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Investigations on Isolation and Characterisation of Unicellular Cyanobacteria in Uzungöl and Cernek Lakes, Samsun, Türkiye

Kübra Özkul[1], Haydar Karakaya[1]

[1]Ondokuz Mayıs University, Faculty of Science, Department of Biology, Samsun, Türkiye

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Abstract

Isolation of unicellular cyanobacteria as pure cultures were carried out from the water samples collected from Uzungöl and Cernek lakes in Kızılırmak delta, Bafra, Samsun. Diluted water samples were spread onto a solid BG11 medium which was positively selective for cyanobacteria and isolated single colonies were observed after incubation under continuous light. Twenty colonies composed of unicellular cells were selected as unicellular isolates. These single colonies from each isolates were transferred into a liquid BG11 medium to grow as liquid cultures under continuous light. Characteristics of the cells in liquid cultures of each isolate were used to ıdentify subsection and form-genus of *Cyanobacteria* they might be included. The results showed that all the isolates were settled in Subsection 1 and form-genus *Synechococcus*. To support this identification, genomic DNAs were isolated from each of the isolates, 16S rRNA gene regions were amplified, and each amplicon was digested with *Eco*RI. Restriction fragment lengths were found to be consistent with those of the standard cyanobacterial strain *Synechococcus* sp. PCC7942. Since restriction fragment lengths and cultural characteristics were consistent, all the isolates were, therefore, evaluated to be included into Subsection 1 and form-genus *Synechococcus.*

Keywords: cyanobacteria, synechococcus, uzungöl, cernek lake

Uzungöl ve Cernek Göllerindeki (Samsun, Türkiye) Tek Hücreli Siyanobakterilerin İzolasyonu ve Karakterizasyonu Üzerine Araştırmalar

Öz

Samsun, Bafra Kızılırmak deltasında yer alan Uzungöl ve Cernek göllerindenden alınan su örneklerinden tek hücreli siyanobakterilerin saf kültürler şeklinde izolasyonu gerçekleştirilmiştir. Seyreltilmiş su örnekleri siyanobakteriler için seçici katı besiyeri yüzeyine ekilerek sürekli ışık altında inkübasyona bırakılmış ve izole koloniler elde edilmiştir. Tek hücrelerden oluşmuş 20 koloni tek hücreli izolatlar olarak seçilmiştir. Her bir izolata ait bu tek koloniler sıvı besiyerlerinde sürekli ışık altında sıvı kültürler şekline üretilmiştir. Bu sıvı kültürlerdeki hücrelere ait karakterler incelenerek hangi siyanobakteriyel altbölüme ve hangi form-cinse dahil oldukları belirlenmiştir. Bu çalışmalar sonucunda bütün izolatların Subsection 1 ve *Synechococcus* form-cinsine dahil olduğu sonucuna varılmıştır. Bu sonucu desteklemek için her bir izolattan genomik DNA izole edilmiş, 16S rRNA gen bölgesi çoğaltılmış ve her bir amplikon *Eco*RI ile kesilmiştir. Elde edilen fragment büyüklüklerinin standart *Synechococcus* sp.PCC7942 suşunun fragment büyüklükleriyle uyumlu olduğu belirlenmiştir. Dolayısıyla restriksiyon fragment büyüklükleri ve kültürel özellikler uyumlu olduğundan izolatların tamamının Altbölüm 1 ve *Synechococcus* form-cinsinin üyeleri olduğu sonucuna varılmıştır.

Anahtar Kelimeler: siyanobakteri, synechococcus, uzungöl, cernek gölü

Sorumlu Yazar / Corresponding Author: Haydar Karakaya haydar.karakaya@omu.edu.tr

Introduction

Cyanobacteria are a diverse group of Bacteria domain (Castenholz et al., 2001; Palinska & Surosz, 2014; Rippka et al., 1979). They have abilities to fix carbon dioxide through a photosynthetic apparatus similar to that of higher plants, using water as an electron donor and producing oxygen as by product. Nitrogen fixation is performed by some of the cyanobacterial strains in special sites inside the cell or in heterocysts which are specialized cells for nitrogen fixation (Wolk, 1996). Thus they play an important role in carbon and nitrogen cycles especially in marine and terrestrial aquatic ecosystems. Estimates show that prokaryotic photosynthetic organisms are responsible for %30 of primary production worldwide (Affe et al., 2018; Pang et al., 2018).

Cyanobacteria exhibit a wide morphological diversity from unicellular to filamentous forms (Stanier & Cohen Bazire, 1977; Whitton & Potts, 2012). Unicellular forms may be present as single cells or as aggregates of cells. Filamentous forms may be a row of single cells with or without differentiated cells like heterocysts and akinetes. Filaments may be branched or not. Cyanobacterial cells may divide in single or two or multiple planes producing cells in equal or unequal sizes. Systematic positions of the cells were determined traditionally by using these cellular properties along with cell sizes, presence or absence of thylakoid membranes and mean G-C content (Castenholz et al., 2001). Recently phylogenetic properties based on nucleotide sequences of certain genes or whole genomes have become common for systematics of cyanobacteria along with phenotypic and cultural properties (Whilmotte & Herdman, 2001).

Cyanobacteria were classified under botanical code for a long time due to their ability to photosynthesize in a manner like plants and were called blue-green algae. Based on phenotypic characteristics several genera and species were identified (Whitton, 1992; Wilmotte, 1994). Eventually, cyanobacteria were proposed to be classified under Bacteriological Code rather than Botanical Code due to their prokaryotic cellular structure (Stanier et al., 1978; Rippka et al., 1979; Oren, 2004). Cellular properties are essential for identifying of cyanobacterial strains under the Bacteriological Code, all the morphological, physiological, biochemical and genetic characteristics are described in axenic cultures of strains while description of phenotypic properties under a microscope and a dried specimen are essential under Botanical Code. Many species were identified under Botanical Code according to the morphological properties of specimens from environmental samples resulting in many of synonyms depending on the phenotypic plasticity of the characters due to various environmental conditions (Wilmotte, 1994). However, cultural identification of cyanobacteria comprise limitations like difficulties in culturing cell from natural samples, maintenance of axenic cultures and accumulation of mutations in the cells of axenic cultures (Rippka, 1988). Efforts to conciliate differences between bacteriological and botanical approaches have been made to establish a settled cyanobacterial systematics (Komárek et al., 2016; Oren, 2004; Schoch et al., 2020; Wilmotte & Herdman, 2001).

Phylogenetic properties were commonly used to identify cyanobacterial isolates and strains also. Certain molecular signature sequences like 16S rRNA gene, *psbBA*, *rpoC1*, ITS of 16S-23S rRNA genes and whole genome sequence if available are used to identify cyanobacterial isolates and strains (Moreira et al., 2020). It is widely agreed that the only acceptable method for the taxonomic evaluation of cyanobacteria is a polyphasic approach, including phenotypic, ultrastructural, biochemical and molecular methods (Palinska & Surosz, 2014). Such a combined method was used for cyanobacterial systematics in Bergey's Manual of Systematic Bacteriology (Castenholz et al., 2001). Cyanobacteria are organized as a class *Cyanobacteria* and five subsections. Subsection 1 and 2 comprise single or aggregates of unicellular strains; Subsection 2 filamentous, non heterocystous; Section 4 filamentous, heterocystous; and Section 5 branched filamentous, heterocystous (Castenholz et al., 2001; Rippka et al., 1979). Genera of cyanobacteria in this volume of Bergey's Manual of Systematic Bacteriology were given as form-genera due to a lack of sufficient cultural and phylogenetic data of strains (Castenholz, 2001). Most of the isolates were given with their strain numbers because of a lack of data to group them in species level. Phylogenetic analyses of 16S rRNA

gene sequences showed that some strains were not clustered with the groups they included according to their phenotypic and cultural characteristics. This situation is shared among the strains placed in Subsection 1 and 2 (Wilmotte & Herdman, 2001).

To settle the systematics of such a group with a wide diversity like cyanobacteria, more data are required especially about cultural and phylogenetic characteristics. Isolation and description of new isolates of cyanobacteria will contribute not only the settlement of cyanobacterial systematics and also determination of cyanobacterial biodiversity in aquatic ecosystems. Therefore, this study was aimed at isolation and characterisation of unicellular cyanobacterial strains from two lakes (Uzungöl and Cernek lakes) in the Kızılırmak delta, Samsun. Unicellular cyanobacteria are especially selected due to the uncertainty of their systematic positions and their significant role as primary producers in aquatic ecosystems. Twenty unicellular cyanobacteria were isolated and identified at form-genus level according to their cultural characteristics and RFLP profiles of the 16S rRNA gene.

Material and Methods

Research Area and Sampling

To isolate unicellular cyanobacteria, Cernek (41.64. 23.25N/36.07.36.14E) and Uzungöl lakes (41.57.09.34N/36.09.75.20E) were chosen as research areas, both the lakes were located in the Kızılırmak delta in Samsun, Türkiye. Water samples were taken from subsurface of the water column in sterile sample bottles in September and January. After transfer to the laboratory, samples were filtered through sterilised Watmann papers to remove macroscopic organic or inorganic matter. Standard unicellular cyanobacterial strain *Synechococcus* sp. PCC7942 and *Synechocystis* sp. PCC6803 were used as positive control strains during isolation and molecular procedures.

Media

BG11 (Blue-Green) medium was used to grow of cyanobacterial strains (Rippka, 1988). BG11 salt solution was solidified by adding 0.7% agarose after separately autoclaved. Filter sterilised cycloheximide (50 µg/ml) was added to the liquid and solid medium after cooling at about 50°C to eliminate eukaryotic microorganisms.

Isolation Procedures

Isolation processes were carried out according to the method described by Rippka (1988) with some modifications. Filtered samples were first diluted serially (10^{-1} to 10^{-3}). A 100 µl of diluted samples were spread onto BG11 plates and incubated at 28°C under continuous light at a density of 40 µmol photons m^{-2} s^{-1} in an incubator for about 20 days. Each colony on the plate was examined under light a microscope to find colonies with unicellular forms. Unicellular colonies were then taken and streaked on a new fresh BG11 solid medium. Single colonies, grown on these plates, were re-streaked another fresh solid BG11 medium again. Cell mass from this medium were transferred into liquid BG11 medium supplemented with 50 µg ml^{-1} cycloheximide and incubated in a shaker incubator under continuous light with the same density above. These liquid cultures were used for microscopic examinations of cellular properties and genomic DNA isolations.

Microscopic Examination

Cyanobacterial cells from single colonies on plates were examined under a light microscope (Nikon) at magnification rates of 400X and 1 000X. Cells and cell clusters in isolated pure liquid cultures were examined in a binocular microscope (Leica) with magnifications of 400X and 1 000X. Cells from each isolated culture were photographed with a camera (Leica DFC290) using the software Leica Application Suite.

Molecular Biological Techniques

General molecular biological techniques were carried out according to Sambrook and Russel (2001). Genomic DNAs from 10-12 day-old log phase liquid cultures were extracted according to the method described previously (Lind et al., 1985). DNA amount and purity were measured using a nanodrop (ND-1000) spectrophotometer. Using genomic DNAs as template, 16S rRNA genes of each isolate were amplified using previously designed primers 16S27F (5'AGAGTTTGATCCTGGCTCAG3') and 16S1494R (5'GTACGGCTACCTTGTTACGAC3') (Taton et al., 2003). To avoid pipetting mistakes, a reaction mixture was prepared including primers, buffer and dNTPs. After sharing the reaction mixture to PCR reaction tubes, certain amounts of genomic DNA as a template, enzyme solution and $MgCl_2$ solutions were added. Final volume was adjusted to 25 µl with pure water. *Taq* DNA polymerase (ThermoScientific) was used for annealing reactions. Reactions were optimized in terms of template DNA, *Taq* DNA polymerase and $MgCl_2$ amounts. Amplification was started with a first denaturation step at 94°C for 3 min, followed by 30 cycles of amplification reactions each with three steps (denaturation at 92°C for 15 secs, annealing at 55°C for 30 secs and extension at 72°C for 1.5 mins). Amplicons were kept at 4°C after a last extension step at 72°C for 5 mins. Amplification products were purified with a Wizard SV Gel PCR Clean-up kit (Promega). 10 µl aliquot of each amplicon was digested with *Eco*RI (Promega) according to the manufacturer's recommendations. Amplicons and digestion product of them were run in 0.7 % agarose gel with 0.5 µg ml^{-1} ethidium bromide and examined under UV light on a transilluminator (Vilber-Lourmat). 1 kb GeneRuler (ThermoScientific) and 50 bp DNA ladder (ThermoFisher) were also run as molecular marker alongside amplicons and *Eco*RI digested samples. The agarose gels with ethidium bromide stained DNA bands were photographed in Bio Imaging System (Syngene). 16S rRNA gene sequences of selected cyanobacterial strains were downloaded from the database Cyanobase (2022) and analysed in pDraw32 DNA analyse software (AcaClone, 2022).

Results

Isolation of Unicellular Cyanobacteria from Water Samples

Water samples from Uzungöl and Cernek lakes were first filtered through Whatman paper to omit organic or inorganic macro-particles including multicellular organisms. The part of water samples passed through the filter carrying microbial cells was used to isolate unicellular cyanobacterial organisms. These filtered water samples were diluted at rates of 10^{-1}, 10^{-2} and 10^{-3}. BG11 medium used as isolation medium was supplemented with cycloheximide to inhibit peptidyl transferase activity of 60S ribosomal subunit of eukaryotic cells. Thus eukaryotic microorganisms including photosynthetic algae were unable to grow on the medium. BG11 medium contains no organic carbon and only photosynthetic microorganisms can grow. Therefore, such growing media, liquid and solid BG11, supplemented with cycloheximide become positively selective for photosynthetic prokaryotes. These selective BG11 plates allow the growth of photosynthetic prokaryotic microorganisms represented predominantly by cyanobacteria. Heterotrophic prokaryotes cannot grow in this medium because of the absence of any organic carbon sources.

A 100 µl aliquot of undiluted and diluted samples was spread on BG11 plates solidified with agarose and supplemented with cycloheximide. Plates were incubated at 25°C about three weeks. One set of plates was incubated under continuous light as described above while another set was incubated at a lower light intensity by shadowing the light with a paper tissue. After the incubation period, cyanobacterial growth appeared on the plates. Especially all or most of the surface of the plates incubated with undiluted and less diluted samples covered by cyanobacterial cells. However, single cyanobacterial colonies appeared on the plates incubated with more diluted samples (Figure 1). Even on such plates some of cyanobacterial growth zones were spread through the surface. The colonies located in a clear area were selected for further isolation processes.

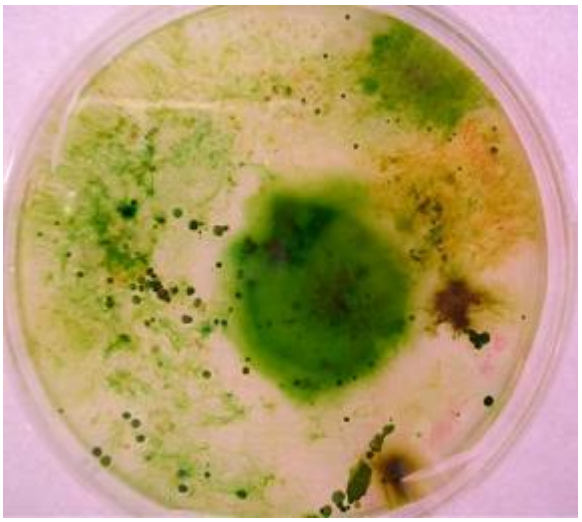

Figure 1. Cyanobacterial Colonies Grown on Selective BG11 Solid Medium After Spreading Diluted Water Sample

For further purification, 20 of single colonies were taken from the surface of the plates with a tip of sterilised toothpick and streaked on a fresh BG11 medium as short lines and incubated at 25°C for further growth (Figure 2a). After this second incubation, cells from each streak that grown from 20 of the colonies were taken with a loop and streaked on a fresh BG11 plate to produce single colonies (Figure 2b). These single colonies were streaked onto fresh BG11 plates again and these plates were used as cell source for liquid cultures. Each of 20 isolates originated from a single colony was taken as pure cultures and named as UCG1-UCG20.

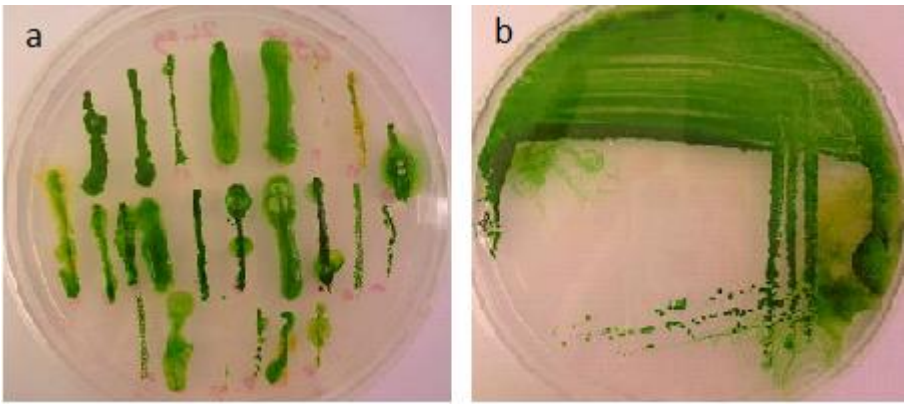

Figure 2. Growth of the Isolates on Selective BG11 Solid Medium

a) Growth of single colonies (isolates) transferred from previous plates (Figure 1) and b) growth of an isolates streaked on selective BG11 solid medium to obtain single colonies.

Each of the isolates on BG11 plates was transferred to BG11 liquid media to grow the isolates as pure liquid cultures. These liquid cultures were used both the morphological identification and chromosomal DNA isolation.

Identification of the Isolates

All the logarithmic phase liquid cultures of 20 isolates were examined under microscope to observe cell division style, cell size and aggregation state. Each isolate was photographed at 400X and 1 000X magnifications (Figure 3). These characteristics were used for identification of the isolates (Table 1). For subsection level identification, the key prepared for subsections of *Cyanobacteria* (Castenholz, 2001) was used. None of the isolates exhibited multiple internal fission and no daughter cell was found smaller than mother-cell. Therefore all of the isolates were included into Subsection I. Subsequently the isolates were identified at form-genus level using a key prepared for Subsection I

previously (Herdman et al., 2001a). While G+C contents of the strains were not measured in this study, the other characteristics were used for morphological identification. Due to fact that all the isolates contained thylakoids, exhibited no budding and no sheath and had relatively small cell size, all the isolates were included in group III-B in the key comprising form-genera *Cyanobacterium*, *Cyanothece*, *Dactilococcopsis*, *Cyanobium* and *Synechococcus*. The isolates were not included into the form-genus *Cyanothece* and *Dactilococcopsis* because their cell sizes were bigger than the isolates. Since *Cyanobacterium* was a rarely represented form-genus and there was great difference between their typical habitats and Kızılırmak delta (Rippka et al., 2001a), the strains were not included into this form genus. Even sizes of the isolates were consistent with the cell sizes of form-genus *Cyanobium* (Rippka et al., 2001b) the strains were not included in this form-genus because it is mainly contains marine strains. Finally all isolates were included into form-genus *Synechococcus* (Herdman et al., 2001b) due to having similar cell sizes and represented as single cells or aggregates.

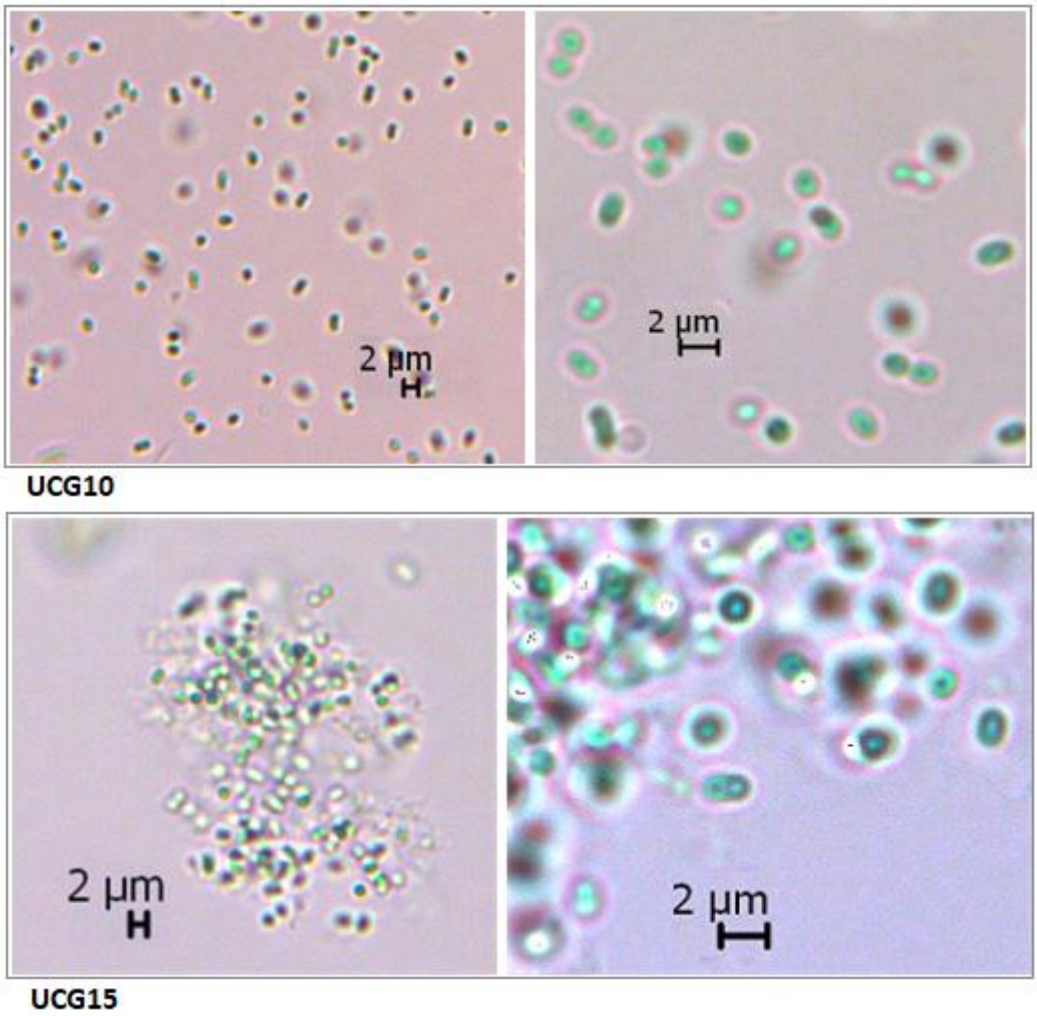

Figure 3. Photographs of Representative Strains UGC10 and UGC15

Magnification of left panels was 400X, that of right panels 1 000X. A 2 µm size indicator bar was added inside of each panel.

Table 1. Morphological Properties and Recognized Identities of the Isolates

Isolate	Cell size (µm)	Cell shape	Aggregation	Identity
UCG1	1–1,5	Coccus	+	*Synechococcus* sp.
UCG2	1–1,5	Coccus	+	*Synechococcus* sp.
UCG3	1–1,8	Coccus	+	*Synechococcus* sp.
UCG4	1–1,8	Coccus	+	*Synechococcus* sp.
UCG5	1–1,8	Coccus	-	*Synechococcus* sp.
UCG6	1,8–1,9	Coccus	+	*Synechococcus* sp.
UCG7	0,8–1,8	Coccus	-	*Synechococcus* sp.
UCG8	0,8–1	Coccus	+	*Synechococcus* sp.
UCG9	1x1,5–1,5x2,3	Rod	+	*Synechococcus* sp.
UCG10	1,2x2,5–1,5x2,5	Rod	-	*Synechococcus* sp.
UCG11	1–1,5	Coccus	+	*Synechococcus* sp.
UCG12	0,8–1,5	Coccus	+	*Synechococcus* sp.
UCG13	1–1,5	Coccus	+	*Synechococcus* sp.
UCG14	1–1,8	Coccus	+	*Synechococcus* sp.
UCG15	1x2,2–1,5x2,2	Rod	+	*Synechococcus* sp.
UCG16	1–1,5	Coccus	+	*Synechococcus* sp.
UCG17	1–1,5	Coccus	+	*Synechococcus* sp.
UCG18	1,2–1,5	Coccus	+	*Synechococcus* sp.
UCG19	1–1,5	Coccus	+	*Synechococcus* sp.
UCG20	1x1,2–1x1,5	Rod	+	*Synechococcus* sp.
PCC7942	0,8x2–1x3	Rod	-	*Syn.* PCC7942
PCC6803	2,2–2,5	Coccus	-	*Syc.* PCC6803

The isolates were named as UCG1 to UCG20. Properties of standard strains Synechococcus sp. PCC7942 and Synechocystis sp. PCC6803 were also shown

RFLP Analyses of 16S rRNA Genes of Isolates

Identification of the isolates was further tested by their 16S rDNA restriction fragment length polymorphism (RFLP) profiles. Genomic DNA from all of the isolates (UCG1-UCG20) and form standard strains *Synechococcus* sp. PCC7942 and *Synechocystis* sp. PCC6803 was isolated and used as template in the 16S rRNA gene amplification. A primer pair (Taton et al., 2003) was used to cover most part of cyanobacterial 16S rRNA gene (from 27 bp to 1494 bp) with a predicted amplicon size of about 1.45-1.50 kb. After optimization studies, 16S rDNA amplicons from all of the isolates and standard strains were obtained with about 1.5 kb size as predicted (Figure 4).

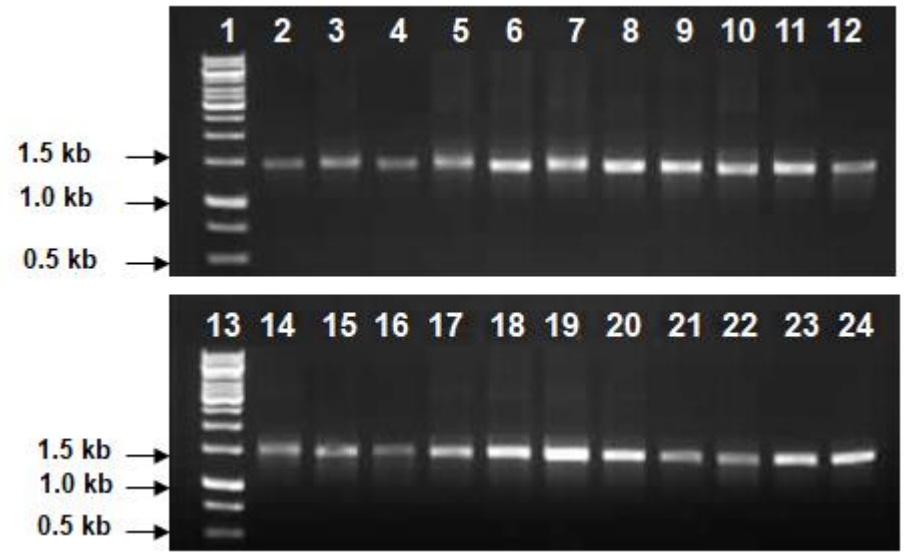

Figure 4. 16S rDNA Amplicons of 20 Isolates Lanes 1 and 13 are 1 kb DNA ladder

Lanes 2 to 12 and 14 to 22 are 16S rDNA amplicons with an expected size of approximately 1.5 kb. Synechocystis sp. PCC6803 (lane 23) and Synechococcus sp. PCC7942 (lane 24) the 16S rDNA gene amplicons were also shown.

To support the identities of the isolates, the 16S rDNA amplicons of each isolates were analysed for any variation in 16S rRNA gene. RFLP properties were used for indication of base pair variations. Initially 16S rRNA gene sequences of *Synechococcus* sp. PCC7942 and *Synechocystis* sp. PCC6803 were downloaded from the cyanobacterial genomic database Cyanobase (2022) and their *Eco*RI restriction fragment lengths were analysed with the software pDRAW32 (AcaClone, 2022). *Eco*RI restriction enzyme produces two fragments from 16S rRNA gene of *Synechococcus* sp. PCC7942 with sizes of 870 bp and 620 bp. The enzyme splits the *Synechocystis* sp. PCC6803 16S rRNA gene into three fragments with sizes of 700 bp, 633 bp and 169 bp. These *Eco*RI RFLP profiles were used as references for the *Eco*RI RFLP profiles of each of the isolates.

Amplicons of the isolates were digested with *Eco*RI and digestion products were separated in an agarose gel (Figure 5). Fragment sizes were compared with those of test strains *Synechococcus* sp. PCC7942 and *Synechocystis* sp. PCC6803. *Eco*RI RFLP profiles of 20 isolates were same or similar to that of *Synechococcus* sp. PCC7942. 15 of the RFLP profiles of isolates were consistent with that of *Synechococcus* sp. PCC7942 as seen in Figure 5. However, five of the isolates exhibited one additional fragment about 670 bp in size along with expected two fragments (870 bp and 620 bp). None of the isolates had an RFLP profile similar to that of *Synechocystis* sp. PCC6803.

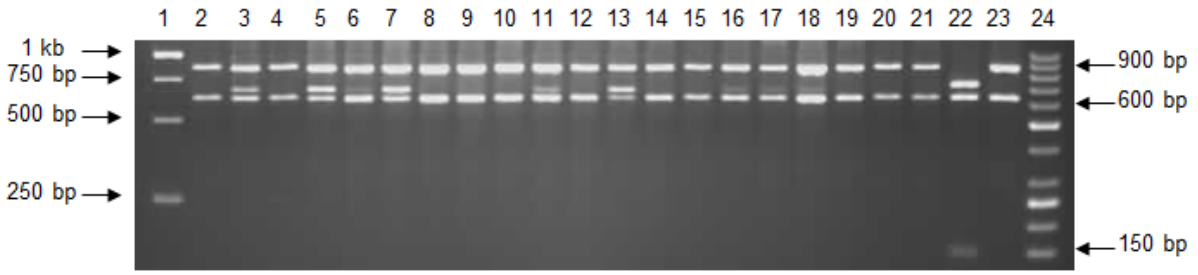

Figure 5. Agarose Gel Electrophoresis Image of *Eco*RI Fragments of 16S rDNA Amplicons of the Isolates Lanes 2 to 24 are *Eco*RI Fragments of the Isolates UCG1 to UCG20, Respectively.

EcoRI fragments of test strains Synechocystis sp. PCC6803 (lane 22) and Synechococcus sp. PCC7942 (lane 23) are also shown. Lane 1 is a 1 kb DNA ladder (ThermoFisher) and lane 24 is a 50 bp DNA ladder (ThermoFisher).

*Eco*RI RFLP profiles of the strain included in the III-B group of the identification key (*Cyanobacterium*, *Cyanothece*, *Dactilococcopsis*, *Cyanobium* and *Synechococcus*) were also analysed in silico using their full 16S rRNA gene sequences deposited in databank.

Cyanobase (2022). *Cyanothece* and *Dactilococcopsis* differed from all of the isolates as they exhibited three fragments. Other three genera exhibited two fragments as the isolates. Size differences of *Cyanobacterium* (866 bp and 610 bp) and *Synechococcus* (870 bp and 620 bp) were little that might not be distinguishable effectively via agarose gel electrophoresis. *Cyanobium* also had two fragments but size of fragments was relatively smaller and distinguishable from that of the isolates. These in silico analyses support that the isolates were not members of form-genus *Cyanobium*.

Discussion and Conclusions

Unicellular cyanobacterial isolates were purified from water samples collected from Uzungöl and Cernek lakes in the Kızılırmak delta in Samsun. Bases of the purification were the elimination of eukaryotic microorganisms, including photosynthetic algae with cycloheximide, and heterotrophic prokaryotes by using a minimal medium BG11 that allows the growth of photosynthetic prokaryotic organisms only. Under such conditions i.e., in BG11medium supplemented with cycloheximide, cyanobacteria and other photosynthetic prokaryotic groups like green and purple bacteria may successfully grow and reproduce. However, only cyanobacterial growth was observed in this study.

All the isolates were included in form-genus *Synechococcus* based on their cell morphology and cell division manner under light microscope. To identify the isolates, identification key (Herdman et al., 2001a) and form-genus descriptions (Herdman et al., 2001b; Rippka et al., 2001a; Rippka et al., 2001b) were used. Because their cells were divided by symmetrically transverse binary fission and they did not exhibit structured sheath, all the isolates were settled in a group of five form-genera. The isolates may not be included into form-genera *Cyanothece* and *Dactilococcopsis* because of their bigger cell sizes than any of the isolates. Cell sizes of the isolates were consistent with the other three form-genera *Cyanobacterium*, *Cyanobium* and *Synechococcus*. However the isolates were included into *Synechococcus* due to the fact that the cells of most of the isolates were represented as united cellular aggregates by mucilage formation. Only three isolates UCG5, UCG7 and UCG10 were did not form cellular aggregates staying unicellular. These three isolates were included into form-genus *Synechococcus* because *Cyanobacterium* is rarely represented form-genus and form-genus *Cyanobium* contains marine strains. Determination of mean G-C base contents would be helpful to ensure the identities of the isolates.

RFLP profiles of the 16S rRNA gene support identification of the isolates as form-genus *Synechococcos* exhibiting two *Eco*RI fragments with the same sizes. rDNAs of all the isolates exhibit two fragments with sizes of about 870 bp and 620 bp, the same as those of standard strains *Synechococcus* sp. PCC7942. Identification of the strains was supported by the results of in silico

analyses of RFLP profiles of various cyanobacterial strains. Also, in silico analyses of RPLF profile showed that the *Cyanobium* 16S rRNA gene was 100 bp shorter than the other strains tested. RFLP profiles of the isolates exhibited no such a short fragment. Therefore, none of the isolates could be included into form-genus *Cyanobium*. Five of the isolates, which were UCG2, UCG4, UCG6, UCG10 and UCG12, showed an additional fragment in the RFLP profile with a size of approximately 670 bp. The origin of this additional fragment may not be expected for certain in this stage unless the full length of 16S rDNA is sequenced. It may be explained by either presence of another contaminant organism in cultures of the isolates or the insertion of an additional fragment into the rRNA gene through lateral gene transfer facilities.

Both the microscopic examination and 16S rDNA RFLP profiles indicate that all of the isolates are members of form-genus *Synechococcus* and members of this form-genus may be expected to be predominantly represented in Uzungöl and Cernek lakes. There were no records about the members of *Synechococcus* in Uzungöl and Cernek lakes in previously conducted studies in the same areas based on microscopic identification of algae from water samples directly (Gönülol & Çomak 1992; Tas et al., 2007). This implies that microscopic identification is not sufficient to determine biodiversity of a certain habitat; culture studies may supply more detailed data about community contents and biodiversity.

Results of this study are derived from the isolation and identification of unicellular cyanobacteria by approaches of microscopic and molecular identification based on cellular properties of pure cultures and RFLP profiles of 16S rRNA gens. With some molecular techniques such as the determination of molecular marker gene sequences will be more accurate however. Thanks to the development of sequencing technologies, whole genome sequences of isolates as well as marker gene sequences will supply precise phylogenetic data about the organism. Such whole genome sequences will also help to settle cyanobacterial systematics. However, not all cyanobacteria are cultivable as are so for other microorganisms. In such cases metagenomics studies would be helpful to detect target DNA sequences like 16S rRNA gene and even whole genomes. Amplification of DNA regions used for phylogenetic relationships like 16S rRNA gen may be amplified from environmental DNA and sequenced by high throughput sequencing. More accurate and more detailed data are possible to be determined about cyanobacteria of Uzungöl and Cernek lakes with such a metagenomics approach.

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Author Contribution

Experimental part of this study was carried out mainly by *Kübra Özkul*. The authors were contributed equally to the other parts of this research

Ethics

There are no ethical issues related to the publication of this article.

Conflict of Interest

The authors declare that there is no conflict of interest.

ORCID

Kübra Özkul https://orcid.org/0000-0001-8958-4185

Haydar Karakaya https://orcid.org/0000-0003-3347-0001

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