

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Abstract

Soil salinity and sodicity is a negative stress widely observed for agricultural production in the world. In saline and sodic soils, inter particulate distances increases by enhancing the repulsive forces because of high Na^+ ion concentration. As a results, this undesirable situation results in dispersion of the soil, loss of porosity, reduction of water permeability in the soil profile. Besides, the crop production of this soil is very poor because of high pH, exchangeable Na^+, deficiency of plant nutrients, high concentration of carbonates and bicarbonates. Considering the big need for the arable lands in the world, the remediation of these soil with effective methods will be the right approach. In this study, it was aimed to isolate and identify the bacteria with carbonate dissolution ability, which had potential to remediate calcerous soils from Dereli/Giresun/Turkey. According to the results, two isolates (*Bacillus* sp. and *Chromohalobacter* sp.) were determined that they were capable of $CaCO_3$ dissolution. Only one isolate

Özet

Toprak tuzluluğu ve sodikliği dünyada tarımasal üretim için yaygın gözlemlenen negatif bir strestir. Tuzlu ve sodik topraklarda, yüksek Na^+ iyon konsantrasyonu nedeniyle itici güçlerin etkisiyle partiküllerin arasındaki mesafe artar. Sonuç olarak, istenmeyen bu durum toprağın dağılmasına, gözeneklerin kaybolmasına, toprak profilinde su geçirgenliğinin azalmasına neden olur. Ayrıca, topraktaki tarımsal üretim yüksek pH, değişebilir Na^+, besin elementlerindeki eksiklik, karbonat ve bikarbonatın yüksek konsantrasyonu nedeniyle çok düşüktür. Dünyada ekilebilir arazilere olan büyük ihtiyaç göz önüne alındığında, bu toprakların etkin yöntemlerle ıslahı doğru bir yaklaşım olacaktır. Bu çalışmada, Dereli/Giresun/Türkiye'den kireçli toprakları ıslah etme potansiyeline sahip olan ve karbonat çözme potansiyeline sahip bakterilerin izolasyonu ve tanılanması amaçlanmıştır. Sonuçlara göre iki izolatın (*Bacillus* sp. and *Chromohalobacter* sp.) $CaCO_3$ çözme yeteneğinde olduğu

(*Halomonas* sp.) exhibited potential of both $CaCO_3$ and $MgCO_3$ dissolution.

Keywords: Calcerous soil, Carbonate, Bacterial dissolution

belirlenmiştir. Sadece bir izolat (*Halomonas* sp.) hem $CaCO_3$ hem $MgCO_3$ çözme potansiyeli göstermiştir.

Anahtar kelimeler: Kireçli toprak, Karbonat, Bakteriyel çözünme

1. INTRODUCTION

The global population is expected to reach 10 billion in 2070 according to the international reports. This rapid population growth has led to an increase in the need for food on a global scale (Goswami et al., 2014). Therefore, not only the effective use of arable land in the world but also the remediation of non-arable land has gained more importance. The total land area of the world is estimated as 149 million square kilometers and only about 27 million square kilometers can be used as arable land because of erosion, slope, excessive pesticide pollution, wrong product selection, and salinity (Sklenicka & Salek, 2008). Salinization and sodification, which are caused by low rainfall, low quality of irrigation water and high evaporations are major problems of agriculture in arid and semi-arid regions of the world. They cause huge losses in crop production all over the world (Ahmad et al., 2006; Shabala & Cuin, 2008). The saline and sodic soils include sodium ($Na+$) and carbonates which are undesirable and toxic for plants. Salinization and sodification reduce the quality, productivity, water of soil and emergence of seedlings, penetration of root and plant growth (Qadir & Schubert, 2002). These soils, especially found in arid and semi-arid regions, generally contain high amount of calcium carbonate ($CaCO_3$), that are poorly soluble (Tamilselvi et al., 2018). There are three polymorphs form of $CaCO_3$ as calcite, aragonite and vaterite. Aragonite is mainly found in shells of aquatic organisms, calcrete profiles and pendant coatings, but rare in soil profiles (Courty et al., 1994). Vaterite is found in shells of aquatic organisms, but as rare in soil profiles as aragonite (Courty et al., 1994). The third polymorphs form of $CaCO_3$, calcite, is most abundant and thermodynamically stable. Magnesian calcite and dolomites are the most known types of calcites, that include magnesium and calcium with carbonates (Tribble et al.,1995; Warren, 2000). Saline and sodic soils exhibit calcareous soil characteristics including magnesian calcite and dolomites (Whipkey et al., 2002).

The researchers study many applications to remediate these saline-sodic-calcareous soils. The remediation tehcniques are application of chemicals such as gypsum, elemental sulfur and phytoremediation by using different plants (Ahmad et al., 2006; Abdel-Fattah, 2012;

Hasanuzzaman et al., 2014; Murtaza et al., 2009). However, these techniques have some disadvantages such as high cost, difficulty of suitable plant selection, and time consumption. Therefore, there have been still a great need for alternative remediation techniques (Tamilselvi et al., 2018). According to the literature, the carbonates are slowly and continually dissolved by microorganisms in the nature (Efe et al., 2020; Farrag et al., 2021; Friis et al., 2003; Orhan et al., 2017; Yanmis et al., 2015). Therefore, the studies about soil remediation with microorganisms dissolving carbonates have been recently gained great attention.

In this study, it was aimed to isolate and identify the soil bacteria with the potential to remediate saline-sodic- calcareous soils by dissolving calcium carbonate.

2. MATERIALS and METHODS

2.1. Soil Samples

The soil samples used for bacterial isolates were collected from Pınarlar Köyü/Dereli/Giresun in sterile glass bottles as the soil of the village (Pınarlar Köyü) was significantly calcareous. The soil samples were supplied from subsurface layer (-5 to -20 cm) and they were transferred in sterile glass bottles to the laboratory.

2.2. Isolation and Purification of Halophilic Bacteria with Carbonate Dissolution Ability

1 g of soil samples were diluted with sterile saline water (0.9 %) by using ten-fold serial dilutions. The samples were shaken for 30 min at 150 rpm, and plated on medium. The isolation of bacterial strains was performed at 25-30 °C on moderate halophile (MH) medium. MH medium include NaCl (8.1 %), $MgCl_2$ (0.7 %), $MgSO_4$ (0.96 %), $CaCl_2$ (0.036 %), KCl (0.2 %), $NaHCO_3$ (0.006 %), NaBr (0.0026 %), peptone (0.5 %), yeast extract (1.0 %), glucose (0.1 %) and agar (1.5 %) (Orhan et al., 2017). In this study, 17 bacterial strains were chosen taking their morphological difference (colonies that differ in their size, shape texture and colour) into account and were stored at –86 °C in 15% glycerol and Luria Bertani Broth (LB) for further studies. The screening of the bacterial isolates for $CaCO_3$ and $MgCO_3$ dissolution potential was performed with Deveze-Bruni's $CaCO_3$ (DBC) agar and Deveze-Bruni's $MgCO_3$ (DBM) agar media, respectively. The medium used for $CaCO_3$ dissolution potential was as follows (per liter): 20 g glucose, 10 g NaCl, 3 g $MgCl_2$, 0.5 g $MgSO_4.7H_2O$, 0.4 g KCl, 0.2 g $(NH_4)_2SO_4$, 15 g agar, and 15 g $CaCO_3$. The medium employed to determine $MgCO_3$ dissolution potential of the bacteria was used which modified by Orhan et al. (2017). The medium used for $MgCO_3$ dissolution potential was same as the medium used for $CaCO_3$ dissolution potential, however,

$MgCO_3$ was added instead of $CaCO_3$. The bacterial strains were incubated at 25 °C for 2 weeks on DBC agar and DBM agar media for $CaCO_3$ and $MgCO_3$ dissolution potential, respectively. The presence of $CaCO_3$ and $MgCO_3$ in the medium results in a blury appearance of the medium. When the bacterial strain dissolves the $CaCO_3$ and $MgCO_3$ a clear zone may form. The clear zone around the colonies was considered as positive for $CaCO_3$ and $MgCO_3$ dissolution capabilities (Orhan et al., 2017).

2.3. Amplification of 16 S rDNA Gene Region by PCR

Genomic DNA extractions of the bacterial isolates with carbonate dissolution ability were performed with a DNA extraction kit (Promega). Then, amplification of 16S rDNA region were performed by PCR using universal primer [UNI16S-L: (5'-ATTCTAGAGTTTGATCATGGCTCA-3') and the reverse primer UNI16S-R: (5'-ATGGTACCGTGTGACGGGCGG TGTGTA-3')] (Orhan et al., 2017). The reaction mixture contained genomic DNA (50 ng) (3 µL), water (15.3 µL) of, 10X buffer (100 mM Tris–HCl, pH 8.30; 500 mM KCl) (3 µL), $MgCl_2$ (25 mM) (1.8 µL), dNTP's mixture (dATP, dCTP, dGTP, dTTP at 10 mM concentration) (0.6 µL), each primer (20.0 pmoles/µL) (3.0 µL) and Taq DNA polymerase (0.30 µL). The PCR was carried out with the reaction steps as a primary heating step (for 2 min at 95 °C); 36 cycles of denaturation (for 1 min at 94 °C), annealing (for 1 min at 54 °C), and extension (for 2 min at 72 °C); a final extension step (for 5 min at 72 °C). The PCR products were screened on 1% agarose gel including ethidium bromide (0.5 mg/mL) and 1 kb DNA molecular weight marker. The PCR products were sequenced by Macrogen (Seoul, Republic of Korea). The sequence data of all the isolates were blasted with the NCBI GenBank sequence database and the bacterial isolates were identified.

3. RESULTS and DISCUSSION

The United Nations declared 17 sustainable development goals (SDGs), under the 2030 Agenda for Sustainable Development and emphasized the importance of environmental sustainability dimensions for socioeconomic development. Especially, according to the 2030 Agenda it has been aimed '*to combat desertification, restore degraded land and soil, including land affected by desertification, drought and floods, and strive to achieve a land degradation-neutral world*' (Toth et al., 2018). Soil degradation due to salinity and sodicity is one of the most important environmental problems that has negative effects on agricultural productivity and sustainability in arid and semi-arid regions (Suarez, 2001). In this context, it is of great importance to remediate saline and sodic lands for agricultural production. The saline and sodic soils are mostly calcareous in arid and semiarid regions of the nature (Qadir et al., 2007). The researchers have developed many strategies to use these soils for agricultural production. The strategies such as chemical application, phytoremediation have some limitations. Therefore, carbonate dissolution with microorganisms have gained acceptance to ameroliate the soil, which is unsuitable for agriculture (Tamilselvi et al., 2016). There are many studies about isolation and identification of bacteria with carbonate dissolution ability (Gulluce et al., 2014; Li et al., 2007; Lian et al., 2008; Orhan et al., 2017; Orhan et al., 2021). In this study, it was decided to isolate bacteria with carbonate dissolution ability, as the application of halophilic bacteria would alleviate soil salinity by increasing decomposition of organic matters and dissolving carbonates ($CaCO_3$ and $MgCO_3$) and these bacterial activities may result in improved soil quality and replacement of Na^+ on exchange site. In this study 17 bacterial strains were isolated from soils of Dereli/Giresun/Turkey. The isolates were evaluated in terms of $CaCO_3$ and $MgCO_3$ dissolution potential by using Deveze-Bruni's carbonate medium (Figure 1). According to the results, three isolates exhibited ability to dissolve $CaCO_3$ (D1, D4, D8) and only one isolate exhibited ability to dissolve both $CaCO_3$ and $MgCO_3$ (D1).

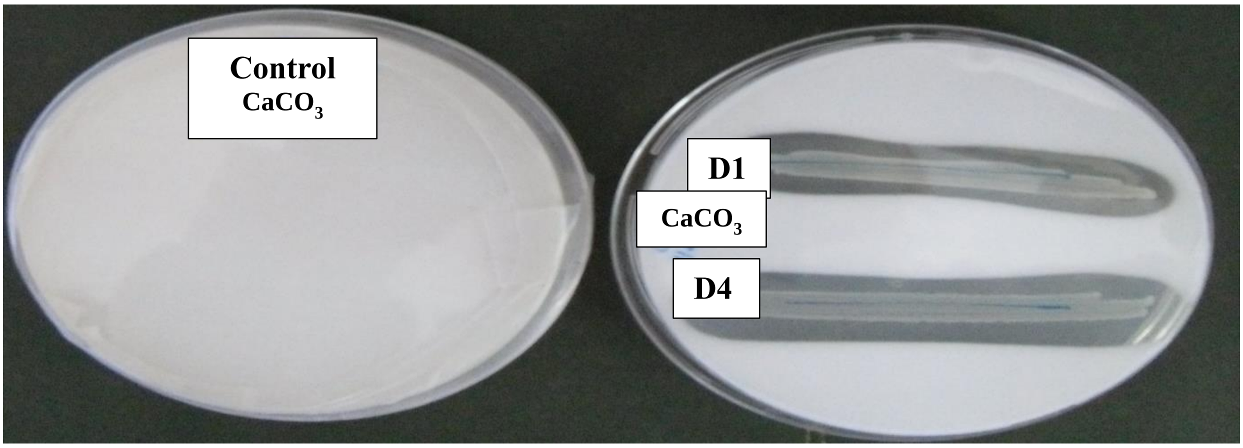

Figure 1. Screening of the bacteria in terms of $CaCO_3$ dissolution ability

These three isolates (D1, D4, D8) were identified by using 16 S rDNA gene region. As a result, they were identified as *Halomonas* sp. (D1), *Bacillus* sp. (D4), and *Chromohalobacter* sp. (D8).

Table 1. Identification Results and Genbank Numbers of Bacterial Isolates

Isolation Number	Identification Results	Genbank Acession Number	Similarity Rate (%)	Base Length (nucleotide)
D1	*Halomonas* sp.	OL913979	99	906
D4	*Bacillus* sp.	OL913980	100	952
D8	*Chromohalobacter* sp.	OL913981	100	884

It was previously reported that *Halomonas* sp. with the dissolution ability of was isolated from from salt-affected soil $CaCO_3$ and $MgCO_3$ (Orhan et al., 2017). According to the literature, there were many *Bacillus* species including *Bacillus subtilis* (Fris et al., 2003; Tamilselvi et al., 2018), *Bacillus* sp. (Gulluce et al., 2014; Orhan et al., 2017) with $CaCO_3$ dissolution ability. In another study, it was reported that two species of *Chromohalobacter* sp. with $CaCO_3$ dissolution ability were isolated from magnesite ore. The results of this study were in accordance with the literature. It is thought that the bacteria isolated and identified in this study could have remediation potential for calcerous soil. The bacteria dissolve carbonate compounds due to their metabolism products such as inorganic acids, organic acids, and some extracellular enzymes (Efe et al., 2020). In further study, it is planned to determine the mechanisms of carbonate dissolution, plant growth promoting (PGP) properties of the bacterial isolates.

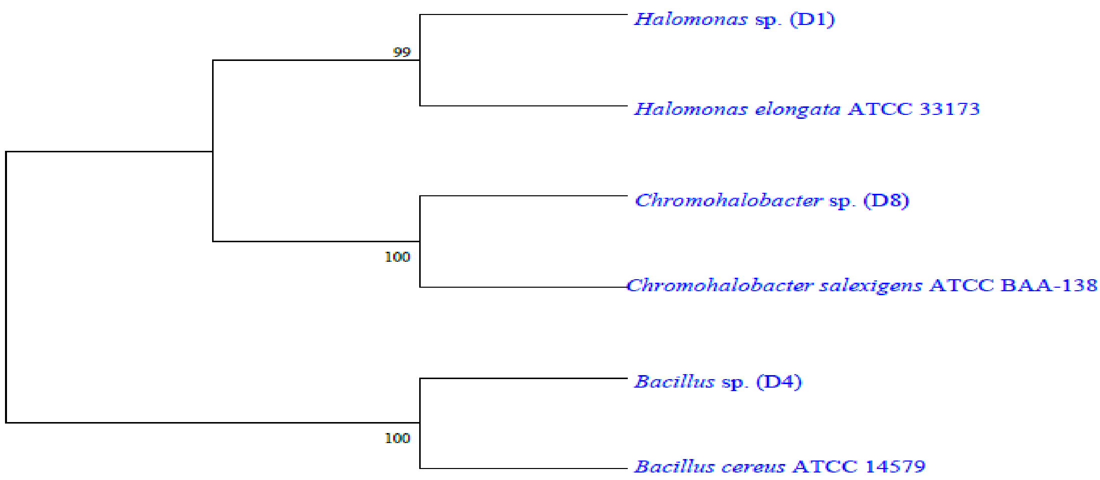

Figure 2. Evolutionary relationships of bacterial isolates. The evolutionary history was inferred by using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per site. Evolutionary analyses were conducted in MEGA7. The type strains are *Halomonas elongate* ATCC 33173, *Choromohalobacter salexigens* ATCC BAA-138, *Bacillus cereus* ATCC 14579.

4. CONCLUSION

Considering the increasing demand for agricultural production, it has gained significant importance to ameroliate saline and calcerous soils, which are non-arable. The halophilic bacteria isolated and identified in this study, which have ability of carbonate dissolution, can provide significant output for soil remediation. Since, adding a new salt to soils that already have excess salts can be prevented due to these bacteria. However, field application of these bacteria should be investigated in order to determine their potentials for salt-affected soil amelioration and soil fertility improvement.

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