Assessment of microbial community diversity in lakes of İğneada floodplain forest by metabarcoding approach

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ABSTRACT

This paper aims to contribute to the understanding of bacterial community patterns of the lakes of İğneada Floodplain Forest by metabarcoding approach. Within this scope, surface water samples were collected from three lakes located in the area namely Mert Lake, Hamam Lake, and Saka Lake, and the bacterial diversity was assessed by a high throughput sequencing of the 16S rRNA gene. Chao1 richness and Shannon diversity were higher in Saka Lake indicated a more diverse bacterial community. Proteobacteria was by far the most abundant phyla in all lakes. Although Bacteroidetes and Actinobacteria also dominated the community, their abundances differed in each lake. While the family Burkholderiaceae represented 25% of the bacterial community in Saka Lake, the abundances were 9% and 4% in Hamam Lake and Mert Lake, respectively. This study is one of the first investigations specifically focused on the bacterial communities in three lakes of İğneada Floodplain by next-generation sequencing platform and gave a prescreening of the bacterial diversity. Further studies are required to determine the biotechnological potential of this unique habitat.

Keywords: Amplicon Sequencing, Bacterial community, İğneada floodplain forest

Introduction

Floodplain forests, longos, are unique habitats mostly covered with freshwater seasonally or permanently and consisted of sand resulted from various streams. These ecosystems are like rainforests and have particular structures and functions (Arekhi et al., 2019). In the Thrace region in Turkey, İğneada has seasonally flooded forests which have critical importance at national and international levels (Arekhi et al., 2019) due to their high water purification potentials and diverse communities (Tecimen & Kavgaci, 2010). The area received a National Park status in 2007. Whereas, in the southern part of the National Park, there are Saka Lake, Deniz Lake, Hamam Lake, Pedina Lake, Mert Lake, the northern part of the National Park consists of Erikli Lake surrounding longos forests (URL 1). These unique habitats can be a major area of interest for biotechnological applications holding the potential to be a source for specific microbial communities, novel enzymes, and biologically important biomolecules.

Bacterial communities are at the center of ecosystem well-being, contribute to the biogeochemical cycles by breaking up organic matter, fixing nutrients, regulating carbon flux etc. (Diao et al., 2017; Ozbayram et al., 2021; Zhang et al., 2020). The community structures alter from one location to another due to a wide variety of factors such as the distinctive environmental conditions, land usage, human activities etc. (Ung et al., 2019). Besides their crucial roles in the ecosystem, only a minor portion of the microbial communities (<10%) can be determined by culture-dependent techniques which limit the revealing functions of the particular environments (Steen et al., 2019). Thus, using culture-independent tools is important to assess the community structures providing valuable insights into metabolic functions and genetic diversity (Riesenfeld et al., 2004; Lloyd et al., 2018). The emerged platforms using next-generation sequencing technology are powerful tools for revealing complex diverse community compositions along with advanced metagenomic databases and powerful bioinformatics and statistical tools (Michán et al., 2021). A considerable amount of literature has been published on bacterial diversity of different freshwater habitats using various next-generation sequencing platforms such as in eutrophic lakes (Ozbayram, et al., 2021; Zhang et al., 2020), flood-pulse tropical lake (Ung et al., 2019), alkaline lake (Kambura et al., 2016), alpine lakes (Llorens-Marès et al., 2020).

Although some researches on these lakes carried out for examining the biodiversity in the lakes of İğneada Floodplain (Altinsaçli, 2001; Güher, 1999, 2003), the bacterial diversity in the lakes has not been investigated. Recent developments in next-generation technology and lower analysis costs have facilitated to uncover the diversity of these ecosystems. Thus, the aim of this prospective study was to explore the bacterial community diversity of these lakes and reveal the unique community structures.

Material and Methods

Sample Collection & Physical Measurements

Sampling was performed in October 2020. One sampling point was determined in each lake and the samples were collected from the surface water of the shores (Figure 1). The water temperature, pH, and dissolved oxygen were measured by the multi-parameter analyzer (Hach Lange, Germany). The measurements were done in triplicates and the average results were presented in the study.
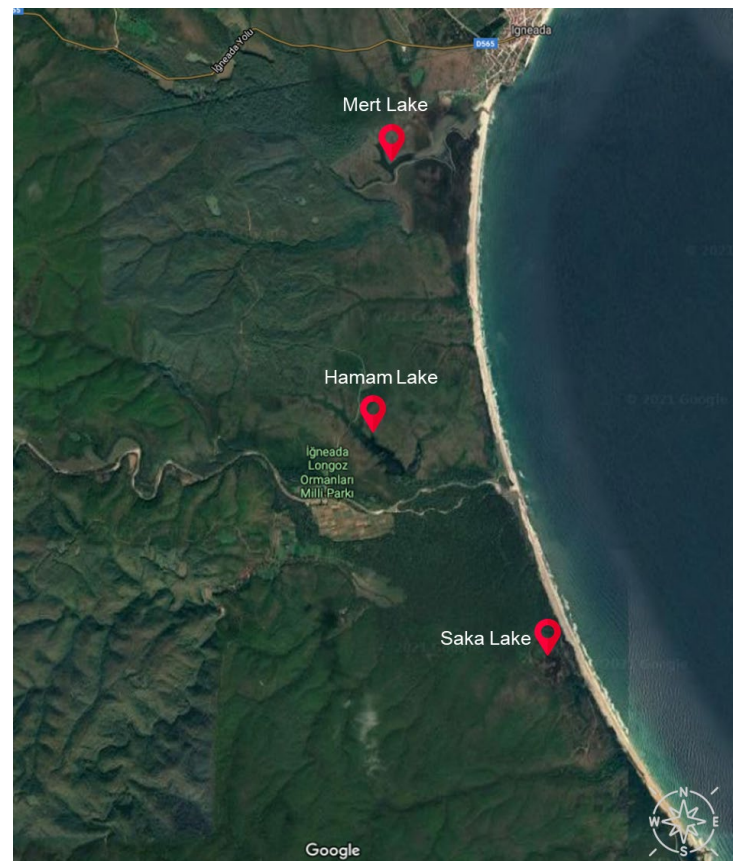

Figure 1. Study area and sampling points

DNA Extraction and Amplicon Sequencing

To concentrate the samples, waters were filtered from a 0.22 µm filter at the sampling site, and the filters were stored under cold conditions during their transfer to the lab. The DNAs were isolated from the filter paper using the NucleoSpin® Soil Kit (Macherey-Nagel, Germany) following the manufacturer's instructions and quantification was done using NanoDrop 1000 (Thermo Fisher Scientific, Inc., DE, USA).

The bacterial community diversity was assessed by 16S rRNA gene-targeted sequencing using Illumina® MiSeq™ platform. Amplicon sequencing library was prepared using bacteria-specific primers targeting the V3-V4 region of the 16S rRNA gene (341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3'). Prepared libraries were purified, quantified, and further sequenced on the MiSeq instrument (Illumina, USA) using 300 bp paired-end chemistry. CASAVA data analysis software was used for demultiplexing and clipping of sequence adapters from raw sequences (Illumina, USA). The fragments with any mismatches to the barcodes or primers were excluded. Data analysis was performed through Quantitative Insight Into Microbial Ecology (QIIME2) v2020.2 (Bolyen et al., 2019). PCR primers were removed from sequences using cutadapt plugin (Martin, 2011). Paired-end reads were joined (vsearch join-pairs) and quality filtered (quality-filter q-score-joined). Then, sequences were denoised using deblur (deblur denoise-other) (Amir et al., 2017). Taxonomy was assigned to each amplicon sequence variant (ASV) using 'feature-classifier classify-sklearn' plugin against the pre-trained Naive Bayes classifier (classifier_silva_132_99_16S_V3.V4_341F_805R.qza) (Comeau, Douglas, & Langille, 2017). The final ASV table was used to calculate alpha diversity metrics. The bacterial diversity was visualized by Krona interactive metagenomic visualization (Ondov et al., 2011).

Results and Discussion

The physical characteristics of the lakes in the sampling period are presented in Table 1. While Mert Lake showed an alkaline characteristic, the lowest pH was determined for Hamam Lake. Water temperature was in the range of 20.1-24.0°C. The dissolved oxygen level in Mert Lake was quite high, in which the lake was covered by aquatic plants in the sampling period. The high oxygen level most probably resulted from the high photosynthesis rate of these aquatic plants. On the other hand, the dissolved oxygen concentrations were measured as 5.75 mg/L and 6.35 mg/L in Hamam Lake and Saka Lake, respectively. The results are in accord with the previous study obtained by (Güher, 2003) confirming the high pH and oxygen saturation in Mert Lake and similar profile for Hamam Lake. On the other hand, (Altinsaçli,

2001) reported different measurements for Saka Lake in the autumn (pH: 7.13; dissolved oxygen: 8.9 mg/L; water temperature: 16°C). Since the water temperature measured in this study was higher than that of (Altinsaçli, 2001), this contradiction may be due to the changing environmental conditions in the lake since 2001.

Table 1. Physical characteristics of the Lakes

Parameter	Mert Lake	Hamam Lake	Saka Lake
pH	9.10	7.30	7.72
Water temperature (°C)	24.0	23.2	20.1
Dissolved oxygen (mg/L)	13.75	5.75	6.35

The bacterial community pattern of Mert Lake is shown in Figure 2. The most dominant phyla were Proteobacteria, Bacteroidetes, and Actinobacteria, they represented 73% of the total reads. The highest abundance of the Cyanobacteria was detected in Mert Lake among the samples (11%). Together with Epsilonbacteraeota, Verrucomicrobia, and Patescibacteria represented 10% of the total sequences. In the community, Bacteroidia and Gammaproteobacteria were the most abundant classes. At the family level, 13% of the total reads were assigned to Nitriliruptoraceae, followed by Flavobacteriaceae (9%). These were the highest levels determined among the samples.

The results in Figure 3 show the bacterial community in Hamam Lake. The major share of the total reads was assigned to Proteobacteria species as in the Mert Lake. Actinobacteria was detected as the second dominant phyla representing 20% of the bacterial community. 7% of the total reads comprised of Bacteroidetes members followed by, Firmicutes (6%) and Verrrucomicrobia (5%). Proteobacteria classes Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria were the most abundant ones. On the other hand, Sporichthyaceae (11%) was by far the most abundant family followed by Burkholderiaceae (9%).

The bacterial community composition of Saka Lake is depicted in Figure 4. Proteobacteria was accounted as the most abundant phylum representing 60% of the bacterial community which was also the highest among samples. The majority of the Proteobacteria reads were assigned to Betaproteobacteria, especially the family Burkholderiaceae (25%). Whereas Firmicutes was the second dominant phylum (12%), Bacteroidetes comprised 11% of the total reads. Together with Bacteroidia, Clostridia species represented 20% of the sequences at the class level. Different from the other two lakes, 4% of the sequences were assigned to Rumminococcaceae species.

Figure 2. Bacterial community composition in Mert Lake

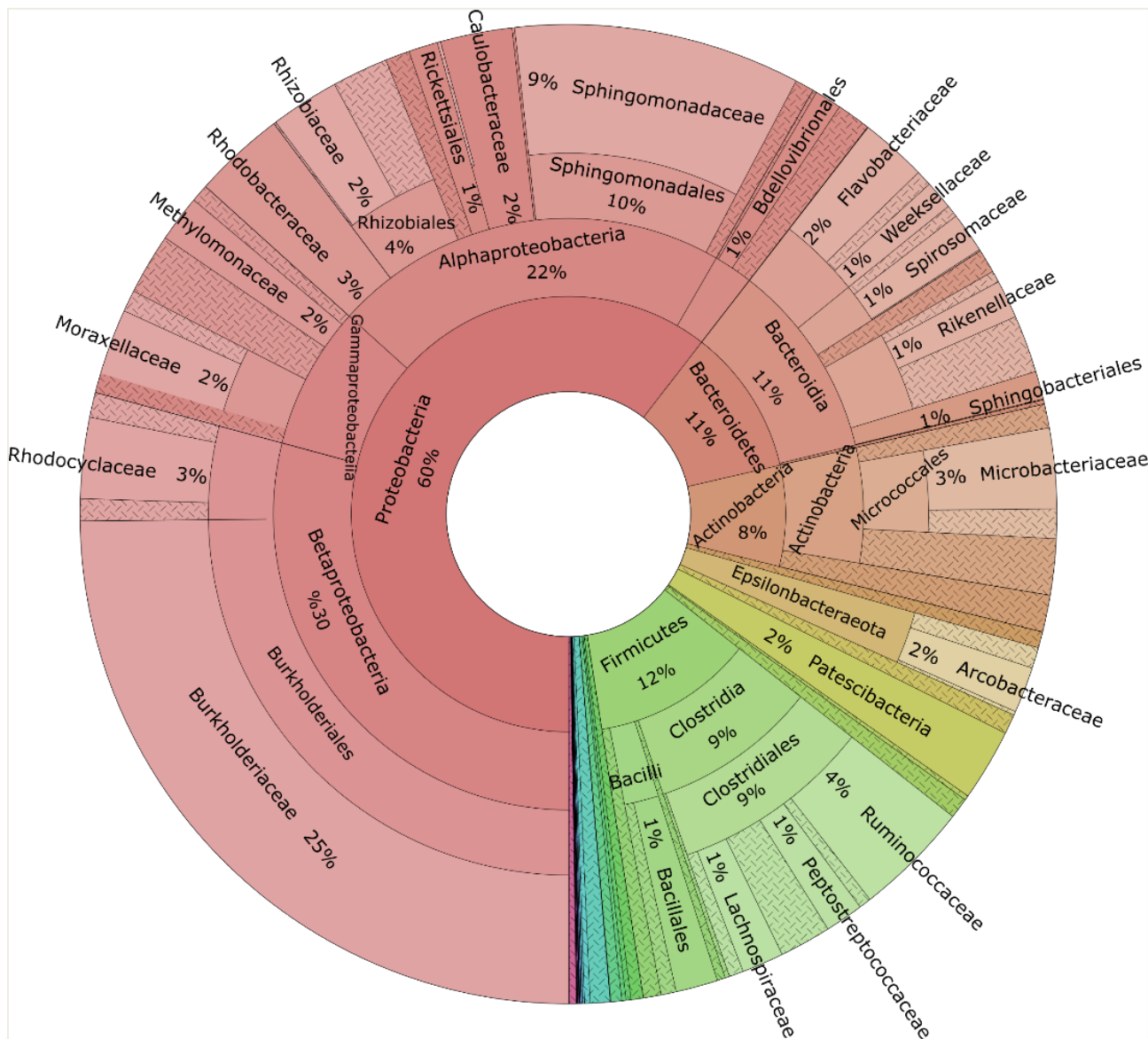**Figure 3.** Bacterial community composition in Hamam Lake

Figure 4. Bacterial community composition in Saka Lake

Table 2 provides an overview of the alpha diversity indices of the samples. Whereas the Chao index was used to reveal the richness of the bacterial community, the Shannon index was used for comparison of diversity. Although Saka Lake was represented with the least number of phyla, it has the highest number of OTUs. Moreover, Shannon and Chao1 indices pointed more diverse bacterial community and bacterial richness in Saka Lake. Among all three samples, Mert Lake had the lowest diversity and species richness.

Table 2. Alpha diversity indices of the samples

Sample	No. of OTUs	Shannon	Chao1
Mert Lake	915	8.2	918
Hamam Lake	1854	9.2	1863
Saka Lake	2309	9.7	2330

The taxonomic analysis revealed that Proteobacteria species were dominated the bacterial communities in the three lakes as well as Actinobacteria members. These results are in keeping with previous observational study, which show these two phyla are the most abundant ones in the freshwater ecosystems (Nakatsu et al., 2019). Betaproteobacteria, one of the well-studied taxonomic classes in freshwater habitats (Newton et al., 2011), was detected as the predominant class in Saka Lake. It was also abundant in Hamam Lake. Some members of this class are enriched at circumneutral to alkaline pH conditions and live in the surface part of the water bodies. This may explain their abundance on those lakes (Newton et al., 2011). Most of the Betaproteobacteria reads were assigned to the family Burkholderiaceae. This family

comprises diverse organisms from aerobic and facultative anaerobic chemoorganotrophs to obligate and facultative chemolithotrophs (Coenye, 2014), occupying a diverse ecological niche. On the other hand, most of the sequences could not be accounted for any genera and the abundance of the assigned genus was lower than 1% of the total reads which was not representative. Whereas Flavobacteriales species were found abundant in Mert Lake, the abundance was lower in Hamam Lake and Saka Lake. The members of this order inhabit the freshwater where the phytoplankton blooms take place frequently (Newton et al., 2011). They can break up wide variety of macromolecules and carbohydrates as well as toxins produced during the phytoplankton proliferation (Ozbayram, et al., 2020). On the other hand, Actinobacteria was one of the dominant phyla in the bacterial communities in all lakes. However, at the family level, the dominant families differed in each lake. Whereas Mert Lake dominated by the heterotroph, alkaliphilic, Nitriliruptoraceae species which can use organic nitriles (Sorokin et al., 2009), facultative anaerobic Sporichthyaceae was abundant in Hamam Lake and Microbacteriaceae which includes mesophilic or psychrophilic bacteria (obligately aerobic/facultatively anaerobic) (Evtushenko & Takeuchi, 2006) was found in Saka Lake. The highest abundance of Firmicutes was determined in Saka Lake. Whereas most of the Firmicutes reads were assigned to Clostridia species, Bacilli was also detected. Firmicutes members are quite diverse and have a role in a wide variety of ecosystem functions. Moreover, they have various mechanisms to survive in different environments such as the formation of endospores. The endospore-forming Firmicutes members can be enriched in the environments where a single/multiple stressors occurred for other bacterial taxa (Filippidou et al., 2016). Thus, to better understand the reason for the abundance of Firmicutes members, periodic monitoring should be performed on this lake.

It is known that bacterial communities serve a function in aquatic ecosystems and have interaction between other components of the food web (Kiersztyn et al., 2019). However, to investigate these interactions a comprehensive study should be carried out considering the vertical diversity and other organisms in the foodwebs. Besides this multifarious relationship, the diversities are dependent on various factors including geographical settings and hydrological characteristics (Zwirglmaier et al., 2015) which give them specific microbial structures. Since the size and depth of these lakes are different, giving them particular characteristics, careful attention should be paid to the comparison of community diversity between these different aquatic environments.

The use of bacteria in the biotechnological field has attracted particular attention from the scholarly community for a long period and they have a wide range of application area such as wastewater treatment, green energy production, decomposition of hardly degradable substances, rehabilitation of contaminated sites etc. (Yong et al., 2021). Besides their direct application in the processes, the novel enzymes and bioactive compounds they produced have also great importance in the industry and commercial products. To contribute the technological innovations, the search for new species and novel metabolites are needed be investigated in the undiscovered areas.

Conclusion

In this study, pre-screening of the bacterial communities İğneada Floodplain Forest lakes, Mert Lake, Hamam Lake, and Saka Lake was completed by 16S rRNA targeted amplicon sequencing. Although the current study is based on a small number of samples, the findings suggest an overview of the bacterial diversity in these three lakes.

The results of this research will serve as a base for future studies. Further research should be undertaken to investigate the hidden biotechnological potential of these unique ecosystems.

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