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Research Article

PHYLOGENETIC DIVERSITY OF NOCARDIA SP. OBTAINED FROM DIFFERENT WATER ENVIRONMENTS

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ABSTRACT. The aim of this study was to investigate the isolation and typing of Nocardia species, which are found in many water resources and have microbial importance. Agent isolations were carried out from sediment samples taken from different points determined around Iğdır Fish Lake. DNA isolations were carried out from the isolated Nocardia species. Subsequently, 16S rRNA gene region sequence analyzes were performed. Sequence analyzes for each isolated bacteria were aligned and these results determined similarities and differences at the level of group and genus of different origins and individual populations. As a result of the study, 2 different types of bacteria were isolated. In the GenBank database, the isolated bacteria were found to have 99.78% and 99.30% similarity with the species with 99% and higher sequence matching. Nucleotide sequencing differences were found to be 0.3% with reported to be isolated from different water sources. Different studies are needed to determine the differences at the gene level of the same type of bacteria isolated from different sources, and to determine the pathogenicity and virulence characteristics among the isolates.

Keywords: *Nocardia* sp., *Real-Time PCR, Sequencing, Phylogenetic diversity*

INTRODUCTION

Microorganisms are living things that have many beneficial properties as well as having harmful effects for other living things. Throughout their life, they benefited from many types of microorganisms in search of food sources. In recent studies involving nutrition and the form of nutrition, microorganisms containing healthy ingredients for the contents of foods and our metabolic activities have been observed. Natural products obtained from plants and microorganisms are used for the treatment of many infections [1].

Actinobacteria have pharmaceutical importance, such as antibacterial, antiviral, anticancer, and immunosuppressive. These species are microorganisms that can produce secondary metabolites [2, 3]. Nocardia species are a complex group of organisms thought to belong to aerobic actinomycetes [4]. In line with the studies conducted for the isolation and identification of this bacterial species, it was also stated that the same type of bacteria isolated in different geographical regions have different genotypic characteristics. At the same time, the differentiation of the same type of bacteria exposed to different environmental conditions has been informed by various researchers. Determining these differences is very important, especially in examining the intra-species changes of bacteria. In this study, the isolation of Nocardia species from the fish lake region of Iğdır province, determination of their morphological characteristics, molecular identification,

genetic similarity rates of species isolated from the same region and phylogenetic affinity with isolates isolated from different regions were investigated.

MATERIALS AND METHODS

Sampling

Nocardia species were isolated from sediments taken from different parts of the Fish Lake in Iğdır province. The pH and humidity of the sediment samples were determined. Isolation of Nocardia species was done by dilution and spreading method. Inoculation was performed on Bennet's agar medium [5].

Bacteria isolation

Planting was carried out on Bennet's Agar for bacteria isolation from samples taken from sediment. Colonies that developed after an incubation period of 15 days at 27 °C were selected considering their morphological characteristics. These isolated colonies were transferred to Bennet's Agar medium by streak plate method. Pure isolates were stored at -20 °C in cryogenic tubes containing 20% glycerol [6].

Colorimetric differences of bacteria

Bacteria growing on Bennet's agar medium were cultivated in Oatmeal Agar medium for color grouping after morphological selection. After 15 days of incubation at 27 °C, differences were determined according to color formation [7].

DNA isolation

High molecular weight DNA was isolated with the automated QIAcube accompanied by the DNeasy Mini kit (Qiagen) as described by the manufacturer. The concentration of total cellular DNA was determined by QIAxpert (Qiagen) [8].

Sequence analysis

Bidirectional sanger sequencing was performed for the 16S rRNA gene using the isolated DNAs using universal bacterial primers. The molecular identification of the isolates was performed by blasting the nucleic acid sequences obtained as a result of the sequence analysis in the GenBank. Then, by performing alignment analysis with the obtained sequences, the differences between the same species of Nocardia isolated from the same region were determined. Finally, the genetic distances of the isolates used in the study were determined according to the bacteria isolated from different regions by comparing the overlapping sequence data of the same species present in the gene bank with over 95% overlapping sequence data [9].

RESULTS AND DISCUSSION

Study area and sampling

The pH rates of the samples taken from Iğdır province fish lake, where the sampling was carried out, were measured as 7.65 and 7.70. The humidity rate was measured as 58.6% -60.1%. Images of the sampled area are given below (Fig. 1).



Fig.1. *Pictures of the Fish Lake where bacteria isolation was carried out*

The samples taken from the sediment of 2 different points shown in fig 2 on the lake in the sampling area are given in the following.

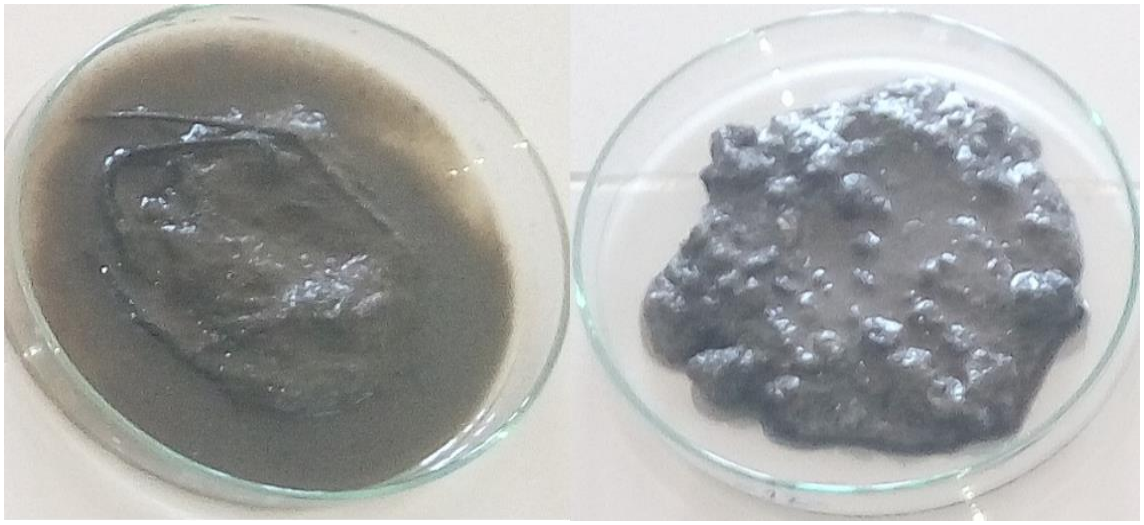


Fig.2. *Samples taken from the sediment where bacteria isolation is carried out*

Isolation of bacteria

Pictures of the isolates planted on Bennet's Agar in sediment samples and isolated and purified at the end of the 27 °C incubation period are given below (Fig. 3).



Fig.3. *Images of Nocardia species isolated in the study growing on Bennt's agar*

DNA isolation – Sequence analysis

A260 / 280 nm nano-spectrophotometric purity ratios of DNA isolated in the study were found to vary between 1.87-1.91. The fact that the DNA purity rates of the samples are close to each other and the degree of purity are in a reliable range show that the isolations performed with the automatic isolation robot give healthier results.

Molecular identifications of the bacterial isolates used in the study were performed as a result of sequencing using bacterial universal primers with isolated DNAs. Percentage identification results of bacteria isolated according to the sequence results are given below.

Table 1. *Identification results of isolated Nocardia* sp.

Isolate	ID	Max ID	Percent %	*Acc number
A11	Nocardia sp	*Nocardia niifigatensis*	99.73	NR_117402
B11	Nocardia sp	*Nocardia wallacei*	99.30	KC113168

* The sequences of bacteria isolated in this study were blasted in the gene bank and sequences with 98% or more similarity to each isolate sequence were selected.

As a result of the sequence analysis, the results of the alignment analysis showing the intraspecies similarity rates of the bacteria isolated and identified in this study are given below. While alignment analysis was performed, due to the difference in the number of nucleic acids read as a result of each sequence and the differences in the sequence lengths loaded into the gene bank, the gap and locus gaps formed at the beginning and end of the sequences were deleted and alignment analysis was performed according to the sequence data of the same length. This also eliminated any similarities or differences due to sequence length or shortness.

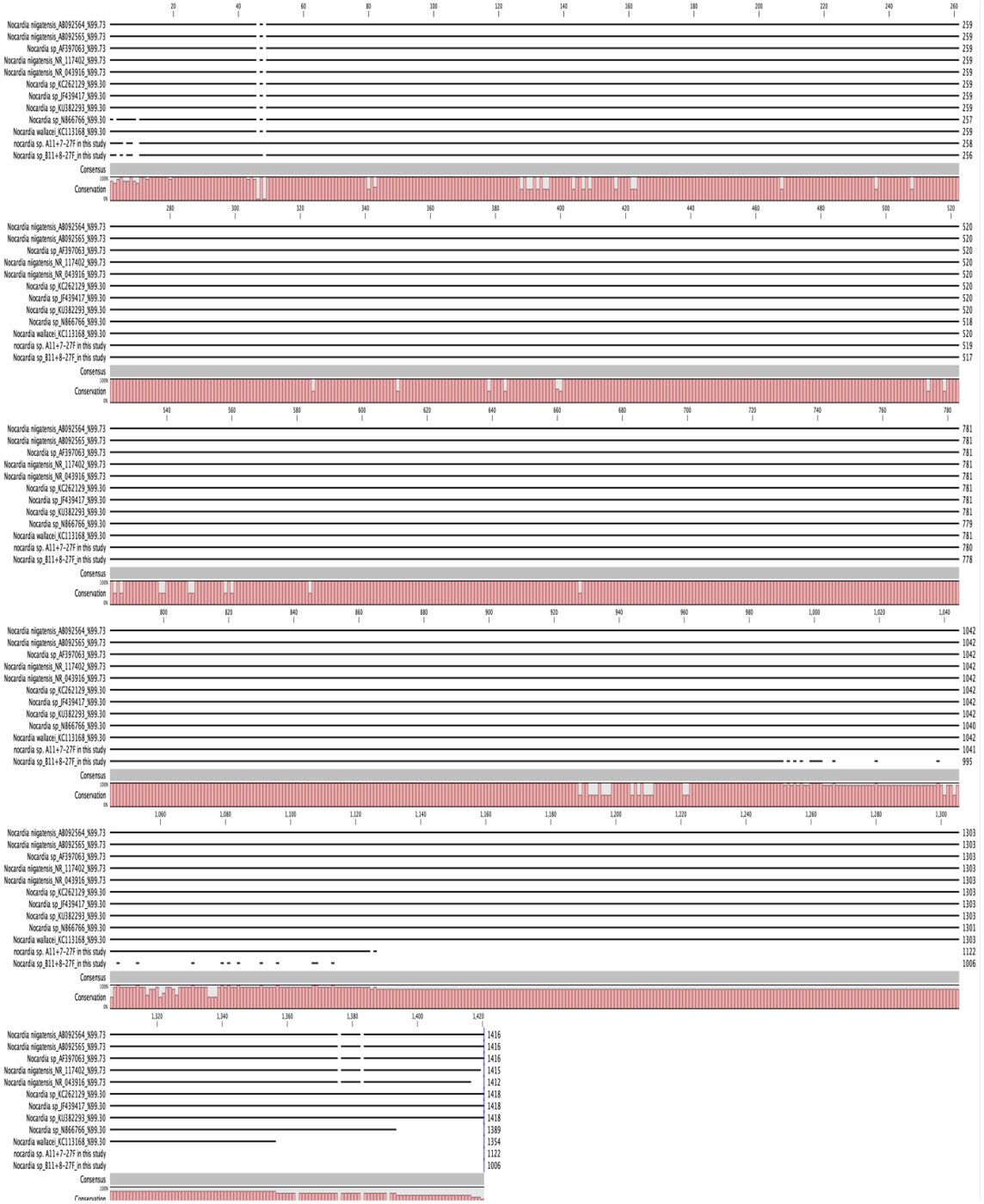


Fig.4. *Alignment analysis of isolated Nocardia sp and other NCBI sequences*

The clodogram showing the affinity relation of the bacteria isolated in the study with bacteria isolated from different regions in the gene bank is given below.

It is seen that the same type of bacteria isolated from the same region are the most similar to each other. In this study, it was observed that the bacteria isolated from the same region were the closest to each other, and other isolates differed at different rates according to their similarity for their own species.

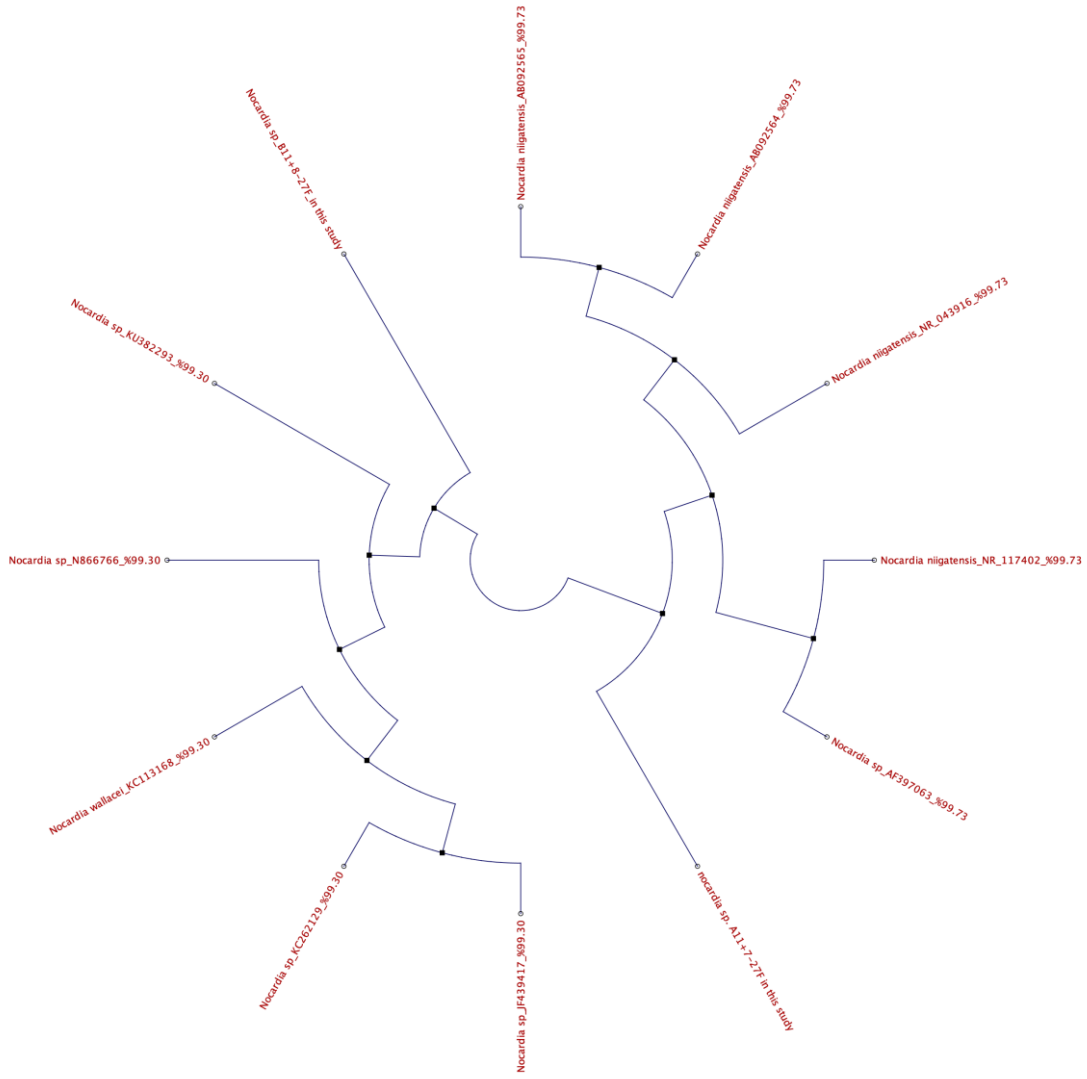


Fig.5. *Dendrogram of isolated bacteria and other related sequences in GenBank*

Nucleotide sequencing differences were found to be 0.3% with reported to be isolated from different water sources.

CONCLUSION

Turkey's highest-altitude lake fishing in the lake has an altitude of 2241 meters. It is home to many fish and bird species. This lake, which has a natural ecosystem, has an important place in terms of geography and biodiversity. Especially the high altitude allows for the detection of new species.

Nocardia species have the capacity to synthesize bioactive molecules with various functions by cell biotransformation [10]. While Nocardia species are mostly isolated from host creatures, some species have also been isolated from areas such as soil and water [11]. Sequence analysis of 16S rRNA to identify and characterize Nocardia species is among the molecular identification methods [12, 13].

The increasing use of Nocardia species in scientific studies makes the diagnosis and diagnosis of these bacteria important. In this context, there is a need for bacteria species

that can produce new active ingredients against increasing antibiotic resistance, especially in the field of health. In our study, Bennet's Agar was used for the isolation of Nocardia species. Some researchers reported that they performed the isolation with the same medium for the purpose of Nocardia isolation [14]. Nocardia strains, better known as clinical isolates, are abundant in the soil, but it has been reported that they can be isolated from places such as cave, marine sediment, activated sludge and rotted plant residues [15].

Sequence analysis method was used in this study for Nocardia identification. Today, sequence analysis is one of the most reliable and accurate methods in molecular-based studies, especially with bacteria. NGS sequencing is seen as the most reliable sequencing method known today. It has been reported in some studies that NGS method was used for the identification of Nocardia species [16]. In recent studies, it has been reported that there are many nocardia species isolated from different ecological areas [17].

As a result of the study, 2 different types of bacteria were isolated. In the GenBank database, the isolated bacteria were found to have 99.78% and 99.30% similarity with the species with 99% and higher sequence matching. Nucleotide sequencing differences were found to be 0.3% with reported to be isolated from different water sources. Different studies are needed to determine the differences at the gene level of the same type of bacteria isolated from different sources, and to determine the pathogenicity and virulence characteristics among the isolates.

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