Isolation and Identification of Bacteria with silicium dissolution ability from boron clay

Derya EFE, Bengü ERTAN, Mehmet SAVAŞ, Furkan ORHAN

Abstract— Boron is naturally found ore in some soils, sedimentary rocks and oceans. Boron has been widely used in production of glass and ceramics, insulation fiberglass, textile fiberglass, enamels, glazes, detergents, bleaches, alloys, metals, fire retardants, agricultural fertilizer, adhesives and various chemicals. The fundamental boron reserves are located in Turkey (72%), Russia, Chile, China and Peru. About 50% of the boron reserves of Turkey is found in Emet and Hisarcık Region. Bor mineral supplied from Emet-Hisarcık Region is defined cholemanite. After enrichment process of colemanite, half of the ore emerges as waste. However, there are very valuable trace elements such as lithium, rubidium, cesium, titanium in colemanite wastes. To obtain these valuable trace elements, the silisium in the ore has to be dissolved. In this study, it is aimed to isolate and identified the bacteria with silisium dissolution ability from boren clay. The bacteria possessing silisium dissolution were identified as *Enterobacter* sp. strain, *Klebsiella* sp., *Leclercia* sp., *Leclercia adecarboxylata* by PCR of 16S rDNA gene region. This is the first study to isolate and identified bacteria with silisium dissolution ability from boron reserves. In the further study, it is planned to apply the isolated bacteria on boron clay for the recovery of the trace elements by a nature friendly method.

Index Terms— Boron clay, silicium dissolution, boron enrichment, environmentally-friendly method, 16S rDNA gene region

1 INTRODUCTION

Boron, symbolised as 'B', is a metalloidic element in the lithosphere occurring in trace quantities in the major reservoirs of the Earth. Boron has been used in many industrial areas including glassand ceramics, insulation and textile fiberglass, enamels, glazes, detergents, bleaches, alloys, metals, fire retardants, agricultural fertilizer, wood treatments, production of insecticides and microbiocides [1]. Generally, the commercial form of Boron are borates. Borates are defined as salts or esters of boric acid or any compound containing B_2O_3. Besides, the term of borate is used for any compound containing boric oxide [1,2], several minerals contain boric oxide. However, the most common and important minerals of them are borax, ulexite and colemanite because of their abundance and commercial importance. Borates were first extracted and used over a thousand years ago. According to the reports, the Babylonians had supplied boron from the Far East by importing over 4000 years ago for fluxing gold. The ancient Egyptians had used boron for mummifying, medicinal and metallurgic applications. The borax was used by European goldsmiths in 12th century. The countries having boron reserves are Turkey, USA, Russia, Chile, China and Peru. However, Turkey is the leading country both in reserves and production for borate. About 50% of the boron reserves of Turkey is found in Emet and Hisarcık Region [3,4].

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Boron mineral supplied from Emet-Hisarcık Region is defined as colemanite and this colemanite mineral have been exposed to many process steps to obtain borate.

After these process steps, half of the ore emerges as waste including many valuable trace elements such as lithium, rubidium, cesium, titanium.

Researchers used some physical and chemical methods like precipitation, solvent extraction, adsorption, roasting and leaching etc. to recover these trace elements from boron wastes [5,6,7]. Some of these methods are costly and the others are harmful for environment and human health. On the other hand, it is difficult to extract these elements in the wastes. These metals are mainly found in boron clay and contain significant amount of silicium. It is assumed that these metals exist as sillicate form in boron clay. So we thought that if silicium dissolution can be achieved, the metal extraction can be succeeding with high efficiency. There are many studies to evaluate alternative ways to recover these trace elements from boron wastes [8]. One of the significant problem for valuable elements recovery from boron wastes is the high amount of silicium in boron wates. Many resesarchers have studied to leach boron wastes by using various solutions [9,10,11]. However, these methods have many side effects on environment health. Bioleaching is used to recovery heavy metals by the application of microorganisms [12]. In other words, bioleaching is described as 'the dissolution of metals from the mineral sources by microorganisms and the conversion of solid valuable metal into their water soluble forms' [13,14]. For example, as in the example of the copper, copper sulfide is microbially oxidized to copper sulfate and valuable metals are present in the aqueous phase and the remaining solids are discarded. 'Biomining', 'bioextraction', and 'biorecovery' are the other terms which are used to describe the

mobilization of elements from solid materials mediated by bacteria or fungi or planktonic residues. The biotechnological applications in mining have been significantly investigated by various research organizations and industries for the past 60 years. The bioleaching processes as biotechnological methods are now established as a worldwide biotechnological process [14].

In this study, it is aimed to isolate and identify silicium solubilising bacteria from boron clay for further studieds. It is thought that if the silium dissolution is succeed by using bacteria, the trace elements recovery will be performed by an environmentally-friendly method. In the next step of our study, the effect of the isolated and identified bacteria will be investigated for boron enrichment process.

4 MATERIAL AND METHOD

Substrats and Chemicals

Chemicals used in this study were purchased commercially from Merck AG (Darmstadt, Germany), Sigma (St. Louis, MO, USA), Fluka Chemie AG (Buchs, Switzerland), Acumedia Manufacturers (Baltimore, MD, USA), and Aldrich-Chemie (Steinheim, Germany). The Wizard Genomic DNA Purification Kit, Taq DNA Polymerase, dNTP were purchased from Promega (Madison, WI, USA). All chemicals were reagent grade and all solutions except soil extract agar (SEA) were made with distilled and deionized water.

Isolation of bacterial isolates

The samples of boren clays were aseptically collected from different areas of Emet Boren Establishing in Kütahya and taken to the Recep Tayyip Erdoğan University Molecular Biology and Genetics Laboratory for bacteria isolation. The samples were bathed in physiological saline solution in test tubes and shaked at room temperature and 150 rpm for 4 hours. Then, the serial dilutions of physiologycal saline solutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}) were streaked onto nutrient agar (NA) and trypticase soy agar (TSA) and incubated at 25 °C for 1-3 days. During the incubation time, representative colonies with different morphological shapes were selected and purified. The purified colonies were stored at –80 °C in storage solution (NB with 15% glycerol) for further studies [15]. After isolation of the bacteria, it is aimed to investigate the silicium dissolution ability of the isolates.

Screening of the bacterial isolates in terms of silicium dissolution ability

The isolated bacteria were incubated at 25 °C for one day on NA. Then, each of the bacteria were incubated on soil extract agar (SEA) medium in petri dishes at 25 °C for one week. SEA was prepared by using 1 gr glucose, 0.5 gr K_2HPO_4, 100 ml soil extract, 20 gr agar and 900 ml tap water and the pH of SEA was adjusted at pH 7.0. To prepare soil extract, 500 gr soil was added into 1000 ml tap water and the mixture of soil and water was sterilized at 121 °C, 1,5 atm for 21 min by using autoclave and soil extract was separated into 100 ml aliquots for further use. The isolates with silicium dissolution ability were determined by observing transparent zones around the linear bacterial growth on soil extract agar [16].

İdentification of bacterial isolates with silicium dissolution ability by 16S rDNA-PCR

The bacteria with silicium dissolution ability were identified by 16S rDNA-PCR. Total genomic DNA isolation of the bacteria with silicium dissolution ability were carried out by using Promega DNA isolation Kit according to the manufacturer recomendations. Then, 16S rDNA gene regions of the bacteria were amplified by PCR. The PCR mixtures were prepared as 50 µl contained 20 pmol of univerisal primers for 16S rDNA. The sequence of the forward primer was defined as UNI16S-L:(5′-ATTCTAGAGTTTGATCATGGCTCA-3′) corresponding to positions 11–26 of *Escherichia coli* 16S rDNA, and the sequence of the reverse primer was defined as UNI16S-R:(5′-ATGGTACCGTGTGACGGGCGG TGTGTA-3′) corresponding to the complement of the positions 1411–1393 of *E. coli* 16S rDNA)]. Besides, the mixtures contained 200 µM each deoxynucleoside triphosphate, PCR buffer, and 0.5 U of *Taq* polymerase (Boehringer), 25 mM $MgCl_2$. Approximately 200 to 300 ng of DNA of the studied bacterial isolate. PCR was performed with thermal cycler (Biorad). The thermal cycler was programmed to perform 40 cycles consisting of 95°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min followed by a final extension step of 10 min at 72°C. PCR products were visualized by agarose gel electrophoresis. 7 µl of PCR products mixed with 6X gel loading buffer. The mixture was loaded on agarose gel (1% w/v) in Tris-Borate-EDTA Buffer (TBE). Then the gel was subjected at 90 V for 90 min for electroforesis application. The gels were stained in ethidium bromide solution (2 µl EtBr/100 ml 1XTBE buffer) for 40 min. The PCR products were visualized by using the Bio Doc Image Analysis System with Uvisoft analysis package (Cambridge, UK). The PCR products were sequenced (Macrogen, Amsterdam, the Netherlands). The results of 16S rRNA gene sequencing were analyzed using the GenBank (http://blast.ncbi.nlm.nih. gov/blast.cgi) server. The sequence was compared with the other sequences contained within GenBank by employing a BLAST search model. The species of the studied isolate was identified according to the 16S rDNA gene sequence of the species which was most closely related to the studied strains from the concerned database [17, 18].

5 RESULTS AND DISCUSSIONS

The amount of waste materials of boron has readily increased because of increasing boron exploitation. The large amounts of released waste materials may be harmful to the environment [19]. Therefore, the United States has taken necessary measures to prevent environment from damages of boron wastes [6]. Although our country is very rich in boron reserves and the large quantities of boron wastes are discharged at the end of the processing of boron in concentrators, necessary measures have not been taken, yet. However, many researchers have recently carried out investigations for the evaluations of wastes. These investigations showed that the most suitable way for the waste evaluation is to utilize the remainder of the mainly clay minerals in suitable sectors such as cement, con-

crete, ceramic and brick, etc. and to recover boron from boron wastes by leaching using various solutions [6, 8, 11]. Besides, there are many reports showing that the boron wastes can be use for biosorption [6]. There are many studies indicating the trace elements in the structure of the boron minerals and clays. Helvacı et al. differentiated Emet borate deposits and they reported high Ca borate (colemanite) levels, very low Na levels and relatively high concentrations of Mg, Sr, As and S [20]. The clay-mineralogy and whole-rock chemistry of the borate-bearing layers of the Hisarcık and Espey mines were examined and it has been reported the clay fraction contained predominantly Li-bearing saponite (60–90 wt.%) by Çolak et al. [21]. Büyükburç et al. aimed to reduce the cost of processes by extracting lithium from boron clays using natural (limestone–clay) materials and the wastes of boric acid were used as a source of gypsum [22]. Ertan et.al. studied the exraction of rubidium from boron clays [6]. According to the study performed by Ertan et. al. silicium is the most significant problem in recovering valuable trace elements. In this study, the bacteria were isolated and purified from boron clay. Then, the purified bacteria were screened for silicium dissolution ability. The bacteria having silicium dissolution potential were identified by their 16S rDNA regions. Their 16S rDNA regions were amplified by PCR, the PCR products were sequenced and analyzed using the GenBank (http://blast.ncbi.nlm.nih.gov/blast.cgi) server. According to the results, the bacteria were identified as *Enterobacter* sp. strain, *Klebsiella* sp., *Leclercia* sp., *Leclercia adecarboxylata*. The genus' of *Enterobacter*, *Klebsiella* and *Leclercia* belong to the family Enterobacteriaceae and they are both nosocomial pathogens listed in the most recent edition of Manual of Clinical Microbiology. *Leclercia* sp. has been rarely isolated from environmental and clinical specimens. Some strains of these genus' can be nonpathogen [23, 24, 25, 26, 27]. It is thought that the bacteria are not pathogen because of being isolated from soil. However, there is a great obligation to perform pathogenicity test for these bacteria before industrial application. The identified bacteria maty have potential to be pathogen according to the literature. Recently, silicate solubilising bacteria draw great interest due to their roles in dissolution of silicate minerals. These bacteria supply silica and potassium for crop uptake and reduce the use of potash fertilizer [28]. Also the researchers have studied silicate solubilising bacteria because of their role in desilication of ores like bauxite. Studies have shown that these bacteria dissolve silica and release phosphate, potassium, iron and calcium from the minerals including silicate [29,30,31,32]. With this approach, it is thought that the bacteria obtained from boron mineral will be usefull in method improvement for obtaining valuable elements in boron mineral by dissolving of silicium. It is planned to apply the isolated bacteria on boron with high silicium content in liquid medium and to determine how much silicium content in boron decrease for the next step of the research. Then, it is aimed to determine the effect of bacteria on the yield of trace elements which are intended to be obtained from boron mineral.

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