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RESEARCH ARTICLE

Cyanobacterial Communities in Mucilage Collected from Çanakkale Strait (Dardanelles): Metagenomic Approach

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Cyanobacteria

Abstract: In this study, cyanobacterial communities in mucilage samples collected from three stations in the Dardanelles Strait were analyzed with a metagenomic approach. Mucilage samples were collected at the beginning of June 2021 from three points of the Dardanelles (Station 1: 40°6'42.78"N, 26°23'57.00"E; Station 2: 40°9'8.09"N, 26°24'16.19"E; Station 3: 40°6 '21.62"N, 26°22'41.25"E). The dominant cyanobacteria were *Prochlorococcus marinus* (39.17%), *Synechococcus* sp. (20.85%), *Lyngbya* sp. (12.00%), *Trichodesmium erythraeum* (7.33%), *Aphanocapsa* sp. (4.33%) and *Leptolyngbya* sp. (3.33%), which constituted 87.00 % of the total number of sequences. In this study, cyanobacteria species that can cause harmful algal blooms and have toxic effects on the mucilage structure have been determined. The Marmara Sea and the Dardanelles Strait, which have been affected by serious disturbances, including industrial activities, anthropogenic impacts, tourism and artificial lighting, will never be fully restored to their former ecological state. In addition, cyanobacteria species in the mucilage may cause harmful algal blooms and have toxic effects that threaten the future well-being of coastal populations and ecosystem stability. Thus, the government and local authorities should pay more attention to combating the mucilage.

Çanakkale Boğazı'ndan Toplanan Müsilajda Siyanobakteri Toplulukları: Metagenomik Yaklaşım

Anahtar kelimeler:

Müsilaj
Deniz agregaları
Deniz karı
Metagenomik
Siyanobakteriler

Öz: Bu çalışmada Çanakkale Boğazı'nda üç istasyondan toplanan müsilaj örneklerindeki siyanobakteri toplulukları metagenomik yaklaşımla analiz edilmiştir. Müsilaj örnekleri, Çanakkale Boğazı'nın üç farklı noktasından (İstasyon 1: 40°6'42.78"K, 26°23'57.00"D; İstasyon 2: 40°9'8.09"K, 26°, 24'16.19"D; İstasyon 3: 40°6 '21.62"K, 26°22'41.25"D) Haziran 2021 başında toplanmıştır. Toplam dizi sayısının %87.00'sini oluşturmuş baskın siyanobakteriler *Prochlorococcus marinus* (%39.17), *Synechococcus* sp. (%20.85), *Lyngbya* sp. (%12.00), *Trichodesmium erythraeum* (%7.33), *Aphanocapsa* sp. (%4.33) ve *Leptolyngbya* sp. (%3.33) olarak belirlenmiştir. Bu çalışma ile müsilaj yapısında zararlı alg patlamalarına neden olabilecek ve toksik etkilere sahip olabilecek siyanobakteri türleri tespit edilmiştir. Endüstriyel faaliyetler, insan kaynaklı etkiler, turizm ve yapay aydınlatma dahil olmak üzere ciddi rahatsızlıklardan etkilenen Marmara Denizi ve Çanakkale Boğazı, hiçbir zaman eski ekolojik durumuna tam olarak geri döndürülemeyecektir. Bu nedenle, devlet ve yerel yönetimler müsilajla mücadeleye daha fazla önem vermelidir.

Introduction

Cyanobacteria is a group of prokaryotic organisms, and they are essential for the water environment. Although cyanobacteria are commonly found in freshwater lakes and reservoirs, climate change and anthropogenic pressure may cause their populations to increase in marine environments (Bobrova et al., 2016; Alvarenga et al., 2017). Cyanobacteria blooming wildly in lakes and marine environments with high concentrations of phosphorus may produce cyanotoxins in concentrations that will poison or kill mainly fish and shellfish, even humans (Stewart et al., 2006; Backer et al., 2015).

The presence of different cyanobacteria genus and/or algal blooms in marine environments in Turkey and nearby geography have been reported (Uysal, 2001; Taş et al., 2006; Spatharis et al., 2012; Kalaitzidou et al., 2015; Teneva et al., 2015; Uysal, 2016; Vinogradova et al., 2017). Previously, cyanobacteria communities were observed in the form of mucilage in the Northeast Atlantic Ocean (Lampitt et al., 1993), in the Sargasso Sea (Lundgreen et al., 2019) and in the Marmara Sea (Toklu-Alicli et al., 2020) as well.

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Anabaena, Aphanoteche, Blennothrix, Blennothrix, Calothrix, Chroococcus, Dermocarpa, Gloeocapsa, Gloeocapsopsis, Gomphosphaeria, Heteroleibleinia, Leibleinia, Leptolyngbya, Lyngbya, Merismopedia, Microcoleus, Microcystis, Oscillatoria, Phormidium, Planktothrix, Pseudanabaena, Rivularia, Schizothrix, Spirocoleus, Spirulina, Symploca, Synechococcus, Trichocoleus, Coelosphaerium, Scytonematopsis, Scytonema, Pannu, Entophysalis, Xenococcus, Tapinothrix, Trichodesmium, Microchaete, Dichothrix, Isactis, Prochlorococcus and *Nostoc* members have been reported among the cyanobacteria recorded in Turkish seas (Uysal, 2000; Polat et al., 2000; Develi and Kıdeyş 2000; Uysal, 2001; Taşkın et al., 2001; Aktan and Aykulu 2003; Aysel et al., 2004; Feyzioglu et al., 2004; Okudan and Aysel 2005; Parlakay et al., 2005; Aysel et al., 2005a; Aysel et al., 2005b; Aysel et al., 2005c; Aysel et al., 2005d; Uysal, 2006; Aysel et al., 2006a; Aysel et al., 2006b; Aysel et al., 2006c; Aysel et al., 2006d; Taş et al., 2006; Eker-Develi et al., 2006; Uysal and Köksalan 2006; Bayindirli and Ulysal 2007; Karaçuha and Gönülol 2007; Aysel et al., 2008; Yildirim and Sukatar 2009; Polat and Uysal 2009; Kurt et al., 2010; Kopuz et al., 2012; Aktan and Balkıs 2014; Feyzioğlu et al., 2015; Ulcay et al., 2015; Güreşen et al., 2015; Balkis and Taş 2016; Kısa and Pabuçcu 2016; Yücel et al., 2017; Balci and Balkis 2017; Yücel et al., 2018; Güreşen et al., 2020; Kocum, E. 2020; Balkıs-Ozdelıce et al., 2021).

In this study, cyanobacterial communities in mucilage samples collected from three stations in the Dardanelles Strait were analyzed by means of a metagenomic approach.

Material and Methods

Mucilage samples were collected at the beginning of June 2021 from three points of the Dardanelles (Station 1: 40°6'42.78"N, 26°23'57.00"E; Station 2: 40°9'8.09"N, 26°24'16.19"E; Station 3: 40°6 '21.62"N, 26°22'41.25"E) (Figure 1). The temperature, pH, and dissolved oxygen of sea waters were measured using a portable meter (WTW Multi-parameter portable meter MultiLine® Multi 3620 IDS SET). Samples were collected onboard a coast guard ship using a 5L Niskin bottle from a maximum depth of 2 m according to the ISO 5667-9 method (ISO 5667-9, 1992).

Figure 1. Sampling points in the Dardanelles (Google Earth Map) (Yilmaz et al., 2021)

Mucilage samples were brought to Çanakkale Onsekiz Mart University, Faculty of Marine Sciences and Technology, Ecotoxicology Laboratory under cold chain conditions for DNA isolation. Samples were centrifuged at 10,000 × g for 10 min and separated. Accumulated fresh samples were immediately used for DNA extraction. DNA isolation from mucilage samples was performed with GenElute™ Soil DNA Isolation Kit (Sigma-Aldrich, St. Louis, MO). DNA degradation and concentration were monitored using spectrometry (OD260/280), fluorometry (Qubit® 2.0 Fluorometer), and 1% agarose gel electrophoresis.

The cyanobacterial 16S rRNA gene was amplified by PCR, using the cyanobacterial specific primers CYA359f GGGGAATYTTCCGCAATGGG and CYA781r GACTACWGGGGTATCTAATCCCWTT (Nübel et al., 1997). The first PCR reactions were performed in a triplicate 25 µL mixture containing 12.5 µL of 2X KAPA HotStart ReadyMix (Roche, Switzerland), 5 µL of each primer (1 µM), and 2.5 µL of template DNA. The PCR program was as follows: 95 °C for 3 min followed by 25 cycles of 95 °C for the 30s, 55 °C for 30 s, 72 °C for 30 s, and a 5 min extension at 72 °C and a final hold at 4 °C. The second PCR reactions were performed in a triplicate 50 µL mixture containing 25 µL of KAPA HiFi HotStart ReadyMix, 5 µL Nextera XT1, 5 µL Nextera XT2, 5 µL of cleaned PCR product and 10 µl PCR Grade water. The second PCR program was as follows: 95°C for 3 min followed by 8 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 30 s, and a 5 min extension at 72°C and a final hold at 4°C.

The sequencing (2 × 250 bp) was performed on the MiSeq platform. The processing and quality control was conducted using DADA2 (Callahan et al., 2016). Chimaera check was conducted with DADA2. Amplicons with a quality of ≥Q20 were retained, and amplicons were filtered and trimmed with DADA2. Taxonomic assignment was performed against the SILVA 138 ribosomal RNA gene database (Quast et al., 2013) with a confidence threshold of 70%.

Results

The seawater temperature, pH and dissolved oxygen at the sampling stations are shown in Table 1. The temperature, pH and dissolved oxygen measurements were similar in all stations. The mean temperature, pH and dissolved oxygen values in the surface layers of the three stations were recorded as 21.87±0.65 ºC, 8.17±0.10, and 8.52±0.24 mg/L, respectively.

The dominant cyanobacteria communities at the order level were Synechococcales and Oscillatoriales (Figure 2). Synechococcales levels were recorded as 68%, 67%, and 68% for Station 1, Station 2, and Station 3, respectively. Oscillatoriales levels were recorded as 22%, 17%, and 19% for Station 1, Station 2, and Station 3, respectively.

The dominant cyanobacteria were *Prochlorococcus marinus* (39.17%), *Synechococcus* sp. (20.85%), *Lyngbya* sp. (12.00%), *Trichodesmium erythraeum* (7.33%), *Aphanocapsa* sp. (4.33%) and *Leptolyngbya* sp. (3.33%) which constituted 87.01 % of the total number of sequences (Figure 3). *Prochlorococcus marinus* and *Synechococcus* sp. levels varied between 38%-41% and 19%-23% for the stations, respectively. *Lyngbya* sp., and *Trichodesmium erythraeum* levels varied between 11%-14% and 6%-8% for the stations, respectively. *Aphanocapsa* sp. and *Leptolyngbya* sp. levels varied between 4%-5% and 3%-4% for the stations, respectively.

Table 1. Seawater temperature, pH and dissolved O_2 in the field

Stations	Temperature (ºC)	pH	Dissolved Oxygen (mg O_2/L)
Station 1	21.9	8.06	8.53
Station 2	21.2	8.25	8.75
Station 3	22.5	8.21	8.27

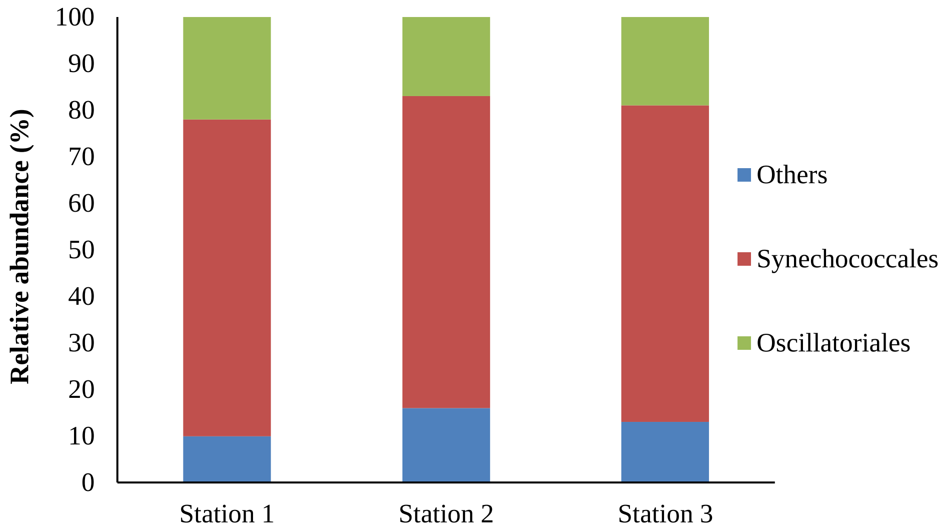

Figure 2. Cyanobacteria communities at the order level in mucilage samples

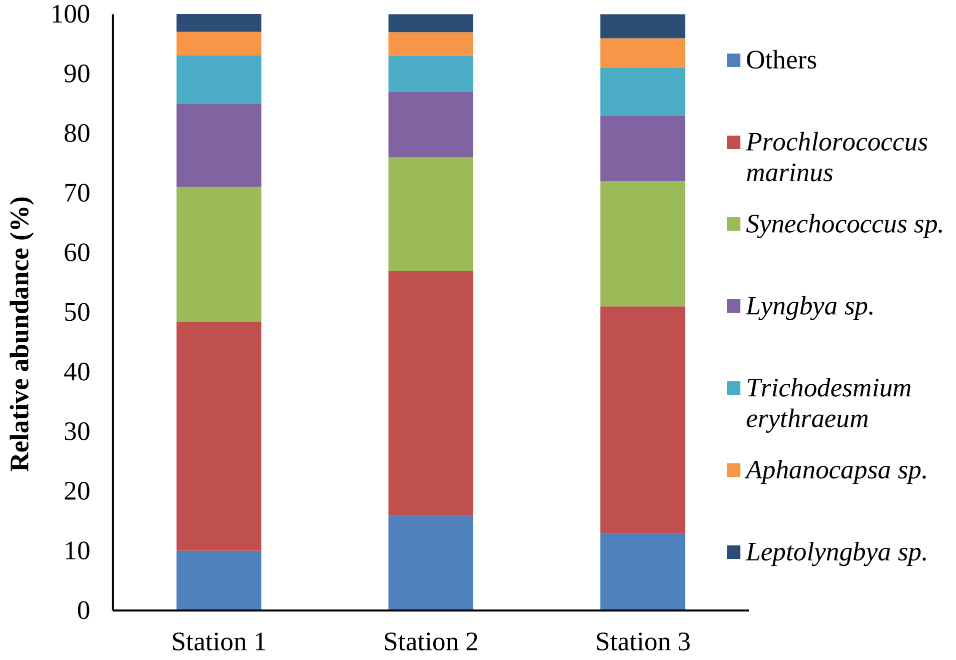

Figure 3. Cyanobacterial communities in mucilage samples

Discussion

In this study, it was determined that cyanobacteria species that may cause harmful algal blooms and have toxic effects are found in the mucilage structure. It is undeniable that humans and other living things shall suffer more in the future due to harmful algal blooms due to the deterioration of the natural balance caused by environmental pollution. *Prochlorococcus marinus* was determined as dominant among the cyanobacteria species in the mucilage structure. *P. marinus* caused an algal bloom for the first time in 2002 in lagoon areas in the Northwestern Sea (Zaitsev and Nesterova 2003; Shiganova, 2008).

Transparent exopolymer particles (TEP), which are extracellular acidic polysaccharides that may form during phytoplankton blooms, are associated with mucilage formation. In laboratory experiments, it has been determined that *P. marinus* has a high TEP production potential (Iuculano et al., 2017). In this study, *Synechococcus sp.* was determined as the second dominant group. It was reported that the mucilage forming ability of *Synechococcus sp.* and *P. marinus*, which are capable of producing TEP, was increased by heterotrophic bacteria (Cruz and Neuer 2019).

Yücel et al. (2018) determined that *Prochlorococcus* is more dominant than small eukaryotes and *Synechococcus* in the Northeastern Mediterranean Sea. Synechococcus density was high during mucilage formation in the Marmara Sea (Gulfs of Bandırma and Erdek) between August 2007 and August 2008 (Toklu-Alicli et al., 2020). In this study, the predominance of *Prochlorococcus* over *Synechococcus* may be associated with the effect of many environmental factors. However, it is known that the density of *Synechococcus* increases more in colder waters (13–16 °C) than *Prochlorococcus*. In our study, the water temperatures in the three sampling stations varied in the range of 21.2-22.5 °C.

The other dominant strains such as *Lyngbya* sp. and *Trichodesmium erythraeum* were detected in mucilage structure in different studies. For example, in the presence of 24-25 °C warm southern water body and 20-22 °C colder northern water body in the Saragossa Sea, *Trichodesmium*, *Synechococcus*, and *Prochlorococcus* distribution in marine snow particles were 13%, 1.8%, and 2.0%, respectively (Lundgren et al., 2019). Metaxatos et al. (2003) determined the cyanobacteria genus as *Microcystis aeruginosa* > *Chroococcus gelatinosus* > *Synechocystis sallensis* > *Trichodesmium erythraeum* > *Lyngbya agardhii*, respectively, according to their density, in the mucilage structure collected at an average water temperature of 23.5 °C in September 1999 in Euboikos Gulf, Aegean Sea.

Trichodesmium erythraeum originates from Indo-Pacific and the Red Sea. For Turkey, the first detection of this organism was made in the Aegean Sea in 1990, where it reached via the Suez Canal and entered the alien species list (Çınar et al., 2011).

In this study, *Aphanocapsa* sp. and *Leptolyngbya* sp. were less abundant in mucilage samples. In the Tuscan Archipelago (between the Ligurian Sea and the Tyrrhenian Sea), between May 1999 and July 2002, the genus *Leptolyngbya*, *Lyngbya* and *Rivularia* were found most frequently in benthic mucilage samples compared to the genus *Oscillatoria*, *Symploca*, *Aphanocapsa and Gloeocapsa* (De Philippis et al., 2005). *Aphanocapsa* caused harmful algal blooms in freshwater reservoirs (de J Magalhães et al., 2019). De Philippis et al. (2005) assumed that this genus, which was detected in a saltwater environment, was previously not reported because it may be challenging to identify in samples containing high levels of suspended matter and therefore, cannot be included in the count. In this study, *Aphanocapsa sp.* was found in the mucilage structure in Dardanelles Strait. Previously, *Aphanocapsa litoralis* (Ulcay et al., 2015) was recorded in the Eastern Mediterranean Sea (Northern Cyprus), and different *Aphanocapsa* genus was recorded in the Black Sea (Aysel et al., 2004).

In this study, cyanobacteria genus identified metagenomically in the mucilage structure have been shown to exhibit toxic effects on different organisms. Some genera may cause other lethal effects, disorders in preference behaviours, and changes in behaviour mobility in fish (Hamilton et al., 2014). For example, *P. marinus* isolated from the Sargasso Sea was found to have the ability to produce the neurotoxic non-proteinous amino acid β-Methylamino-L-alanine (Cox et al., 2005). It was observed that the marine Synechococcus genus might have a toxic effect on marine invertebrates (Martins et al., 2007).

Lyngbya caused acute toxic effects on zebrafish embryos (Berry et al., 2004) and dermatitis in humans (Osborne et al., 2001). *T. erythraeum* produces toxins carried by some fish, and its soluble toxins in seawater may pose a health hazard to humans (Endean et al., 1993). It was also reported that *T. erythraeum,* which has cytotoxic and genotoxic effects, damages human lymphocyte DNA and has a toxic effect on *Artemia salina* (Narayana et al., 2014).

Leptolyngbya genus causes pathogenic microbial mat black band disease, which infects corals worldwide and produces toxins (Myers et al., 2007). *Crossbyanol B*, a toxic brominated polyphenyl ether isolated from *Leptolyngbya crossbyana*, had a harmful effect on *Artemia salina* (Choi et al., 2010).

Conclusion

An investigation of the diversity and ecology of cyanobacteria in the mucilage obtained from Dardanels was conducted. *Prochlorococcus marinus* was the most common cyanobacterial species, followed by *Synechococcus* sp.. The Marmara Sea and Dardanelles Strait, impacted by severe disturbances, including industrial activities, anthropogenic influence, tourism and artificial illumination, have never been completely restored to their former ecological state. In addition, cyanobacteria species in the mucilage may cause harmful algal blooms and have toxic effects that threaten the future well-being of coastal populations and ecosystem stability. Thus, the government and local authorities should pay more attention to combating the mucilage using coastal monitoring tools.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

S. Yılmaz and M.A. Küçüker collected the Mucilage samples. S. Yılmaz analysed the data. All authors contributed to the study conception, design and writing.

Ethics Approval

Ethics committee approval is not required for this study.

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