Acta Oceanol. Sin., 2018, Vol. 37, No. 10, P. 4–10
DOI: 10.1007/s13131-018-1297-y
http://www.hyxb.org.cn
E-mail: hyxbe@263.net

Morphology, ultrastructure and phylogeny of *Cyanothece* sp. (Cyanobacteriaceae: Cyanophyceae) isolated from the eastern Indian Ocean

ZHANG Xiaodong[1, 2], YANG Shuang[1, 2], SUN Jun[1, 2*], WU Chao[3], WANG Jing[1, 2], ZHANG Guicheng[1, 2], DING Changling[1, 2]

[1] Tianjin Key Laboratory of Marine Resources and Chemistry, Tianjin University of Science and Technology, Tianjin 300457, China

[2] Research Centre for Indian Ocean Ecosystem, Tianjin University of Science and Technology, Tianjin 300457, China

[3] Institute of Marine Science and Technology, Shandong University, Jinan 250110, China

Received 19 September 2017; accepted 23 June 2018

Abstract

One strain of unicellular greenish algae embedded by mucilage was successfully isolated from equatorial area in the Indian Ocean. Microscopic observation, ultrastructure features and genetic identification confirmed that the strain was closely related to *Cyanothece* sp., which was a cyanobacteria species with great ecological significance. Cells were solitary with oval or bacilliform shapes. Diameters of this strain were relatively small, ranging from 2.5 to 6.5 μm on average. Ultrastructure of cells was simple. Thylakoids were arranged parietal and keritomized content were observed in the thylakoid region. Various electron-transparent granules with low electron-dense region as well as cyanophycin or glycogen granules-like organelle and carbonxysomes were also observed. For pigment composition, the dominant pigments were chlorophyll *a*, β-Carotene, Zeaxanthin and an unknown pigment, contributing 23.8%, 26.1%, 14.7% and 15.7% to total pigments respectively. The phylogenetic analysis of 16S rRNA gene and *nif*H gene confirmed that Strain EIO409 was closely related with *Cyanothece* sp. .

Key words: *Cyanothece*, cyanobacteria, morphology, 16S rRNA gene, *nif*H gene, Indian Ocean

Citation: Zhang Xiaodong, Yang Shuang, Sun Jun, Wu Chao, Wang Jing, Zhang Guicheng, Ding Changling. 2018. Morphology, ultrastructure and phylogeny of *Cyanothece* sp. (Cyanobacteriaceae: Cyanophyceae) isolated from the eastern Indian Ocean. Acta Oceanologica Sinica, 37(10): 4–10, doi: 10.1007/s13131-018-1297-y

1 Introduction

The cyanobacteria are the most ancient group of algae in the world, with definite fossil remains dating back about 2 700 million years (Lee, 2008). Till now at least 1 899 taxa was described according the data from WoRMS (World Register of Marine Species, http://www.marinespecies.org/index.php) with many species processing very important ecological significance. The cyanobacteria comprise one class (Cyanobacteriaceae) and a group of genus with uncertain taxonomic position (Cyanobacteria incertae sedis). All the cyanobacteria species thrive in variable aquatic and terrestrial environments, indicating great morphological, biochemical, and metabolic diversity (Dor, 1998). Some well-known taxa, such as *Microcystis* and *Anabeana* could cause blooms in eutrophic lakes and some can even produce toxin which is lethal for the plankton, fish and even humans (Hayes et al., 2007). In the tropical and subtropical oceanic waters, species of genus *Trichodesmium* could form blooms which may play a key role in global new production and nitrogen cycle (Galloway et al., 2004).

The genus *Cyanothece* are a kind of unicellular cyanobacteria that are morphologically and ecologically diverse. Traditionally, species of *Cyanothece* were classified in the widespread genus *Synechococcus* Näg, 1849 which are very vital primary producers and play a key role in global carbon cycle (Komárek, 1976; Rippka and Cohen-Bazire, 1983; Waterbury et al., 1986). *Synechococcus* are ubiquitous in various habitats including coastal area and open ocean with morphological and ecological diversity. For some strains, multiple algae cells could be embedded by a thin mucilage layer and other members of this genus are totally unicellular. Both of these suggested that the genus *Synechococcus* needed reexamination at that time. Komárek (1976) erected the genus *Cyanothece* after studying different botanical species included in genus *Synechoccoccus* (Komárek, 1976) based on the morphological and cytological characteristics of the type species, *Cyanothece aeruginosa* (Näg). Rudi then supported this classification using molecular analysis (Rudi et al., 1997). For the type species *Cyanothece aeruginosa*, cells are surrounded by a thin mucilage layer and the shapes are oval, solitary and large, with diameters ranging from 10 to 24 μm (Komárek, 1976). Thylakoids are arranged radially and occasionally fasciculate and the thylakoid region is full of keritomized content (Komárek and Cepák, 1998); Loose structure of nucleoid, filling the entire cell volume, could be observed after DAPI staining (Cepák, 1993). According to the WoRMs Database, after several species was re-

Foundation item: The National Natural Science Foundation of China under contract Nos 41876134, 41676112, 41276124, 41406155 and 41506182; the Natural Science Foundation of Tianjin under contract No. 17JCZDJC40000; the Exploration Program of Ocean with Science and Technology of Tianjin under contract No. KJXH2013-22; the Changjiang Scholar Program of Chinese Ministry of Education of China to Jun Sun.

*Corresponding author, E-mail: phytoplankton@163.com

classified into genera *Cyanobium* and *Cyanobacterium*, the genus *Cyanothece* possesses five species: *Cyanothece aeruginosa* (Nägeli) Komárek, 1976, *Cyanothece halobia* Roussomoustakaki and Anagnostidis, 1991, *Cyanothece lineata* Komárek and Komárková-Legnerová, 2002, *Cyanothece major* (Schröter) Komárek, 1976, and *Cyanothece shiloi* (Campbell and Golubic) Komárek and Anagnostidis, 1995.

The Indian Ocean is the third largest ocean in the world and strong stratification induced by monsoon suppresses up-welling and mixing of the deep waters, making it a typical oligotrophic area (Fine et al., 2008; Kumar et al., 2009; Rixen et al., 2009). Within this oligotrophic area region, nitrogen fixation plays a key role in the biochemical cycle and recent discoveries proved the great contributions of unicellular cyanobacteria to nitrogen fixation in marine environments. Studies over the last decade have established members of this genus to be important components of the marine ecosystem, and contribute significantly to the nitrogen and carbon cycling (Bandyopadhyay et al., 2011). Various strains of the *Cyanothece* have been proved to possess the function of nitrogen fixation (Reddy et al., 1993; Welsh et al., 2008; Bandyopadhyay et al., 2011; Park et al., 2014) and such character make *Cyanothece* particularly attractive for studying the interaction between nitrogen fixation and photosynthesis (Reddy et al., 1993).

In May 2015, one strain of greenish algae was successfully isolated from equatorial area of the Indian Ocean. Numerous cells were embedded by mucilage and formed a large colony. No free single cell was observed in the culture. Microscopic observation, ultrastructure features and genetic identification (16S rRNA gene and *nif*H gene) confirmed that the strain was closely related to *Cyanothece* sp. .

2 Materials and methods

2.1 *Isolation and culture*

Fifty mililiters water sample was collected from surface water of Sta. 1409 (0°0.013′N, 88°0.026′E) at equatorial of the eastern Indian Ocean. The water was added with enriched nutrients in an autoclaved transparent flask and incubated under natural light in room temperature. After brought back to the lab, the water was incubated under a 12 h:12 h light: dark cycle at 2 500 lx with YZ21RR16/G cool-white fluorescent light tubes (Sunshine 117 Company, China) at 25°C and the algae was grown in the f/2 medium (Guillard and Ryther, 1962). Single cell was isolated several weeks later when the greenish cells aggregation was observed. The isolated strain was then cultured in the f/2 medium.

2.2 *Molecular identification and phylogeny analysis*

Genomic DNA was extracted with a plant DNA extraction kit (Sangon, China), following the manufacturer's protocol. For 16S rRNA gene amplification, the primers (CYA106F and CYA781Ra/b) and PCR programs were following Nübel et al. (1997). For *nif*H gene, two round nest-PCR was applied followed Zehr et al. (1998) with the primers *nif*H3/4 and *nif*H1/2 as the first and second round PCR respectively. The PCR products were purified using a gel purification kit (Axygen, USA) and the purified products were sequenced directly in the Beijing Genomics Institute by the same pairs of PCR primers. The sequence was blasted in the Genebank to confirm the closest identity. Alignment of sequences was conducted and maximum likelihood phylogenic tree was constructed by MEGA Version 5 (Tamura et al., 2011).

2.3 *Morphology observation*

The vegetative cells aggregation were collected and fixed with 2.5% glutaraldehyde for more than 24 h. The fixed cells were washed with 0.1 mol/L PBS buffer (pH 7.4) twice for 5 min. The cell aggregation was post-fixed with 1% osmium tetroxide solution (OsO_4) overnight after being rinsed in 0.1 mol/L PBS buffer (pH 7.4). Dehydration of fixed cells was conducted through a gradual ethanol series (10%, 30%, 50%, 70%, 90% and 100%, 10 min at each step) followed by 100% acetone. Spurr's resin (Spurr,

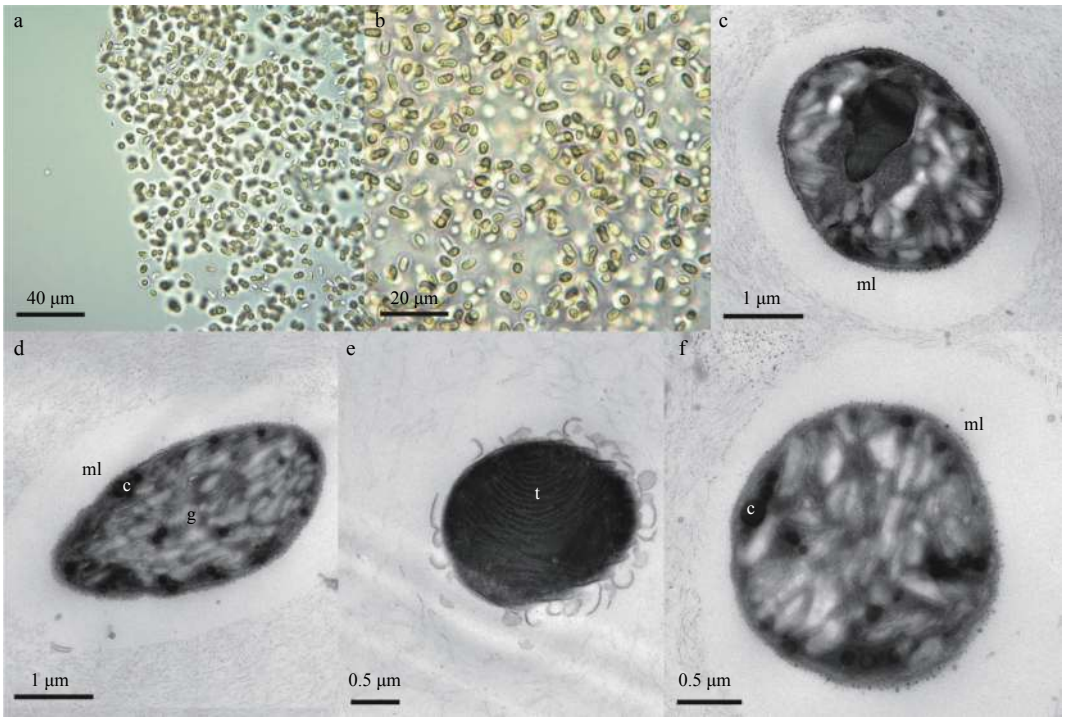

Fig. 1. Morphology and ultrastructure of the *Cyanothece* sp. (Strain EIO409) isolated from the eastern Indian Ocean. Abbreviation c represents cyanophycin or carbonxysomes, g electron-transparent granules, t thylakoids, and ml mucilage layer.

1969) was applied to embed the dehydrated cells which was then polymerized and sectioned. The ultrathin sections were stained with uranyl acetate and lead citrate, and examined under a HITACHI 7000 Electron Microscope (Hitachi, Japan).

2.4 *Pigments composition analysis*

The pigments composition was measured by high-performance liquid chromatography (HPLC, Agilent Technologies, 1260 Infinity, USA) following the classic method (Zapata et al., 2000). The cells were collected by filtering through 0.7 μm GF/F glass fiber membranes under a low pressure. The samples were then frozen at –80°C immediately until further analysis. Pigments were extracted in 3 mL pure methanol and kept at –20°C for one hour after ultrasound treated for 30 s with ice-bath. The extraction was filtered through 0.22 μm nylon membrane to remove the detritus and mixed with equal volume of tetrabutyl ammonium acetate before analysis. Pigments were then identified by retention time and absorption spectra. Quantification was performed with standards from DHI Water and Environment, Hørsholm, Denmark.

3 Results

3.1 *Isolation, cultivation and mophology observation*

In this study, one strain of green color algae forming colony was successfully isolated from surface water of Sta. 1409 located in equatorial area in the Indian Ocean and it was named as Strain EIO409. Cells of the strain were solitary with oval or bacilliform shapes and they formed non-motile aggregation embedded in mucilage layer (Figs 1a, b, c, d, f). Size of this strain was ratively small, ranging from 2.5 to 6.5 μm on average of measurement of

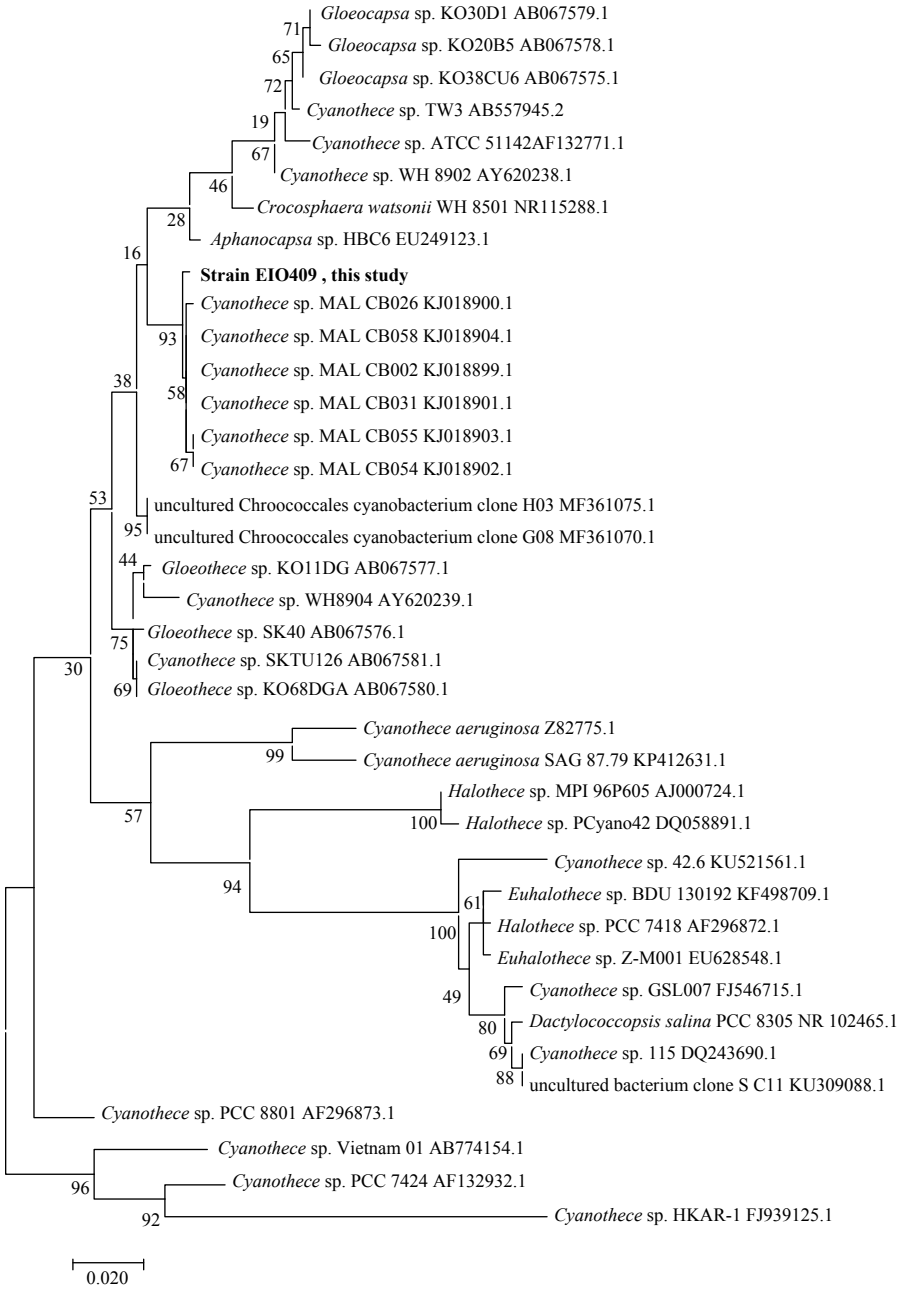

Fig. 2. Maximum likelihood phylogenetic tree showing the phylogenetic associations of *Cyanothece* sp. strain EIO409 (highlighted in bold) with closely related sequences based on the 16S rRNA gene sequences. Bootstrap numbers were based on 1 000 replicated trees.

more than 30 cells. Numerous short thylakoids were arranged parietal and keritomized content were observed in the thylakoid region (Fig.1e). Thin fibers that emanated from the outer membrane perpendicularly were found occasionally (Fig. 1e). Multiple electron-transparent granules with low electron-dense region were arranged in some cells (Figs 1c, d, f). Besides, cyanophycin or glycogen granules-like organelle and carbonxysomes were also observed (Figs 1d, f).

3.2 *Phylogenetic analysis*

Maximum likelihood method was applied to construct the phylogenetic tree based on 16S rRNA gene and *nif*H gene (Figs 2 and 3). The phylogenetic analysis of both 16S rRNA gene and *nif*H gene confirmed that the isolated Strain EIO409 was closely related with *Cyanothece* sp.. However, it is difficult to figure out which species it is. Most closely related strains were isolated from Taean, Korea with the similarity of 99%, suggesting this strain with this genotype can adapt completely different niches. Within this phylogenetic tree of 16S rRNA gene, two clusters were separated clearly. One cluster is the *Halothece* cluster (Cluster 3) comprised of strains which could grow at higher salinity (>3%). The other cluster is comprised of fresh water strains cluster (Cluster 2). Our strain of *Cyanothece* was grouped in a subcluster containing *Gloeocapsa* and *Aphanocapsa* in the Cluster 3 and could be separated from the *Euhalothece* subcluster and *Halothece* subcluster, indicating the far relationship with *Euhalothece*, the newly established genus (Mogany et al., 2018). However in the phylogenetic tree of *nif*H gene, the relationship between *Cyanothece* and *Gloeocapsa* is not as close as that in 16S rRNA gene. Due to the limitation of *nif*H gene sequences in the GeneBank, the *nif*H gene provided a relatively low resolution to classify the strain.

3.3 *Pigments composition*

From the HPLC measurement, 14 kinds of pigments (including unknown pigments) were detected. The recognized pigments were shown in Fig. 4. The dominant pigments were chlorophyll *a*, β-Carotene, zeaxanthin and an unknown pigment, contributing 23.8%, 26.1%, 14.7% and 15.7% to total pigments respectively. Violaxanthin was also detected in this strain, with a low concentration. Some unknown pigments with different peak sizes were also detected. No chlorophyll other than chlorophyll *a* and chlorophyll *a* derivatives was found.

4 Discussion

Komárek (1976) first established the genus *Cyanothece* which was separated from a traditional genus *Synechococcus* NÄG, 1849 based on the form of cells, structure of cell content and biological characteristics of the type species *C. aeruginosa*. The algae strains possessing large, oval and unicellular cells with "keritomized" cell content were removed into the genus *Cyanothece*. The classification was supported lately by the following ultrastructur-

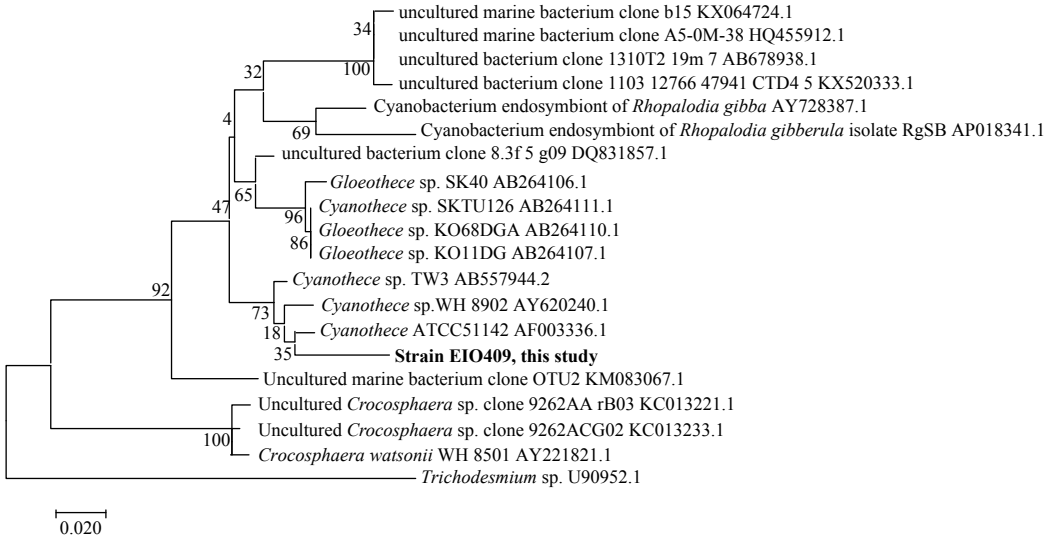

Fig. 3. Maximum likelihood phylogenetic tree showing the phylogenetic associations of *Cyanothece* sp. strain EIO409 (highlighted in bold) with closely related sequences based on the *nif*H gene sequences. Bootstrap numbers were based on 1 000 replicated trees.

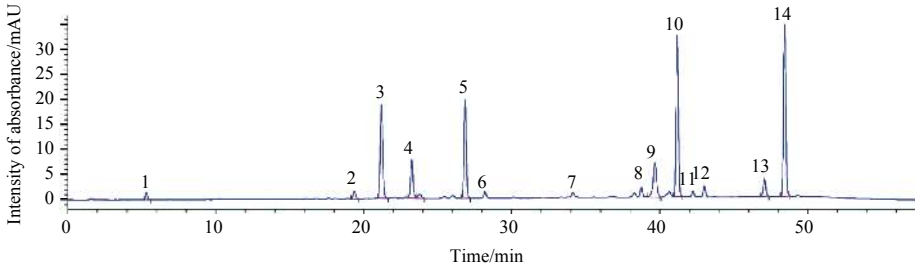

Fig. 4. Chromatograms of HPLC pigment analysis for *Cyanothece* Strain EIO409. Peak 1 represents unknown pigment, 2 violaxanthin chlorophyll *a* derivative, 3 unknown pigment, 4 alloxanthin, 5 zeaxanthin, 6–9 unknown pigment, 10 chlorophyll *a*, 11 unknown pigment, 12 and 13 unknown pigment, and 14 β-carotene.

Table 1. Comparison of characteristics (morphology and ultrastructural features) of Strain EIO409 and related species/strains

	Strain EIO409	*Cyanothece* Kom, 1976	*C. aeruginosa*	*C. halobia*	*Cyanothece* 115	*Halothece* (unvalid)	*Halothece* PCC 7418	*Euhalothece* sp. nov.	*Euhalothece* sp. Z-M001
Isolation sites	Equatorial Indian Ocean	marshes with water plants, peaty bogs	acidic marshes and peaty bogs	halophilic saltwork	coastal water of China	shallow heliothermal hypersaline ponds	Solar Lake, Sinai, Egypt	Lake St Lucia, South Africa	Soda Lake Magadi
Tolerance	halotolerant	–	–	halotolerant	marine	halotolerant	halotolerant	halophile	extreme natronophile
Salinity	34	–	–	–	1–5	–	3–6	6–12	3–10
Temperature/°C	29	–	–	–	28	–	25–37	25–45	35
Gas vesicles	–	–	–	no	no	–	yes	yes	–
Thylakoid organisation	parietally arranged	numerous, radially oriented	radially oriented	large intrathylakoidal spaces	–	irregular arrangement	numberous, radially oriented thylakoids	irregular arrangement	densely packed at the cell periphery
Cell morphology	oval or bacilliform shapes	oval to widely oval	oval	wide oval	rod shaped	oval	rod-round	oval-cylindrical	round
Cell size/μm (limits)	2.5–6.5	(7.2)12–40(100)×(6)7–25(76)	(9)21–40(50)×7(20)–25(36)	7–12(15)×(6)7–12(13.5)	2.5–5×7.5–10	2.5–12.6×2.6–7.8	5–7.4	8–14	2.7–4
Cell division	binary fission	–	single plane	–	binary fission in a single plane		single plane	binary fission divides into two identical cells	divides into two daughter cells
Mucilage layer	thick	narrow	narrow	–	–	without mucilaginous envelopes	–	thin, fine slime and loose layer around them	–
Reference	this study	Margheri et al. (2008)	Komárek et al. (2004), Komárek and Cepák (1998)	Komárek et al. (2004)	Zhang et al. (2007)	Margheri et al. (2008)	Cohen et al. (1975)	Mogany et al. (2018)	Mikhodyuk et al. (2008)

al and molecular analyses (Rippka and Cohen-Bazire, 1983; Komárek and Cepák, 1998). Rippka and Cohen-Bazire (1983) separated two other genera *Cyanobacterium* and *Cyanobium* closely related with *Cyanothece* from *Synechococcus* mainly based on biochemical criteria. The classification was further supported by cytological and molecular analyses (Rudi et al., 1997; Komárek et al., 1999; Castenholz et al., 2001). Although a lot of gene markers including the rRNA sequences and some functional genes like *rpoC*1 gene and *nif*H gene were applied in the phylogenetic analysis of cyanobacteria, the sequences of *Cyanothece* in GeneBank are still limited, resulting in some difficulties in phylogenetic analysis (Mogany et al., 2018). In this study, it is difficult to identify this strain to the species level even with the morphologic, ultrastructural as well as molecular evidences.

Species in the genus *Cyanothece* are morphologically and ecologically different with each other (Komárek and Cepák, 1998). *Cyanothece* species inhabit multiple ecological environment including mashes, peaty bogs, clear lakes, mountain soils (Komárek, 1976), intertidal sands (Reddy et al., 1993), coastal port and rice field (Bandyopadhyay et al., 2011), indicating great diversity of physiological characteristics. Besides, this genus is highly diverse in shape, size and growth rate (Rippka and Cohen-Bazire, 1983; Welsh et al., 2008; Bandyopadhyay et al., 2011). Some strains of *Cyanothece* possess lengthwise thylakoid arrangement and others have radical one (Komárek and Cepák, 1998). A corresponding cell structure to *Cyanothece aeruginosa* was found in the ecologically different *Cyanothece halobia* (Roussomoustakaki and Anagnostidis, 1991; Cepák, 1993), for which large intrathylakoidal spaces are particularly characteristic. The size of intrathylakoidal spaces, number of thylakoids and limits in cell dimensions are the main cytological differential features between these two species. The other characteristic *Cyanothece* species, that differ from each other mainly in quantitative features in cell morphology (but are still not studied by EM procedures), are *C. maior* (Schröter) Kom, 1976 and *C. shiloi* (Campb and Golubic) Kom and Anagn, 1995. All these species evidently belonging to genus *Cyanothece* differ from each other in cell shape and cell size, in modifications in several intracellular structures, and in ecological characters.

However, the described five species could not represent the diversity of this species well. Previous studies divided *Cyanothece* species and strains into three clusters based on their morphological and ecological characteristics including cell size and salt tolerance (Garcia-Pichel et al., 1998; Garrity et al., 2001; Mogany et al., 2018). Within these three clusters, Clusters 1 and 2 comprises of freshwater strains, whereas strains of Cluster 3 “Halothece” inhabit in the environment with salinity higher than 3%. The Cluster 3 Halothece could also be divided into two subclusters: *Euhalothece* and *Halothece*. Mogany et al. (2018) reported a strain isolated from Lake St. Lucia, the largest hypersaline estuarine lake in Africa and established it as a new genus and species *Euhalothece* sp. after examining the morphological characteristics features, physiological data, pigment compositions as well as the genomic data. In contrast, the establishment for the genus *Halothece* is still unvlid due to the absence of the Latin diagnosis and description of physiological character difference (Oren, 2009; Mogany et al., 2018). However, within the subcluster *Euhalothece* possesses a high phylogenetic diversity. Up to present, *Cyanothece* is still taxonomically problematic with discrepant results from traditional morphological studies and experiment on both natural populations and cultures. Comparison of characteristics (morphology and ultrastructural features) of our strain and related species/strains was shown in Table 1. However, obviously it is difficult to distinguish the strain with other species or strains and give it a very solid taxonomic position with these morphological data. Further study about its ecological characteristics and genetic information is still needed.

Cyanothece Strain EIO409 was isolated from oligotrophic water located in equatorial area in the Indian Ocean which is important for global nitrogen cycle (Wang et al., 2017). Previous studies on some strains of *Cyanothece* proved that the cyanobacteria possess the nitrogenase gene and ability to carry out aerobic nitrogen fixation which can help *Cyanothece* to serve as diazotroph in the ocean (Reddy et al., 1993, 1996; Zehr et al., 1998; Welsh et al., 2008; Bandyopadhyay et al., 2011). With the ability of nitrogen fixing, *Cyanothece* plays a significant role in the global biochemical cycle. The cells of *Cyanothece* can create an anoxic intracellular environment at night, allowing oxygen-sensitive processes to take place in these oxygenic organisms (Bandyopadhyay et al., 2011). The morphology of our strain showed that the cells were embedded by mucilage layer which may help the cells generate the anoxic intracellular environment for the nitrogen fixing activity. However, although the *nif*H gene was detected, the nitrogen fixing activity of our strain is still unknown. Besides, due to the diverse morphological andecological characteristics of this genus, further study about the nitrogen-fixing of *Cyanothece* is needed.

References

Bandyopadhyay A, Elvitigala T, Welsh E, et al. 2011. Novel metabolic attributes of the genus *Cyanothece*, comprising a group of unicellular nitrogen-fixing cyanobacteria. mBio, 2(5): e00214–11

Castenholz R W, Wilmotte A, Herdman M, et al. 2001. Phylum BX. Cyanobacteria. In: Boone D R, Castenholz R W, Garrity G M, eds. Bergey's Manual® of Systematic Bacteriology. New York: Springer, 473–599

Cepák V. 1993. Morphology of DNA containing structures (nucleoids) as a prospective character in cyanophyte taxonomy. Journal of Phycology, 29(6): 844–852, doi: 10.1111/j.0022-3646.1993.00844.x

Cohen Y, Padan E, Shilo M. 1975. Facultative anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica*. Journal of Bacteriology, 123(3): 855–861

Dor I. 1998. A checklist of cyanophyta (cyanobacteria) of Israel and adjacent regions. Israel Journal of Plant Sciences, 46(3): 239–254, doi: 10.1080/07929978.1998.10676733

Fine R A, Smethie W M Jr, Bullister J H, et al. 2008. Decadal ventilation and mixing of Indian Ocean waters. Deep Sea Research Part I: Oceanographic Research Papers, 55(1): 20–37, doi: 10.1016/j.dsr.2007.10.002

Galloway J N, Dentener F J, Capone D G, et al. 2004. Nitrogen cycles: past, present, and future. Biogeochemistry, 70(2): 153–226, doi: 10.1007/s10533-004-0370-0

Garcia-Pichel F, Nübel U, Muyzer G. 1998. The phylogeny of unicellular, extremely halotolerant cyanobacteria. Archives of Microbiology, 169(6): 469–482, doi: 10.1007/s002030050599

Garrity G, Boon D R, Castenholz R W. 2001. Bergey's Manual of Systematic Bacteriology: Volume 1. the Archaea and the Deeply Branching and Phototrophic Bacteria. 2nd ed. New York: Springer

Guillard R R L, Ryther J H. 1962. Studies of marine planktonic diatoms: I. *Cyclotella nana* Hustedt, and *Detonula conferraceae* (Cleve) gran. Canadian Journal of Microbiology, 8(2): 229–239, doi: 10.1139/m62-029

Hayes P K, El Semary N A, Sánchez-Baracaldo P. 2007. The taxonomy of cyanobacteria: molecular insights into a difficult problem. In: Brodie J, Lewis J, eds. Unravelling the Algae: The Past, Present, and Future of Algal Systematics. Boca Raton, FL: CRC Press

Komárek J. 1976. Taxonomic review of the genera *Synechocystis* Sauv. 1892, *Synechococcus* Nag. 1849, and *Cyanothece* gen. nov. (Cy-

anophyceae). Archiv für Protistenkunde, 118: 119–179
Komárek J, Cepák V. 1998. Cytomorphological characters supporting the taxonomic validity of *Cyanothece* (Cyanoprokaryota). Plant Systematics and Evolution, 210(1–2): 25–39, doi: 10.1007/BF00984725
Komárek J, Cepák V, Kaštovský J, et al. 2004. What are the cyanobacterial genera *Cyanothece* and *Cyanobacterium*? Contribution to the combined molecular and phenotype taxonomic evaluation of cyanobacterial diversity. Algological Studies, 113(1): 1–36, doi: 10.1127/1864-1318/2004/0113-0001
Komárek J, Kopecký J, Cepák V. 1999. Generic characters of the simplest cyanoprokaryotes *Cyanobium*, *Cyanobacterium* and *Synechococcus*. Cryptogamie Algologie, 20(3): 209–222, doi: 10.1016/S0181-1568(99)80015-4
Kumar S P, Narvekar J, Nuncio M, et al. 2009. What drives the biological productivity of the Northern Indian Ocean?. In: Wiggert J D, Hood R R, Naqvi S W A, et al., eds. Indian Ocean Biogeochemical Processes and Ecological Variability. Washington, DC: American Geophysical Union, 33–56
Lee R E. 2008. Phycology. 4th ed. Cambridge: Cambridge University Press, 547
Margheri M C, Ventura S, Kaštovský J, et al. 2008. The taxonomic validation of the cyanobacterial genus *Halothece*. Phycologia, 47(5): 477–486, doi: 10.2216/07-87.1
Mikhodyuk O S, Gerasimenko L M, Akimov V N, et al. 2008. Ecophysiology and polymorphism of the unicellular extremely natronophilic cyanobacterium *Euhalothece* sp. Z-M001 from Lake Magadi. Microbiology, 77(6): 717–725, doi: 10.1134/S0026261708060106
Mogany T, Swalaha F M, Allam M, et al. 2018. Phenotypic and genotypic characterisation of an unique indigenous hypersaline unicellular cyanobacterium, *Euhalothece* sp. nov.. Microbiological Research, 211: 47–56, doi: 10.1016/j.micres.2018.04.001
Nübel U, Garcia-Pichel F, Muyzer G. 1997. PCR primers to amplify 16S rRNA genes from cyanobacteria. Applied and Environmental Microbiology, 63(8): 3327–3332
Oren A. 2009. Problems associated with the taxonomic validation of the cyanobacterial genus *Halothece* by Margheri et al. 2008, Phycologia 47: 477–486. Phycologia, 48(4): 313–314, doi: 10.2216/09-33.1
Park J W, Nam S W, Kim H S, et al. 2014. Enhanced photobiological H_2 production by the addition of carbon monoxide and hydrogen cyanide in two unicellular N2-fixing cyanobacterial strains isolated from Korean coasts. Ocean Science Journal, 49(1): 11–18, doi: 10.1007/s12601-014-0002-0
Reddy K J, Haskell J B, Sherman D M, et al. 1993. Unicellular, aerobic nitrogen-fixing cyanobacteria of the genus *Cyanothece*. Journal of Bacteriology, 175(5): 1284–1292, doi: 10.1128/jb.175.5.1284-1292.1993
Reddy K J, Soper B W, Tang J, et al. 1996. Phenotypic variation in exopolysaccharide production in the marine, aerobic nitrogen-fixing unicellular cyanobacterium *Cyanothece* sp.. World Journal of Microbiology and Biotechnology, 12(4): 311–318, doi: 10.1007/BF00340206
Rippka R, Cohen-Bazire G. 1983. The cyanobacteriales: a legitimate order based on the type strain *Cyanobacterium stanieri*?. Annales de l'Institut Pasteur/Microbiologie, 134(1): 21–36
Rixen T, Ramaswamy V, Gaye B, et al. 2009. Monsoonal and ENSO impacts on particle fluxes and the biological pump in the Indian ocean. In: Wiggert J D, Hood R R, Naqvi S W A, et al., eds. Indian Ocean Biogeochemical Processes and Ecological Variability. Washington, DC: American Geophysical Union, 365–383
Roussomoustakaki M, Anagnostidis K. 1991. *Cyanothece halobia*, a new planktic chroococcalean cyanophyte from Hellenic heliothermal saltworks. Algological Studies/Archiv für Hydrobiologie, (64): 71–95
Rudi K, Skulberg O M, Larsen F, et al. 1997. Strain characterization and classification of oxyphotobacteria in clone cultures on the basis of 16S rRNA sequences from the variable regions V6, V7, and V8. Applied and Environmental Microbiology, 63(7): 2593–2599
Spurr A R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. Journal of Ultrastructure Research, 26(1–2): 31–43, doi: 10.1016/S0022-5320(69)90033-1
Tamura K, Peterson D, Peterson N, et al. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution, 28(10): 2731–2739, doi: 10.1093/molbev/msr121
Wang Jing, Kan Junjun, Zhang Xiaodong, et al. 2017. Archaea dominate the ammonia-oxidizing community in deep-sea sediments of the eastern Indian ocean—from the equator to the bay of Bengal. Frontiers in Microbiology, 8: 415
Waterbury J B, Watson S W, Valois F W. 1986. Biological and ecological characterization of the marine unicellular cyanobacterium *Synechococcus*. Canadian Bulletin of Fisheries and Aquatic Sciences, 214: 71–120
Welsh E A, Liberton M, Stöckel J, et al. 2008. The genome of *Cyanothece* 51142, a unicellular diazotrophic cyanobacterium important in the marine nitrogen cycle. Proceedings of the National Academy of Sciences of the United States of America, 105(39): 15094–15099, doi: 10.1073/pnas.0805418105
Zapata M, Rodríguez F, Garrido J L. 2000. Separation of chlorophylls and carotenoids from marine phytoplankton: a new HPLC method using a reversed phase C8 column and pyridine-containing mobile phases. Marine Ecology Progress Series, 195: 29–45, doi: 10.3354/meps195029
Zehr J P, Mellon M T, Zani S. 1998. New nitrogen-fixing microorganisms detected in oligotrophic oceans by amplification of nitrogenase (*nif*H) genes. Applied and Environmental Microbiology, 64(9): 3444–3450
Zhang Yunyi, Chi Zhenming, Lu Weidong. 2007. Exopolysaccharide production by four cyanobacterial isolates and preliminary identification of these isolates. Journal of Ocean University of China, 6(2): 147–152, doi: 10.1007/s11802-007-0147-x