Isolation and Molecular Characterization of Bacterial Strains with Magnesite Enrichment Potential from Gümüşhane Soil

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Abstract— Magnesite ($MgCO_3$) is the most important source used in the production of pure magnesium and various magnesium components and has become an important economic element for countries due to its versatile industrial use. However, pollutants with calcium derivatives, depending on the geological characteristics of the region, significantly limits the use of mines. Enrichment studies using physical and chemical methods for the removal of calcium pollutants cause high cost, low efficiency and negative effects on the environment. Therefore, scientific communities have required to overcome the pollutant effects with newly developed approaches based on microorganisms. For this purpose, soil samples were taken from different regions of Gümüşhane province and brought to the research laboratory aseptically to carry out bacterial isolation studies. In the laboratory, calcium carbonate and magnesium carbonate dissolution potentials were tested for each isolate and isolates which were able to dissolve calcium carbonate but not magnesium carbonate were identified. Then, morphological, physiological and biochemical identification studies of active bacterial isolates were performed according to the general microbiology procedures. According to the results of the sequence data, active isolates MT 17 and MT 24 were identified as *Stenotrophomonas maltophilia* and *Bacillius* sp., respectively. In conclusion, the results of this study clearly offer two valuable bacterial strain for the development of new microbiological based approaches to eco-friendly removal of calcium pollutants from the magnesite ores
Keyword; Bacteria, isolation, magnesite mine.

1 INTRODUCTION

MINES are accumulations of minerals in the earth's crust, which can occur as a result of long processes. The mines, which are considered a mystery of nature, are classified in various ways according to their physical and chemical properties, formation forms and sectors in which they are used. There is a direct relationship between the economic strengths and development levels of countries and their mineral reserves, the processing and efficient use of these reserves. Although the United States is the leading mining country in the world, Canada, Australia and the United States are the most investing countries in mining exploration. Turkey in terms of geological and tectonic structure is home to many mineral deposits. There are about 90 different minerals production in the world and 60 of them can be done in Turkey. In terms of magnesite production Turkey ranks second after North Korea [1-6].

Magnesite is a magnesium mineral and has a wide range of uses. Magnesite mineral which has great importance in the world economy in the recent years. It has many crucial applications agriculture, construction, chemical, rubber, plastic, paper, automotive and aircraft industries. It is an important raw material especially in the refractory industry. Since the main material of the refractory industry is sinter magnesite, more than 90 % of the produced magnesite is converted to sinter and caustic calcined magnesite [7-11].

Although magnesite contains 47.62 % magnesium oxide (MgO) and 52.38 % carbon dioxide in its structure, it may also contain foreign minerals such as silicon, aluminum, iron and calcium depending on the geological characteristics of the region. These foreign minerals cause some positive or negative effects depending on the application area where magnesite will be used. Calcium oxide is the most important pollutant factor the use magnesite in the Turkey. The presence of more than 1 % of calcium oxide in the structure of the magnesite to be used in refractory production is undesirable since it reduces the resistance of the brick against weathering [12-13]. In order to remove the magnesite from the pollutants in its structure and to increase its economic value, ore is subjected to enrichment process. Today, the most commonly used traditional methods in magnesite enrichment are; magnetic separation, electrostatic separation, manual separation (triage), optical enrichment and enrichment using chemicals. Consist of physical and chemical processes traditional methods have some major disadvantages. Research of new, effective and lower cost environmentally friendly methods that can eliminate these disadvantages has been the priority of the scientific World [14-16].

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Research in the world literature shows that low grade ore and ore wastes can be recovered by bioleach method based on the recovery of metals. however, the application time of the biolic mechanism is much longer than that of other applications and is difficult to control, therefore it has led to the search for an alternative mechanism in this mechanism [5], [13], [17].
In the light of these studies, the bacteria that can dissolve $CaCO_3$ from isolates obtained from soil samples taken from different regions of Gümüşhane province, but cannot dissolve $MgCO_3$, which are very valuable as industrial raw materials, were isolated and the physical and chemical properties of these bacteria isolates were determined by conventional methods. In addition to conventional methods, 16S rDNA protected gene regions were amplified by PCR technique and these regions were identified by molecular analysis.

2 Materials and Methods

2.1 Sampling and isolation of strains

In this study; Soil samples were taken from different parts of Gümüşhane province and transferred to the laboratory. Each soil sample was weighed to 1 g and homogenized by shaking in 9 ml sterile water for 2 hours. Two sets of dilution sets were prepared up to 10^{-6} and seeded on nutrient agar (NA) medium for each dilution and incubated for 2-7 days. Colonies that have been differentiated during the incubation period selected and purified by repeated line sowing on NA and was then stored at -86 °C in 100 ml Nutrient Broth (NB) medium containing 15% glycerol [5], [13],

2.2 Investigation of $CaCO_3$ and $MgCO_3$ Dissolution Potential

All bacterial isolates for determination of calcium carbonate dissolution capabilities; 12 g of yeast extract, 20 g of dextrose, 5 g of $CaCO_3$ and 15 mL of YDC-agar medium containing 15 g of agar in 1 L of distilled water were spread in a line and incubated at 30 °C for 2 hours. At the end of the period, positive bacteria that converted the food medium from white to transparency were selected and the same procedure was repeated on YDM (Yeast Dextrose $MgCO_3$) agar medium modified from YDC agar medium. After 2 weeks; $MgCO_3$ solubility of the isolates were examined. The isolates which can be used in the enrichment process which can dissolve $CaCO_3$ well and which cannot dissolve $MgCO_3$ were determined [6], [18].

2.3 Morphological and Physiological Characterization

Some physical, morphological and biochemical properties of the isolates were determined according to the methods described by Harley and Prescott (2002) [19].

2.4 DNA Isolation

Genomic DNA isolation was performed according to the modified method described by Adıgüzel (2006) [20].

2.5 PCR amplification of 16S rDNA gene regions of bacterial isolates

The 16S rDNA region, which is important for bacterial systematics from genomic DNA obtained from test isolates, was amplified by PCR method using universal primers [21-22],

2.6 Amplification of 16S rDNA region from Bacterial Isolates

For this study 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-CGGTTACCTTGTTACGACTT-3') forward and reverse universal primers were used respectively.

2.7 PCR Amplification of 16S Sequence

PCR reaction was carried out in a 30 µl reaction mixture containing (1.2 µl of dimethyl sulfoxide (DMSO), 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 25 pmoles of forward primer and reverse primer, 50 ng DNA template and 5 U Taq DNA polymerase along with reaction buffer). The reaction in 95 °C for 2 min, 36 cycles of 1 min at 94 °C, 1 min at 53 °C, 2 min at 72 °C and final 5 min extension step at 72 °C, then brought down to 4 °C [21].

2.8 Anaysis of PCR products

The amplified PCR products were analyzed with the QIAxcel advanced analysis system and 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 and the gene sequences were sent to GenBank® to obtain access numbers.

3 Results and Discussion

In this study, 15 bacteria were isolated from soil samples taken from different regions of Gümüşhane province. In order for the magnesite mine to be industrially valuable, it must be purified from Ca pollutant without losing Mg content. Therefore, $CaCO_3$ solubility of isolated bacteria strains was determined in YDC agar medium and $MgCO_3$ solubility was determined in YDM agar medium. It was determined that 2 of 15 bacterial isolates could be used in magnesite enrichment studies by dissolving $CaCO_3$ and not dissolving $MgCO_3$ (Fig. 1).

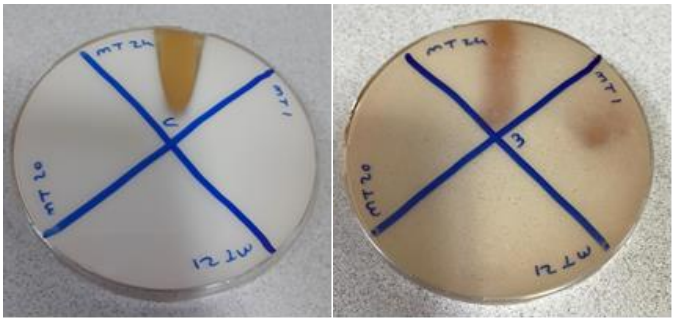

Fig. 1. The image of forming zone of isolated strains in the YDC agar medium.

2 bacterial isolates were first identified by morphological and physiological tests by classical methods; temperature and pH living conditions were investigated. Table 1. (a and b). The literature shows that traditional methods are not sufficient for the diagnosis and characterization of bacterial isolates. There-

fore, diagnosis and characterization should be made by molecular methods in addition to traditional methods [20-25].

TABLE 1
MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERIZATION RESULTS FOR BACTERIAL ISOLATES (a and b).

Isolate code	Salt tolerance (%)	pH range
MG 17	%10	5-9
MG 24	%5	5-7

a

Isolate code	Gram Staining	Endospore	Motility	Size	Cell Morphology	Gelatine
MG 17	+	TERMINAL	-	1	Coccus	-
MG 24	-	TERMINAL	-	1-2	Basil	-

b

In our study, one of the molecular identification methods 16S rDNA molecular sequence analysis method was used. From the genomic DNA purified from the test isolates, the 16S rDNA region, which is important for bacterial systematics, was amplified by PCR *in vitro* using universal primers and sequence analysis were conducted. Molecular analyzes and analyzes using conventional methods were compared. According to the results MT17 *Stenotrophomonas maltophilia,* MT24 *Bacillus* sp.

When the findings obtained from this study and previous studies are evaluated, it is clear that Bacillus microorganisms has an important potential for industrial applications and can be used in magnesite enrichment studies. However, it is scientifically important that *Stenotrophomonas* is newly isolated microorganism for remove to $CaCO_3$ pollutants from magnesite ores and has not been studied before. In addition, the use of microorganisms in natural soil flora, being an environmentally friendly method, being a low-cost and low-energy biological method have increased its scientific importance. In the light of all these studies, it is envisaged to remove enviromental pollution and increase the value of the mine economically by using biological methods in the removal of $CaCO_3$ pollutant in the structure of magnesite.

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