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The Characterization of Prokaryotic Diversity in Lake Beyşehir Using a 16s Metagenomics Study

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Research Article

Abstract−Lake Beyşehir, located in the Central Anatolia Region, is the largest lake in the region, providing drinking water and irrigation. It is Turkey's third-largest lake and the largest freshwater lake. Although large populations use lakes in our country, there are not enough studies on prokaryotic diversity, so revealing the diversity is essential for lakes and the people around lakes. A metagenomic analysis allows for the determination of the species of microorganisms obtained through DNA isolation of sample directly taken from the environment without culturing, as well as the genetic structure and functional roles of these microorganisms and their impact on human and ecological health. We utilized NGS high-throughput methods for the metagenomic analysis of the Lake Beyşehir sequences of 16S rDNA (regions V3–V4). Illumina NovaSeq technology was used to sequence the variable regions. Metagenomic analysis was performed on these sequences using MOTHUR software, and prokaryotic diversity was explored, revealing the phyla Proteobacteria, Verrucomicrobia, Bacteroidetes, and Actino-bacteria.

Keywords: *16S rRNA, Illumina sequencing technology, metagenomic, mothur, prokaryotic diversity*

1. Introduction

Lake Beyşehir, located in Central Anatolia, is the largest lake that provides the drinking and irrigation needs of the Central Anatolia Region. Lake Beyşehir Basin is the critical freshwater source of the Konya Closed Basin, the biggest closed basin in the Central Anatolia Region (Şener & Taştekin, 2019). The lake is still used for multiple purposes, particularly for the drinking water supply (Beyşehir District and seven cities), agricultural irrigation (the Konya Plain, Şarkikaraağaç, and Kıreli pumped irrigation), fishing, and tourism (Dinç & Öztürk, 2013). All prokaryotic diversity studies on Lake Beyşehir have been done with traditional cultural and taxonomic procedures.

Worldwide, freshwater lake ecosystems have been investigated in terms of their biome, e.g., Toolik Lake, Alaska, USA; Adirondack Lake, New York, USA; Lake Loosdrecht, The Netherlands; Crystal Bog Lake and Sparkling Lake, USA; Lake Cadagno, Switzerland; and Lake Fuchskuhle, Germany (Hobbie et al., 1999; Hiorns et al., 1997; Zwart et al., 1998; Fisher & Triplett, 1999; Bosshard et al., 2000; Glöckner et al., 2000). These freshwater lake ecosystems provide important ecosystem services to many millions of people, such as water supply, food production, recreation, and biodiversity conservation. However, freshwater lake ecosystems are also facing multiple threats from human activities, such as pollution, eutrophication, habitat loss, overexploitation, invasive species, and climate change (Zhang et al., 2018). Therefore, understanding the

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structure and function of freshwater lake ecosystems and their responses to environmental changes is essential for their sustainable management and conservation.

Metagenomics is the direct analysis of DNA from natural environments without the need to culture microorganisms in the sample. It is done by isolating their DNA and analysing using next-generation sequencing techniques (Kolbert & Persing, 1999). The metagenomic approach outperforms microbial culture in terms of assisting in the discovery of metabolic pathways and unique gene sequences of unculturable bacteria straight from the environment. This is the main advantage of metagenomics because culturing microbes is time-consuming or not all microbes can be cultured. Previous research on culture-independent microorganisms found that the species richness of organisms in brackish lakes is exceptionally high compared to that of freshwater lakes (Tang et al., 2015; Wang et al., 2012).

Next-generation DNA sequencing technologies, which have emerged as one of the most important technologies developed in recent years and which are used today, can be used in many studies such as transcriptome analysis, determination of ploidy level, development of molecular markers, and determination of mRNA profile because of the technology's high accuracy and ultra-fast sequencing capabilities. In addition to these studies, next-generation DNA sequencing technologies can also be used for metagenomics and ecology research, which aim to reveal the diversity and dynamics of microbial communities in various environments (Oulas et al., 2015) (Miller et al., 2022).

QIIME is extensively used in metagenomic investigations to process data generated by sequencing the highly variable sections of the 16S rRNA gene, a commonly targeted area for finding bacteria in environmental samples (Oulas et al., 2015) (Kuczynski et al., 2011). MOTHUR is an open-source software program that analyzes and compares microbial communities from 16S rRNA gene data from next-generation sequencing (NGS) (Schloss et al., 2009).

MOTHUR software allows to control and customize every stage of the analysis. MOTHUR uses the SILVA database, which is a more up-to-date and comprehensive database for the classification of bacterial and archaeal communities. MOTHUR uses the Vsearch algorithm, which is faster and less memory-intensive than USEARCH. This enables processing larger data sets in a shorter time. MOTHUR offers different methods for OTU clustering. For example, the optiClust method uses an algorithm to optimize OTU clustering (López-García et al., 2018).

Despite some studies on fish species living in the lake (Özparlak, Arslan, G., & Arslan, E., 2012), the prokaryotic diversity of Lake Beyşehir remains unknown. Bacteria are part of the carbon cycle and the food chain in lake ecosystems (Tank et al., 2009). Therefore, we aimed to obtain detailed information on prokaryotic diversity in Lake Beyşehir by employing the 16S metagenomic technique. In addition, since this is the first work done on the lake, it will be a source for researchers in other disciplines for future studies completed in and around Lake Beyşehir.

2. Materials and Methods

2.1. Location of the Study and Sampling

Lake Beyşehir (37.679638N, 31.718719E 1.121 m) (Figure 1) water sample was collected in sterile containers from the lake in October 2019. The water sample was taken from the surface layer (1 m depth), the water column's middle segment, and the bottom layer (1 m above the sediment). A sample of 1 L of water in equal proportions was collected and held at 4 °C in the dark for no more than 24 hours before the DNA extraction. The sample's salinity was measured using a hand refractometer (Hach HQ40d), and the pH was determined using a pH meter (SM 4500 H+ B.). The elements in the water sample were identified using Koski's ICS and ICP MS (SM 4110 B / ICS and SM 3125 B. / ICP MS) devices (Konya Water and Sewage Administration).

Figure 1. Map of Türkiye Showing Geographical Locations of Lake Beysehir with Their Respective Altitude Levels (Google Maps, 25.12.2022, https://www.google.com/maps/place/Bey%C5%9Fehir+G%C3%B6l%C3%BC/@37.7792921,31.2339629,10z).

2.2. DNA Extraction and Quantification

In the study, a lake water sample of 1 L was filtered using a 0.22 μm filter paper (Merck Millipore, Darmstadt, Germany), and this filter was used for metagenomic DNA extraction (Mutlu et al. 2008). After the filter paper was placed inside a 2 ml Eppendorf tube, DNA extraction from filter paper was performed using the DNeasy PowerWater Kit (Gilbert et al., 2011). The DNA concentration was measured using a NanoDrop 2000 fluorometer from Thermo Scientific (Cseke et al., 2003). The DNA was transferred to a commercial laboratory for 16S rDNA sequencing and metagenomic investigation (Gen Ova, Istanbul, Türkiye).

2.3. Sequencing and Bioinformatic Analysis

The amplicon produced by the primers (341F: 5′-CCTACGGGNGGCWGCAG-3′/805R: 5’-GACTACHVGGGTATCTAATCC-3’) intended to target the V3 and V4 regions of 16S rDNA is about 465 bp long. The amplified library is utilized for paired-end read sequencing on the NovaSeq platform (2x250 bp)(Klindworth et al., 2013).

The collected data were examined using MOTHUR v1.48.0 (Schloss et al., 2009). Forward and reverse readings were assembled into contigs, filtered, and processed. We filtered out the examination of any sequences that contained ambiguities or homopolymers greater than 8 bp. After filtering, the sequences were deduplicated and aligned to the SILVA v132 reference small subunit rRNA gene alignment database (Quast et al., 2013). Using a 95% cut-off, the start and end positions were optimized to get rid of the sequences that did not cover the whole alignment. After the alignments were made, columns with gaps or dot characters were taken out, and the sequences were reduplicated a second time. Denoising was accomplished by clustering sequences with less than one difference per 100 bp, and chimeras were eliminated by utilizing the MOTHUR version of the VSEARCH method (Rognes et al., 2016). The Wang technique (Wang et al., 2007) was used to classify sequences with the naive Bayesian classifier against the SILVA v132 reference taxonomy database and a 70% bootstrap threshold. Chloroplast, Mitochondria, and Eukarya sequences were eliminated. The optiClust method (Westcott & Schloss, 2017) was used to estimate clusters and OTUs with 99% identity using the obtained sequences. Based on the most prevalent sequence within each cluster, consensus classifications and representative sequences were produced for every OTU. Following data processing in MOTHUR, OTUs were further filtered. The composition of bacterial communities was investigated across a range of taxonomic scales.

The KRONA tool (Ondov et al., 2011) was used to build interactive charts displaying the taxonomic diversity of the sample.

3. Results and Discussion

3.1. Physicochemical Parameters

Lake Beyşehir is an essential freshwater reserve for Turkiye's three largest cities as well as Konya and Isparta. As a result, their physicochemical properties were also examined. The measurements of the common and heavy metals found in the freshwater lake are shown in Table 1, along with other water quality characteristics (i.e., Lake Beysehir). The pH values of the sample ranged from 7.0 to 7.3, and the total dissolved solids (TDS) salinity and conductivity levels from Lake Beyşehir were within suitable limits. (Table 1).

Table 1
Lake Beyşehir Physico-Chemical Parameters

	Characteristics of Water				Common Metals					
Sample Name	pH	TDS Mg/L	Salinity/EC (µS/cm)	Conductivity (µS)	Mg (mg/L)	Zn (µg/L)	Ca (mg/L)	B (µg/L)	Fe (µg/L)	Mn (µg/L)
Lake Beyşehir	8.2	180	267	314	26.6	<12	16.53	<48	7.21	3.84

Heavy Metals (µg/L)										
Sample Name	Pb	Hg	Al	Cr	Co	Ni	Cu	As	Cd	Ba
Lake Beyşehir	0.6	0.05	<9.6	<0.3	0.103	<0.54	3.93	8.07	<0.3	19.93

3.2. Taxonomic Structure

The Beyşehir freshwater environment showed a high level of bacterial community diversity. From the sample we took from Lake Beyşehir, 41.6 million readings were obtained. After these reads were filtered according to the quality of the sequences, 31.18 million reads were obtained. Metagenomic contigs were analyzed by SİLVA (Keegan et al., 2016). As a result, 11 phyla were identified. The results show that the microbial composition of Lake Beyşehir is as follows: Proteobacteria (44%) predominated in Lake Beyşehir; Bacteroidetes (27%) was the second most dominant phylum; and Actinobacteria (20%), Verrucomicrobia (3%), and Firmicutes (3%) were the least common phyla. Figure 2 shows the phylum distribution in Lake Beyşehir.

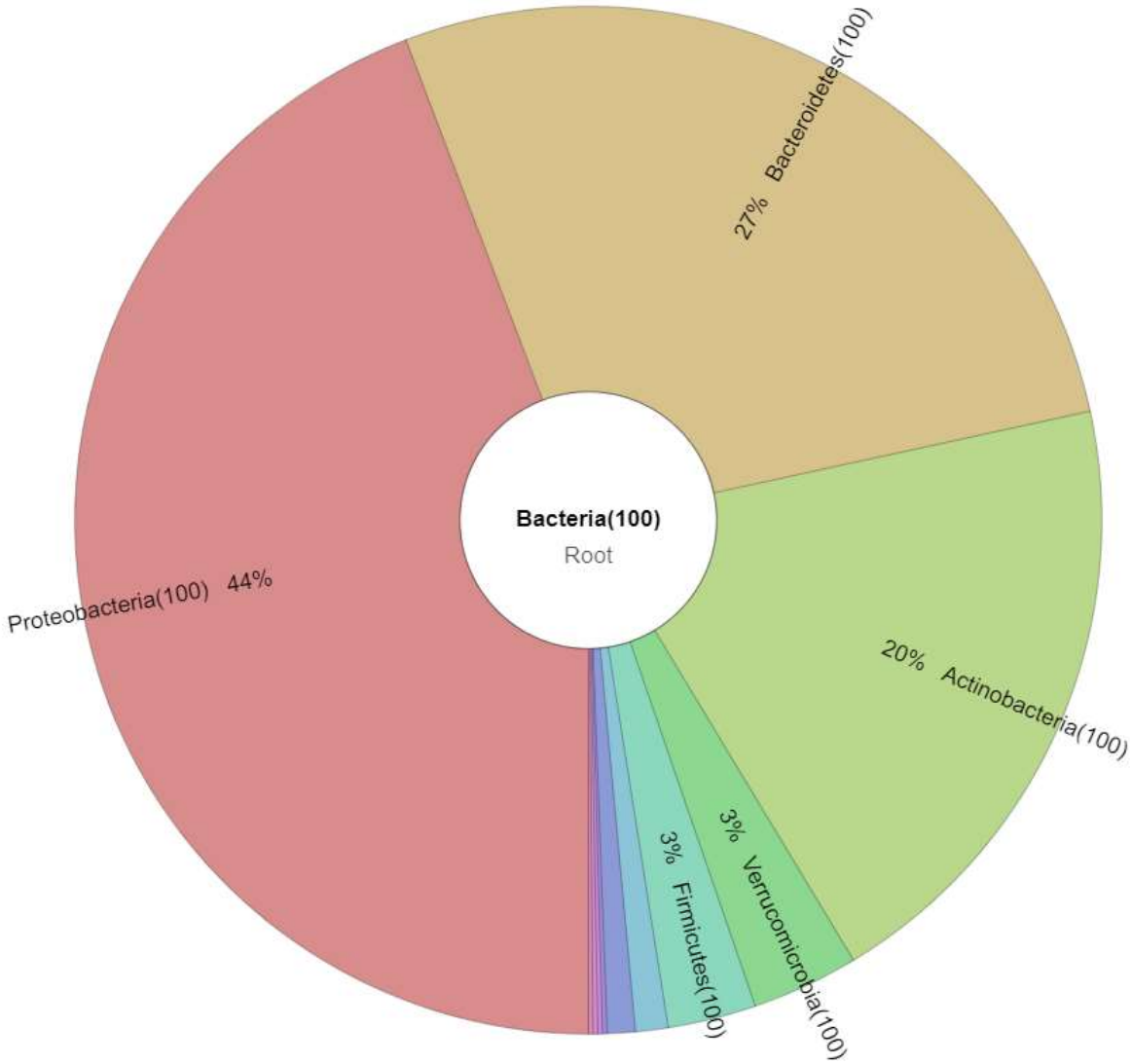

Figure 2. Lake Beysehir Microbial Community Structure at the Phylum Level (Fewer than one are not shown.)

To further evaluate the bacterial diversity present in the sample, the contigs were annotated at the bacterial class level. Significant differences in bacterial classes were found between the samples; Betaproteobacteria (28%) was predominant in Lake Beyşehir. The second most abundant bacterial class in Lakes Beyşehir was Sphingobacteria (22%) followed by Actinobacteria (20%) and Alphaproteobacteria (15%). This is shown in Figure 3.

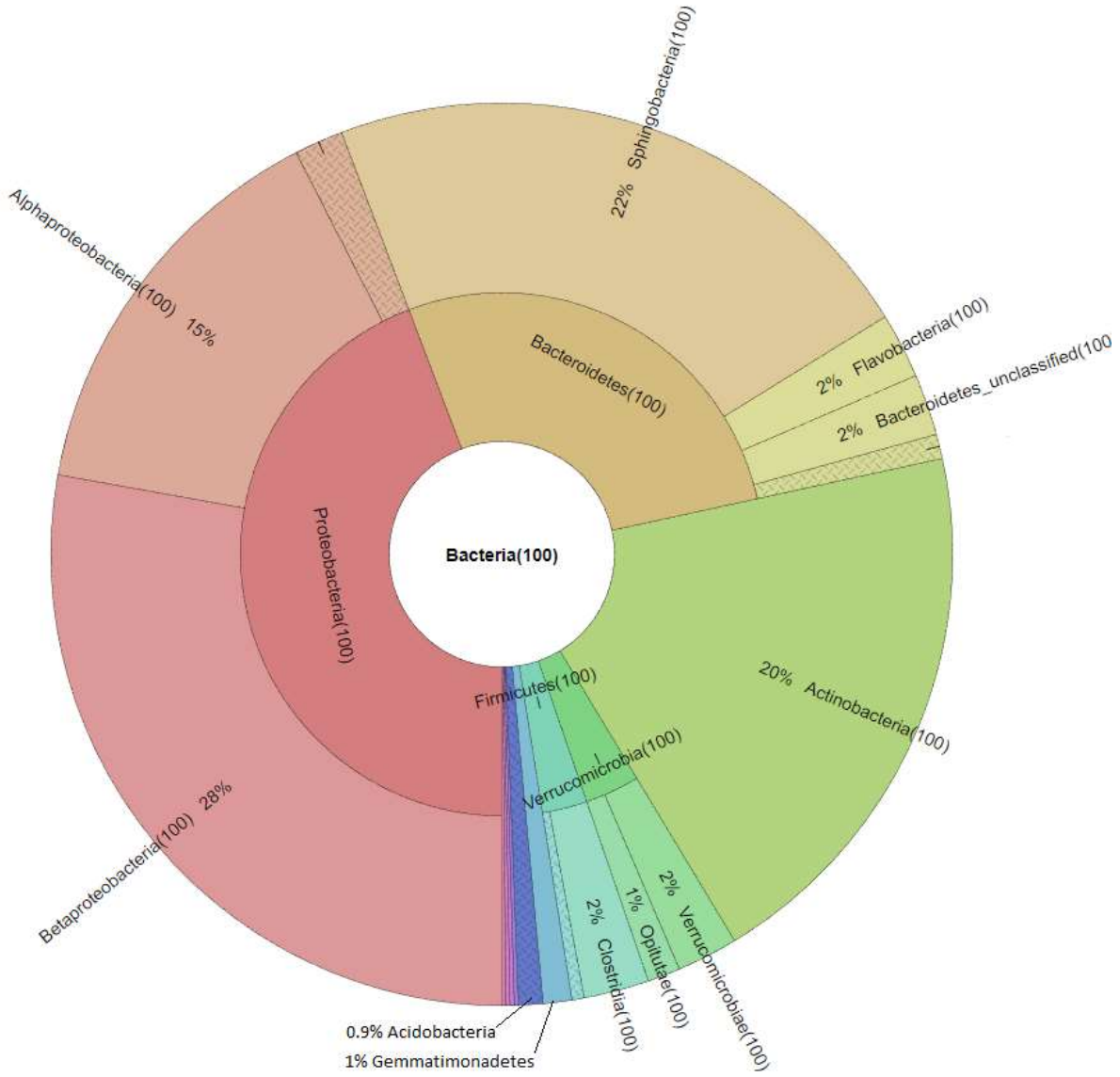

Figure 3. Lake Beyşehir Microbial Community Structure at the Class Level (Fewer than one are not shown.)

3.3. Differential Bacterial Profiling at the Family and Genus Level

Figure 4 demonstrates the top 52 bacterial families characterized in the sample. The majority of the families found are typical freshwater environment inhabitants. Nevertheless, several families were unique to a particular sample. Several genera included pathogenic species. However, Lake Beyşehir showed a more significant concentration of families that contained these species, including Alcaligenaceae, Burkholderiaceae, and Enterobacteriaceae. Sequences related to Micromonospora were found in Lake Beyşehir. In Lake Beyşehir, the sequences associated with the genus *Mycobacterium* were discovered. The dominant bacteria in the bacterial community of the Lake Beyşehir sample were also identified as belonging to bacterial species that use organic substances for sustenance. These genera included *Methylophilus* and *Sphingomonas*. (Figure 5)

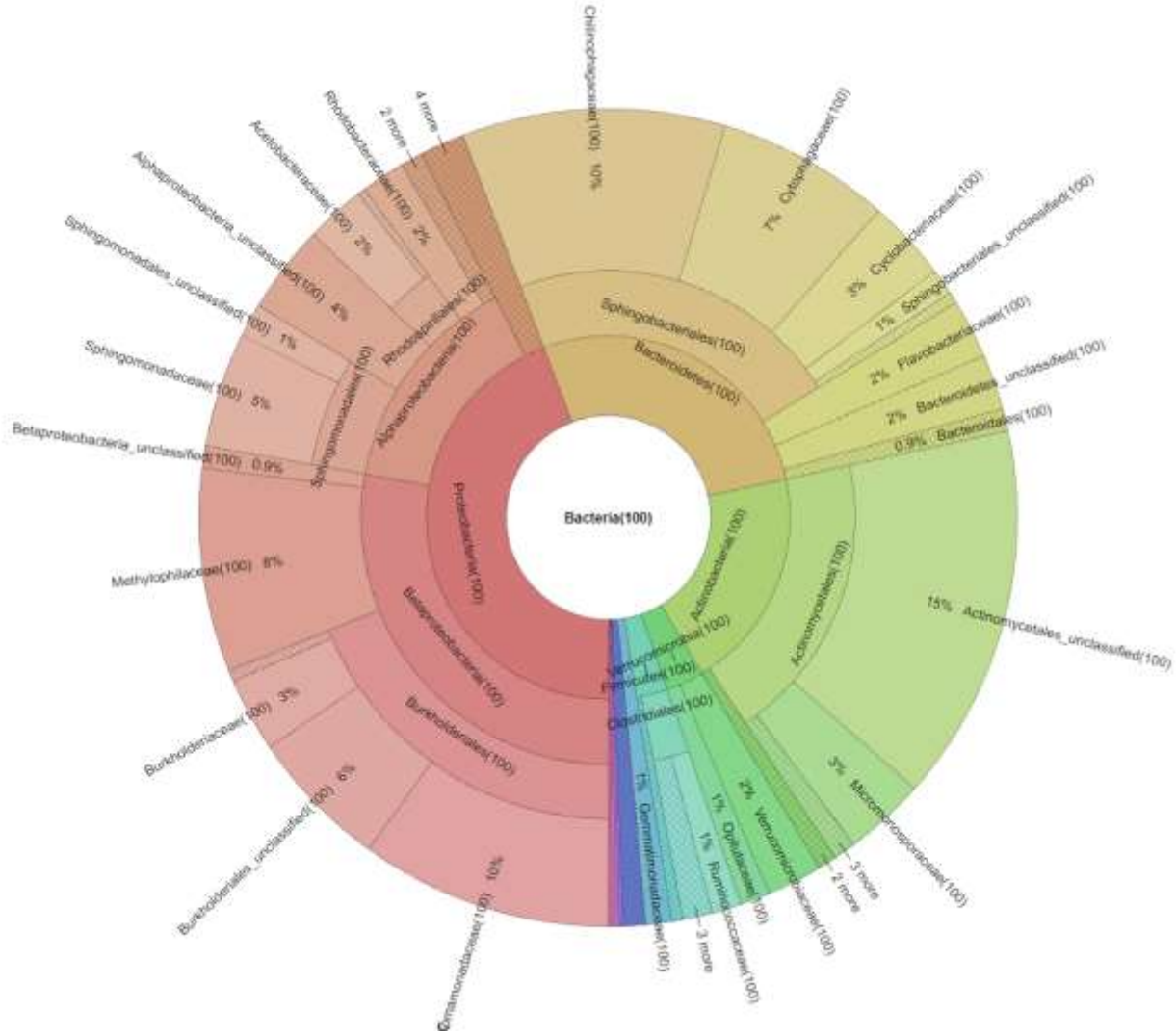

Figure 4. Lake Beyşehir Microbial Community Structure at the Family Level (Those fewer than one are not shown.)

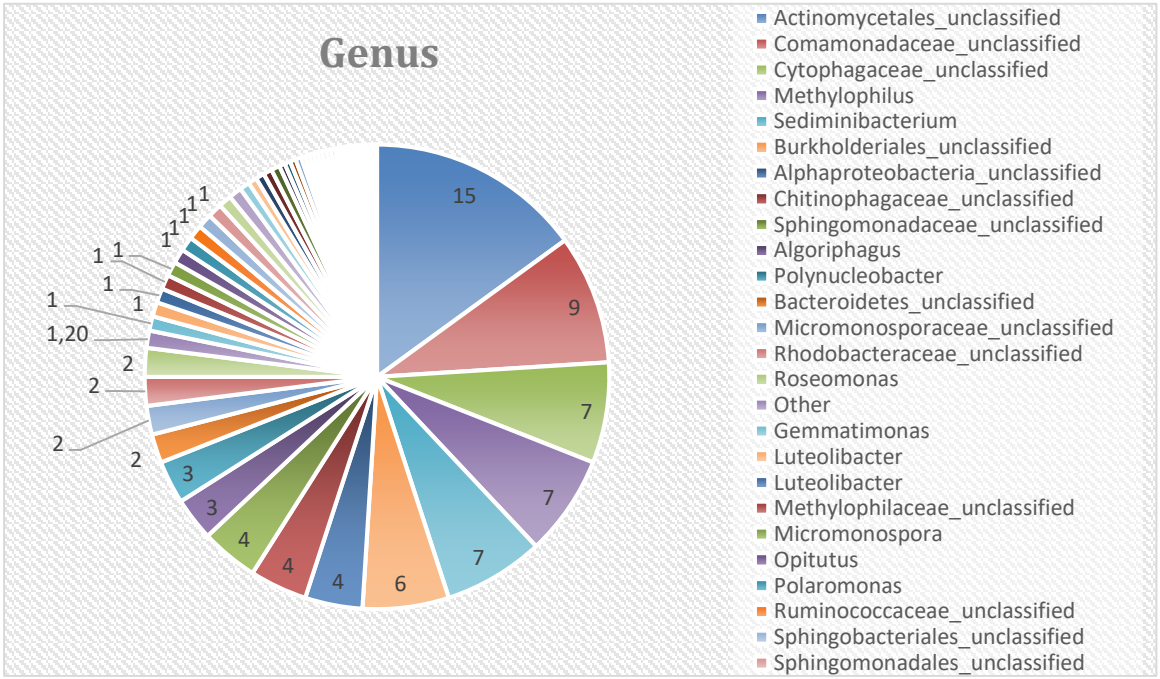

Figure 5. Lake Beyşehir Microbial Community Structure at the Genus Level (Those fewer than one are not shown.)

There have only been a few phylogenetic studies focused on Lake Beyşehir which is a freshwater lake. There has been no previous study on the prokaryotic diversity of Lake Beyşehir. Studies on Lake Beyşehir have been limited to the fish species in the lake. The most prevalent phylum in Lake Beyşehir was Proteobacteria. The dominant phyla after Proteobacteria in Lake Beyşehir were Bacteroides, Actinobacteria, and Verrucomicrobia. Metagenomic data showed that these lakes differed from those in Pakistan, Bulgaria, and North and Central America (Iliev et al., 2017; Oh et al., 2011; Carvalho et al., 2013). The microbial difference between Lake Beyşehir with freshwater lakes in other countries has been observed. Examining the difference with other freshwater lakes in Turkey will be useful. The metagenomic study by Saleem et al. (2019) revealed the microbial differences between Keenjhar, Rawal, and Saif-ul-Muluk, three freshwater lakes in Pakistan. According to the 2019 study, microbial differences between Lake Beyşehir and other clean water lakes in Turkey can be studied (Saleem et al., 2019). In the study by Toyama et al. (2016) in Amazon basin lakes, the results were as follows: Proteobacteria (36.1% to 59.5%), Actinobacteria (12.7% to 28.2%), Planctomycetes (0.5% to 3.6%), Bacteroidetes (1.9% to 1.9%) 3.5%), and Firmicutes. (2.3 to 5.5%) This shows that there is a difference in Planctomycetes (0.1%) when compared with the results in our study. In addition, Bacteroidetes was found to be between 1.9% and 3.5% in the study conducted in lakes in the Amazon region, while it was found to be 27% in our studies in Lake Beyşehir.

In Kayani et al. (2018), a metagenomic analysis of basal ice from an Alaskan glacier, DNA and RNA samples were taken from the basal ice of the Matanuska Glacier, and microbial diversity and metabolism analyzes were performed using metagenomic sequencing methods. In the study, Nitrospiraceae and Gallionellaceae families were found to have the highest rate according to 16S rRNA sequence data. This shows that there is a difference according to the results of our study.

Lake Beyşehir requires more detailed information and microbial diversity studies. Extremely important for biotechnology, microorganisms are a part of the food chain, depend upon dead organic materials and inorganic materials as energy and carbon sources, and contribute to the carbon cycle (Fenchel & Jorgensen, 1977). For these reasons, we used next-generation sequencing to learn more about the composition and diversity of prokaryotic communities in Lake Beyşehir.

4. Conclusion

This is the first report on the bacterial diversity of Lake Beyşehir, and the results showed the presence of phyla Proteobacteria, Verrucomicrobia, Bacteroidetes, and Actinobacteria. The study concludes that there should be further detailed studies on the prokaryotic diversity of Lake Beyşehir; this study provides new insights into the microbial ecology of the lake and serves as a reference for future research. It is a multidisciplinary resource for studies that can be done in the future to ensure the continuation of the lineage (species) of Beyşehir endemics and other fish species, especially the species known as Redwing (*Chondrostoma beysehirense*) and Beyşehir siraz (*Capoeta mauricii*), which are in danger of extinction in Lake Beyşehir, the largest freshwater lake in Anatolia. In our study, a genus was detected that is extremely important in terms of biotechnology, such as *Lactobacillus*. Our study shows that Lake Beyşehir is in a position to contribute biotechnologically to the people of the region, in addition to drinking and agricultural irrigation needs.

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Author Contributions

Fahri Pat: Designed the study; collected water samples and designed the analysis studies, writing-editing.

Sultan Fidan Pedük: Conducting experiments, generating data, performing formal analysis, analyzing the results.

Neşe Akçay: Conducting experiments, generating data, performing analysis.

Hatice Kübra Kızıl Pat: Conducted field studies, experiments, data generation, analysis the results.

Ercan Arıcan: Analysis the results, writing-review and editing.

Conflicts of Interest

The authors declare no conflict of interest.

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