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Egypt's unique location and diverse environments support a wide diversity of cyanobacteria and algae. In this study, a novel cyanobacterial strain was isolated from soil near the Unox factory which produces tile and iron oxide pigment in the 10th of Ramadan City, Egypt. The physicochemical properties of the soil were determined, and initial laboratory cultivation revealed that the strain exhibited normal *Nostoc* or *Nostoc*-like morphology. The molecular and phylogenetic analysis based on the 16S rRNA gene distinctly classified the strain within the genus *Desikacharya*. The sequencing of the 16S-23S ITS region and folding of secondary structures gave interesting and unique secondary structures. Consequently, the strain has been officially recognized as a new species within the genus *Desikacharya* and designated as *Desikacharya aegyptiaca* sp. nov. This discovery enhances the taxonomic diversity of cyanobacteria and highlights the potential for identifying novel microbial species in diverse and less-explored environments.

Keywords: Cyanobacterium, 16S rRNA, *Desikacharya*,16S 23S ITS, Nostoc

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INTRODUCTION

Despite extensive research, Egyptian algal diversity remains an abundant yet underexplored resource (Fathi & Zaki, 2003; Shanab, 2006; Saber et al., 2020; Shawer et al., 2022). Cyanobacteria are essential bioresources for biotechnology because they produce a wide range of natural chemicals (Singh et al., 2018). They play a crucial role in Earth's geochemical cycles, converting organic molecules for use by other organisms (Yadav et al., 2021). The common genera include *Anabaena*, *Nostoc*, *Oscillatoria*, and *Chroococcus* (Afify et al., 2018; Zaki et al., 2021). The classification of cyanobacteria, both historically and currently, is based on microscopic findings. Phenotypic classification relies on traits that can overlap or change due to laboratory conditions, such as cultivation in synthetic media, temperature, pH, or light intensity. All these factors affect the external shapes of algae, making identification challenging. Despite the limitations of morphological classification, it facilitates identification and is cost-effective and easy to perform. However, these morphological characteristics sometimes do not distinguish cyanobacterial taxa (Shih et al., 2013). The development of tools for assessing physiology, cellular composition, and 16S rRNA genes resulted in the discovery of new taxa and updated cyanobacterial classification (Komarek & Anagnostidis, 1986; Shih et al., 2013). Still, these methods were likewise insufficient for determining species-level correlations (Boyer et al., 2001). Recently, trends have shifted, and researchers are using polyphasic methods that combine information from genetics, morphology, ecology, and biochemistry. The 16S–23S rRNA internally transcribed spacers (ITS) are a highly conserved region. Accordingly, it is now feasible to detect and demonstrate the evolutionary relationships of very similar species (Martins & Branco, 2016). Researchers have adopted a polyphasic approach, integrating genetic, morphological, ecological, and biochemical data for more precise taxonomy (Oren et al., 2022; Strunecký et al., 2023). This has led to the discovery of novel taxa, including *Desikacharya*, a genus introduced in 2018 and placed within the Nostocaceae family (Saraf et al., 2019). While *Desikacharya* has been reported from Iran, Brazil, Thailand, and India, little is known about its diversity in Egypt. This study aims to document and characterize a newly discovered species of *Desikacharya* from the 10th of Ramadan City, Egypt. Given Egypt's unique geographical position at the intersection of Europe, Africa, and Asia along with its diverse habitats that support a rich array of cyanobacterial and algal taxa. This study uses a combination of morphological and genetic identification methods to provide a precise characterization of the new species and emphasize its role in global algal diversity.

MATERIALS AND METHODS

Chemicals

The chemicals used in the Z medium (Staub, 1961*)* for this investigation were of analytical grade purity and were procured from Sigma-Aldrich.

Sampling, isolation, and purification of the Cyanobacterial strain

A soil sample was collected from the 10th of Ramadan city in July 2019, the soil sample was collected from the vicinity of the Unox factory, which specializes in the production of artificial iron oxide pigment as well as calcium carbonate, which is used in the construction industry in the form of interlock tiles and tiles, cement blocks, etc. The color of the soil sample may be affected by the production of pigment. The soil's hue ranges from white to light red due to the significant amounts of calcium carbonate and the diverse oxide products. These oxides, emitted from within the factory, diffuse beyond its walls, impacting the soil's coloration. After removing the uppermost layer of soil with a sterile spatula, about 2-3 cm below the ground's surface, gathered and placed in clean plastic bags. Within 24 hours of collection, the labeled sample bags specifying the location and date were delivered to the laboratory. The strains were isolated and cultivated using conventional techniques (Staub, 1961; Shawer et al., 2023). Pure cultures were obtained by performing dilutions and plating on a Z medium. Ampicillin (25–50 $\mu g \cdot ml^{-1}$) was added to prevent bacterial contamination without affecting the algal isolate. The culture was then cultivated at 25˚C to 30˚C with constant illumination (20–50 µmol m^{-2} s^{-1}). The newly formed isolated single colonies were moved to a fresh Z medium after they grew in one to two weeks. The pure algal strain was kept on Z medium in a culture room with a 12/12-hour light/dark cycle at 28 ± 2°C.

Physical and chemical analysis of soil samples

The soil sample was subjected to physical and chemical analysis at the Soil Microbiology Department, Soil, Water and Environmental Research Institute, Agriculture Research Center, Giza, Egypt. The saturation percentage (SP) was determined according to USDA (1969). Cation Exchange Capacity was determined according to Rhoades (1982) Carbonates, and bicarbonates were estimated to corresponding to the method of Rowell (1994). Electric conductivity (EC) was measured according to USDA (1969). Sulfates were measured as the difference between the total measured soluble cations and the total measured soluble anions. Ca, Mg, Cl, K, and Na were measured according to Jackson (1967). Available nitrogen content was extracted with 2 N KCl (1 g soil: 10 ml KCl solution) and determined according to Page et al., (1982). Organic matter (OM) was determined by oxidizing the organic matter in air-dried soil with ferrous sulfate (0.5 N) and using potassium dichromate (1 N) as an indicator according to Walkley (1947). The trace elements (available content of Fe, Zn, Co, and Cr) in the soil samples were measured according to Soltanpour & Schwab, (1977).

Identification of Cyanobacterial strain
Morphological identification

Identification of alga was carried out according to classical identification keys using cell color, morphology, and the dimensions of vegetative cells, heterocyst, and spores (Castenholz, 2015).

Molecular Techniques

Approximately 1 ml of exponentially growing 15–20-day old culture of Algal isolate E4 from liquid medium was taken in a 1.5 ml microcentrifuge tube and washed 6-7 times with sterile deionized water before DNA separation. The Fast DNA Spin Kit for soil (MP Biomedicals, Santa Ana, CA, USA) was subsequently utilized to separate genomic DNA. Using Thermo Fisher Scientific NanoDrop TM One/One C Microvolume UV-Vis Spectrophotometer, DNA was measured after being eluted in 60 µl of elution buffer included with the kit and observed on a 0.8% agarose gel. Primer 1 (5'-CTCTGTGTGCCTAGGTATCC-3') and primer 2 (5'-GGGGAATTTTCCGCAATGGG-3'**)** were used to amplify the 16S rRNA gene and 16S-23S ITS region (Wilmotte et al., 1993; Nübel et al., 1997; Boyer et al., 2002). The polymerase chain reaction was conducted using Bio-Rad T100TM thermal cycler in a reaction mixture of 25 µl having 1x Taq Buffer, 0.5 µM of each primer, 250 µM of dNTP mix, 1 U/µl of Taq Polymerase, 15-20 ng of genomic DNA and autoclaved deionized water in a sterile 0.5 ml microcentrifuge tube. The amplification cycle consists of an initial denaturation at 95°C for 50 seconds. followed by 30 cycles at 95 °C for 40 s, 50°C for 1min, 72°C for 1min, and a final extension at 72°C for 10min. 100 µl of the final PCR product was visualized on a 1% agarose gel. The bands were excised under a UV illuminator, and the extracted gel products were purified using the QIAquick Gel Extraction Kit (Qiagen GmbH, Hilden, Germany). Sanger dideoxy sequencing was used to sequence the purified amplicons via a 3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA).

Phylogenetic analysis

A BLAST search was conducted to identify the 16S rRNA gene and 16S-23S ITS region sequences related to our strain. Subsequently, these sequences were manually aligned to ensure that the alignment

accurately reflected the conserved secondary structures of the 16S rRNA gene. The phylogenetic tree was constructed using the aligned 16S rRNA gene sequences through the Maximum likelihood (ML) method using the general time reversal model respectively, in MEGA X 10.2.6 (Tamura et al., 2011). Phylogenetic analysis was done using the bootstrap method with 1000 replicates to provide nodal support for the ML tree. The entire analysis consisted of 312 sequences. All sequences were aligned using MAFFT (Katoh & Standley, 2013) with the defaulting alternatives. BMGE optimized alignment gaps and parsimony uninformative characters (Criscuolo & Gribaldo, 2010). MEGA X (version 10.2.6) was used to conduct maximum-likelihood (ML) and maximum-parsimony (MP) phylogenetic analysis (Kumar et al., 2018). By using 1000 bootstrap replications, the most frugal trees' resilience was assessed (Felsenstein, 1985). The optimal nucleotide substitution model for ML analysis was identified using Model Test 3.7 and the Akaike Information Criterion (AIC) (Posada & Crandall, 1998). MEGA X (version 10.2.6) was used to construct the phylogenetic tree, and the generated tree was then modified and saved in TIF format (Al-Bedak & Moubasher, 2020).

16S-23S ITS region analysis

Different conserved and variable regions of the 16S-23S ITS of *Desikacharya aegyptiaca* sp. nov along with the related strains were annotated using different colors. The areas relating to D1-D1', V2, Box-B, and V3 helices were then analyzed for their folded secondary structures employing Mfold on the UNAFold Web Server (Zuker, 2003).

RESULTS

The soil's physicochemical properties were determined and listed in Table 1. Results show the electrical conductivity (EC) which represents 6.29 ds/m. Organic matter (O.M) content suggests moderate organic matter content (1.41 %). A moderate cation exchange capacity (9.56 meq/100 g soil indicates that the soil can hold and exchange essential cations. pH is 8.3. Total nitrogen (0.07%) indicates low nitrogen content that can limit the vegetative growth of algae. Results of Sodium (0.516) and Potassium (0.0168) ppm represent moderate levels of elements. Additionally, the low presence of bicarbonate (0.066 ppm) suggests limited availability of carbon sources, while the ratios of chlorides and sulfate were (0.6272 ppm) and (0.7526 ppm), respectively. The result shows high iron levels (Fe) (4661 ppm) as the soil sample was collected from the vicinity of the Unox factory, which specializes in producing artificial iron oxide pigment.

Table 1. Physicochemical properties of the soil sample.

Parameter	Value
EC (dS/m)	6.29
pH	8.30
Sp%	38.0
O.M%	1.41
C.E.C (meq/100 g soil)	9.56
Total Nitrogen%	0.070
Ca+2 (ppm)	0.8158
Mg+2 (ppm)	0.0972
Na+ (ppm)	0.516
K+ (ppm)	0.0168
CO_3^{2-} (ppm)	0
$HCO3^-$ (ppm)	0.066
Cl (ppm)	0.6272
SO_4^{2-} (ppm)	0.7526
Zn (ppm)	37
Co (ppm)	0
Cr (ppm)	12
Fe (ppm)	4661

Isolation and morphological characterization of cyanobacterial strain

Upon initial examination, the pure isolate was obtained using Z medium agar plates. The morphological characteristics of the cyanobacterial isolate: Trichomes are dark green and consist of a single row of cells. The vegetative cells may be curved or entangled with other trichomes. The vegetative cells are disc or barrel-shaped, sometimes spherical or semi-spherical, constricted at cross-wall. True branching may be from one side or both sides, and the mother and lateral branches have the same diameter. Akinetes are oval, cylindrical, or semi-spherical and are found in series far from heterocyst. The terminal heterocyst is spherical or rectangular, and its diameter is larger than that of vegetative cells. The intercalary heterocyst is also oblong or cylindrical with rounded ends and are sometimes found in pairs. It exhibited morphological characteristics resembling *Nostoc* but differs in several key aspects: the constriction at the cross-wall of vegetative cells, and the presence of true branching (Figure 1). The branching can be either perpendicular or lateral in an unusually striking way, and the number of heterocyst, particularly multiple terminal heterocyst. The vegetative cells measure 2.40 -3.72 µ in diameter and 2.64-4 µ in length, spores measure 5.6-9 µ in diameter and 4-9 µ in length. These differences suggested that the genus might be distinct from *Nostoc*, yet like the newly described genus of *Desikacharya* within the

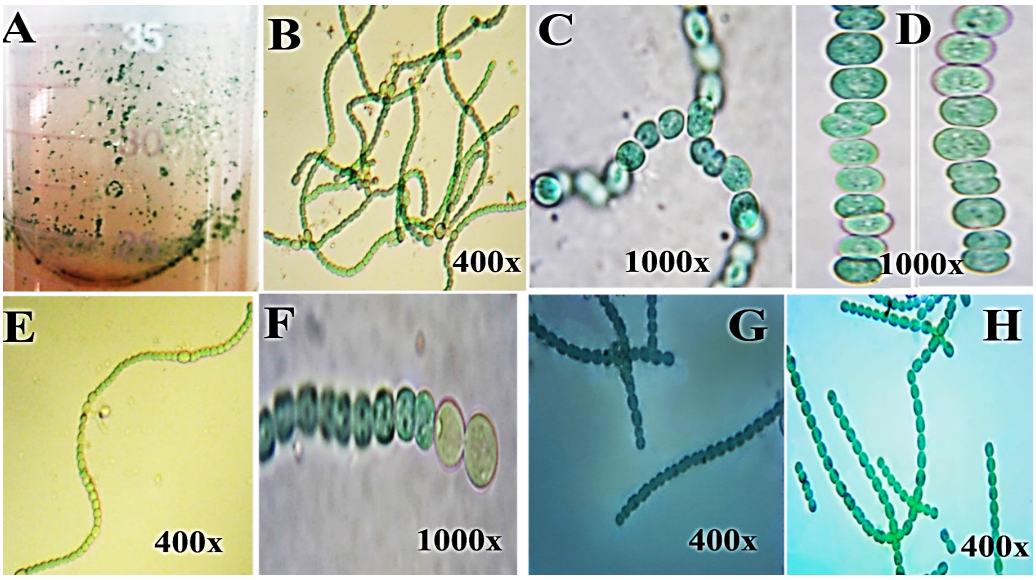

Figure 1. Morphology of Cyanobacterial isolate E4 (A-H); (A) Alga on solid medium. (B) Coiling filaments with intercalary and terminal heterocyst. (C) True branching. (D) Spores. (E) Filament with terminal and intercalary heterocyst. (F) Two terminal heterocysts. (G) Typical lateral branching pattern. (H) The nature of trichomes and a typical branching pattern.

Table 2. Comparative morphological, ecological, and physico–chemical assessment of *Desikacharya* strains.

Types of *Desikacharya* Described	*Desikacharya thermotolera* (Saraf et al., 2019)	*Desikacharya nostocoid* (Saraf et al.,2019)	*Desikacharya soli* (Saraf et al., 2019)	*Desikacharya aegyptiaca* sp. nov.
Intercalary vegetative cell	3.63–4.92 µm (length) 3.46–3.81 µm (width) Usually barrel-shaped	3.83–5.09 µm (length) 3.44–5.33 µm (width) Usually, barrel-shaped	3.58–4.93 µm (length) 3.79–4.48 µm (width) Usually barrel-shaped	2.64 - 4 µm (length) 2.40 -3.72µm(width) disc or barrel-shaped, sometimes spherical or semi-spherical
Intercalary heterocyst	4.74–6.00 µm (length) 4.45–5.27 µm (width) Spherical shape	4.72–6.32 µm (length) 4.98–6.03 µm (width) Spherical shape	4.91–6.89 µm (length) 4.17–5.70 µm (width) Spherical shaped	3.39-5.68 µ(length) 3.22-5.35 µm(width) oblong or cylindrical with rounded ends are sometimes seen in pairs.
Akinete	Absent	Absent	Absent	5.6-9 µm (length) 4-9 µm (width) oval, cylindrical, or semi-spherical
Locality	Mandsaur, India	Mandsaur, India	Mandsaur, India	10th of Ramadan City, Egypt
Habit	Soil	seepage	Soil	Soil
pH	7.34	7.36	7.36	8.3
Temperature (c̊)	43.20	43.20	43.11	38

same family. Such characters sparked our interest in discovering this new species. This prompted us to conduct genetic analyses to potentially identify a new species and classify the organism based on a polyphasic approach.

Molecular studies and phylogenetic analysis

According to a Mega blast examination in the NCBI database using a sequence of 16SrRNA of the E4 isolate, the most similar strains were *Fischerella* sp. RV14 [(GenBank accession number DQ786173; identities = 1391/1543 (90.0%); Gaps = 47/1543 (3%)]. Compared with sequences of the type strains, the closest hits were *Desikacharya constricta* SA10 [GenBank accession number NR_172580 (type material); identities = 1109/1155 (96.02%); Gaps = (9/1155 0%)]. To ascertain the taxonomic identity of *Desikacharya* sp. isolate E4 with the most closely related species listed in GenBank, a phylogenetic study of the 16S rRNA dataset was utilized and evoked the presence of 35 sequences in the 16S RNA collection. Out of 1579 characters in the determined uniformity data, 1110 were matched successfully (no gaps, no N). The remaining 143 characters were

classified as variable characters, meaning they were uninformative (12.9 % of determined characters), and the remaining 107 characters were classified as uniform informative (9.6 % of constant). The genetic record was related using the combination of the Maximum Likelihood and Maximum Parsimony methods. The most parsimonious tree with a tree length of 931, the highest log likelihood (-6939.40), consistency index of 0.385429, retention index of 0.650635, and composite index of 0.250773, for all sites and parsimony-informative sites, is shown in Figure 2. In the phylogenetic tree, *Desikacharya* sp. isolate E4 in this investigation was in a single, long distinct lineage, demonstrating its originality and named *Desikacharya aegyptiaca* Strain E4 Egypt.

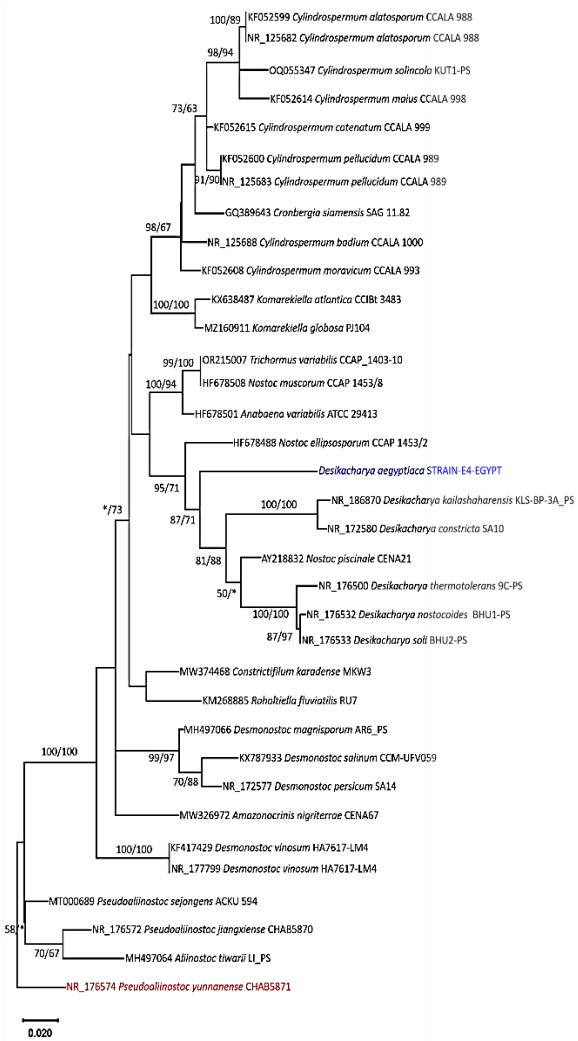

Figure 2. phylogenetic tree based on 16S rRNA gene of the strain E4 :(1000 replications) of *Desikacharya aegyptiaca* isolate E4 (in bold blue color) compared with other 16S rRNA sequences of similar strains in GenBank. Bootstrapping support values for ML/MP ≥50% are displayed next to the suitable nodes. The phylogenetic tree was deep-rooted to *Pseudoaliinostoc yunnanense* CHAB5871 (NR_176574) as outgroup (in red).

Analysis of 16S-23S ITS secondary structure

The coiled secondary structure of the D1–D1' helix region in the E4 strain was sequenced and compared to the previously reported structures of other *Desikacharya* species. The findings of this study's morphological, phylogenetic, and 16S–23S ITS secondary structure analysis offered compelling indications for our hypothesis that the strain E4 constitutes a new species of *Desikacharya* (Figure 3).

DISCUSSION

Soil microbiota plays a crucial role in preserving soil structure, characteristics, and functionality. Cyanobacteria and microalgae are the main contributors that are ubiquitous in soils and pioneers in their development of biocrusts, however, their biodiversity and characteristics remain mostly unknown in comparison with their aquatic equivalents (Weber et al., 2022). The soil conditions create a selective environment favoring adaptable and tolerant algae species. Collecting soil samples from environments contaminated with solid waste for algal cultivation represents a pioneering effort at the Egyptian level.

The physicochemical properties of the soil were determined. The E.C. level divided the soils into two groups: strong and very strong salinity levels. The presence of Cyanophyta is a matter of tolerance and adaptability (Metting, 1981). According to An & Jones, (2000) cyanobacterial cell density exhibited positive correlations with EC. In addition, Aly et al., (2019) reported that Cyanobacteria's optimum EC range is 4.16 – 5.5 as they live in saline and hypersaline environments. Algal isolation can tolerate and adapt to prevent dehydration due to the presence of gelatinous sheaths. Among soil properties, pH is a very important factor in the growth, establishment, and diversity of cyanobacteria, which have been reported to prefer neutral to slightly alkaline pH for optimum growth (Kaushik, 1994). The obtained results revealed that the pH of the soil was 8.3. According to earlier research by Brock (1973), blue-green algae were absent at low pH levels, nevertheless, Chlorophyta and other eukaryotic algae flourished. Numerous research later validated it as well (Salama & Kobbia, 1982; El-Gamal, 1995; El-Attar, 1999). The O.M. content suggests moderate organic matter content. As mentioned by Al-Fredan & Fathi (2007) and El-Hameed et al., (2007), the soil's organic matter concentration is thought to be a major determinant of the number of algae in the soil.

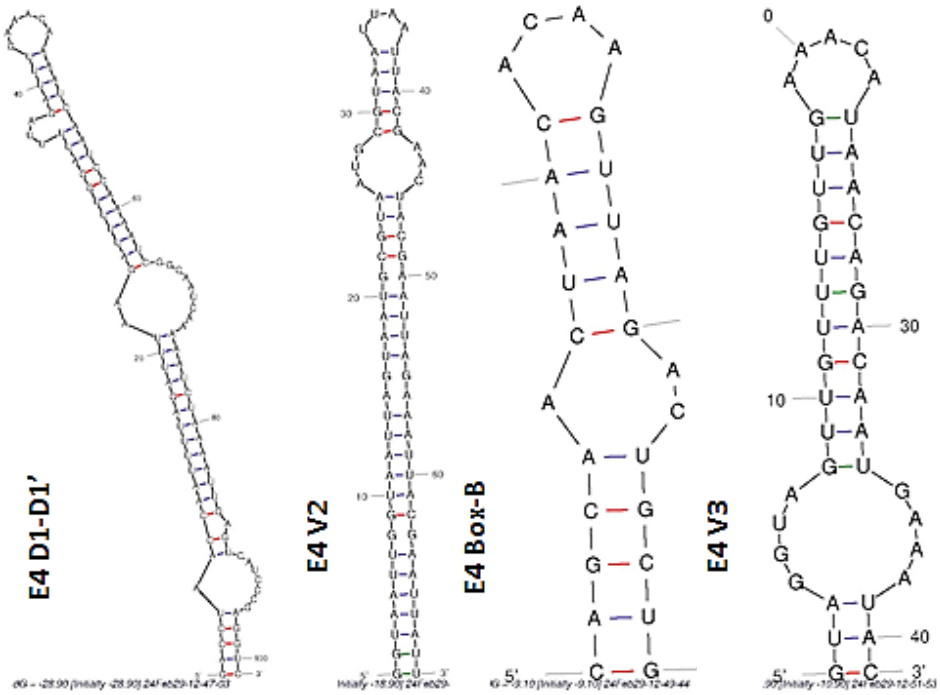

Figure 3. Secondary structures of the D1-D1', V2, Box-B, and V3 helices of strain E4

A moderate CEC (9.56 meq/100 g soil indicates that the soil can hold and exchange essential cations. As stated by Hastings et al., (2014), more monovalent and divalent cations can be bound by soils with high CECs owing to accessible sites in the soil and OM particles. Nitrogen is considered one of the most critical nutrients for growth since it is a constituent in all structural and functional proteins such as peptides, enzymes, chlorophylls, energy transfer molecules, and genetic materials in algal cells (Cai et al. 2013). The concentration of nitrogen in culture medium considerably affects both cell growth rate and biochemical compositions of microalgae (Wang et al., 2013). Total nitrogen (0.07%) indicates low nitrogen content that can limit the vegetative growth of algae. Sodium has been shown to inhibit reductase activity in cultures of blue-green algae and blue-green algae have an absolute requirement for sodium (Ward & Wetzel, 1975). Also, the result shows high iron levels. In recent years, the importance of iron in microalgae growth has been studied by many researchers. Behrenfeld et al., (2006) confirmed that iron had a key function in regulating phytoplankton biomass. Iron is essential for cyanobacteria, serving as a critical component in various cellular processes, particularly in electron transport mechanisms. The abundance of iron-dependent proteins in cyanobacteria underscores their reliance on this micronutrient (González et al., 2016). According to a related research report by Starks (1979), heavy metals including A1, B, Cd, Cu, Mn, Si, and Zn significantly affected the algal flora in different ways, but overall, they improved it. Our isolation can thrive in iron-rich environments. In short, the present soil sample favors the growth of alkaliphilic algae, particularly certain cyanobacteria. High iron levels benefit many algae such as cyanobacteria, though Chromium toxicity may restrict some species. The isolation was further described by integrating the morphological description and genetic studies. It exhibited morphological characteristics resembling *Nostoc* but differed in several key aspects, which suggested that the genius might be distinct from *Nostoc*, yet like a newly described genus of *Desikacharya* within the same family. *Desikacharya* is a newly established genus of cyanobacteria. The genus *Desikacharya* is named in honor of Desikachary, a renowned Indian cyanobacterial taxonomist. It falls within the family Nostocaceae, which includes several other cyanobacterial genera. Moreover, Saraf et al*.,* (2019) reported the isolation of *Desikacharya* sp. from soil.

According to the Phylogenetic analysis using a sequence of 16SrRNA of the E4 isolate, the closest hit was *Desikacharya constricta* SA10*. Desikacharya* sp. isolate E4 in this investigation was in a single, long distinct lineage, demonstrating its originality, and

named *Desikacharya aegyptiaca* Strain E4 Egypt. *Desikacharya* species exhibit morphological similarities to the genus *Nostoc* but are genetically distinct, as evidenced by their unique 16S-23S ITS region structures and phylogenetic clustering. The primary criterion for establishing a new genus is the distinct phylogenetic clustering based on the 16S rRNA gene (Saraf et al*.,* 2019). Furthermore, the coiled secondary structure of the D1–D1′ helix region for the E4 strain was detected by Mfold and compared to the structures previously published for other Desikacharya species. Our results were in the same manner as that obtained by Zuker (2003) and Saraf et al., (2019) who identified the D1–D1¢ secondary structures, BoxB, and V3 helix regions of the 16S–23S ITS utilizing Mfold. In this study, the first data about a new Egyptian member of the *Desikacharya* genus was presented. Additionally, it highlights the need for further investigation into Egypt's cyanobacterial diversity. Finally, the discovery of *Desikacharya aegyptiaca* in an industrially impacted area holds significant ecological importance. It suggests that this species may possess adaptations to survive in polluted or stressed environments, making it a potential bioindicator of ecosystem health. Its presence could indicate resilience to heavy metals, chemical pollutants, or fluctuating water conditions, offering insights into bioremediation potential. Additionally, studying its physiological and genetic traits might contribute to understanding how microorganisms adapt to anthropogenic stress, aiding in the development of strategies for ecosystem restoration and environmental monitoring. We anticipate that many more new cyanobacteria from Egypt will be identified soon.

CONCLUSION

The classification and discovery of new algae species present significant challenges due to their vast diversity, morphological similarities, and the need for advanced molecular and genetic analysis. In the current study, one *Nostoc*-like strain was isolated, purified, cultivated, and identified. Molecular and phylogenetic analyses confirmed their classification within the *Desikacharya* genus. Further sequencing of the 16S-23S ITS region revealed unique structural features, leading to its identification as a novel species, *Desikacharya aegyptiaca sp. nov.* This discovery enhances cyanobacterial taxonomy and highlights the potential for finding new microbial species in unexplored environments.

The polyphasic approach in cyanobacterial taxonomy is crucial as it integrates multiple lines of evidence to achieve a more accurate and comprehensive classification. This method combines morphological, ecological, molecular, and biochemical data, overcoming the limitations of traditional classification methods that rely primarily on morphology.

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ETHICAL APPROVAL

There are no experiments on people or animals in this study.

CONFLICT OF INTEREST

All authors proclaim that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

Conceptualization, R.A.S, A D.E, and A.Y.M.; Methodology, W.M.Q and A.Y.M.; Software, W.M.Q, A.Y.M and A.A.S.; Data analysis, W.M.Q, A.D.E, and A.Y.M.; Writing Review and Editing, W.M.Q, A.D.E, R.A.S. and A.Y.M. All authors have read and agreed to the published version of the manuscript.

AVAILABILITY OF DATA

Data that supports the findings of this study have been deposited in GenBank with the accession codes PQ309038. This investigation offers all the data collected or estimated throughout the study.

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