Miseq sequence identification of bacteria isolated from Çıldır Lake

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Abstract

In this study, it was aimed to identify and phylogenetic evaluation of bacteria isolated from Çıldır lake sediment. Four sediment sampling were done from two different regions. For bacterial isolations, samples were incubated in Bennet's agar at 28 ºC for 7 days. Morphological and microscopic (Scanning Electron Microscopy-SEM) images of the isolated bacteria were taken. After the PCR process performed with universal primers, the isolated bacteria were identified by sequencing the PCR amplicons using the MiSeq sequence method. In the results of working; 2 Nocardia sp and 1 Amycolatopsis sp species were isolated. When looking at the spore chain morphology in the SEM image, it was observed that a knobby structure was formed, the sequence alignment of 16S rRNA longer than 1000 bp and the sequences obtained from GenBank in the phylogenetic tree were closely similar (99 % <). It has been observed that the bacterial fauna of Çıldır Lake has a low bacterial density that does not pose a risk. The high accuracy of the sequence analysis process for the identification of bacteria has been confirmed. We believe that entering the sequence data of the isolated bacterial species as GenBank data set will be important in terms of providing data for other studies with nucleotide sequences.

Keywords:16S rRNA, Bacteria, Çıldır lake, MiSeq sequencing, SEM.

Received:26.06.2021
Accepted:09.10.2021
Published:10.12.2021
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Cite this article as: Ş.Önalan and H. Seçkin. Miseq sequence identification of bacteria isolated from Çıldır Lake, *Eastern Anatolian Journal of Science, Vol.7, Issue 2, 11-15,2021.*

1. Introduction

By identifying the dominant bacteria in fish consumed as human food, it is possible to determine the bacteriological quality of these foods and also to learn the causes of spoilage. For this purpose, skin, gills and organs of marine and freshwater fish are examined. In addition, the presence of bacterial agents in aquatic organisms is investigated by performing bacterial isolation from soil and sediment in the aquatic environment (Fguira, et al., 2005). The most important factor effective on aquaculture is diseases. Bacterial disease factors cause great economic losses in aquaculture (Bilgi, 2006).

Actinomycetes are filamentous bacteria belonging to the Actinobacteria family. Actinomycetes are considered the most invaluable prokaryotes in the medical and biotech industries due to their ability to produce a large number of bioactive molecules, especially for antibiotic compounds. Among the actinomycetes, the Streptomyces genus is thought to be responsible for the production of 60 % of antibiotics. Nocardia spp. isolated from rainbow trout for the first time as a fish infection, was identified and reported to be effective on humans and stimulate the immune system (Chen and Wang, 1993). The genus Nocardia Trevisan 1889 belongs to the suborder Corynebacterineae and currently encompasses about 40 species of mycolic-acid-containing actinomycetes, including the recently described species (Nocardia puris, Nocardia inohanensis, Nocardia yamanashiensis, Nocardia tenerifensis and Nocensardis etebra).

In this study, isolation and identification of bacteria isolated from different sediment sources of Çıldır Lake were performed. Morphological and microscopic (SEM) imaging was performed. PCR amplicons were obtained with DNA isolation followed by universal bacterial PCR primers (27F-1492R). 16S rRNA gene sequencing was obtained

and the similarities and differences between species were revealed.

2. Material and Method

2.1. Sampling and bacteria isolation

Three bacteria were isolated from sediments taken from 2 different regions in Çıldır Lake. In the first region (A), sediment samples were taken from a depth of about one meter. Samples were taken from the shore point of the second region (B). Dilution method was used for bacteria isolation. Inoculation was made on Bennet's agar medium. Then it was kept at 28 ºC for 7 days for incubation. These colonies were transferred to Bennet's agar by line planting method and the colonies were tried to be dropped one by one. Isolates were stored at -20 ºC in cryogenic tubes containing 20 % glycerol (Seçkin and Önalan, 2020).

2.2. Morphological and Microscopic images acquisition

Bacterial columns growing on Bennet's agar were viewed macroscopically under Stereo microscope (Canon). Growing bacterial colonies were displayed with Scanning Electron Microscope (SEM-ZEISS) as EHT: 10.00 keV and Working Distance (WD): 8.9. The branching structures of the bacteria were examined on the basis of families in the images obtained (Seçkin and Önalan, 2020; Önalan and Çevik, 2020).

2.3. DNA isolation

DNA isolations from bacteria isolated in the study were isolated by the automated QIAcube device with the Mericon Bacterial DNA isolation kit (Qiagen) as described by the manufacturer. Total DNA concentration was determined by QIAxpert (Qiagen) (Önalan and Çevik, 2020).

2.4. PCR analysis

PCR process was performed using the bacterial DNAs isolated in the study and using a universal primer set (27F-1492R). For this purpose; Master mix without nucleic acid dye (12 µl), DNA (5 µl), Primer set (1 µl + 1 µl) and water (6 µl) were used. The total volume was set at 25 µl. The PCR protocol continued after 10 minutes of denaturation at 95 ºC, 30 seconds at 94 ºC, 30 seconds at 55 ºC and 45 cycles at 72 ºC. In the last step, the PCR process was terminated with the final extension process at 72 ºC for 7 minutes. PCR amplicons were kept at -20 ºC for sequence analysis (Önalan, 2019).

2.5. Sequence analysis

The samples have been uploaded to the MiSeq sequencer (Illumina). A standard flow cell was placed in the flow cell compartment. The collected samples were diluted with cooled HT1 buffer to a concentration of 2 nmol / l and an equal amount of 0.2 N NaOH was added to denature the sample. Before the samples were loaded into the cooling section of the MiSeq machine, 600 µl of the spiked samples with a final concentration of 8 pmol / l were pipetted into the sample well on the MiSeq consumable cartridge. Sequencing was performed on a MiSeq sequencer with one-way reading including an index run according to manufacturer's instructions (Sikkema-Raddatz et al., 2013).

3. Results

3.1. Sediments' sampling results

Three bacteria were isolated from sediments taken from 2 different regions in Çıldır Lake. Images of sediment samples taken for use in the study are given below (Figure 1). Nocardia sp-1 and Nocardia sp-2 species were isolated from the sediment samples taken from the first region (A) and Amycolatopsis sp from the samples taken from the second region (B).

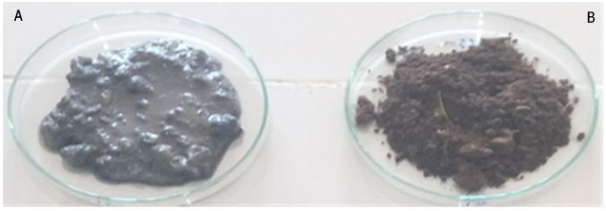

Figure 1. Sediment samples used in the study (A- Sediment sample from which Nocardia sp-1 and Nocardia sp-2 species were isolated and taken from a depth of about one meter. B- The sample from which Amycolatopsis sp was isolated and taken from the shore point).

3.2. Bacteria isolation results

As a result of bacteria isolation from sediment, 2 Nocardia sp and 1 Amycolatopsis sp were isolated. The bacteria isolated at the end of 7 days

incubation period at 28 ºC in Bennet's Agar from sediment samples are given below (Figure 2).

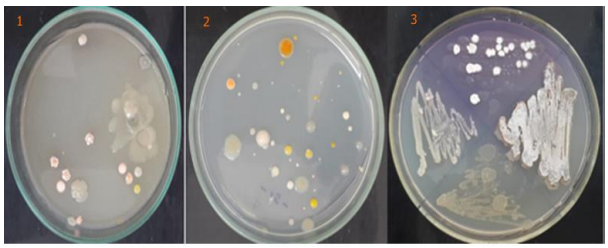

Figure 2. Isolated bacteria from sediment samples (Picture-1; Medium from region A from which Nocardia species is isolated. Picture-2; The medium from which the Amycolatopsis strain from zone B is isolated. Picture-3; Color grouping image of species isolated from regions A and B).

3.3. Electron microscope screening results

Looking at the morphology of the bacteria isolated, it was seen that a knobby structure was formed. This specific appearance has been identified as the microscopic image belonging to the Nocardia and Amycolatopsis families (Figure 3).

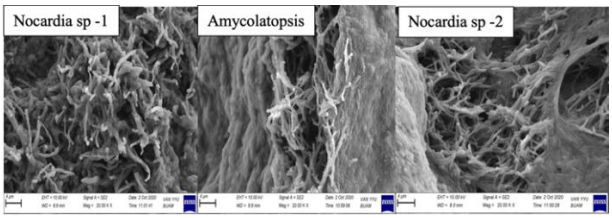

Figure 3. SEM images of Nocardia (1-3) and Amycolatopsis sp species (Knotted structure image of Nocardia sp-1 and Nocardia sp-2 strains isolated from region A and Amycolatopsis sp strain isolated from region B obtained as a result of SEM).

3.4. DNA isolation and sequencing analysis results

It was observed that the A260 / 280 nm nano-spectrophotometric purity ratios of the isolated DNA varied between 1.9-2.0. The fact that the DNA purity rates of the samples are close to each other and the purity level is within a reliable range shows that the isolations made with the automatic isolation robot give healthier results. The molecular identification of the bacterial isolates used in the study was performed by sequencing using isolated DNAs and bacterial universal primers. Percent identification results of bacteria isolated according to sequence results are given below. As a result of the sequence analysis, the results of the dendrogram analysis showing the intra-species similarity rates of the bacteria isolated and identified in this study are given below (Figure 4).

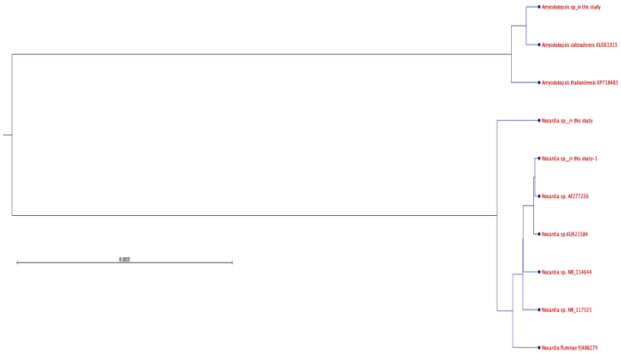

Figure 4. Dendrogram analysis result of isolated bacteria.

When performing the alignment analysis, the gap and locus gaps formed at the beginning and end of the sequences were deleted due to the difference in the number of nucleic acids read as a result of each sequence and the length of the sequence loaded into the gene bank, and alignment analysis was performed according to the sequence data of the same length.

4. Discussion and Conclusion

Classification of microorganisms (morphological, physiological, biochemical) based on traditional microbiological methods cannot provide a clear determination of the taxonomic positions of microorganisms. For this reason, an advanced approach, which is now called polyphasic taxonomy, is used to evaluate morphological and biochemical data together with the information obtained through molecular techniques (Prakash et al., 2007). Nocardia species were isolated from region A taken from a depth of about one meter and showed a wide distribution in the medium. Nocardia species have been isolated from the aquatic environment. Amycolatopsis sp was isolated from the coastal region, that is, from a point with a lower water content than the first region.

Because of Nocardia and Amycolatopsis bacteria have a complex structure, species

identification is difficult. According to Bergey's Systematic Bacteriology Handbook, it was reported that unknown Nocardia species can be identified first with the 16S rRNA gene (Goodfellow et al., 2012). The 16S rRNA gene plays a crucial role in species identification and phylogenetic relationships of prokaryotes, including Nocardia (Wei et al., 2019). 16S rRNA gene sequence analysis has allowed the identification of many new Nocardia species and have been precise methods to reliably identify these isolates at the species level (Conville et al., 2006). In our study, the similarity dendrogram of Nocardia and Amycolatopsis species isolated from Çıldır Lake was obtained by using 16S rRNA gene region.

Similarly, in some studies, it has been reported that molecular methods are used after sediment sampling. Based on the 16S rRNA sequence from sediments taken from the Algerian Lake Fetzara, wetland ecosystem, it has been reported that four different actinomycetes (Actinomadura, Nocardia, Nonomuraea and Micromonospora) have been isolated (Benhadj et al., 2019). Similarly, it has been reported that bacteria of the genus Streptomyces, Nocardia and Nocardiopsis were isolated and identified from samples taken from underground lakes in Badzheyskaya and Okhotnichya caves in Siberia (Voytsekhovskaya et al., 2018). In a different study, it was reported that *Amycolatopsis nivea* was isolated from the sediment sample taken from the Yellow River of China (Niu et al., 2020). In a different study, it was reported that bacterial isolation and molecular identification were carried out from sediment samples taken from different points around Iğdır Fish Lake (Seçkin and Önalan, 2020). In another study, phylogenetic analysis of 16S rRNA sequence of an actinomycetes strain isolated from the marine environment of India was reported to be 100 % similar to *Amycolatopsis alba* (Dasari and Donthireddy, 2011).

In conclusion, bacteria isolation and phylogenetic analysis from soil samples taken from sediment in different regions of Çıldır Lake were performed in this study. According to the phylogenetic analysis of the test isolates completed with 16S rRNA sequence analysis, priority is given to the isolates that show the most nucleotide difference with their closest relatives, and it is planned to be identified by microbiological and molecular methods and to be included in the literature in future studies.

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