

# Comparing Microbial Communities in Mucilage and Surrounding Seawater: Metagenomic Insights into Mucilage Formation in the Marmara Sea

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## Research Article

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# Abstract

In 2021, the rapid emergence of mucilage in the Marmara Sea raised concerns about its environmental impact. This study compares mucilage and seawater samples from 16 stations across the Marmara Sea using shotgun metagenomic sequencing and bioinformatics. Sampling was conducted from April to September 2021 across varying oceanographic conditions, including different temperatures and salinity levels, to identify changes in the microbial community. Results showed that 58% of the reads in mucilage samples could not be assigned to any taxon, indicating significant microbial "dark matter." Clean seawater was characterized by a higher presence of Euryarchaeota, Proteobacteria, and Rhodothermaeota, while Chlamydiae and Fusobacteria were dominant in mucilage. Pre-mucilage seawater samples showed microbial compositions similar to mucilage, suggesting a link between microbiome imbalance and mucilage formation. These findings provide a comparative metagenomic analysis highlighting the need for further studies to understand mucilage's environmental impact and develop early warning strategies.

## 1. Introduction

Marine environments exhibit a wide variety of naturally occurring phenomena that influence ecosystem dynamics. Two notable phenomena often conflated are marine snow and mucilage. Marine snow consists of small, sinking aggregates of organic and inorganic material, including dead phytoplankton, fecal pellets, and detritus. These aggregates, ranging from millimeters to several centimeters in size, serve as a primary food source for deep-sea organisms (Alldredge & Silver, 1988). In contrast, mucilage refers to a thicker, gelatinous layer that can form surface accumulations or spread through the water column. It consists of a combination of organic matter, polysaccharides, and various microbial communities (Danovaro et al., 2009). The two phenomena differ not only in size and structure but also in their ecological roles and impact on the marine environment. While both involve organic material, mucilage often leads to ecological disruptions and environmental concerns, particularly in the Marmara Sea.

Although mucilage formation has occurred periodically in the Marmara Sea since the 1990s, the 2021 outbreak reached dimensions that may threaten human health (Luglie et al., 2008; Tufekci et al., 2010; Aslan et al., 2021; Karadurmus & Sari, 2022; Kavzoglu & Goral, 2022). Understanding the underlying mechanisms is a crucial first step in eliminating mucilage. An examination of existing reports on the microbial load of mucilage reveals that metagenomic-scale studies are quite limited. Thus, the precise microbial content of mucilage, the taxonomic and functional diversity of the mucilage microbiome, and the ecological associations that drive its development and progression remain unanswered research questions.

The formation of mucilage is a multifactorial process influenced by natural and anthropogenic factors. Rising sea temperatures due to climate change can alter the stability of the water column and increase stratification, fostering conditions favorable for mucilage development (Danovaro et al., 2009). Increased

urbanization and industrialization have led to higher levels of nutrient runoff into coastal waters, promoting eutrophication and subsequent algal blooms that contribute to the mucilage matrix (Nikolaidis et al., 2006). Overfishing disrupts marine ecosystems and may shift the balance toward species that produce mucilage (MacKenzie et al., 2002).

The concept of microbial imbalance, in this context, refers to significant shifts in the composition of microbial communities that favor mucilage-forming organisms. This imbalance often involves an increase in polysaccharide-producing bacteria and a decrease in species that contribute to the degradation of organic matter, creating conditions suitable for mucilage proliferation (Bongiorni et al., 2007; Zoppini et al. 2005). Understanding these shifts is critical to developing comprehensive disposal strategies, which encompass physical removal, identification, and cultivation of bacteriophages targeting mucilage-forming bacteria, and the use of specific microbial consortia to degrade mucilage (Wright et al., 2021).

High-throughput sequencing has become indispensable for understanding the diversity and ecology of microbial communities within mucilage. Metagenomic sequencing identifies previously unrecognized microbial species and functional genes involved in mucilage formation, offering insights into their interactions and ecological roles (Giovanni et al., 2005). Mapping the microbiome at the metagenome scale allows researchers to identify the presence of unknown taxa ("dark matter") and examine the interactions between mucilage-forming organisms and their environment (Frias-Lopez et al., 2008). These insights help develop early warning strategies and intervention methods to mitigate the impact of mucilage on marine ecosystems.

In this study, we aim to compare the microbial communities in mucilage and surrounding seawater from the Marmara Sea to understand the processes contributing to mucilage formation and the potential impacts on the marine environment. By conducting metagenomic-scale comparative analyses, we aim to uncover the taxonomic and functional diversity of mucilage-forming organisms and explore strategies for effective identification and disposal.

## **2. Experimental Procedures**

### **2.1 Fieldwork**

Mucilage samples collected from 11 different stations (MS1-MS11) in the Marmara Sea between April and September 2021 were included in the current study. Approximately 15 mL of mucilage sample was taken in sterile falcon tubes containing DNA/RNA shield buffer (Zymo Research) from ten stations, and one lyophilized mucilage sample (20 g) was obtained for one station.

At the onset of mucilage formations in April 2021, two pre-mucilage seawater samples (Msu-Y1, Msu-Y2) were collected from areas where mucilage had not reached. To represent mucilage-free seawater (referred to hereafter as clean seawater), surface seawater samples were taken from three different stations in May 2022, while mucilage was absent both on the surface and throughout the water column.

The additional stations included Saros Bay (SAR), the Çanakkale Strait (CB), and the Special Environmental Protection Area located within the borders of Gökçeada (GEB). Water samples from all five locations were collected using a sterile Nansen water sampler with a 5-liter capacity and were filtered in the field using a vacuum pump through a 0.22µM filter (Steripak-GP20 filter, Millipore, Bedford, MA) (Frias-Lopez et al., 2008). Subsequently, the filters were placed into 50 ml Falcon tubes filled with DNA/RNA shield buffer (Zymo Research). The coordinates for all 16 stations were recorded using GPS and the coordinates are provided in Supplement 1.

## 2.2 Wet laboratory processes

Total DNA isolation was conducted using the Qiagen PowerSoil DNA isolation kit (Qiagen, Germany) following the manufacturer's protocols. For mucilage samples, approximately 0.2 g of sample was collected with sterile tweezers in a BSL2 level biosafety cabinet and transferred to GarnetBead tubes provided by the Qiagen PowerSoil DNA isolation kit. For water samples, after the filter papers were cut into small pieces with a sterile scalpel in the BSL2 cabinet, they were placed into GarnetBead tubes. These tubes were then subjected to treatment at 65°C and 95°C for 10 minutes, followed by DNA isolation according to the kit's procedure. The quality of the isolated DNAs was assessed using a NanoDrop (Shimadzu BioSpec-nano), and the quantification of DNA samples in terms of double-stranded DNA (dsDNA) amounts was determined using the Qubit fluorometric system (Qubit 2.0 Fluorometer, Life Technologies) with the Qubit dsDNA-BR assay kit.

Shotgun metagenome sequencing was carried out on a total of 10 mucilage and 5 water samples using Illumina platforms, following the guidelines of the Nextera XT DNA Library Preparation Kit. Library construction involved enzymatic fragmentation of DNA, followed by adapter ligation, amplification, and quality control. Subsequently, the sequencing libraries were loaded onto the Illumina Next Seq 550 system for high-throughput sequencing, generating comprehensive metagenomic datasets for downstream bioinformatics analysis.

## 2.3 Dry laboratory processes

### 2.3.1 Processing of raw shotgun metagenomics data

In order to conduct *de novo* analysis, firstly the raw data of the samples were pooled and collectively subjected to metagenome assembly. After removing the adapters and low-quality sequences with Trimmomatic v0.39 (Bolger et al., 2014), IDBA-UD assembler (Peng et al., 2012) was used for this process and contigs were obtained. In order to determine the relative abundances of the genomic contigs, the total cluster of contigs was indexed with BWA-mem2 (Vasimuddin et al., 2019) tool, and the raw reads were mapped onto the contigs, and the average reads per fragment were determined at the sequence level per million. In order to annotate the genes in the contigs; First, open reading frames were detected by running Prodigal gene detection algorithm (Hyatt et al., 2010) in metagenome mode, then each detected frame was annotated at the gene ontology level with eggNOG-mapper (Huerta-Cepas et

al., 2017) and converted to an ontology, as well as functional groups. Unmapped open reading frames were neglected on the assumption that they are not coding genes.

## 2.3.2 Taxonomic analysis

In order to determine the relative abundance in the contigs obtained in the previous step, Kaiju (Menzel et al., 2016) (with nr\_euk database and default parameters) and Kraken2 (Wood et al., 2019) (with nt Database and default parameters) taxonomic classification programs were used. The assignment of Kaiju was considered to be the preferred classifications in cases that both methods disagreed. The relative abundance of an assigned taxon was estimated by mapping the sequencing reads onto the associated contigs. Normalization using reads per million base pairs concluded the final relative abundance.

## 2.3.3 Comparative Functional analysis

For functional analysis, genes predicted on the contigs by Prodigal were aligned against eggNOG database using Diamond (Buchfink et al., 2015). All eggNOG annotated genes were evaluated for relative abundance for each sample and enriched at the pathway level. As a result of these enrichments, KEGG (Kyoto Encyclopedia of Genes and Genomes) ontologies, CAZy (Carbohydrate-Active enZymes Database) (Cantarel et al., 2009) and protein families classification (PFAM) (Bateman et al., 2004) functional group annotations and their relative abundances were organized using previously estimated contig relative abundances. In order to detect biosynthetic gene clusters, the biosynthetic units in the samples were determined using BiG-MEx (Biosynthetic Gene cluster METagenomic eXploration) modules a tool for mining biosynthetic gene clusters' domains and classes) metabolic cluster mapping tool (Andreu et al, 2021).

The comparisons were primarily conducted with alpha and beta-diversity analyzes on both taxonomic and functional profiles. Bray-Curtis (Bray and Curtis, 1957) dissimilarity were used as beta-diversity index and Shannon Index (Wang et al., 2015) were used as alpha-diversity measurement. In order to examine the biodiversity differences between mucilage and clean seawater samples, alpha-diversity measurements were subjected to Mann-Whitney U test and beta-diversity values were subjected to one-way ANOVA test. Mann-Whitney U test and multiple hypothesis test were applied for the relative abundance values of each taxon and functional structure detected in the samples. The differences at taxonomic or functional biodiversity level between the clean seawater and mucilage samples were multiply-tested for statistical significance using Mann-Whitney U test. In order to visually observe the genotypic characteristics among the geographic distributions as well as clean seawater-mucilage difference, the relative abundance vectors were ordinated in 2D using Principal Component Analysis (PCA) performed by Python SciPy library.

## 3. Results

Due to insufficient quality and quantity of isolated dsDNA, next-generation sequencing couldn't be conducted for the lyophilized mucilage sample (MS1). Consequently, shotgun metagenome sequencing

was carried out for 15 samples, generating a total of 58.4 Gbp of read data. Detailed sequencing data statistics for all samples are provided in Table 1.

Table 1  
Statistics of sequencing data

Sample	Reads	Contigs	N50*	Longest Contig	Average Contig Length	Total Contig Length	N80**
MS2	21.984.724	240.447	799	88.951	697	167.812.811	426
MS3	63.881.640	650.720	1257	442.235	940	612.212.260	537
MS4	22.691.910	28.712	377	12.301	364	10.470.825	320
MS5	21.714.280	249.062	823	251.373	744	185.410.840	441
MS6	23.347.442	244.907	982	107.527	823	201.558.903	479
MS7	22.656.394	223.788	940	215.273	787	176.135.579	465
MS8	78.719.956	795.630	1169	373.301	889	707.541.004	510
MS9	21.926.708	203.865	1411	233.250	969	197.667.564	528
MS10	21.991.972	302.937	670	123.372	661	200.402.569	408
MS11	24.276.176	287.548	754	73.798	685	197.096.919	422
Msu_Y1	27.062.660	278.911	687	6.1121	646	180.243.434	405
Msu_Y2	24.864.772	314.331	583	78.131	582	183.115.265	384
GEB	11.592.104	132.904	1838	219.676	1.108	147.321.065	614
SAR	8.759.208	110.834	1005	66.910	812	90.087.134	497
CB	2.040.918	37.702	953	41.650	835	31.507.945	506
<b>* When all Contig lengths are listed, the contig length corresponding to the 50th percentile</b>							
<b>** When all Contig lengths are listed, the contig length corresponding to the 80th percentile</b>							

Based on taxonomic assignment, the study found that on average,  $58.2 \pm 9.6\%$  of reads from mucilage samples,  $68.9 \pm 9.6\%$  from pre-mucilage water samples, and  $35.4 \pm 2.4\%$  from clean seawater samples couldn't be attributed to any taxon. The taxonomic assignments for the assigned reads at the species and phylum levels are provided in Supplement 2 and Supplement 3. It was observed that the predominant bacteria in the samples originated from the environment, with only MS4 harboring several human pathogens.

n terms of alpha diversity, clean seawater samples exhibited a more diverse range of microbial communities compared to mucilages, as illustrated in Fig. 1. Statistical tests conducted for taxonomic

assignments revealed significant differences in beta diversity between mucilage and clean seawater (with p-values of 0.000024 at the species level and 0.00164 at the phylum level).

The analysis revealed that mucilage and the clean seawater samples exhibited distinct groupings, whereas the pre-mucilage water samples (Msu-Y1 and Msu-Y2) clustered closely with the mucilage group, as demonstrated in Fig. 2.

Table 2 displays the most frequently assigned phyla for both clean seawater and mucilage samples.

Table 2  
The relative abundance of the most common phyla found in both mucilage and clean seawater samples is expressed as a percentage.

Phylum	Mucilage	Clean seawater
Proteobacteria	20.8 ± 9.6	47.7 ± 4.1
Bacteroidetes	13.5 ± 8.8	6.9 ± 1.7
Verrucomicrobia	1.7 ± 1.5	2.9 ± 1.01
Planctomycetes	1.2 ± 1	0.33 ± 0.2
Cyanobacteria	0.5 ± 0.4	1.1 ± 0.3
Rhodothermaeota	0.015 ± 0.012	0.4 ± 0.2

The statistical tests conducted to determine if there were significant differences at the taxonomic level between the samples revealed statistically significant variations ( $p < 0.05$ ) for numerous taxa. However, after error correction (FDR - false discovery rate correction), no statistically significant results were found between groups at the species level. At the phylum level, following the FDR correction, it was found that the phyla observed in higher abundance in clean seawater samples were Euryarchaeota ( $p = 0.014$ ), Proteobacteria ( $p = 0.019$ ), and Rhodothermaeota ( $p = 0.034$ ). On the other hand, within the mucilage group, Chlamydiae ( $p = 0.014$ ) and Fusobacteria ( $p = 0.034$ ) were reported to be in excess. Additionally, the readings that couldn't be assigned to any phyla (Unclassified) were found to be significantly higher ( $p = 0.015$ ) in the mucilage group compared to clean seawater samples.

## 3.2 Comparative Functional analysis

When comparing the clean seawater and mucilage samples, no statistically significant protein families and KEGG ontologies were detected after the p-value correction. However, significant differences were observed in the presence and amounts of carbohydrate processing enzyme classes (CAZy) between clean seawater and mucilage samples. The results revealed that certain enzyme classes were highly present in the mucilage samples, whereas these classes were almost absent in mucilage sample MS4, where human pathogens were dominant.

Two enzyme families (GH3 and GH13,  $p = 0.014$ ) belonging to the glycoside hydrolase (Glycoside Hydrolase, GH) family, three enzyme families (GT8, GT19 and GT28,  $p = 0.015$ ) belonging to the GlycosylTransferase Family (GlycosylTransferase Family, GT) and one family (CE10,  $p = 0.015$ ) from the carbohydrate esterase family (Carbohydrate Esterase Family, CE) were found to be significantly greater in clean seawater than mucilage samples. One enzyme family (GH31,  $p = 0.022$ ) belonging to the glycoside hydrolase family, four different enzyme families (GT3 ( $p = 0.038$ ), GT30 ( $p = 0.014$ ), GT35 ( $p = 0.016$ ) and GT81 ( $p = 0.014$ ) belonging to the GlycosylTransferase Family one of the enzyme families (CBM 48,  $p = 0.014$ ) with carbohydrate binding activity (carbohydrate-binding modulated) and one family (CE1,  $p = 0.014$ ) from the carbohydrate esterase family were found to be significantly more abundant in mucilage than clean seawater samples (Supplement 4).

The analysis conducted for BiG-Mex is presented in Fig. 3. Among the biosynthetic classes consisting of 25 different gene clusters, terpenes were found to be significantly higher ( $p = 0.03$ ) in clean seawater. In domains consisting of 93 different gene pathways, cyanobactin ( $p = 0.006$ ), siderophore ( $p = 0.007$ ), nrps ( $p = 0.007$ ), bacteriocin ( $p = 0.011$ ), hserlactone ( $p = 0.017$ ), t3pks ( $p = 0.017$ ), phosphonate ( $p = 0.021$ ), ectoine ( $p = 0.025$ ), and t1pks ( $p = 0.025$ ) were detected significantly more in mucilage. Conversely, sactipeptide was more prevalent in clean seawater ( $p = 0.025$ ).

## 4. Discussion

Mucilage formation, which involves the buildup of a gelatinous substance on the water's surface and throughout the water column, has been documented for more than 200 years across various marine environments (Fonda et al., 1989; Schilling & Zessner, 2011). Despite this long history, there have been relatively few studies exploring the microbial content of mucilage (Vojvoda et al., 2014; Kiliyas et al., 2014; Rouaud et al., 2019). The current study aimed to investigate the microbial composition of mucilage and compare it to surrounding marine water.

Taxonomic analysis of the sequencing data revealed that the majority of reads from mucilage and pre-mucilage water samples could not be assigned to any taxonomic group. Despite employing a wide range of sequencing data from public and private databases, over half of the reads from mucilage (58%) and pre-mucilage water (69%) remained unclassified, known as "dark matter." The high proportion of "dark matter," highlights both the presence of novel microbial species and potential technical limitations in the current taxonomic databases. These unidentified reads may represent previously unknown taxa or unique metabolic pathways that haven't been characterized. This significant amount of unexplored genomic content aligns with earlier research on similar marine formations (Frias-Lopez et al., 2008). This scarcity of identifiable taxa likely reflects the lack of datasets and the limited number of metagenomic studies available, as well as the low prevalence of culture-based studies on mucilage samples. In a mucilage sample (MS3) no assignment has been made for a contig over 145k bp and it strengthens the existence of unidentified or new microbial taxa in mucilage microbiome. Further characterization of this genomic "dark matter" is crucial, as these novel organisms could have unique ecological roles and metabolic functions that contribute significantly to mucilage formation and persistence.

Alpha diversity analysis showed that clean seawater samples had a wider range of microbial communities than mucilage. This discrepancy is likely due to the high proportion of "dark matter." A previous research has confirmed that the microbial species in mucilage often differ significantly from those in the surrounding seawater, showing that mucilage aggregates contain exclusive microbial diversity, including pathogenic species absent in surrounding seawater (Danovaro et al., 2009). Similarly, in another study it was demonstrated that mucilage communities are enriched with specific microorganisms not present in surrounding seawater, highlighting the unique microbial habitat that mucilage provides (Rouaud et al., 2019). This study also found a statistically significant difference in beta diversity between mucilage and clean seawater, consistent with these earlier findings. Interestingly, pre-mucilage water samples exhibited microbial diversity more similar to mucilage than to clean seawater, highlighting a potential ecological transition. This transition could serve as an early warning indicator of impending mucilage formation and aid in predicting and managing future outbreaks.

Certain phyla, including Euryarchaeota, Proteobacteria, and Rhodothermaeota, are found in high levels in soils (Hollister et al., 2010; Canfora et al., 2014; Zhao et al., 2018) and hypersaline environments (Fernandez et al., 2014; Xie et al., 2017). While these phyla were less abundant in pre-mucilage water than in clean seawater, their prevalence declined further following the onset of mucilage formation. The decrease in Proteobacteria and halophilic bacteria in mucilage samples may reflect the loss of microbial diversity in response to deteriorating marine hemostasis.

Chlamydia and Fusobacteria were found in higher abundances in mucilage samples compared to clean seawater. Chlamydia species, commonly known as intracellular parasites, have been linked to several diseases in humans and animals (Moulder J., 2005; Burnard & Polkinghorne 2016). Although it has been detected in freshwater, seawater, and wastewater (Benamri et al., 2021) their presence with high proportion in mucilage raises potential public health concerns, particularly if these pathogens proliferate and contaminate water systems. Fusobacteria are often associated with oil-contaminated sediments or anaerobic environments (Gutierrez et al., 2016), and their occurrence in mucilage suggests that similar environmental conditions may be present in this gelatinous matrix. Their ecological roles in mucilage, while not fully understood, likely include influencing its formation and persistence through microbial interactions and contributing to its ecological complexity (Kilias et al., 2014). Thus, both Chlamydia and Fusobacteria may play ecological roles in mucilage formation and progression, although their precise contributions remain unclear.

The comparative analyses between mucilage and clean seawater samples reveal important functional differences. Mucilage samples displayed a higher abundance of enzymes related to polysaccharide biosynthesis and glycosyltransferase families, indicating an increased capacity to synthesize complex carbohydrates (Voiniciuc et al, 2018; Zhang et al., 2023). The overrepresentation of carbohydrate-active enzyme families, such as glycoside hydrolases and carbohydrate-binding modules, suggests that the mucilage microbiome is adapted for the accumulation and maintenance of the mucilage matrix. This carbohydrate synthesis capacity could explain the thick, gelatinous consistency of mucilage. Clean seawater samples, on the other hand, were richer in enzymes responsible for carbohydrate degradation

and terpenoid biosynthesis, indicating a different metabolic profile (Wright et al., 2021). The differences in biosynthetic gene clusters, particularly those involved in secondary metabolism, emphasize the distinct metabolic pathways active in mucilage and suggest its growth and persistence are supported by specialized biosynthesis. Significant differences emerged between mucilage and clean seawater for carbohydrate-processing enzymes and secondary metabolite biosynthetic gene clusters. While polysaccharide accumulation is crucial in mucilage formation, terpene-producing gene clusters were less abundant in mucilage than in clean seawater. Biosynthetic pathways like siderophore biosynthesis could also contribute to the formation of unstable organic compounds, which might influence mucilage persistence (Del Negro et al., 2005).

In comparison to other regional studies, this research offers one of the most comprehensive comparative analyses between mucilage and clean seawater microbial communities in the Marmara Sea. Previous studies primarily focused on individual aspects of phytoplankton composition or specific mucilage samples without directly comparing them to surrounding seawater (MacKenzie et al., 2002). Our findings expand the understanding of the microbial diversity and functional potential of mucilage, confirming that it hosts a distinct community with unique metabolic activities. The difference in metabolic pathways has critical implications for developing early warning systems and management strategies for mucilage outbreaks in the Marmara Sea and other regions. Furthermore, the findings underscore the need for targeted intervention strategies, possibly through microbial consortia or phage therapy, to address the specific metabolic activities of mucilage-associated organisms.

## Conclusions

This study provides valuable insights into the relationship between the microbiome and mucilage formation in the Marmara Sea. The high proportion of unidentified reads ("dark matter") in mucilage and pre-mucilage water samples underscores the significant role of previously unknown or poorly understood microbial taxa in mucilage formation. The overrepresentation of enzymes involved in polysaccharide biosynthesis and glycosyltransferase families in mucilage samples suggests that the mucilage microbiome is specialized for synthesizing complex carbohydrates, contributing to the dense, gelatinous structure of mucilage. Meanwhile, the reduced abundance of known phyla like Proteobacteria and halophilic bacteria in mucilage indicates a shift in the microbial community composition that accompanies mucilage proliferation.

Early detection of microbial community shifts in pre-mucilage water samples, which appear more similar to mucilage than clean seawater, could serve as a critical warning sign for impending mucilage outbreaks. Monitoring these transitional communities and their enzyme activities may help develop proactive strategies to manage mucilage formation.

Additionally, the detection of pathogenic taxa like Chlamydia and Fusobacteria in mucilage highlights the importance of managing potential health risks associated with mucilage proliferation. Given the significant knowledge gaps revealed by the large proportion of unidentified taxa, comprehensive

research efforts are needed to better characterize the mucilage microbiome. Such efforts should include large-scale metagenomic analyses, advanced culture-based studies, and more complete microbiome datasets to uncover unknown taxa and their metabolic pathways. Developing this understanding is crucial for implementing early warning systems and devising effective management strategies to minimize the ecological and health impacts of mucilage formation.

In summary, this study highlights the need for comprehensive large-scale cultureomic and microbiome data to identify unknown taxa in mucilage and to develop early warning strategies and mitigation methods for its potential ecological impacts.

## **Declarations**

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### **Ethical Approval**

Not Relevant

### **Consent to Participate**

No human subjects were involved in this research. No data or information pertaining to humans was utilized in the study.

### **Consent to Publish**

The authors have declared their consent for publication.

### **Competing Interests**

The authors have declared that there are no competing interests.

### **Author Contributions**

Conceptualization: Aycan Gundogdu, Ozkan Ufuk Nalbantoglu, and Herdem Aslan; Investigation: Gizem Karis, İlknur Sarıkaya, Meryem Nisa Erdogan, Mehmet Hora and Herdem Aslan; Data analysis: Aycan Gundogdu, Ozkan Ufuk Nalbantoglu, Gizem Karis, İlknur Sarıkaya, and Mehmet Hora; Original draft preparation: Aycan Gundogdu; Writing-review and editing: Aycan Gundogdu, Ozkan Ufuk Nalbantoglu and Herdem Aslan.

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## Data Availability Statement

The data presented in this study are stored at the Erciyes University Genome and Stem Cell Center, Metagenomics & Bioinformatics Laboratory's data repository and can be provided upon request from the corresponding author.

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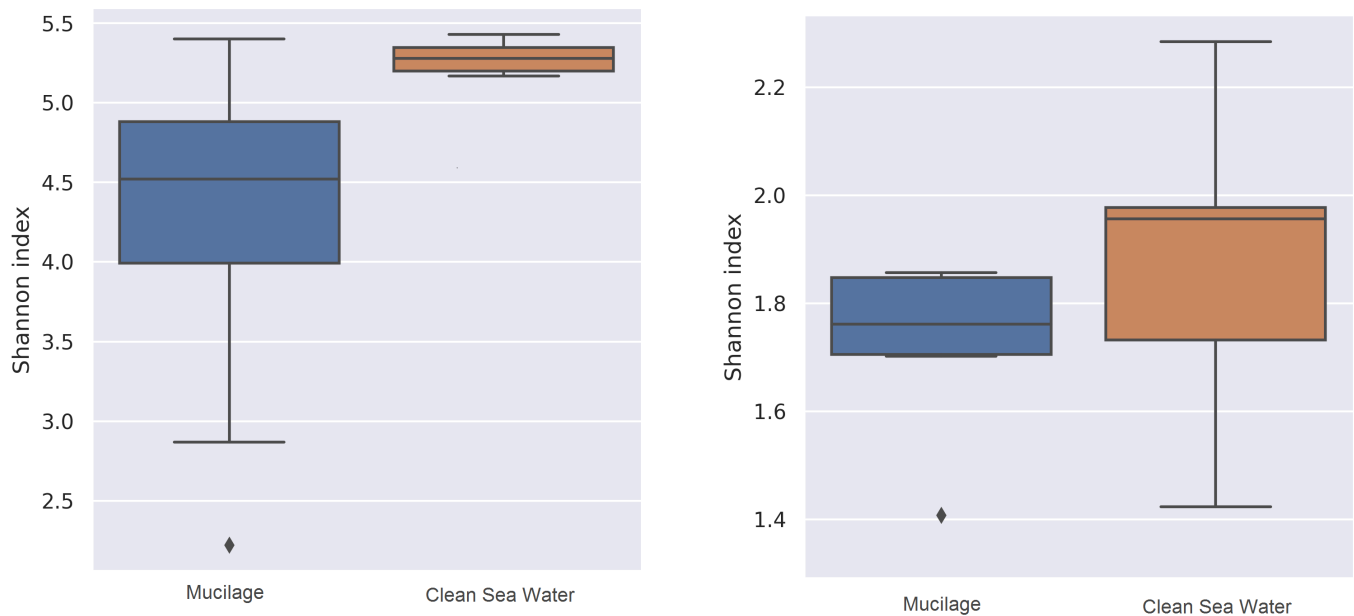
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## Figures



**Figure 1**

Comparison of alpha diversity at different taxonomic levels between the mucilage and clean water samples revealed the following: a. At the species level, there was a significant difference ( $p = 0.0188$ ). b. At the phylum level, the difference was not statistically significant ( $p = 0.17$ ).



Distribution of biosynthetic gene clusters (BiG-Mex) per sample a. Class-based distribution, b. Domain-based distribution

## Supplementary Files

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