Egyptian Journal of Aquatic Biology and Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 27(5): 661 – 675 (2023)
www.ejabf.journals.ekb.eg

Morphological and Molecular Identification of Four Blue-Green Algae Isolated from Some Water Bodies in Basrah Governorate, Southern Iraq

Mustafa T Hatem [*], **Emad Y.A Al-Sultan**
Department of Biology, College of Education for Pure Sciences, University of Basrah, Iraq
[*]**Corresponding Author:** muss200059@gmail.com

ARTICLE INFO

Article History:
Received: Sept. 11, 2023
Accepted: Oct. 3, 2023
Online: Oct. 13, 2023

Keywords:
Blue-green algae,
Molecular identification,
Gloeocapsa calcarea,
16S rRNA gene,
PCR

ABSTRACT

The identification of blue-green algae is a paramount importance step due to their significant role in various ecosystems and potential implications for human health. Four species of blue-green algae were molecularly and morphologically identified; three species of which were identified for the first time locally in the water bodies in Basrah southern the Republic of Iraq, represented by *Cyanobacterium aponinum*, *Leptolyngbya halophile* and *Chroococcidiopsis cubana*. The fourth species, *Gloeocapsa calcarea*, is registered for the first time in the GenBank on the NCPI website. A phylogenetic tree was designed based on the *16S rRNA* gene sequences using the Neighbor-Joining method through molecular evolutionary genetics analysis Version 11. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test are displayed next to the branches. The tree showed that the strains were closely related to their respective reference strains.

INTRODUCTION

Blue-green algae, also known as cyanobacteria, represent a diverse group of prokaryote organisms belonging to the kingdom Monera, especially the bacteria division Eubacteria and the class cyanobacteria (**Yamada, 2020; Vanlalsangi & Lalmuanpuii, 2022;**).

The cyanobacteria are among the oldest living organisms capable of photosynthesis and oxygen production for the Earth's atmosphere. They have been playing an active role in this process since the early ages, most likely for over 3.5 billion years (**Rasmussen *et al*., 2008; Chittora *et al*., 2020**). This group of algae is commonly distributed in a wide range of terrestrial and aquatic habitats. Their habitats include ponds, rivers, lakes, estuaries and coastal waters (**Blaha *et al*., 2009; Schmidt *et al*., 2014; AL-Sultan & Hatem, 2018**). Furthermore, they can adapt to hot springs and extreme environments such as deserts (**Whitton & Potts, 2012).**

Blue-green algae are known to form extensive and widespread accumulations in surface waters, a phenomenon referred to as blooming an effect of severe climate changes

Indexed in Scopus ELSEVIER DOAJ IUCAT

and excessive nutrient levels beyond natural limits. This, in return, constitutes a significant threat to freshwater ecosystems and the quality of drinking water **(Blahova *et al*., 2008; Nezbrytska *et al*., 2022).**

Cyanobacteria in aquatic environments produce a diverse range of toxic secondary compounds known as cyanotoxins. These toxins diverge into hepatotoxins (toxins affecting the liver), neurotoxins (toxins affecting the nervous system), cytotoxins (toxins affecting cell tissue cultures), dermatotoxins (toxins affecting the skin), and irritating toxins (**Wiegand & Pflugmacher, 2005; Bláha *et al*., 2009; Al-Sultan & Hatem, 2019; Gdemi & Awad, 2022**). Molecular identification is necessary for determining relationships, origin and evolution among living organisms. The features of this molecular target that make it a good tool in genetics also make it beneficial for detecting organisms with prokaryotes. Studying the gene sequence of *16S rRNA* can identify described strains and potentially lead to the identification of uncultivated species **(Clarridge, 2004; Matsumoto & Sugano, 2013; Stanojković *et al*., 2022).**

In recent years, there has been an increasing trend towards the use of modern techniques including DNA sequencing due to their ability to rapidly and accurately diagnose prokaryotic genera and species. The method of amplifying the *16S rRNA* gene has been used for identifying these prokaryotic, primarily because this gene is present in all prokaryotic organisms and remains relatively unchanged among different species. The *16S rRNA* gene consists of multiple nucleotides containing specialized regions that allow for the identification of these species and genera using specific primers, which amplify the desired gene **(Jenkins *et al.*, 2012).**

The Shatt al-Arab River is the primary water source in the Basrah Governorate southern of Iraq and a vital artery because its water is utilized in numerous agricultural, industrial, fishing and navigation movements, and it can also be treated and desalinated for drinking purposes through filtration and purification plants (**Mohammed *et al*., 2014; Alwaeli & Athbi, 2021**). However, the water at the estuary of Shatt al-Arab faces the risk of pollution from industrial facilities found along its banks such as domestic activities and human settlements (**Al-Saad *et al*., 2017**). Due to the scarcity of environmental studies related to the molecular identification of algae, specifically blue-green algae, in the Iraqi environment, this study was conducted to identify some of the cyanobacteria present in the waters of Shatt al-Arab and the rivers associated with it.

MATERIALS AND METHODS

Sample collection and isolation of blue-green algae

Water samples were collected from several water bodies in Basrah Governorate in the Republic of Iraq, represented by the Shatt al-Arab River, Shatt al-Basrah River, Al-Ashar River, and Al-Khindak River, in November 2021. (Table 1 & Fig. 2). Samples

were obtained at a depth of 30cm below water surface using a plankton collection net and then put in clean, sealed plastic containers. Samples were immediately transferred to the laboratory of the biology departments, College of Education, Basrah University for the isolation and culturing of the cyanobacterial species, according to the dilution method of **Stein (1975).**

A volume of 1ml of water sample was mixed with 9ml of distilled water by centrifugation at 3000 rpm for 10 minutes. The deposit was the series diluted with 10ml of distilled water, and slides were prepared from this solution to identify the cyanobacterial species using an Olympus CX21 optical microscope. Unialgal cultures of cyanobacterial species were obtained by taking 5ml of the algal solution, and repeatedly washing with distilled water using a TLE-Danger centrifuge at 3000 rpm for 10 minutes. Subsequently, one ml of the washed samples was transferred into test tubes at dilutions, and the volume was adjusted to 10ml using sterile liquid media (BG-11). The cultures were then incubated in a growth chamber with a light and dark cycle of 14:10 under a light intensity of 130-150 $\mu E^2 Sec^{-1}$, as described in the study of **Stein (1975)**.

Purification of unialgal cultures

Obtaining axenic cultures of blue-green algae was made according to the method of **Wiedeman *et al.* (1964).** The purification process involved several steps: First, the unialgal cultures of the algae were washed with sterile distilled water by centrifuging them at 3000 rpm for 5 minutes. The resulting sediment was washed with sterilized distilled water and repeated 12 times to get axenic algal cultures. These cultures were tested to be free from bacteria, fungi and contamination by other microorganisms, according to **Stein (1975).**

Morphological and phenotypic

The blue-green algae were morphologically identified by a Leica optical microscope with magnifications of x4, x10, x40 and x100 according to **Desikachary (1959)** and **Prescott (1975)** using website www.algaebase.org. Additionally, research studies of **Moro *et al.* (2007), Kim *et al.* (2015), Will *et al.* (2019)** and **Zare *et al.* (2020)** were considered.

Extraction of genomic DNA and PCR amplification

An amount of 1ml from each of the four blue-green algal cultures was transferred and centrifuged at 3,000 × g for 5min at ambient temperature. The pelleted cells were lysed with 200µl of buffer GST plus 20µl of proteinase K solution (gSYNC™ DNA Extraction Kit). The suspension was vigorously vortexed and incubated at 60°C overnight for thorough cell lysis. DNA extraction was then carried out as per the kit's protocol. Each genomic DNA (gDNA) extract was finally eluted with 100µl of Elution Buffer, and gDNA concentration was measured spectro-photometrically (NanoPhotometer; Implen,

NanoPhotometer™ N50, Germany). PCR-based molecular characterization was achieved using a pair of primer set CYANO 16S rDNA 27F1: AGAGTTTGATCCTGGCTCAG and 781R1: GACTACTGGGGTATCTAATCCCTTT specific for segment of blue-green algae *16S rRNA* (**Jungblut *et al.*, 2005**). For PCR, the regions of genomic DNA were amplified targeting the portion of the blue-green algae *16S rRNA* gene. *16S rRNA* PCR reaction consists of 50µl that includes 5µl at a concentration higher than 20ng/ µl of template DNA, 2µl of each primer 25µl Master Mix (Promega) and 16µl of Nuclease free water. Reactions were performed in a Thermal Cycler (DALB) programmed for 35 cycles. The initial denaturation step was at 95˚C for 5min, followed by 34 cycles of 94˚C (30sec), 58˚C (30sec), and then 72˚C (90sec). An additional one cycle of extension was determined at 72˚C (5min). The PCR products were in electrophoresis in 1.5 % agarose gels stained with ethidium bromide (1 µg/ml) using TPE electrophoresis buffer. Sizes of amplified products were compared with the DNA ladder 1000+pb (BioNEER) under ultraviolet radiation to determine the length of amplified products. The gel was a photography done with a digital camera.

The nucleotide sequencing analysis

The product PCR of amplification by *16 S rRNA* gene was sent to Macrogen Company in South Korea. A volume of 20 microliters of each primer and 10ul of the PCR product were placed in small PCR tubes.

The phylogenetic tree

A phylogenetic tree was constructed using the Neighbor-Joining method based on the DNA sequencing data obtained from Macrogen Company. The *16S rRNA* gene sequences of four isolated and purified strains of blue-green algae in the current study were approximated to the exact gene sequences from reference strains registered in GenBank, utilizing the MEGA Molecular Evolutionary Genetics Analysis Version 11 software.

RESULTS

١ henotypic classification (Morpho-taxonomy)

The four species were morphologically identified according to the morphological characteristics, as follows:

Division: Cyanophyta

Class: Cyanophyceae

Order (1): Chroococcales

Family: Cyanobacteriaceae

Genus: *Cyanobacterium*

Species: ***Cyanobacterium aponinum*** Ivano Moro, 2007

Order (2): Leptolyngbyales

Family: Leptolyngbyaceae

Genus: *Leptolyngbya*

Species: ***Leptolyngbya halophile*** Alois Hansgirg, 1893

Order (3): Oscillatoriales

Family: Oscillatoriaceae

Genus: *Oscillatoriales*

Species: ***Gloeocapsa calcarea*** Edward Tilden, 1898

Order (4) : Chroococcidiopsidales

Family: Chroococcidiopsidaceae

Genus: *Chroococcidiopsis*

Species: ***Chroococcidiopsis cubana*** KomarekandHindak, 1965

2. Morphological description

2.1. **Cyanobacterium aponinum** *Ivano Moro, 2007*

The algal *Cy. aponinum* appears under the light microscope as small, non-motile, single or paired oval-shaped cells. The length of the cells ranges from 1.8 to 4µm, while the width ranges from 2 to 1.8µm. These cells are related together to form chains of 2 or 4 cells. Binary fission is honored during the asexual growth phase. This species represents the first record in Iraq (Fig. 1A).

2.2. **Leptolyngbya halophila** *Alois Hansgirg, 1893*

The species *L. halophila* appears under the light microscope as dark green-blue. It has long, wavy, flexible, intertwined filaments with rounded ends. The filaments are surrounded by a very thin sheath. The filaments have no transparent zones. The filaments are composed of barrel-shaped cells that are approximately equal in dimensions, ranging from 2.4 to 1.3µm. This species represents the first record in Iraq (Fig. 1B).

2.3. **Gloeocapsa calcarea** *Edward Tilden, 1898*

The algal *Gl. calcarea* is a unicellular alga that appears under the light microscope as regular colonies with quadrilateral lobes surrounded by a very thin and colorless sheath. Colonies consist of 4- 8 cells, with the cells within the colony separated by a thin colourless membrane. The cells are spherical or oval-shaped, green-blue in color, with 2- 4µm in diameter. During the early stage of asexual growth, the alga appeared as single cells in binary and quaternary colonies. After entering the stationary phase, the algae form stacked colonies contain 8 cells (Fig. 1C).

***2.4.* Chroococcidiopsis cubana** *Komarek and Hindak, 1965*

The algal *Ch. cubana* is a unicellular alga that appears in irregular colonies called palmelloid colonies. The cells are spherical or oval-shaped, non-motile, with a diameter ranging from 2.3 to 8.4µm, depending on the growth stage. They are surrounded by a sheath. They are known for the presence of gas vacuoles. During the early stage of asexual growth, the alga appears as small, variably-sized spherical cells. The process of binary fission is observed during the asexual growth stage, where colonies of two- and four-celled cells can noticed under the light microscope. At the beginning of the stationary phase, the cells separate into individual cells within the colony. This species represents the first record in Iraq (Fig. 1D).

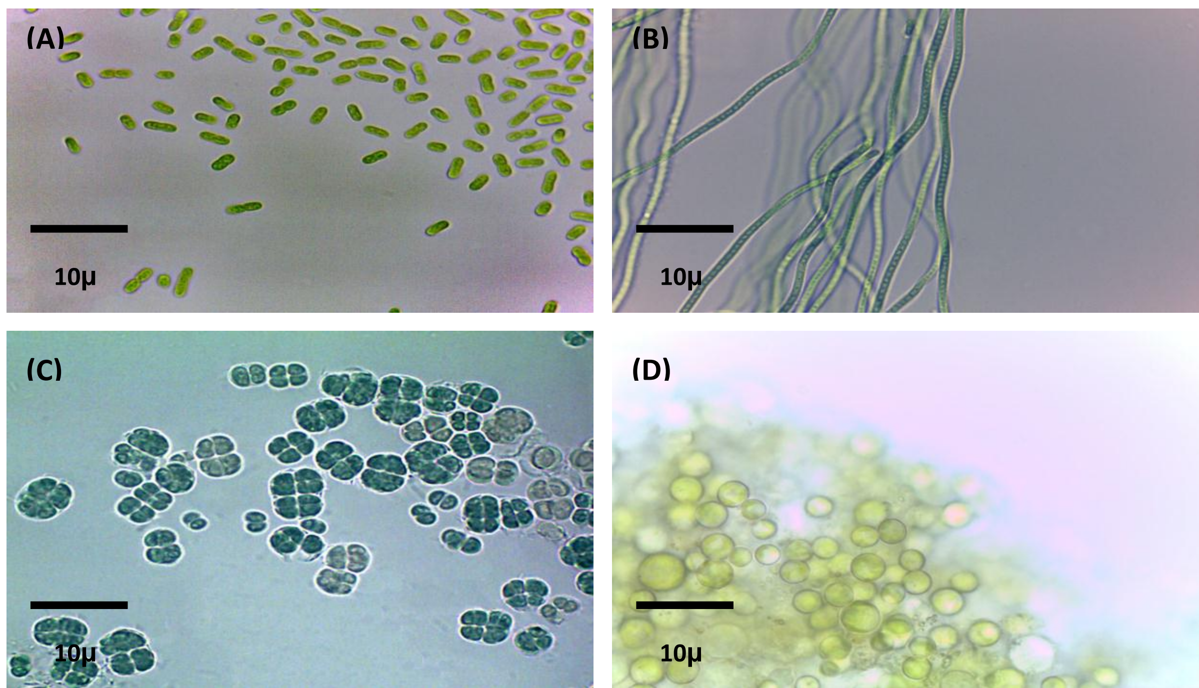

Fig. 1. Light microscopy micrographs of blue-green algae X100 A- *Cy. aponinum* B- *L. halophila* C- *Gl. calcarea* D- *Ch. Cubana*

Table 1. Water sampling sites and their coordinates

Sampling	Sampling sites	Latitude	Longitude
Cy. aponinum	Al-Ashar River	N:30˚ 30′ 47. 4″	E: 47˚ 49′ 57. 0″
L. halophila	Shatt al-Basra River	N:30˚ 27′ 03. 5″	E: 47˚ 45′ 22. 6″
Gl. calcarea	Al-Khindak River	N:30˚ 30′ 41. 5″	E: 47˚ 49′ 24. 8″
Ch. cubana	Shatt al-Arab River	N:30˚ 30′ 41. 2″	E: 47˚ 51′ 03. 5″

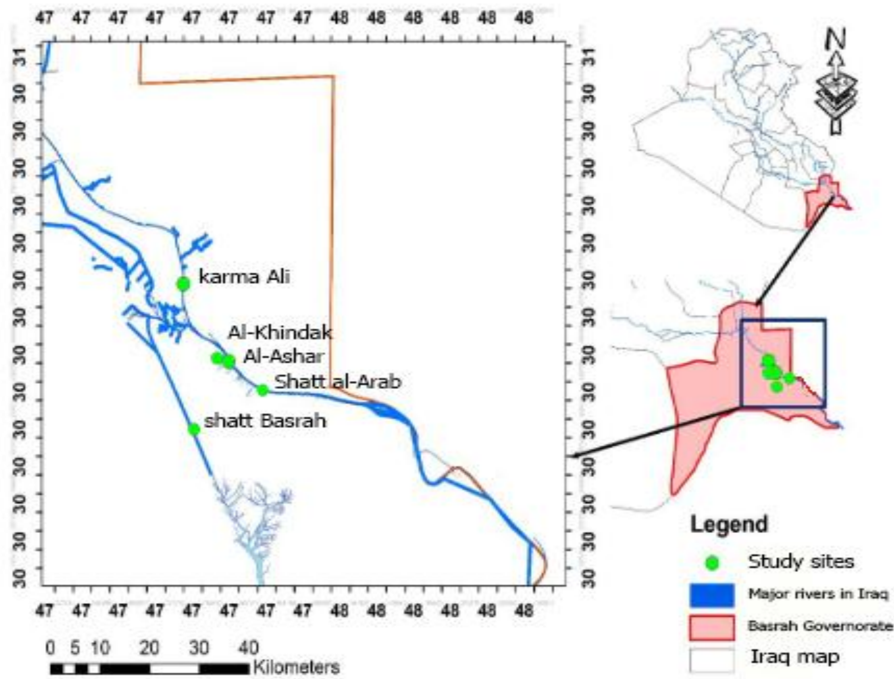

Fig. 2. A map showing the locations of sampling collection sites

3. The molecular identification using the *16S rRNA* gene

The amplified DNA results of the *16S rRNA* gene for the isolated algae showed positive results using the polymerase chain reaction (PCR) technique. Upon examination of the agarose gel under UV light, it was observed that the size of the gene produced by electrophoresis migration ranged between 700 and 800 bp. A single band appeared for each sample as compared to the DNA ladder. This indicates that all the samples studied possess this gene, as illustrated in Fig. (3).

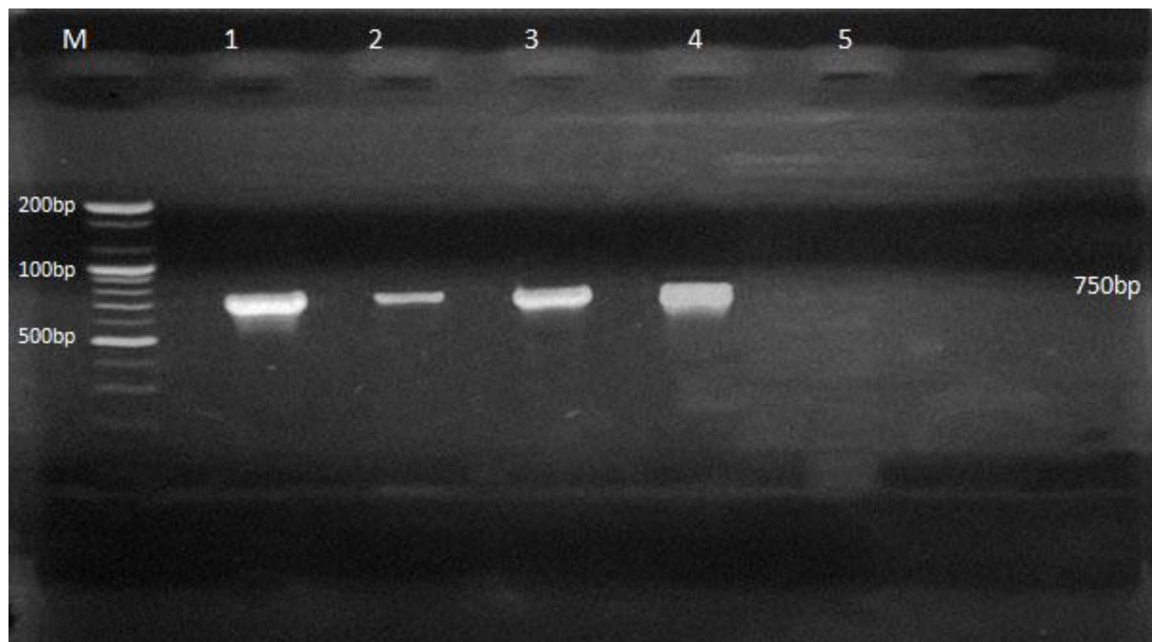

Fig. 3. Electrophoresis results of blue-green alga species by PCR amplification of *16S rRNA* using primer pairs 27F1/781R1 M: Ladder 1- *Cy. aponinum* 2- *L. halophila* 3- *Gl. calcarea* 4- *Ch. Cubana* 5- Control

4. The molecular identification of algal

The results of molecular identification based on nucleotide sequencing analysis using the *16S rRNA* gene diagnostic region for the five species of algae show a similarity between the local and global strains and those globally registered in GenBank. In the mentioned Table (2) and (Fig. 4), a significant similarity is observed between the registered strain *Cy. aponinum* NR_102443.1 and the studied strain number (1) *Cy. aponinum* OR269463 isolated from the Al-Ashar River, with a 99.43% match in their nucleotide sequences. The studied strain number (2), *L. halophile* OR269464 shows a 99.27% match the globally registered strain *L. halophila* OL310629.1. Furthermore, the studied strain number (3), *Gl. calcarea* OR269466 isolated from the Khandaq River shows a 99.58% match with the registered strain *Gloeocapsa* sp. MG822739.1 A significant similarity is also observed between the strain *Ch. cubana* MH208396.1 and the studied strain number (4), *Ch. cubana* OR269467, with a 98.29% match in their nucleotide sequences. The isolated strains of algae registered in the GenBank and alignment are performed for the *16S rRNA* gene sequences between the reference species and the sequences of the current study samples.

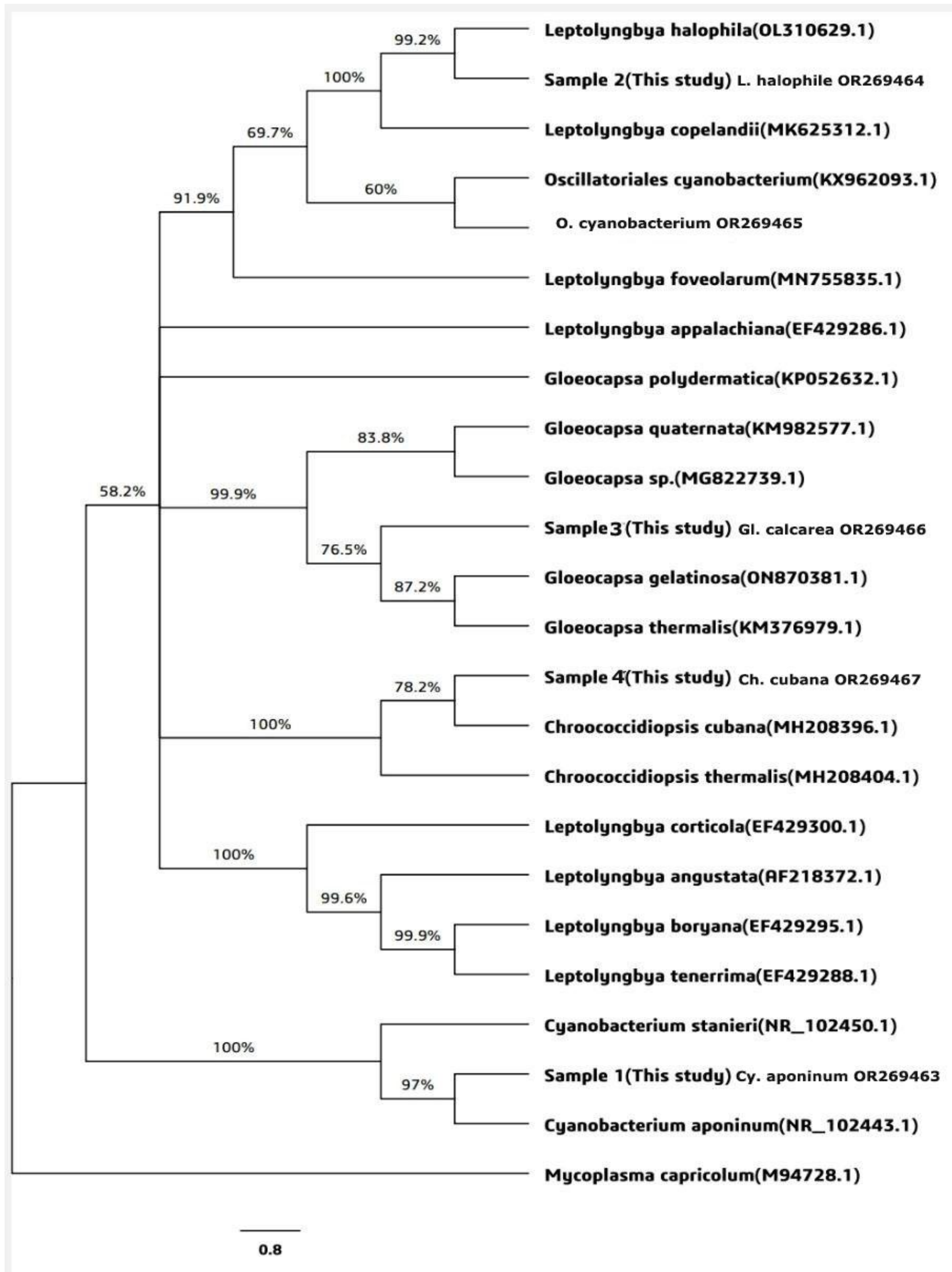

Fig. 4. The evolutionary tree by using the Neighbor-Joining method through molecular evolutionary genetics analysis Version 11. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are displayed next to the branches.

Table 2. Sequence homology between the *16S rRNA* gene sequences for the species of cyanobacterial by using *16S rRNA* sequences from the NCBI GenBank database

no	strain	Accession no.	ID of nearest match; Accession no.	%ID
1	*Cy. aponinum*	OR269463	*Cy. aponinum* NR_102443.1	99.43%
2	*L. halophile*	OR269464	*L. halophile* OL310629.1	99.27%
3	*Gl. calcarea*	OR269466	*Gloeocapsa sp*. MG822739.1	99.58%
4	*Ch. cubana*	OR269467	*Ch. cubana* MH208396.1	98.29%

DISCUSSION

Morphological identification

Blue-green algae have recently gained significant attention as one of the most groups extensively studied among aquatic organisms due to the increasing occurrence of harmful algal blooms affecting other organisms (**Sandrini-Neto *et al*., 2016; Sha *et al*., 2021**).

To shed light on previously unisolated species of algal from aquatic habitats in Iraq. The current study focused on isolating, characterizing, purifying, and propagating four species belonging to four genera. This supplied strong evidence for the environmental diversity of blue-green algae in the Iraqi aquatic environment. Four of these species were recorded for the first time in Iraq, compared to previous studies and the checklist of identified algae in Iraq by **Maulood *et al*. (2013)**.

These species include the algal *Ch. Cubana*, *Cy. aponinum* and *L. halophila*,. The study also included the isolation, characterization, purification, and cultivation of the blue-green alga *Gl. calcarea*, as cited in **Maulood *et al*. (2013)**, without details about its isolation or characteristics. Therefore, the current study is locally the first of its kind that isolates these four species of algae. Numerous earlier local studies mainly focused on isolating known algal species, especially the algal *M. aeruginosa*, such as the studies of **Al-Rekaby (2002)**, **Al-Hilfi (2007)** and **AL-Shaheen (2011)**.

The studies of **Aubaeed (2017)** and **Al-Sultan (2007)** agree with the present study concerning that the Iraqi environment is a suitable climate for the growth of different and new species of blue-green algae and the possibility of laboratory isolation, identification, purification and cultivation. The study of **Al-Sultan (2007)** isolated and identified four species of blue-green algae from the water bodies in the Basrah Governorate: *Calothrix parietina*, *Microcystis aeruginosa*, *Hapalosiphon Welwitschii*, in addition to the species *Microcystis flos-aquae*, which was locally recorded for the first time. The study of **Aubaeed (2017)** included the isolation, identification, purification and cultivation of eight species of blue-green algae from water bodies in the Basrah

Governorate, such as the rivers of Shatt al-Arab, Al-Ashar and Al-Khandaq. These species are *Merismopedia glauca*, *Microcystis flos-aque*, *Nostoc commune*, and *Oscillatoria pseudogeminata*, four of which were locally recorded for the first time, including *Lyngbya rubida*, *Pseudanabaena limnetica*, *Phormidium laysanense*, and *Stigonema informe*.

The apparition of new species of algal in the aquatic environment of Basrah Governorate could be the result of several factors, and among those contributing factors are climate changes that affect water temperatures, salinity levels, and the system of river currents, as these changes may create new environments or change conditions. Climatic conditions of aquatic areas allow new species of algae to emerge. Likewise, new species of algae may be transmitted through polluted water or commercial ships and containers from one region to another in what is known as biological invasions in the area. Environmental pollution resulting from industrial, agricultural and domestic waste also plays a role in contributing to change the water properties and create a favorable environment for some new species of algae.

Molecular identification

The results of the current study confirmed the identification of certain species by comparing them with reference strains in the GenBank database. The strain *Cy. aponinum* was molecularly identified, showing a 99.43 matching percentage with the reference strain of *Cy. aponinum* NR_102443.1. This finding is consistent with the studies conducted by **Meng *et al.* (2018)** and **Celikoglu *et al.* (2022)**, which described *Cy. aponinum* isolated from water through *16S rRNA* gene sequencing. Sequence analysis using the BLAST program showed significant similarity and alignment with *Cy. aponinum* strains in the USA (CP003947) and India (KM275589), as other *Cy. aponinum* strains in the GenBank database.

The algal *L. halophile* showed a similarity of up to 99.27% with the reference strain of *L. halophile* OL310629.1. This finding coincides with the results of **Pramanik and Mukherjee (2019)** and **Tiwari *et al.* (2020)**. In these studies, ten isolates of *16S rRNA* gene amplification were performed using PCR, and the results indicated that they belong to Leptolyngbya sp.

The species *Gl. calcarea* showed a similarity percentage of up to 99.58 to reference strains, including *Gloeocapsa* sp. MG822739.1. It is important to note that all the strains recorded in the GenBank have been identified as the genus *Gloeocapsa* without specifying the species *Gl. calcarea* as the strain *Gloeocapsa* sp. PKUAC-GDTS1-6 Accession no. MG822738 in Poland and the strain *Gloeocapsa* sp. PCC 7428 Accession no. CP003646 in the USA. Therefore, this study represents the first record of determining

the nucleotide sequence of the Gl. calcarea species using the S16 rRNA gene in the GenBank, based on the morphological characteristics that match the description of *Gl. calcarea* according to the study of **Prescott (1975)**.

The species *Ch. cubana* showed a similarity of 98.29% to the reference strains *Ch. cubana* MH208396.1 and MK484708. This finding aligns with a study conducted by **Will *et al.* (2019)**, where they isolated five species of blue-green algae in Germany. The molecular characterization was accurately determined using *16S rRNA* gene sequencing and implementing a comparison with those in the GenBank. The amplification, sequencing, and comparison of the *16S rRNA* gene revealed that one of the isolated species belongs to the *Ch. cubana* species.

The identification of blue-green algae through the *16S rRNA* gene is a widely used technique for taxonomic classification and phylogenetic analysis. This gene region is highly conserved among bacteria and cyanobacteria, making it a suitable target for species identification and differentiation. It is important to note that the results presented in this study provide evidence of the presence of the *16S rRNA* gene in the studied samples, indicating the potential presence of blue-green algae; however, further analysis and sequencing of the amplified DNA fragments would be necessary for a more detailed and accurate identification of the blue-green algae species present in the samples through sequence analysis and comparison of the results with the GenBank on the NCBI website, and this is indeed what was done in the current study. Moreover, this molecular identification approach using the *16S rRNA* gene can contribute to comprehension of the genetic diversity and distribution of blue-green algae in the studied environment. Comparing the obtained results with existing databases and previous studies can help establish connections and identify similarities or differences in the identified blue-green algal species. This, in turn, may have implications for ecological assessments, water quality monitoring, and potential harmful algal bloom investigations in the studied area. In summary, the use of PCR amplification and agarose gel electrophoresis targeting the *16S rRNA* gene region has provided strong evidence for the presence and classification of blue-green algae in the studied samples.

CONCLUSION

This study effectively employed morphological and molecular identification of four cyanobacterial species; two of them were determined as a first record locally, and the species, *Gloeocapsa calcarea* was recorded for the first time in the whole world in the GenBank, which was isolated from several water bodies in Basrah Governorate, southern Iraq.

REFERENCES

AL-Hilfi, W.A. The Study of some biological effects for the toxin produced by alga *Microcystis aeruginosa* Kutz. (Cyanobacteria).Thesis.College of Sciences.University of Basrah; 2007 (Iraq). 58 pp.

AL-Rekaby, W.J. Ecophysiological Study for *Microcystis aeruginosa* Kuetz. Thesis.College of Sciences.University of Babylon; 2002 (Iraq). 84 pp.

Al-Saad, H. T; Al-Timari, A. A. K., Douabul, A. A. Z; Hantoush, A. A; Nasir, A. M. and Saleh, S. M. (2017). Status of oil pollution in water and sediment from Shatt Al-Arab Estuary and North-West Arabian Gulf. *Mesopotamian Journal of Marine Sciences*, *32*(1): 9-18.

AL-Shaheen, M. A. G. (2011). Removal of Microcystins from Aqueous Cells Extract of some toxic Cyanobacterial species by using activated carbon. *Marsh Bulletin*, *6*(1): 82-97.

AL-Sultan, EY. Bioassay of some toxic microalgae.Thesis.College of Education for Puresciences.University of Basrah; 2007 (Iraq) 129 pp.

Al-Sultan, E. Y. A. and Hatem, M. T. (2018). Isolate and cultivate three species of blue-green algae from soil in southern Iraq and study the effect of purified microcystins from alga Oscillatoria Pseudogeminata on seed germination of tomato plant Lycopersicon Esculentum. *Journal of Biology, Agriculture and Healthcare*, *8*(16): 27-36.

Alwaeli, A. and Athbi, A. M. (2021). New records of algal species from the Shatt Al-Arab River, Southern Iraq. *Mesopotamian J Mar Sci*, *36*(1): 79-87.

Al-Sultan, A. and Hatem, M. T. (2019). Toxic Effects of Purified Microcystins from Soil Blue-Green Alga Oscillatoria pseudogeminata on Tomato Plant Lycopersicon esculentum. *Baghdad Science Journal*, *16*(1): ١٦٩-١٧٧.

Aubaeed, M. A. The capability of some blue-green algae isolated from some water bodies in AL-Basrah Governorate / Southern Iraq to the production of toxins.Thesis.College of Education for Pure Sciences.University of Basrah; 2017 (Iraq). 113 pp.

Blaha, L.; Babica, P.; and Marsálek, B. (2009). Toxins produced in cyanobacterial water blooms-toxicity and risks. *Interdisciplinary toxicology*, 2(2): 36 -41.

Blahova, L.; Babica, P.; Adamovský, O; Kohoutek, J.; Maršálek, B.; and Bláha, L. (2008). Analyses of cyanobacterial toxins (microcystins, cylindrospermopsin) in the reservoirs of the Czech Republic and evaluation of health risks. *Environmental Chemistry Letters*, *6*: 223-227.

Celikoğlu, E.; Cankılıç, M. Y. and İdil, Ö. (2022). Isolation and Identification of Tersakan Stream (Amasya, Suluova TÜRKİYE) Cyanobacteria and Investigation of the Presence of the Microcystin-LR. *Genetics of Aquatic Organisms*, *7(1)*: GA538

Chittora, D.; Meena, M; Barupal, T.; Swapnil, P. and Sharma, K. (2020). Cyanobacteria as a source of biofertilizers for sustainable agriculture. *Biochemistry and biophysics reports*, *22*: 100737.

Clarridge, J. E. (2004). Impact of *16S rRNA* gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clinical microbiology reviews, 17*(4): 840-862.

Desikachary, T.U. Cyanophyta. Indian Council of Agricultural Search, New Delhi; 1959, 700.

Gdemi, H. D. and Awad, E. Y. (2022). The effect of some environmental factors on the production of hepatotoxins (Microcystin) in the Shatt al-Arab waters in Basrah Governorate southern Iraq. *Eurasian Research Bulletin*, *14*: 103-117.

Jenkins, C.; Ling, C. L.; Ciesielczuk, H. L.; Lockwood, J.; Hopkins, S.; McHugh, T. D. and Kibbler, C. C. (2012). Detection and identification of bacteria in clinical samples by *16S rRNA* gene sequencing: comparison of two different approaches in clinical practice. *Journal of Medical Microbiology, 61(4)*: 483-488.

Jungblut, A. D.; Hawes, I.; Mountfort, D.; Hitzfeld, B.; Dietrich, D. R.; Burns, B. P. and Neilan, B. A. (2005). Diversity within cyanobacterial mat communities in variable salinity meltwater ponds of McMurdo Ice Shelf, Antarctica. Environmental microbiology, 7(4): 519-529.

Kim, J. H; Choi, W.; Jeon, S. M.; Kim, T.; Park, A.; Kim, J.; Heo, S. J.; Oh, C.; Shim, W. B. and Kang, D. H. (2015). Isolation and characterization of Leptolyngbya sp. KIOST- 1, a basophilic and euryhaline filamentous cyanobacterium from an open paddle- wheel raceway Arthrospira culture pond in Korea. *Journal of Applied Microbiology*, *119*(6): 1597-1612.

Matsumoto, T. and Sugano, M. (2013). *16S rRNA* gene sequence analysis for bacterial identification in the clinical laboratory. Rinsho byori. *The Japanese journal of clinical pathology*, *61(12)*: 1107-1115.

Maulood, B. K.; Hassan, F. M.; Al-Lami, A. A; Toma, J. J.; and Ismail, A. M. (2013). Checklist of algal flora in Iraq. *Ministry of Environment, Baghdad*, 94.

Meng, F.; Cui, H.; Wang, Y. and Li, X. (2018). Responses of a newly isolated *Cyanobacterium aponinum* strain to temperature, pH, CO 2 and light quality. *Journal of Applied Phycology*, *30*: 1525-1532.

Mohammad, B. M.; Ali. M.A.; Hadi. M.; Nematollah. J.; Javad. A. and Houshang. H. (2014). Evaluation of Karun River water quality Scenarios using simulation model results. *International Journal of Advanced Biological and Biomedical Research, vol. 2(2)*: 339- 358.

Moro, I.; Rascio, N.; La Rocca, N.; Di Bella, M. and Andreoli, C. (2007). *Cyanobacterium aponinum*, a new Cyanoprokaryote from the microbial mat of Euganean thermal springs (Padua, Italy). *Algological Studies*, *123*(1): 1-15.

Nezbrytska, I.; Usenko, O; Konovets, I.; Leontieva, T.; Abramiuk, I.; Goncharova, M. and Bilous, O. (2022). Potential use of aquatic vascular plants to control cyanobacterial blooms: A review. *Water*, *14*(11): 1727.

Pramanik, P. J. and Mukherjee, J. O. Y. D. E. E. P. (2019). Euryhalinema mangrove gen. nov; sp. nov. and Leptoelongatus litoralis gen. nov; sp. nov.(Leptolyngbyaceae) isolated from an Indian mangrove forest. *Phytotaxa*, *422*(1): 058-074.

Prescott, G .W. Alage of the western Great Lake area. 6th ed; William C. Brown Co. Publishers. Dubuque, Towa;1975, 977.

Sandrini-Neto, L.; Pereira, L.; Martins, C. C.; de Assis, H. C. S.; Camus, L. and Lana, P. C. (2016). Antioxidant responses in estuarine invertebrates exposed to repeated oil spills: effects of frequency and dosage in a field manipulative experiment. *Aquatic Toxicology*, *177*: 237-249.

Schmidt, J. R.; Wilhelm, S. W. and Boyer, G. L. (2014). The fate of microcystins in the environment and challenges for monitoring. *Toxins*, *6*(12): 3354-3387.

Sha, J.; Xiong, H.; Li, C.; Lu, Z.; Zhang, J.; Zhong, H.; ... and Yan, B. (2021). Harmful algal blooms and their eco-environmental indication. *Chemosphere*, *274*: 129912.

Stanojković, A.; Skoupý, S.; Hašler, P.; Poulíčková, A. and Dvořák, P. (2022). Geography and climate drive the distribution and diversification of the cosmopolitan cyanobacterium Microcoleus (Oscillatoriales, Cyanobacteria). *European Journal of Phycology*, *57*(4): 396-405.

Stein, JR. Handbook of the phycological method. Cambridge University Press. Cambridge; 1975, 445 pp.

Tiwari, O. N.; Bhunia, B.; Muthuraj, M.; Bandyopadhyay, T. K.; Ghosh, D. and Gopikrishna, K. (2020). Optimization of process parameters on lipid biosynthesis for sustainable biodiesel production and evaluation of its fuel characteristics. *Fuel*, *269*: 117471.

Vanlalsangi, R. and Lalmuanpuii, R. Cyanobacteria-derived bioactive compounds, A beneficial aspects. In: *Expanding Horizon of Cyanobacterial Biology*. Academic Press; 2022. 195- 208.

Whitton, B. A. and Potts, M. Introduction to the cyanobacteria. In: *Ecology of cyanobacteria II: their diversity in space and time*. Dordrecht: Springer Netherlands; 2012. 1-13.

Wiedeman, V. E; Walne, P. L. and Trainor, F. R. (1964). A new technique for obtaining axenic cultures of algae. *Canadian journal of botany*, *42*(7): 958-959.

Wiegand, C. and Pflugmacher, S. (2005). Ecotoxicological effects of selected cyanobacterial secondary metabolites a short review. *Toxicology and applied pharmacology*, *203*(3): 201-218.

Will, S. E.; Henke, P; Boedeker, C.; Huang, S.; Brinkmann, H.; Rohde, M.; ... and Petersen, J. (2019). Day and night: metabolic profiles and evolutionary relationships of six axenic non-marine cyanobacteria. *Genome Biology and Evolution*, *11*(1): 270-294.

Yamada, T. Cyanobacteria and Aigae. In: Recombinant microbes for industrial and agricultural applications. 1st . CRC Press; 2020, 701-712.

Zare, M.; Bahador, N. and Baserisalehi, M. (2020). Phylogenetic analysis of isolated Phormidium sp. and *Cyanobacterium aponinum* from Kor River. *Iranian Journal of Fisheries Sciences*, *19*(5): 2649-2659.