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Biodiversity, morphology and taxonomy of cyanobacteria isolated from soils with different texture

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ABSTRACT

Blue-green algae, also known as cyanobacteria, play a significant role in soil habitats as they impact the growth and development of plants. They serve as Plant Growth Promoting Rhizobacteria (PGPR) by conducting photosynthesis, fixing nitrogen, and enhancing soil mass growth and nitrogen levels. This research aimed to isolate cyanobacteria from various Governorates including Kafr El-Sheikh, El-Gharbia, El-Dakahlia, Damietta, and El-Behiera, and identified 50 different cyanobacterial isolates through morphological characterizations such as thallus color, morphology and dimension, heterocyst size, vegetative and reproductive cells. The majority of isolates were identified as *Nostoc* sp., *Anabaena* sp., *Calothrix* sp., and *Oscillatoria* sp., while the lesser frequent ones included *Calothrix* sp., *Oscillatoria* sp., *Phormidium* sp., and *Pseudanabaena* sp. Those isolates that exhibited the most favorable properties in fixed nitrogen and mass production were identified using molecular 16S rRNA and were found to belong to *Nostoc lichenoides, Nostoc indistinguendun* and *Nostoc favosum*.

Keywords: Cyanobacteria, morphology, taxonomy

INTRODUCTION

Cyanobacteria are a remarkable group of photosynthetic prokaryotes that are often the dominant microalgae found in soils (Zimmerman, 1992). They can have various morphologies, ranging from unicellular to filamentous thalli (Kumar *et al.,* 2013), and some common genera include Anabaena, Noctoc, Oscillatoria, and Chroococcus (Afify *et al.,* 2018; Zaki *et al.,* 2021). Although they were initially thought to be algae due to their ability to carry out photosynthesis, they are now recognized as belonging to the subclass of Gram-negative prokaryotes (Stanier and Cohen-Bazire, 1977). Cyanobacteria are ubiquitous phototrophic prokaryotes that have found a wide range of applications in industry due to their ability to fix elemental nitrogen and make it available to plants, thus increasing nitrogen in soils and contributing to algal blooms (Metting, 1981; Afify *et al.,* 2022 a&b). Their importance is summarized by their diverse characteristics, including being autotrophic or heterotrophic, free-living or symbiotic, psychrophilic or thermophilic, acidophilic or alkylotrophic, and planktonic or epiphytic (Alam et al. 1989). Cyanobacteria have been shown to encourage nitrogen and soil organic carbon and have been used as inoculants to improve soil structure and fertility (Sepehr *et al.,* 2019; Pandey et al. 2005; Afify *et al.,* 2023). Additionally, they excrete extracellular polymeric secretions mainly composed of polysaccharides (Hu *et al.,* 2003). Therefore, this study aimed to investigate the biodiversity, morphology, and molecular identification of cyanobacteria isolates collected from soils at different locations in Egypt with a focus on their ability to fix nitrogen and promote biomass growth.

MATERIALS AND METHODS

Isolation of cyanobacteria

A laboratory experiment was conducted at the Agriculture Faculty, Al-Azhar University, Cairo, Egypt, in 2021 **(**Afify*, et al.*, 2023). To isolate cyanobacteria, samples of soil were obtained from various locations at Kafr El- Sheikh, (Sakha), El-Gharbia (Qutor), El-Dakahlia (Meetghamr), Damietta (Kafrsaad) and El-Behiera (Elghtatba), Governorates. To obtain the cyanobacterial isolates were applied using collected soil samples in the two following media: Sterilized liquid BG11 medium and Sterilized 0.7% agarized Z medium and modified Watanabe medium at 30ºC under continuous light of 120 cm long white fluorescent lamps with a light intensity of 2500 Lux according to El-Ayouty and Ayyad (1972).

Purification of cyanobacteria

The cultures of cyanobacteria were purified as described by Pringsheim (1949) colored growth was picked up, subcultured and streaked many times in a new agarized Watanabe medium plate.

Bacteria-free cyanobacterial cultures

To obtain cyanobacteria free cultures, the procedures were used as described by washing (Hoshaw and Rosowski, 1973) and tested for purity according to Mercuric chloride treatment (Gupta *et al.,* 1959).

Maintenance of cyanobacteria

On the agar slants of Modified Watanabe Medium (El-Nawawy *et al.,* 1958) the stock cultures were maintained in a refrigerator at 5ºC.

Identification of the cyanobacterial isolates

The purified cyanobacterial isolates were identified by using morphological characterization carried out according to the following parameters: thallus color, thallus morphology and dimension, size of heterocyst, and vegetative and reproductive cells (Castenholz, 2015).

Determination of growth activities and **N_2-fixing of all cyanobacterial isolates**

To determine the cyanobacterial growth activities and their capacities for N_2-fixation. A growth curve experiment was conducted for fifty cyanobacterial isolates and cultivated for 35 days.

Preparation of standard cyanobacterial inoculum

The inoculum from the fifty cyanobacterial isolates morphologically identified isolates were prepared in Modified Watanabe liquid medium with incubation at 28-30ºC under continuous illumination (2500 lux) for 21 days.

Determination of total nitrogen content

Total nitrogen was determined for the fifty cyanobacterial isolates cultures by using the micro-Kjeldahl method according to Jackson (1973).

Selection and molecular identification of the most efficient cyanobacterial strains

At the Agricultural Genetic Engineering Research Institute (AGERI) Agriculture Research Center (ARC), PO Box12619, Giza, Egypt, the use of molecular 16S rRNA genes, which are approximately 1500bp in length, enabling the identification of the most efficient cyanobacterial strains based on dry weight and fixed nitrogen amount. This approach also allows for the determination of the species identity of an unknown bacterium by analyzing its unique rRNA gene sequence. The cyanobacterial isolates were subjected to DNA extraction using the improved method of Cheng and Jiang (2006), which is a modification of the standard phenol/chloroform method described by Neumann et al. (1992)**.**

16S rRNA sequencing analysis

The sequences were analyzed using BLAST program (http://www.ncbi.nlm.nih.gov/BLAST) Sequences were aligned using Align Sequences Nucleotide BLAST.

Statistical analysis

A completely randomized design was performed. The collected data were subjected to the analysis of variance (ANOVA) according to Steel and Torrie (1980). The differences between means were compared using the least significant difference (LSD) at 5%.

RESULTS

Isolation, purification, and identification of cyanobacteria

The isolation and purification of cyanobacteria dominated the soil samples collected from different sites in various Governorates. Fifty cyanobacterial isolates were obtained in pure cultures. Table (1) showed the distribution of these isolates in the five Governorates in Egypt.

Table 1. Distribution of cyanobacterial genera in different Governorates in Egypt.

Governorate (origen soil samples)	Cyanobacteria genera	No. of Isolates	Genera Frequency (%)
Kafr El-Sheikh (K) (34%)	*Anabaena* sp.	5	10
	Nostoc sp.	10	20
	Calothrix sp.	1	2
	Oscillatoria sp.	1	2
El-Gharbia (E) (16%)	*Anabaena* sp.	4	8
	Nostoc sp.	3	6
	Oscillatoria sp.	1	2
El-Dakahlia (Da) (16%)	*Anabaena* sp.	3	6
	Nostoc sp.	5	10
Damietta (D) (14%)	*Anabaena* sp.	4	8
	Nostoc sp.	3	6
El-Behiera (El) (20%)	*Anabaena* sp.	6	12
	Oscillatoria sp.	2	4
	Phormidium sp.	1	2
	Pseudanabaena sp.	1	2
Total number of isolates = 50			

General characters of isolated cyanobacterial genera

Genus: Anabaena

This genus is present in all governorates (34%), the genus characterizations are thallus green or blue-green or yellowish brown and gelatinous or fluid gelatinous. Microscopic examination revealed that trichomes were not-ramified, uniseriate, and of cylindrical cells. Trichomes are short, single straight or bent, cells barrel-shaped. Heterocysts were of single occurrence and mostly produced intercalary or terminal. Terminal heterocysts conical and longer. In some spp. heterocytes are cylindrical to ellipsoid. Akinete in long chains away from heterocysts, ellipsoidal or oblong, akinetes is remarkable in the genus and 3-6 in series, sub-spherical, 5-6 µ broad, 6-7 µ long.

Genus: Nostoc

The second genus is distributed in governorates at a rate of 21%. The culture of this genus is characterized by being dark or greenish brown, yellowish green, and later becoming brownish. The colonies are macroscopic, forming a mucilaginous and amorphous structure. Microscopically, the thallus is small and appears as a thin layer on the agar surface, with flexuous, pale green young cells that turn brown or pale yellowish as they age. The trichomes, which do not have a sheath, come in three different sizes and shapes, with square shapes rarely observed, particularly in the terminal parts of the trichomes, while the apical cells may be slightly larger and oval. Few heterocysts were observed, which occurred singly and had two positions, either intercalary or terminal, and were similar in size to vegetative cells. Hormogonia, which may or may not have heterocytes, formed tightly coiled masses of four to twenty cells in a sheath, which became hardened into small pustules on the thallus surface. No spores were noted. The trichomes initially appeared globose and then released the hormogonia, which had a terminal heterocyst and a firm sheath. Young trichomes and hormogonia then developed again to form large colonies, which were enveloped in a strong periderm. Akinetes, if present, paired or formed short moniliform sequences in trichomes, and were larger in size than vegetative cells, appearing subglobose to ovate. Akinetes were found in long rows between heterocytes or trichomes without heterocytes. Ripe akinetes were oval, with granular content and a smooth surface, and had a slightly brownish epispore.

Genus: Calothrix

Calothrix is a genus that is exclusively found in Kafr El-Sheikh governorate (2% distribution). Morphologically, the thallus is filamentous, with heteropolar filaments having a wider basal part. Heterocysts are located basally, and

the trichomes are not constricted at the cross walls and always taper terminally. Firm sheaths are always present, and cells may be narrowly elongated towards the end. Heterocysts are spherical in shape and basal. Akinetes are ellipsoidal and appear above basal heterocysts, developing from a vegetative portion of a trichome.

Genus: Oscillatoria

Oscillatoria, on the other hand, is distributed by 4% and is isolated from various governorates. The thallus is expanded, blackish-green, with filaments that are more or less straight, free, solitary or parallel to each other and constricted at the cross walls. End-cells are capitate.

Genus: Phormidium

Thallus dark blue-green, flexuous, slightly constricted at the cross-walls; trichomes 1.6-1.8 µ width, (2.1-) 2.6-3 (-2.5.3.9) µ length. In the present form, the cells are slightly longer than in the type species.

Genus: Pseudanabaena

Trichomes actively moving by gliding movement, cylindrical, straight with polar gas-vacuoles; sheath fine, thin, diffluent, occasionally present under settling of the samples in solid media; cell length > breadth 1.5-1.8 µ width, 3.9- 4.6 µ length. **Table (2)** presents the cultural and morphological features of all the cyanobacterial isolates, which were identified**.**

Table 2. Cultural and morphological characteristics of cyanobacterial isolates from soils

Isolates Code	Thallus Color	Thallus Morphology	Vegetative Cell			Heterocyst's				Akinetes			Cyanobacteria Identified Name
			Shape	Width (µm)	Length (µm)	Site	Width (µm)	Length (µm)	Shape	Shape	Width (µm)	Length (µm)	
K_1,E_1,D_1, Da_1&El_1	Green	Filaments	Short angular	4-4.5	2-3.5	Intercalary	5-6	6.5-7.5	Barrel	-	-	-	***Anabaena cylindrica***
K_2,E_2,D_2, Da_2&El_2	Oliveceous green, blue green	Filaments	Cylindrical	3.6-4.1	4.1-5	Terminal or intercalary	4.2-5	4.2-5.4	Spherical	Ellipsoidal or oblong	5-6.3	5-8.1	***Anabaena variabilis***
K_3,E_3,D_3 Da_3&El_3	Yellowish brown gelatinous	Filaments	Cylindrical	2.5-3	1-2.5	Terminal or intercalary	2.5-3	7-8	Cylindrical to ellipsoid	Cylindrical ellipsoid or reniform	4.5-5.5	8.5-12	***Anabaena gelatinosum***
K_4,E_4,D_4 Da_4&El_4	Green	Filaments	Barrel	4-5	4-5	Terminal-ntercalary	3-3.5	4-4.5	Subspherical conical	3-6in series, sub-spherical	5-6	6-7	***Anabaena oryzae***
K_5&El_5	-	Trichome single, straight or bent	Barrel	6	5		5	7	Subspherical	spherical	8	9	***Anabaena fertilissima***
El_6	-	Trichome single, straight	Subquadrate	2.5-3	2-3	Intercalary	3-4	5-7	Cylindrical,round apex	Ellipsoidal	5-6	7-15	***Anabaena orientalis***
K_1,E_1,D_1& Da_1	Dark green	Filaments	Barrel, granular, yellowish	5-6	5.5-7	Terminal-intecalary	-	-	-	-	-	-	***Nostoc muscorum***
E_2,D_2& Da_2	Dark green	Gelatinous to rubbery	Cylindrical	2.3-2.7	2.7-5	Terminal-intecalary	3.2-4.1	3.8-5.9	Subspherical	Spherical or subspherical	3.6-5.4	2.7-6.3	***Nostoc calicola***
K_3,E_3,D_3&Da_3	Greenish brown	Parallel to long axis tube	Subglobose to doliform	3.5-4	4-6	Intercalary falsely terminal	6	8.5	-	-	-	-	***Nostoc commune***
K_4&D_4	Green to blackish	Trichome with sheath	Barrel to subglobose	0.8-1.2	2.4-4.8	Intercalary falsely terminal	5-6	6-7.2	Ovate	Subglobose to ovate	1.5	5	***Nostoc verrucosum***

Cont. Table 2. Cultural and morphological characteristics of cyanobacterial isolates from soil

Isolates Code	Thallus Color	Thallus Morphology	Vegetative Cell			Heterocysts				Akinetes			Cyanobacteria Identified Name
			Shape	Width (µm)	Length (µm)	Site	Width (µm)	Length (µm)	Shape	Shape	Width (µm)	Length (µm)	
K_5&D_5	Dirty blue green	Filaments	Depressed quadratic or oblong	3.3-4.5	3.5-5.8	One sige	1.8-5.8	2.1-3.8	Spherical or convex	-	-	-	***Nostoc edaphicum***
K_6	Yellowish green, later brownish	Trichome	Barrel	2.7-3.6	2.7-4.1	Terminal	4.1-4.5	4.1-5	Spherical	Spherical	3.6-7.2	4.1-6.5	***Nostoc entophtum***
K_7	Young cells palegreen, olderones brown	Filaments	quadratic, oblong or barrel	4.1-5.4	3.6-5.4	-	4.5-6.8	5-7.2	-	Terminal or intercalary, cylindrical	-	-	***Nostoc rivulare***
K_9	Dark green	Filaments	apical cells sometimes slightly larger, oval	1.6-1.8	2.6-3	Terminal	4.6-6.2	5.2-8	Intercalary,	Long rows	6-8.5	6.5-11	***Nostoc viride***
K_1	-	Filaments	Hetropolar	-	-	-	-	-	Spherical	Ellipsoidal	-	-	***Calothrix tenella***
K_1,E_1& E_{l1}	Dark green	Solitary straight	Solitary	4-8.2	4-7.1	-	-	-	-	-	-	-	***Oscillatoria brevis***
El_1	Blakish green	Filments	Parallel	23.8-30.6	5.1-10.2	-	-	-	-	-	-	-	***Oscillatoria jenesis***
El_1	Dark blue green	Flexuous		1.6-1.8	2.5-3.9	-	-	-	-	-	-	-	***Phormidium foveolarum***
E_1	-	-	Cylindrical	1.5-1.8	3.9-4.6	-	-	-	-	-	-	-	***Pseudanabaena galeata***

Determination some biological activities of cyanobacterial isolates *in vitro*

1. Nitrogen fixation by cyanobacterial isolates

Results in Figure (1) showed that there were increases in the cyanobacteria fixed-nitrogen (mg N/100 ml-culture) along with the proceeding of experiment, where the most high cyanobacteria fixed-nitrogen were found with all isolates at the log phase 14-28 days of incubation time. Also, results in Fig. (1) showed that all isolates were differed in their capacity in the extracellular nitrogen secreted, where the nitrogen content was lowest with isolate *Oscillatoria* spp. While, the superior of extracellular-nitrogen secreted with the isolates *Nostoc* spp. Extracellular-nitrogen amount increased gradually by cyanobacterial isolates with increasing incubation period.

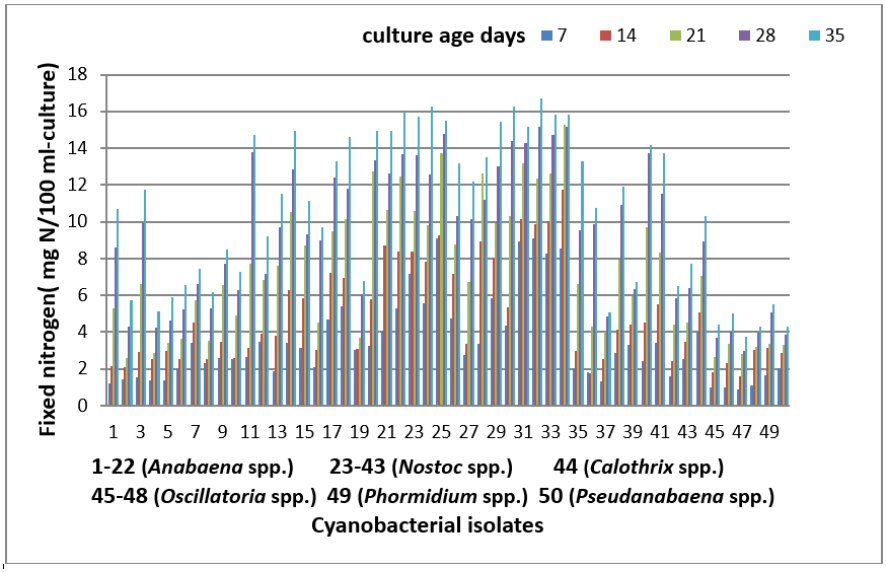


Fig. 1. Amounts of fixed-nitrogen by cyanobacteria isolates (mg N/100ml-culture). With the least significant difference (LSD) at 5% = 3.35.

2. Cyanobacteria biomass

Results in Figure (2) studied the biomass as dry weight in the experiment proceeded, and recorded increases in cyanobacteria where the highest cyanobacteria dry weights with all isolates at the log phase between 14-28 days of incubation time. The study found that the different isolates had varying abilities in producing biomass, with *Oscillatoria* sp. having the lowest amount. As the incubation time increased, the amount of biomass produced by the cyanobacterial isolates also increased, with the highest values observed after 35 days of growth. *Nostoc* spp. had the highest biomass production, recording 348, 318, and 310 mg/100 ml of liquid culture, while *Oscillatoria* spp. had the lowest at 50 mg/100 ml of culture.

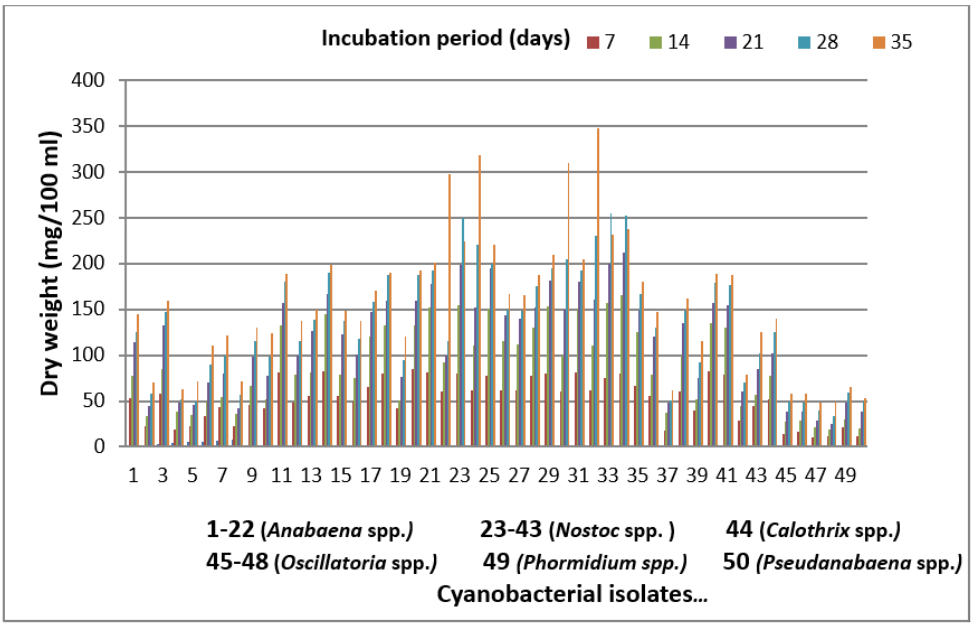
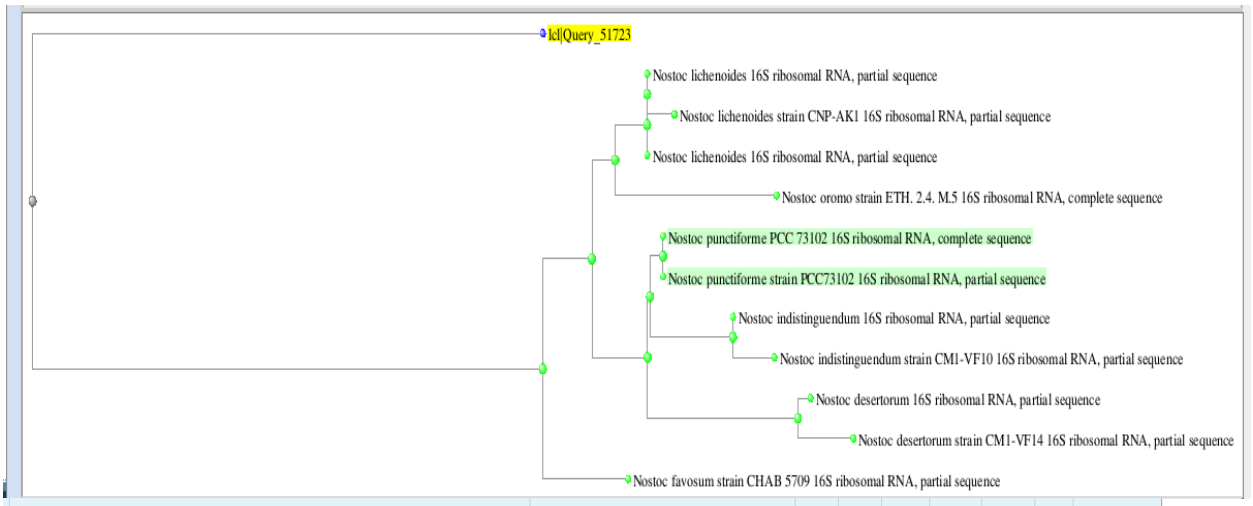


Fig. 2. Dry weight of the cyanobacteria isolates (mg/100-culture). With the least significant difference (LSD) at 5% = 13.20.

Molecular identification of the most efficient cyanobacterial strains

Based on the results, it can be inferred that the most efficient isolates for nitrogen fixation ability and biomass production belonged to all three strains of *Nostoc* spp. Molecular identification revealed that these three isolates contained 16S ribosomal RNA (rRNA) genes that were approximately 1500bp in length. These genes consist of regions of variable DNA sequences that are unique to the species harboring them. Therefore, the identity of an unknown bacterium can be determined from its distinct rRNA gene sequence. To do this, rRNA genes are first amplified using PCR technology. Subsequently, PCR cycle sequencing is conducted, and the rRNA sequence is determined with a capillary sequence analyzer. The resulting sequence is then compared to known rRNA sequences in Gen-Bank® and subjected to a rigorous review process for validation (as shown in Figures 3,4, and 5).



Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Nostoc lichenoides 16S ribosomal RNA, partial sequence	Nostoc lichenoides	1049	1049	96%	0.0	89.81%	1465	NR_177882.1
Nostoc lichenoides 16S ribosomal RNA, partial sequence	Nostoc lichenoides	1049	1049	96%	0.0	89.81%	1465	NR_177881.1
Nostoc punctiforme PCC 73102 16S ribosomal RNA, complete sequence	Nostoc punctiforme PCC 73102	1049	1049	96%	0.0	89.81%	1489	NR_074317.1
Nostoc punctiforme strain PCC73102 16S ribosomal RNA, partial sequence	Nostoc punctiforme	1049	1049	96%	0.0	89.81%	1410	NR_114430.1
Nostoc favosum strain CHAB 5709 16S ribosomal RNA, partial sequence	Nostoc favosum	1045	1045	96%	0.0	89.83%	1487	NR_176571.1
Nostoc lichenoides strain CNP-AK1 16S ribosomal RNA, partial sequence	Nostoc lichenoides	1040	1040	96%	0.0	89.55%	1112	NR_176984.1
Nostoc indistinguendum 16S ribosomal RNA, partial sequence	Nostoc indistinguendum	1023	1023	96%	0.0	89.20%	1463	NR_177880.1
Nostoc desertorum 16S ribosomal RNA, partial sequence	Nostoc desertorum	1021	1021	96%	0.0	89.04%	1462	NR_177879.1
Nostoc indistinguendum strain CM1-VF10 16S ribosomal RNA, partial sequence	Nostoc indistinguendum	1017	1017	96%	0.0	88.93%	1113	NR_176986.1
Nostoc desertorum strain CM1-VF14 16S ribosomal RNA, partial sequence	Nostoc desertorum	1006	1006	99%	0.0	87.76%	1112	NR_176985.1
Nostoc oromo strain ETH. 2.4. M.5 16S ribosomal RNA, complete sequence	Nostoc oromo	996	996	96%	0.0	88.55%	1489	NR_172703.1

Nostoc lichenoides

GTCAACAGCTGGATACGCCTGCTAATACCCAATGTGCCGCAAGGAGAAACATTTATGGCCTGAGGATGAGCTCGCGTCTGATT
AGCTAGTTGGTGGGGTAACGGCTCACCAAGGCGACGATCAGTAGCTGGTCTGAGAGGATGACCAGCCACACTGGGACTGAG
ACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTCCGCAATGGGGGCAACCCTGACGGAGCAAGACCGCGTGG
GGGAAGAAGGTTTGTGGATTGTAAACCCCTTTTGATTGGGAAGAAGACAGTGACGGTACCAATCGAATCAGCATCGGCTAAC
TCCGTGCCAGCAGCCGCGGTAATACGGAGGATGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGTCCGTAGGTGGAAGTT
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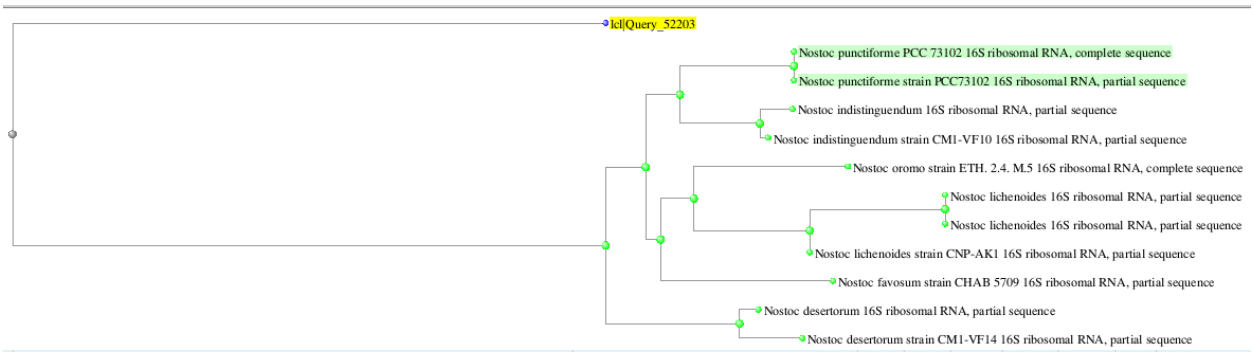
TGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCAGGATTTGACATGTCGC
GAACCTTCTTGAAAGGGAAGGGTGCCTTCGGGAGCGCGAACACAGGTGGTGCATGGCTGTCGTCAGCTCCTGTCGTGGGATG
TTGGGTTAA

Fig. 3. Gentic bioformations background for the identification of *Nostoc lichenoides*



Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Nostoc desertorum 16S ribosomal RNA, partial sequence	Nostoc desertorum	1129	1129	99%	0.0	87.56%	1462	NR_177879.1
Nostoc indistinguendum 16S ribosomal RNA, partial sequence	Nostoc indistinguendum	1120	1120	99%	0.0	87.33%	1463	NR_177880.1
Nostoc punctiforme PCC 73102 16S ribosomal RNA, complete sequence	Nostoc punctiforme PCC 73102	1084	1084	99%	0.0	86.45%	1489	NR_074317.1
Nostoc punctiforme strain PCC73102 16S ribosomal RNA, partial sequence	Nostoc punctiforme	1084	1084	99%	0.0	86.45%	1410	NR_114430.1
Nostoc favosum strain CHAB 5709 16S ribosomal RNA, partial sequence	Nostoc favosum	1079	1079	99%	0.0	86.43%	1487	NR_176571.1
Nostoc lichenoides 16S ribosomal RNA, partial sequence	Nostoc lichenoides	1071	1071	97%	0.0	86.91%	1465	NR_177882.1
Nostoc lichenoides 16S ribosomal RNA, partial sequence	Nostoc lichenoides	1071	1071	97%	0.0	86.91%	1465	NR_177881.1
Nostoc oromo strain ETH. 2.4. M.5 16S ribosomal RNA, complete sequence	Nostoc oromo	1062	1062	99%	0.0	85.95%	1489	NR_172703.1
Nostoc indistinguendum strain CM1-VF10 16S ribosomal RNA, partial sequence	Nostoc indistinguendum	875	875	78%	0.0	87.11%	1113	NR_176986.1
Nostoc desertorum strain CM1-VF14 16S ribosomal RNA, partial sequence	Nostoc desertorum	860	860	78%	0.0	86.69%	1112	NR_176985.1
Nostoc lichenoides strain CNP-AK1 16S ribosomal RNA, partial sequence	Nostoc lichenoides	856	856	78%	0.0	86.55%	1112	NR_176984.1

Nostoc indistinguendun

GTTAGTGGCCGACGGGTGAGTAACGCGTGTGAATCTGCCTTCTGGTCTGGGACAACAGAGGGAAACTTCTGCTAATCCCGGAT
AAGCCTACGGGTGAAAGATTAATTGCCTGGAGATGAGCTCGCGTCTGATTAGCTAGTTGGTAAGGTAAAAGCTTACCAAGGC
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TAGGCAGTGGAAACTACAAAGCTAGAGTCTGCTAGGGGCCAAGGGAATTCCCGGTGTAGCGGTGAAATGCGTAGAGATCGG
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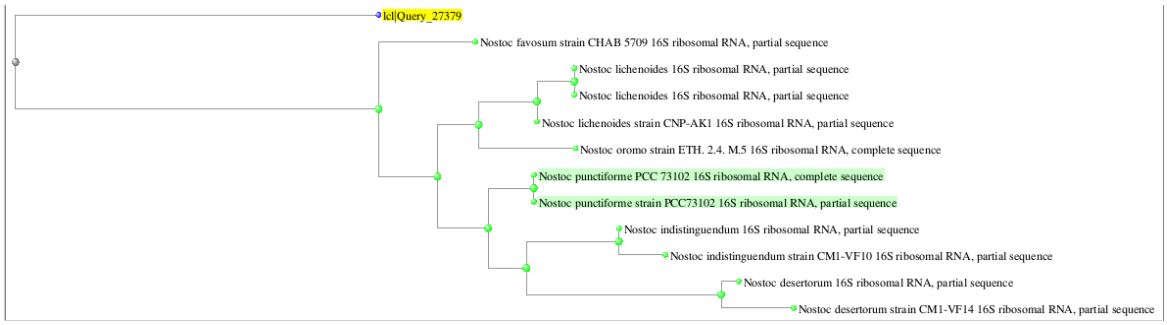
CGATGTAACGTGAAGAACCTTAGCAGGGCTTGACATGTCGCGAATCTAGGGGAAACCTTGGAGTG

Fig. 4. Gentic bioformations background for the identification of *Nostoc indistinguendun*.



Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Nostoc favosum strain CHAB 5709 16S ribosomal RNA, partial sequence	Nostoc favosum	1363	1363	99%	0.0	93.33%	1487	NR_176571.1
Nostoc lichenoides 16S ribosomal RNA, partial sequence	Nostoc lichenoides	1362	1362	100%	0.0	93.02%	1465	NR_177882.1
Nostoc lichenoides 16S ribosomal RNA, partial sequence	Nostoc lichenoides	1362	1362	100%	0.0	93.02%	1465	NR_177881.1
Nostoc punctiforme PCC 73102 16S ribosomal RNA, complete sequence	Nostoc punctiforme PCC 73102	1362	1362	99%	0.0	93.20%	1489	NR_074317.1
Nostoc punctiforme strain PCC73102 16S ribosomal RNA, partial sequence	Nostoc punctiforme	1362	1362	99%	0.0	93.20%	1410	NR_114430.1
Nostoc oromo strain ETH. 2.4. M.5 16S ribosomal RNA, complete sequence	Nostoc oromo	1344	1344	99%	0.0	92.77%	1489	NR_172703.1
Nostoc indistinguendum 16S ribosomal RNA, partial sequence	Nostoc indistinguendum	1326	1326	99%	0.0	92.32%	1463	NR_177880.1
Nostoc desertorum 16S ribosomal RNA, partial sequence	Nostoc desertorum	1308	1308	100%	0.0	91.71%	1462	NR_177879.1
Nostoc lichenoides strain CNP-AK1 16S ribosomal RNA, partial sequence	Nostoc lichenoides	1221	1221	90%	0.0	92.75%	1112	NR_176984.1
Nostoc indistinguendum strain CM1-VF10 16S ribosomal RNA, partial sequence	Nostoc indistinguendum	1180	1180	90%	0.0	91.67%	1113	NR_176986.1
Nostoc desertorum strain CM1-VF14 16S ribosomal RNA, partial sequence	Nostoc desertorum	1166	1166	90%	0.0	91.30%	1112	NR_176985.1

Nostoc favosum

TCACAGACTGGAGCTTAACTCCAGGAAGGCAGTGGATACTGAGGAGCTAGAGTTCGGTAGGGGTAGAGGGAATTCCCAGTGT
AGCGGTGAAATGCGTAGAGATTGGGAAGAACACCAGTGGCGAAGGCGCTCTACTGGGCCGAAACTGACACTCAGGGACGAA
AGCTAGGGGAGCGAAAGGGATTAGATACCCCTGTAGTCCTAGCTGTAAACGATGGATACTAGGCGTAGCTTGTATCGACCCG
AGCTGTGCCGAAGCAAACGCGTTAAGTATCCCGCCTGGGGAGTACGCACGCAAGTGTGAAACTCAAAGGAATTGACGGGGG
CCCGCACAAGCCGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCAGGGCTTGACATTCGGCGAATCTGCTT
GAAAGAGGAGAGTGCCCATTGGGAGCGCCGTAACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAA
GTCCCGCAACGAGCGCAACCCTCGTCCTTAGTTGCCATCATTAAGTTGGGAACTCTAGGGAGACTGCCGGTGACAAACCGGAG
GAAGGTGGGGATGACGTCAAGTCAGCATGCCCCTTACGTTCTGGGCTACACACGTACTACAATGGTAGGGACAAAGGGCAGC
AAACTCGAGAGGGTAAGCGAATCCCATCAAACCCAGCCTCAGTTCAGATTGCAGGCTGCAACTCGCCTGCTTGAAGGAGGAAT
CGCTAGTAATCGCAGGTCAGCATACTGCGGTGAATTCGTTCCGGGGCCTTGTACACACCGCCCGTCTCACCATG

Fig. 5. Gentic bioformations background for the identification of *Nostoc favosum.*

Therefore, these three of the highest strains were selected and identified by using molecular 16S rRNA genes as follows: *Nostoc lichenoides, Nostoc indistinguendun* and *Nostoc favosum* (Figure 6).

Fig. 6. Photographs of microscopic preparation of the three most efficient cyanobacterial strains.

DISCUSSION

Out of the fifty isolates of cyanobacteria, from the various locations at Kafr El- Sheikh, (Sakha), El-Gharbia (Qutor), El-Dakahlia (Meetghamr), Damietta (Kafrsaad) and El-Behiera (Elghtatba), Governorates. Fifty cyanobacterial isolates were obtained in pure cultures; bacteria-free (El-Gamal, *et al.* 2008). The cultural and morphological features of all these cyanobacterial isolates, were identified using the second edition of Bergey's Manual of Systematic Bacteriology (2001). Cyanobacteria are classified into five quasi-taxonomic groups or subsections based on their morphological characteristics, which include the presence or absence of differentiated cells (akinetes and heterocysts) and ramification (true or false) in the trichome morphotype. The identification of these groups was made using a dichotomous key as described by several authors Geitler (1932), Desikachary (1959), El-Ayouty *et al.* (1977), Gupta & Pandey (1979), Prescott (1982), Roger & Ardales (1991), El-Gamal (1995), Naz (2004), Pinevich (2008), Shariatmadari & Riahi (2011) and Komarek *et al.* (2014l). Firstly, Stal (1995) stated that in nature cyanobacterial nitrogen appears different patterns depending on the type of strain and environmental conditions. Singh, *et al.* (2011) found that filamentous cyanobacteria have a specialized structure for nitrogen fixation called heterocysts which contain a key enzyme "nitrogenase" that is responsible and involved in the nitrogen fixation process. Cyanobacteria exhibited the highest dry weight with an increasing incubation period (Taha, 2000). These cyanobacterial strains served as Plant Growth Promoting Rhizobacteria (PGPR) by fixing nitrogen, enhancing soil mass growth and nitrogen levels (El-Zawawy, 2016; Afify, *et al.* 2018; El-Zawawy, *et al*., 2021; Zaki, *et al.* 2021; Afify, *et al.* 2022 a&b and Afify, *et al.,* 2023).

CONCLUSION

Cyanobacteria are often the most important group of oxygenic phytoplankton**.** Besides dominating the phytoplankton community composition, they also frequently bulid up dense populations. This study, indicated that *Nostoc lichenoides, Nostoc indistinguendun* and *Nostoc favosum* were the most predominant cyanobacterial species of dry weight and nitrogen fixation in the soil samples collected from the sites of study. Consequently, *Nostoc lichenoides, Nostoc indistinguendun* and *Nostoc favosum* are stronfly suggested to be effective as Plant Growth Promoting Rhizobacteria (PGPR).

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تنوع السيانوبكتيريا المورفولوجى والتقسيمى المعزوله من الأراضى ذات القوام المختلف

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تعتبر السيانوبكتيريا (الطحالب الخضراء المزرقه) أحد مجموعات البروكاريوتات المهمه حيث توجد فى الأراضى ولها القدره على تطور ونمو النبات. تعرف السيانوبكتيريا ببكتيريا المجال الجذرى المشجعه لنمو النباتPGPR لأن لها القدره على القيام بعملية التمثيل الضوئى وتثبيت النتروجين الجوى وبالتالى تمد التربه بالماده العضويه والنتروجين. فى هذه الدراسه تم الحصول على خمسون عزله من السيانوبكتيريا وتنقيتها بالطرق المعروفه لتنقية السيانوبكتيريا وذلك من أراضى مختلفه لمحافظات مصر تشمل كفر الشيخ والغربيه والدقهليه ودمياط والبحيره. وعند تعريف هذه العزلات مورفولوجيا طبقا شكل ولون الثالوس وحجم الهيتروسست بالإضافه إلى الخلايا الخضريه والتكاثريه كانت أعلى نسبه مئويه لإنتشار السيانوبكتيريا فى أراضى محافظة كفر الشيخ 34% وتمثلها أجناس نوستوك وأنابينا وكلوثريكس وأوسيلاتوريا. بينما أقل نسبه كانت فى أراضى محافظة دمياط 14% وتشمل جنسى نوستوك وأنابينا أما أقل الأجناس إنتشارا سيدوأنابينا وفورميديم وأوسيلاتوريا وكلوثريكس. وطبقا لتقديرات النتروجين المثبت والوزن الجاف لجميع عزلات السيانوبكتيريا أمكن الحصول على أعلى ثلاثة من العزلات فى محتواها من النتروجين والوزن الجاف وتم تعريفها جزيئيا *Nostoc favosum*. and *Nostoc lichenoides, Nostoc indistinguendun*

الكلمات المفتاحية: السيانوبكتيريا ، التصنيف ، التقسيم