

A Preliminary Metabarcoding Study of Prokaryotes in Gökçeada Salt Lake Lagoon, Turkey

Sibel Kucukyildirim Celik¹ 

¹Hacettepe University, Faculty of Science, Department of Biology, Ankara, Turkey

ORCID IDs of the authors: S.K.C. 0000-0003-2241-3060

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ABSTRACT

Objective: Microorganisms play an important role in all ecosystem processes and a growing body of molecular and ecological evidence has shown that microbial biodiversity is much more diverse and complex than previously anticipated. This study aimed to determine the prokaryotic microorganisms present in Gökçeada Salt Lake Lagoon.

Materials and Methods: A metabarcoding approach was used to determine the microbial diversity in Gökçeada Salt Lake Lagoon.

Results: 16S rDNA targeted sequencing revealed 5 Archaea and 31 Bacteria species and Archaea represented 63.2%. The most frequent Archaea genus was *Halorubrum*, which belongs to the Euryarchaeota phylum, and the dominant species of Bacteria was *Halomonas sulfidaeris* (Proteobacteria phylum).

Conclusion: This work will contribute to our understanding of the microbial community structure and composition in coastal lagoons. However, further surveys will improve our knowledge on microbiota in Gökçeada Salt Lake Lagoon.

Keywords: Biodiversity, halophile, 16S rDNA, metabarcoding

INTRODUCTION

Microorganisms are the most abundant taxonomically and metabolically diverse organisms on Earth. It is well established that microbiota plays an important role in ecosystem stability and sustainability (1). However, biodiversity-based research has mainly focused on plants and animals, giving little attention to microorganisms. In environmental samples, the identification of microorganisms at the species level by conventional techniques is costly, time-consuming, and also continues to be resolved. Recently, many studies have been conducted to provide information about the diversity and distribution patterns of microorganisms, however, it is remarkable that there is still much unknown.

Application of DNA sequence-based methods has led to significant progress in molecular taxonomy and systematics over the last twenty years, revealing re-

markably large diversity even in environments that are relatively well studied. For taxonomic purposes, sequencing of the specific regions (i.e. variable regions of 16S, 5S, or 23S rDNA genes) of isolates has resulted in the development of extensive public DNA sequence databases. Moreover, high-throughput sequencing technologies provide an opportunity to generate large amounts of data in a relatively short time. One of the most important advantages of this method is the ability to identify large numbers of species from environmental samples with different characteristics (2,3).

Until recently, microbial biodiversity studies mainly conducted on extreme environments (such as high or low temperature, high or low pH) were yet little explored and particularly unique. In this work, the sampling area was a coastal lagoon located in the southeast of Gökçeada Island (Turkey), called Gökçeada Salt Lake Lagoon. The maximum depth of the lagoon is 2 meters,



Corresponding Author: Sibel Kucukyildirim Celik

E-mail: sibelkucukyildirim@gmail.com

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and its total area is approximately 2 km². Coastal lagoons like Gökçeada Salt Lake Lagoon are considered wetlands and have a special hydrological structure formed by a transition zone between fresh and saltwater. Since each wetland ecosystem has its own physicochemical properties, and community structure should be evaluated separately (4).

This study aimed to determine the prokaryotic community structure in Gökçeada Salt Lake Lagoon using a culture-independent approach that includes metabarcoding based on amplicon sequencing. Metabarcoding is mainly used to characterize species-level diversity in the environmental samples and is considered a method that has the potential to identify rare taxa (5). In addition to the identification of microorganisms at the species-level, information about abundance, distributions, and biological functions of these microorganisms can be obtained by the metabarcoding approach. The high sensitivity and specificity of the method provide an advantage, especially in organisms that cannot be cultured in vitro (6-8). Because previous work is only based on conventional methods (4,9), the prokaryotic diversity in Gökçeada Salt Lake Lagoon is likely limited to only identifiable species. Also, another motivation for this study is that no study focusing on prokaryotic microorganisms in Gökçeada Salt Lake Lagoon has previously been included in the literature. In short, the metabarcoding approach used in this study allows us to determine prokaryotes without prior cultivation and provide a deeper analysis of the prokaryotic diversity in Gökçeada Salt Lake Lagoon.

MATERIALS AND METHODS

Sampling and Physicochemical Analysis

To determine the prokaryotic diversity of Gökçeada Salt Lake Lagoon, three water samples (1 Liter of each) were sampled aseptically in September 2019 (40° 7' 47.2" N 25° 56' 52.1" E) (Figure 1). All samples were stored at 4°C and immediately transported to the laboratory. The analysis of physicochemical parameters (total salinity, major anion and cation concentrations

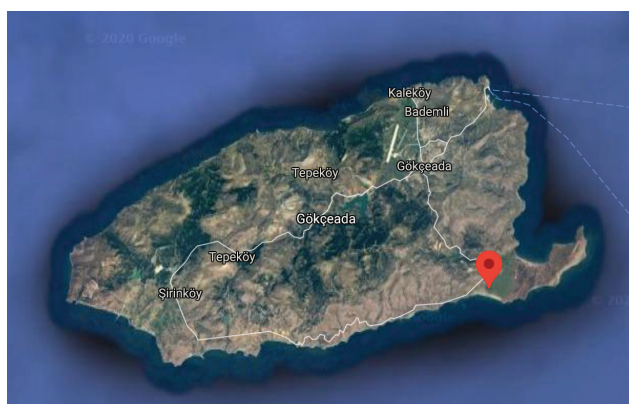


Figure 1. Gökçeada Salt Lake Lagoon in Turkey. The sampling area was shown with a red circle on the map (Satellite imagery: Google/Google Maps (n.d.)).

(pH and Na⁺, K⁺, Ca²⁺, Mg²⁺, Cl⁻, CO₃²⁻, SO₄²⁻ and NO₃⁻) in the samples were performed by Hacettepe University Water Chemistry Laboratory.

DNA Isolation and Sequencing

For DNA isolation, water samples (500 ml each) were filtered through a vacuum filter (0.22 µm filter membrane). Then, filter membranes were cut into small pieces and DNA was isolated using ZymoBIOMICS® DNA Miniprep Kit (Zymo Research, Irvine, CA) according to the manufacturer's instructions. Isolated DNA samples were analyzed on a 1% agarose gel, and DNA quantity was evaluated using Nanodrop 2000 UV-Vis spectrophotometer (Thermo Fisher, USA). Quantified DNA samples were combined based on equal molarity and were stored at -20 °C before analysis.

The sample was processed with the BM Labosis (Turkey) Sequencing Service: Targeted Metabarcoding by using the specific primers that targeting the prokaryotic 16S rDNA gene, (515F (5'-GTGACGCMGCCGCGTAA-3') and 806R (5'-GGACTACNVTGGTWTCTAAT-3')) (10-12). The sequencing protocol of the Earth Microbiome Project was used and adapted to the Illumina MiSeq instrument (11). After the amplicon library was prepared, the product was quantified with qPCR fluorescence reading. The library was cleaned using Select-a-Size DNA Clean & Concentrator™ (Zymo Research, Irvine, CA). Then, qualitative and quantitative measurement of the library was performed with TapeStation® and Qubit®. The sample was sequenced using the Illumina MiSeq instrument following the manufacturer's recommended protocol.

Data Analysis

Raw data were processed and read quality was controlled by FastQC and QIIME2. DADA2 was used to obtain specific amplicon sequences and chimeric sequences were excluded from the analysis using the same program (13). QIIME2 was used to cluster DNA sequence data with more than 97% similarity as operational taxonomic units (OTUs) (14,15) and chimeric OTUs were extracted from the dataset using USEARCH algorithm (16). Taxonomic assignments of OTUs were performed using the QIIME2 pipeline (15) and SILVA database using a 70% confidence level cutoff for assignment (17). Raw sequencing data were recorded in the NCBI database (PRJNA517326). Figure 2 was prepared using Krona (18).

RESULTS AND DISCUSSION

Physicochemical analyses

During sampling, temperature and pH were measured as 20.9°C and 6.98, respectively. The physicochemical properties of the samples are shown in Table 1. Sodium (Na⁺) and Chloride (Cl⁻) ions were found to have the highest concentration in the samples. According to the chemical composition of the Lagoon, it can be expected that halophilic organisms dominate in this environment.

After 16S rDNA sequencing, 57,847 high-quality paired-end reads were obtained. The number of identified prokaryotic

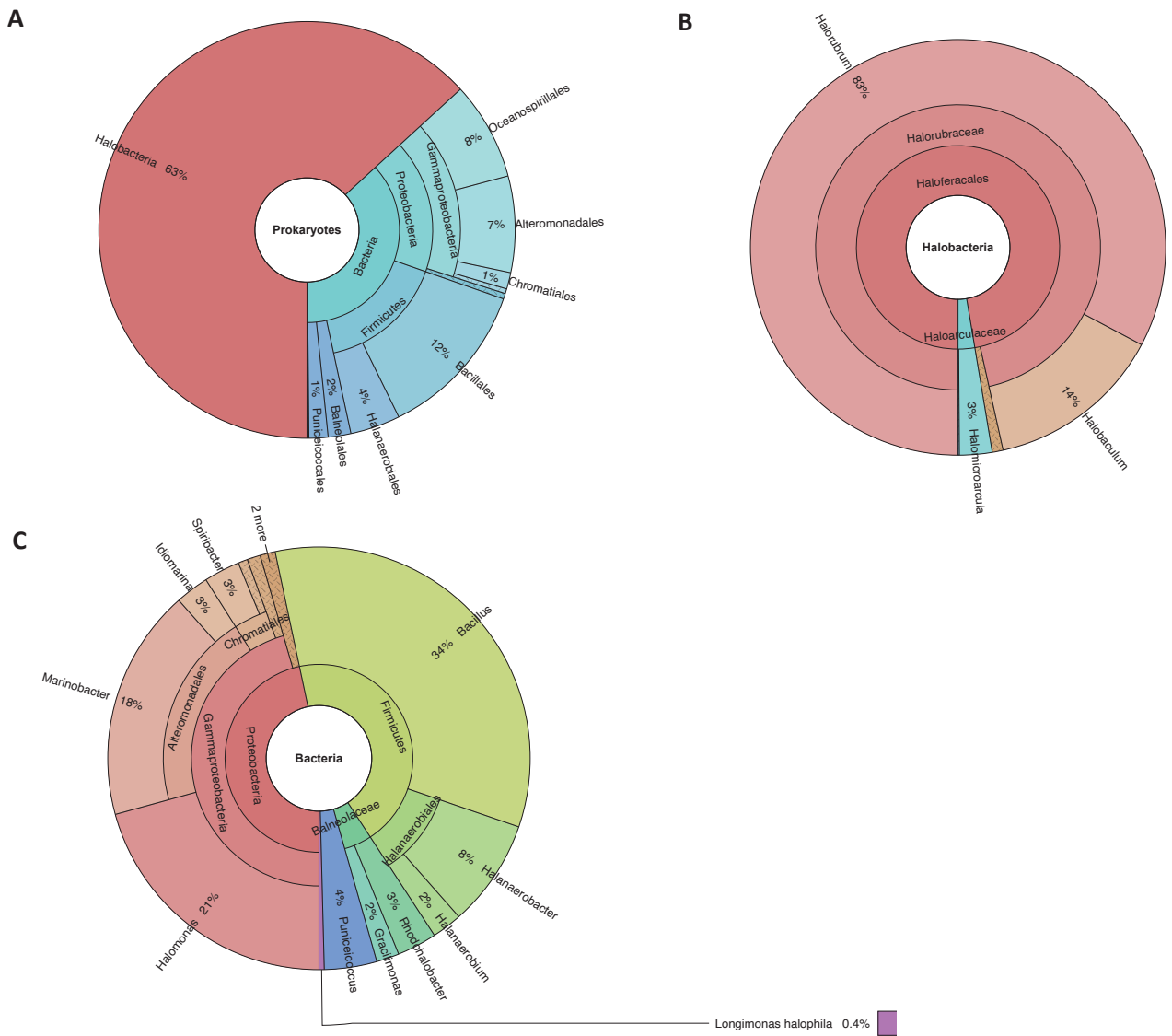


Figure 2. Relative distributions of prokaryotes (A), Archaea (B) and Bacteria (C) as obtained from 16S rDNA metabarcoding data.

Table 1. Physicochemical properties of the samples collected from Gökçeada Salt Lake Lagoon (ion concentrations in g/L).

	Temperature (°C)	pH	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺	HCO ₃ ⁻	Cl ⁻
Average	20.9	6.98	111.63	3.47	2.27	16.07	0.24	225.12

species in the sample was found to be 36 by comparison of OTUs with the SILVA database (Table 2). Despite 63.2% of the sample consisting of Archaea members, only 5 archaeal species were determined (Figure 2A). The most abundant genus of Archaea was *Halorubrum* (82.7%), and the others were *Halobaculum* (13.7%), *Halomicroarcula* (2.5%), *Halobellum* (0.8%), and *Halosimplex* (0.1%) (Figure 2B). Metagenomic

and metabarcoding approaches were previously used to investigate microbial compositions of different hypersaline environments (6, 19, 20), and the Archaea was previously described as the dominant group in these environments (21,22). As observed in this study, *Halorubrum* was previously reported among dominant archaeal genera in salt lakes and salterns (23,24).

Table 2. Species detected in this study via metabarcoding approach

Organism			No. of reads	%
Prokaryotes	Archaea	<i>Halorubrum sp.</i>	3922	52.30
Prokaryotes	Archaea	<i>Halobaculum sp.</i>	652	8.69
Prokaryotes	Bacteria	<i>Halomonas sulfidaeris</i>	462	6.16
Prokaryotes	Bacteria	<i>Bacillus persicus</i>	420	5.60
Prokaryotes	Bacteria	<i>Bacillus litoralis</i>	350	4.67
Prokaryotes	Bacteria	<i>Marinobacter aquaticus</i>	261	3.48
Prokaryotes	Bacteria	<i>Marinobacter flavimaris</i>	168	2.24
Prokaryotes	Archaea	<i>Halomicroarcula sp.</i>	120	1.60
Prokaryotes	Bacteria	<i>Puniceicoccus vermicola</i>	111	1.48
Prokaryotes	Bacteria	<i>Halanaerobacter lacunarum</i>	100	1.33
Prokaryotes	Bacteria	<i>Halomonas fontilapidosi</i>	91	1.21
Prokaryotes	Bacteria	<i>Rhodohalobacter halophilus</i>	84	1.12
Prokaryotes	Bacteria	<i>Halanaerobacter salinarius</i>	78	1.04
Prokaryotes	Bacteria	<i>Spiribacter aquaticus</i>	76	1.01
Prokaryotes	Bacteria	<i>Idiomarina atlantica</i>	71	0.95
Prokaryotes	Bacteria	<i>Bacillus pseudofirmus</i>	49	0.65
Prokaryotes	Bacteria	<i>Halanaerobacter jeredensis</i>	49	0.65
Prokaryotes	Bacteria	<i>Halanaerobium praevalens</i>	47	0.63
Prokaryotes	Bacteria	<i>Marinobacter persicus</i>	47	0.63
Prokaryotes	Bacteria	<i>Bacillus mesophilus</i>	41	0.55
Prokaryotes	Archaea	<i>Halobellus sp.</i>	40	0.53
Prokaryotes	Bacteria	<i>Bacillus thioparans</i>	37	0.49
Prokaryotes	Bacteria	<i>Gracilimonas halophila</i>	34	0.45
Prokaryotes	Bacteria	<i>Bacillus hemicellulosilyticus</i>	30	0.40
Prokaryotes	Bacteria	<i>Hydrogenovibrio halophilus</i>	28	0.37
Prokaryotes	Bacteria	<i>Guyarkeria hydrothermalis</i>	20	0.27
Prokaryotes	Bacteria	<i>Arcobacter group</i>	18	0.24
Prokaryotes	Bacteria	<i>Halanaerobium saccharolyticum</i>	17	0.23
Prokaryotes	Bacteria	<i>Gracilimonas tropica</i>	14	0.19
Prokaryotes	Bacteria	<i>Marinobacter salinus</i>	13	0.17
Prokaryotes	Bacteria	unknown	11	0.15
Prokaryotes	Bacteria	<i>Halomonas glaciei</i>	11	0.15
Prokaryotes	Bacteria	<i>Ruegeria intermedia</i>	8	0.11
Prokaryotes	Bacteria	<i>Halomonas zhaodongensis</i>	8	0.11
Prokaryotes	Bacteria	<i>Desulfohalobium sp.</i>	6	0.08
Prokaryotes	Archaea	<i>Halosimplex sp.</i>	5	0.07
Total			7499	

A total of 31 bacterial species were identified (Table 2). Proteobacteria were the dominant bacterial phylum (47% of bacteria) and followed by Firmicutes (44% of bacteria). *Bacillus* (33.6%), *Halomonas* (20.7%), *Marinobacter* (17.7%), *Halanaerobacter* (8.2%) were the most common and most abundant bacterial genera in the sample analyzed (Figure 2C). Considering the chemical composition of the Lagoon, the presence of halophilic bacterial genera such as *Salinibacter* could be expected (25). However, our analysis did not find any OTU related to *Salinibacter*, which may be a result of the abundance of some haloarchaeal species (e.g. *Halorubrum*), which can inhibit *Salinibacter* growth as previously suggested by Anton et al. (26).

In conclusion, in this study, the prokaryotic diversity of Gökçeada Salt Lake Lagoon was evaluated, and Euryarchaeota, Proteobacteria, and Firmicutes members were found to be essential components of its prokaryotic community. This study allows us to improve our knowledge of the prokaryotic community structure in the sampling area. But a more comprehensive microbiome analysis can be obtained with future metabarcoding/metagenomic analyses by focusing on both prokaryotic and eukaryotic microbes. In addition, although OTUs that cannot be associated with any taxa are relatively few in this study, OTUs still have the potential to contribute to the identification of new organisms. In future studies, it is planned to collect samples from different depths and locations of the lake and elaborate the analyses to enlighten complete microbial diversity and microbial community structure.

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