A taxonomic study of cyanobacteria in wheat fields adjacent to industrial areas in Yazd province (Iran)

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Abstract

Culturing, isolation, purification, and identification of cyanobacteria collected from wheat field soil, in five stations around the industrial areas in Yazd province (Iran) were conducted in this study. Identification of taxa was based on morphology and molecular methods. Cluster analysis and principal component analyses performed using SPSS software and rate of resemblance among the taxa were investigated. The phylogenetic analyses carried out according to 16S rRNA gene sequences data, and the phylogenetic relationships were assessed by Maximum Parsimony, Maximum Likelihood and Bayesian Inference. The results of this study indicated presence of 32 species included in four orders of cyanobacteria. Among these orders, *Oscillatoriales* with three families, eight genera and 13 species showed the highest species diversity, whereas *Chroococcales* with two families, three genera and four species, exhibited the lowest diversity. Among isolated taxa, *Oscillatoria ornata*, *O. engelmanniana*, *Jaaginema pallidum*, *Pseudanabaena minima*, *Stenomitos frigidus*, *Nostoc edaphicum*, and *Gloeothece tepidariorum*, are reported as new records for algal flora in Iran. Distributions and descriptions of the new taxa were also presented.

Keywords: Cyanobacteria, heavy metals, morphological diversity, new record, phylogenetic analyses, 16S rRNA

مطالعه تاکسونومیک سیانوباکتری‌های مزارع گندم مجاور مناطق صنعتی استان یزد*

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خلاصه

در این مطالعه، سیانوباکتری‌های موجود در خاک مزارع گندم در پنج ایستگاه در مجاورت مناطق صنعتی استان یزد پس از طی مراحل کشت و خالص‌سازی مورد شناسایی قرار گرفتند. شناسایی نمونه‌ها براساس ویژگی‌های مورفولوژیک و نیز به روش مولکولی صورت گرفت. همچنین تجزیه خوشه‌ای و تجزیه مولفه‌های اصلی با استفاده از نرم‌افزار SPSS صورت گرفت و قرابت میان آرایه‌ها براساس صفات ریختی بررسی گردید. همچنین آنالیزهای فیلوژنتیک با استفاده از مارکر مولکولی 16S rRNA انجام و روابط فیلوژنتیک آرایه‌ها براساس روش‌های Maximum Parsimony، Maximum Likelihood و Bayesian ارزیابی گردید. حاصل این مطالعه، شناسایی ۳۲ گونه از سیانوباکتری‌ها بود که در چهار راسته مختلف قرار گرفت. راسته *Oscillatoriales* با سه تیره، هشت جنس و ۱۳ گونه و راسته *Chroococcales* با دو تیره، سه جنس و چهار گونه به ترتیب از بیشترین و کمترین سطح تنوع برخوردار بودند. همچنین نتایج حاصل از این تحقیق، نشانگر کارایی نسبی مارکر فیلوژنتیک (سکانس ژنی 16S rRNA) به کارگرفته شده و نیز کارایی مطالعات تاکسونومی عددی (آنالیز تجزیه خوشه‌ای) در جداسازی آرایه‌های مورد مطالعه بود. ارزیابی چگونگی پراکنش گونه‌های شناسایی شده در ایستگاه‌های مطالعاتی و ارایه شرح آرایه‌های جدید برای فلور جلبکی ایران از دیگر یافته‌های این مطالعه است. از میان آرایه‌های شناسایی شده، هفت گونه به اسامی *O. ornata*, *Oscillatoria engelmanniana*, *Jaaginema pallidum*, *Pseudanabaena minima*, *Nostoc edaphicum*, *Gloeothece tepidariorum* و *Stenomitos frigidus* برای نخستین بار از ایران گزارش می‌شوند.

واژه‌های کلیدی: آنالیز فیلوژنتیک، تنوع مورفولوژیکی، سیانوباکتری، فلزات سنگین، گونه جدید، 16S rRNA

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Introduction

Cyanobacteria are the most ancestral lineage of phototrophic organisms which are classified in Eubacteria kingdom due to their prokaryotic structure. Species of this phylum are distributed in various habitats, including the aquatic and terrestrial ecosystems (Lamprinou *et al*. 2013). Their existence in extreme conditions such as saline soil or heavy metal polluted environments shows the adaptation compatibility of this group of algae. More details about metabolic flexibility of cyanobacteria and their functional value have been revealed in recent decades. Species diversity of cyanobacteria in natural habitats is considered as basic knowledge for economic exploitation of these microorganisms.

Terrestrial ecosystems are known as one of the main habitats of cyanobacteria and there are numerous reports about their existence in terrestrial habitats (Hoffmann 1989, Rindi 2007). There are several reports on the presence of cyanobacteria in terrestrial ecosystems of Iran. Ehrenberg (1854) reported 45 species of algae of which, 29 species were validated. In autumn of 1972, the Belgian multi-purpose expedition investigated deserts of the central, eastern and middle parts of Iran, mainly Dasht-e-Kavir, Dasht-e-Lut and Hamun-e Jaz Murian. Leonard, as the botanist of the Belgian expedition team, collected samples of algae which later were studied by Compere (Compere 1981).

In recent years, several studies have been focused on algal flora which resulted in many reports of cyanobacteria in Iran terrestrial ecosystems especially from Gilan and Mazanderan provinces in north of Iran but, stressful regions such as arid or polluted soils have not yet given enough attention. Moghtaderi *et al*. (2009) reported *Microcoleus vaginatus* Gomont ex Gomont from soil crust of Chadormalu desert in Bafgh region (Yazd province, Iran). Jafari *et al*. (2014) reported *Microchaete goeppertiana* Kirchner as a new record of cyanobacteria from the oil-polluted soil in south of Iran. Hokmollahi *et al*. (2016) taxonomically studied blue-green algae based on morphological, physiological and molecular characterization in Yazd province (Iran) terrestrial ecosystems.

Wheat (*Triticum vulgare* L.) is the most important source of grain food for human beings. Similar to other fields, wheat fields are terrestrial ecosystems providing relatively favorable conditions for the growth and propagation of cyanobacteria due to their appropriate moisture. Yazd province has 26,000 hectares of wheat-cultivated fields with annual production of 100,000 tons. The most important industrial activities as well as agricultural fields of Yazd province are located in Yazd-Ardekan area. Contiguity of industries to agricultural lands has caused critical problems for people's health and also affected cyanobacterial flora of these areas. On the other hand, some industrial and anthropogenic activities may result in heavy metals pollutions (e.g. Cadmium and Lead) in adjacent area and fields. Soil cyanobacteria play an important role in bioremediation of various contaminants (Rai *et al*. 1998, Whitton & Potts 2000). They are able to accumulate toxic heavy metals and organic pollutants (e.g. Arsenic) which will result in higher concentrations of these pollutants in their cells compared to surrounding area (Shamsuddoha 2006). Recently, use of cyanobacteria as an economic and low-cost remediable technology has increased worldwidely (Bhatnagar & Kumari 2013).

Regarding importance of this group of microorganisms as well as limited information about soil microflora of agricultural fields in Yazd province; the present investigation is aimed to study soil microorganisms, especially cyanobacteria of the wheat lands adjacent to industrial areas in Yazd province for gaining better understanding about the effects of industrial pollution on agricultural lands and their algal flora.

Materials and Methods

- Field study, sampling and analyses

Soil samples were collected from five wheat farms located in different regions of Yazd province of Iran (Figs 1 & 2) based on Rangaswamy methodology (1966). Soil sampling was done from fields adjacent to industrial areas in Aug. 2013 (Table 1).

Fig. 1. Locations of Yazd province and sampling stations.

Fig. 2. Views of different stations in the studied area.

Table 1. Geographical and some ecological details of the sampling locations

Code	Location	Latitude/Longitude	pH	EC (ds/m)	Pb (ppm)	Cd (ppm)
1	Kalantar farm	32.2 N/54.12 E	7.07	2.98	2.686	0.1>
2	Shamsi	32.18 N/54.0 E	6.70	4.18	1.976	0.1>
3	Torkabad	32.20 N/54.58E	6.53	12.96	4.044	0.1>
4	Tabas three-way	32.11 N/54.5 E	6.34	8.85	2.054	0.1>
5	Sadrabad	32.5 N/54.6 E	6.72	3.03	1.602	0.1>

- Evaluation of heavy metals contents of soil samples

Heavy metals content of the soil samples, such as Lead (Pb) and Cadmium (Cd) were determined by Kavir-e Jonoob Institute (Yazd). Atomic absorption spectrometry was employed for determination and measurement of heavy metals.

- Isolation and identification of cyanobacteria

The collected soil samples were maintained by culturing in BG11 & BG110 media (Rippka *et al*. 1979) and incubated at 28±2 °C with continuous illumination at 70 µmol photon $m^{-2}s^{-1}$. After isolation, samples were purified by several subculturings on solid BG11 media (Kaushik 1987).

Identification of taxa was carried out by light microscopy based on accepted references (Desikachary 1959, Prescott 1962, Komárek & Anagnostidis 2005). The photographs were shot and drawn with camera

(Canon G10) and camera lucida (Olympus, Japan), respectively. The list of taxa is shown in Table 2.

- Morphometric study on cyanobacterial strains

Morphometric studies were performed on five filaments or cells from each species. Totally, 14 quantitative and qualitative morphological characters were studied (Table 3). Characters were selected based on those reported by Nayak & Prasanna (2007), Heidary *et al*. (2013) and author's own field observations.

- Statistical analysis

In order to determine the taxa interrelationships, cluster analysis and principal component analysis (PCA) were performed. For multivariate analyses, the mean of quantitative characters was used, while qualitative characters were coded as binary/multistate characters. Standardized variables (mean=0, variance=1) were considered for multivariate statistical analyses. The Euclidean distance was applied as dissimilarity coefficient in cluster analysis of morphological data (Podani 2000). ANOVA test via SPSS (ver.15) and Excel software were employed for statistical analyses.

- Molecular study on cyanobacterial strains

Seven cultures of purified cyanobacterial isolates were used for phylogenetic studies. The DNA extractions of purified taxa were performed by genomic DNA extraction kit (Thermo Scientific, Lithuania). For DNA amplification, the 16S rRNA gene regions, approximately 600 bp in length, were amplified by PCR using 106F (5'CGG ACG GGT GAG TAA CGC GTG A 3') and 781Rb (5'GAC TAC AGG GGT ATC TAA TCC CTT T 3') primers (Nübel *et al*. 1997). The PCR mixtures contained 5 µl *Taq* commercial buffer, 5 µl purified DNA, 5 pM of each dNTP, 10 µm of each primer and 2.5 U *Taq* polymerase. An initial cycle involved 5 min at 94 °C, and then 34 cycles of amplification were started (1 min at 94 °C, 1 min at 60 °C and 1 min at 72 °C). The termination cycle lasted for 10 min at 72 °C. PCR amplified products were subjected to 1.5% (w/v) agarose gel using TBE buffer stained with 6 µg/ml of DNA safe stains. The sequence was determined by the Pishgam Company (Iran).

- Phylogenetic analysis

Sequences were aligned using CLUSTAL W multiple sequence alignment program. Phylogenetic relationships were assessed by Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI). MP was conducted through use of PAUP* ver. 4.0b 10. Bootstrap analysis was also employed to evaluate the tree topologies by performing 1000 replications.

Results

- Physicochemical properties of soil

The results of physical analysis of the soil samples showed that, pH values in soil extract were mostly between 6 and 7. The highest EC belonged to station 3 (12.96 dS/m) (Table 1). Cd concentrations in soil samples were in acceptable range according to standards (Allaway 1990, Pendias & Pendias 1992).

- Description of taxa

In the present study, 32 taxa of heterocystous and non-heterocystous cyanobacteria were identified and recorded from Yazd province (Table 2), which were classified in four orders (*Oscillatoriales*, *Synechococcales*, *Nostocales*, and *Chroococcales*) including 10 families and 18 genera. Those taxa which are drawn with camera lucida are shown in figures 3–5. Seven species of identified taxa *viz*. *Oscillatoria ornata*, *O. engelmanniana*, *Jaaginema pallidum*, *Pseudanabaena minima*, *Stenomitos frigidus*, *Nostoc edaphicum*, and *Gloeothece tepidariorum* were considered as new records from Iran.

Descriptions of the new records are as follows:

Table 2. List of cyanobacterial species recorded from wheat fields of Yazd

Taxon	Site				
	1	2	3	4	5
Oscillatoria subbrevis Schemidle	●				
O. tenuis Ag. ex Gomont	●	●	●	●	●
O. limosa C. Agardh ex Gomont					●
**O. ornata* Kütz. ex Gomont			●		
**O. engelmanniana* Gaiducov	●				
Lyngbya aestuarii Liebman ex Gomont	●				●
**Stenomitos frigidus* (F.E. Fritsch) Miscoe & J.R. Johansen	●	●	●	●	
Jaaginema sp.$_1$	●				
Jaaginema sp.$_2$	●			●	
Jaaginema sp.$_3$	●			●	
Jaaginema sp.$_4$		●			
**Jaaginema pallidum* (Böcher) Anagnostidis & Komárek	●				
J. pseudogeminatum (G. Schmid) Anagnostidis & Komárek				●	
Aphanocapsa delicatissima West & G.S. West			●	●	
**Pseudanabaena minima* (G.S. An) Anagnostidis	●	●	●	●	●
Phormidium sp.$_1$		●		●	●
Phormidium sp.$_2$			●		●
Pseudophormidium sp.		●			
Symplocastrum sp.			●		
Geitlerinema sp.					●
Planktothricoides sp.		●			
Planktothrix agardhii (Gomont) Anagnostidis & Komárek	●			●	
**Nostoc edaphicum* Kondrateva	●			●	
Nostoc hatei S.C. Dixit		●	●	●	
Nostoc sp.$_1$			●		
Nostoc sp.$_2$				●	
Trichormus sp.	●		●	●	
Calothrix sp.				●	
Chroococcus minimus (Keissler) Lemmermann	●	●	●	●	
Chroococcus sp.			●		
Limnococcus limneticus (Lemmermann) Komárková, Jezberová, O. Komárek & Zapomelová	●	●		●	●
**Gloeothece tepidariorum* (A. Braun) Lagerheim				●	

1. Kalantar farm, 2. Shamsi, 3. Torkabad, 4. Tabas three-way, 5. Sadrabad
∗ Indicative of new records for Iran

1. *Oscillatoria ornata* Kütz. ex Gomont (Fig. 3c)

Thallus blue-green, trichomes straight or irregularly twisted, thin and slightly curved at the end, 6–7 µm wide; cells length shorter than their width (3 µm long), cells granulated, without aerotopes, apical cell rounded and without calyptra.

2. *Oscillatoria engelmanniana* Gaidukov

Trichomes single or in clusters, blue-green to gray and lavender, straight or irregularly twisted, 6–12 µm broad, not constricted at the cross wall; cells length shorter than their width (3–5 µm long), cells granulated; apical cell rounded, usually with small thickness at the cell wall, rarely with calyptra.

3. *Jaaginema pallidum* (Böcher) Anagnostidis & Komárek (Fig. 3f)

Trichomes colorless, less than 1 µm wide, cross walls unclear, not constricted at the cross walls; apical cell rounded.

4. *Pseudanabaena minima* (G.S. An) Anagnostidis (Fig. 3i)

Trichomes single or in clusters, straight or almost straight, blue-green, width 2 µm, clear, constricted at the cross walls; cells length longer than their width (1.5 times or more); homogeneous, cell content without aerotopes; apical cell rounded.

5. *Stenomitos frigidus* (F.E. Fritsch) Miscoe & J.R. Johansen (Fig. 3h, j)

Trichomes with mucilaginous sheath, width 1 µm, clear, constricted at the cross walls, not attenuated at the end; cell cylindrical with rounded ends or somewhat barrel-shaped, cells length equal or twice of their width; apical cell conical-rounded and without calyptra.

6. *Nostoc edaphicum* Kondrateva (Fig. 4c)

Trichomes with mucilaginous sheath; single-layered sheath, spherical or elliptical, trichome size (30)20 × 50(30) µm; heterocysts at both ends of the sheath, spherical, with diameter of 4–5 µm; vegetative cells spherical, with diameter of 4–5 µm.

7. *Gloeothece tepidariorum* (A. Braun) Lagerheim (Fig. 5g)

Colonies in different sizes, cells long, 5(6)–7(9) µm; almost homogeneous, dark blue-green with multi-layered and colorless sheath.

- Morphological study

In morphometric investigation, the morphological variation of cyanobacterial taxa was examined among several species and genera from several families. The most important characteristics of the studied taxa are summarized in table 3.

Table 3. Morphological characters and their character states in studied taxa

Character	Character state
Gelatinous sheath	0) Present, 1) Absent
Mobility	0) Present, 1) Absent
Apical cell shape	0) Rounded, 1) Conical with rounded apex
Trichome color	0) Dark Blue-green, 1) Pale blue-green, 2) Yellowish brown
Vegetative cell shape	0) Spherical, 1) Cylindrical, 2) Discoid
Structure form	0) Unicellular, 1) Filamentous
Constriction at the cross walls	0) Not constricted, 1) Low constricted, 2) High constricted
Granulation	0) Present, 1) Absent
Restriction at the end of trichome	0) Present, 1) Absent
Heterocyst	0) Present, 1) Absent
Akinet	0) Present, 1) Absent
Heterocyst position	0) Terminal, 1) Terminal and middle
Thallus complexity	0) Present, 1) Absent
Trichome width	0) Lower than 6 µm, 1) Higher than 6 µm

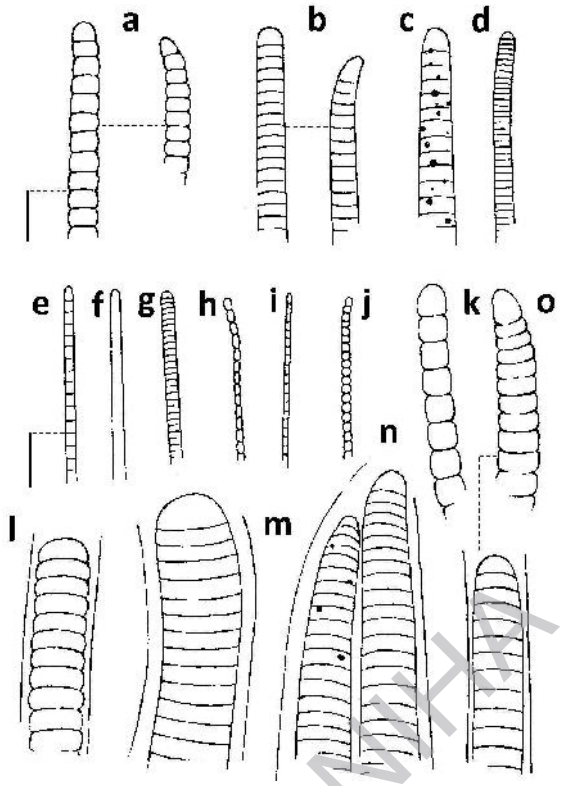

Fig. 3. Schematic view of a. *Oscillatoria tenuis*, b. *O. subbrevis*, c. *O. ornata*, d. *Phormidium* sp.$_1$, e. *Jaaginema* sp.$_2$, f. *Jaaginema pallidum*, g *Jaaginema* sp.$_1$, h., j. *Stenomitos frigidus*, i. *Pseudanabaena minima*, k. *Phormidium* sp.$_2$, o., l. *Oscillatoria* sp., m. *Oscillatoria limosa*, n. *Symplocastrum* sp.

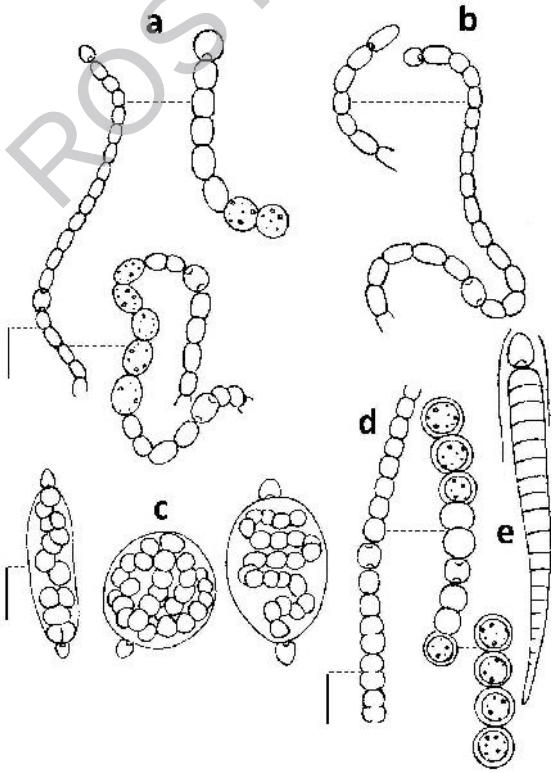

Fig. 4. Schematic view of a. *Nostoc hatei*, b. *Nostoc* sp.$_2$, c. *Nostoc edaphicum*, d. *Trichormus* sp., e. *Calothrix* sp.

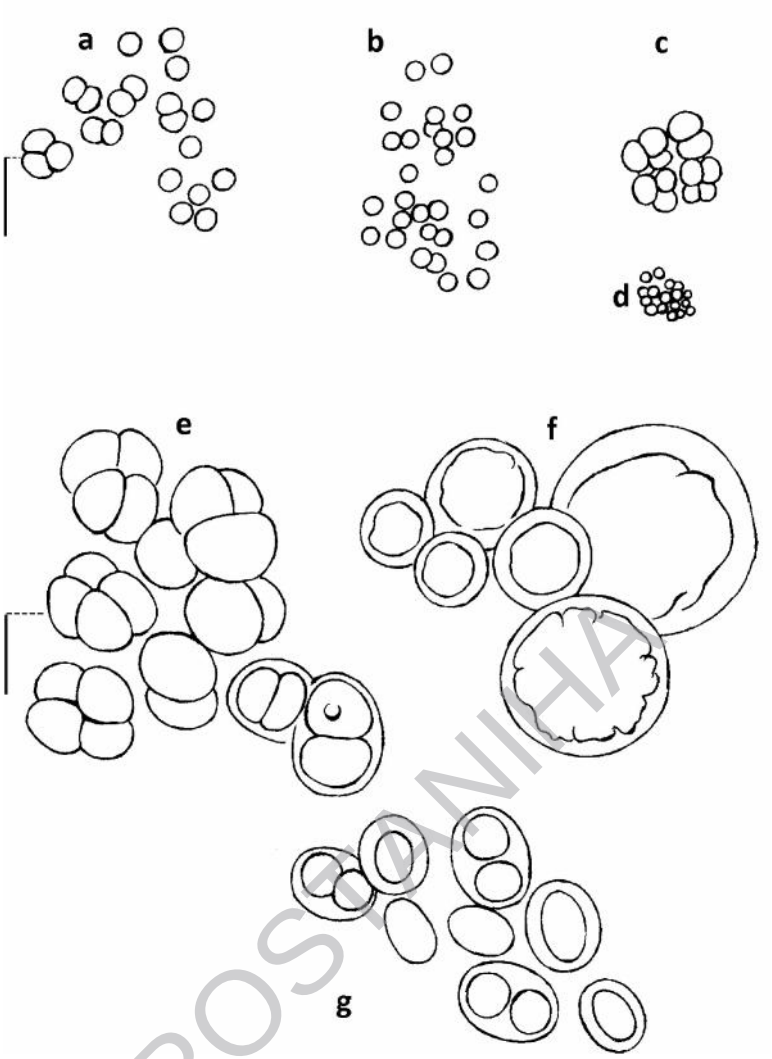

Fig. 5. Schematic view of a. *Chroococcus* sp. 1, b. *Chroococcus minimus*, c. *Chroococcus* sp. 2, d. *Aphanocapsa* sp., e. *Limnococcus limneticus*, f. *Haematococcus* sp., g. *Gloeothece tepidariorum.*

- Phylogenetic study

In the present study, 16S rRNA gene sequences from 28 taxa of cyanobacteria were obtained out of which, 21 sequences were extracted from GenBank (Table 4) used in phylogenetic analyses. The phylogenetic relationship of several taxa from five families of cyanobacteria (*Pseudanabaenaceae*, *Leptolyngbyaceae*, *Coleofasciculaceae*, *Oscillatoriaceae*, and *Nostocaceae*) was evaluated indicating high levels of similarity between the studied samples and species of the same genus recorded in NCBI.

In phylogenetic tree based on 16S rRNA gene sequencing, *Nostocales* taxa were monophyletic linage, while *Oscillatoriales* taxa were paraphyletic group. In this phylogenetic tree, the separation of some genera was distinct, while in some genera such as *Leptolyngbya* and *Pseudoanabaena* this was not clear.

Table 4. Information and GenBank accession number for the studied taxa

Taxon and strain designation	Origin	GenBank accession No.
Bacillus amyloliquefaciens	-	HM016080.1
B. subtilis	-	HQ232422.1
Geitlerinema exile	USA	JN166636.1
Jaaginema sp.$_2$ ISB87	Iran, Yazd, Site 1*	KX911987
Leptolyngbya badia	USA	EF429297.1
L. schmidlei	-	AF355398.1
L. subtilissima	Soil, Southern Africa	KC463197.1
Leptolyngbya sp.	Gujarat, Vatva Industrial Estate, India	FJ410906.1
Leptolyngbya sp.	Portugal	HM217060.1
Leptolyngbya sp.	Paradise Mountain Range, Mojave Desert, USA	HM018680.1
Leptolyngbya sp.	Poland	EU528669.2
Leptolyngbya sp.	USA	EU528665.1
Nodosilinea epilithica	Italy	HM018677.1
Nostoc calcicola	India	GQ167549.1
N. commune	Paddy field of Sambalpur, India Odisha, India	KP792345.1
N. ellipsosporum	Paddy field of Sambalpur, Odisha, India	KP792330.1
N. muscorum	Hailakandi district of southern Assam, northeast India	KP052630.1
Nostoc sp. ISB81	Iran, Yazd, Site 4*	KX911981
Phormidium terebriforme	Spain, mat on sandy surface from Guadarrama river	JN382220.1
Phormidium sp.	Pakistan, fresh irrigation water present in crop fields	FJ839355.1
Phormidium sp.	USA	JN166684.1
Planktothricoides sp. ISB86	Iran, Yazd, Site 4*	KX911986
Pseudanabaena minima ISB83	Iran, Yazd, Site 1*	KX911983
P. minima ISB84	Iran, Yazd, Site 2*	KX911984
P. minima ISB85	Iran, Yazd, Site 5*	KX911985
Pseudanabaena sp.	USA	JN166630.1
Pseudanabaena sp.	USA	JN166652.1
Stenomitos frigidus ISB82	Iran, Yazd, Site 4*	KX911982

* Sites 1. Kalantar farm, 2. Shamsi, 4. Tabas three-way, 5. Sadrabad (geographical positions of sites are mentioned in Table 1)

Discussion

The cyanobacterial diversity has been already studied in several aquatic and wet land ecosystems of Iran (Shariatmadari *et al*. 2015, Saadatnia & Riahi 2009, Shokravi *et al*. 2002), but few studies have been addressed the diversity of these microorganisms in relatively dry soils of central provinces of Iran. In the present study, cyanobacteria were identified in five sites of Yazd-Ardekan district (Yazd province). The results of this research revealed that the wheat fields adjacent to industrial area of this province contain a flora of cyanobacteria resistant to stressful conditions and have less diversity than other reported field like paddy fields. This reduction in diversity can be attributed to some

factors such as climatic conditions in the studied area (low rainfall, strong sunshine and high temperature), special chemical and physical structure of soil (salinity and heavy metals) and also pollution caused by irrigation of plants with industrial waste water.

Among the identified taxa, members of *Oscillatoriales* were dominant. In the current study, *Jaaginema* and *Oscillatoria* had the highest diversity in the soil samples of all sites (Fig. 6). Among the studied sites, site 4 with 17 species and site 5 with eight species possess the highest and the lowest species diversity, respectively.

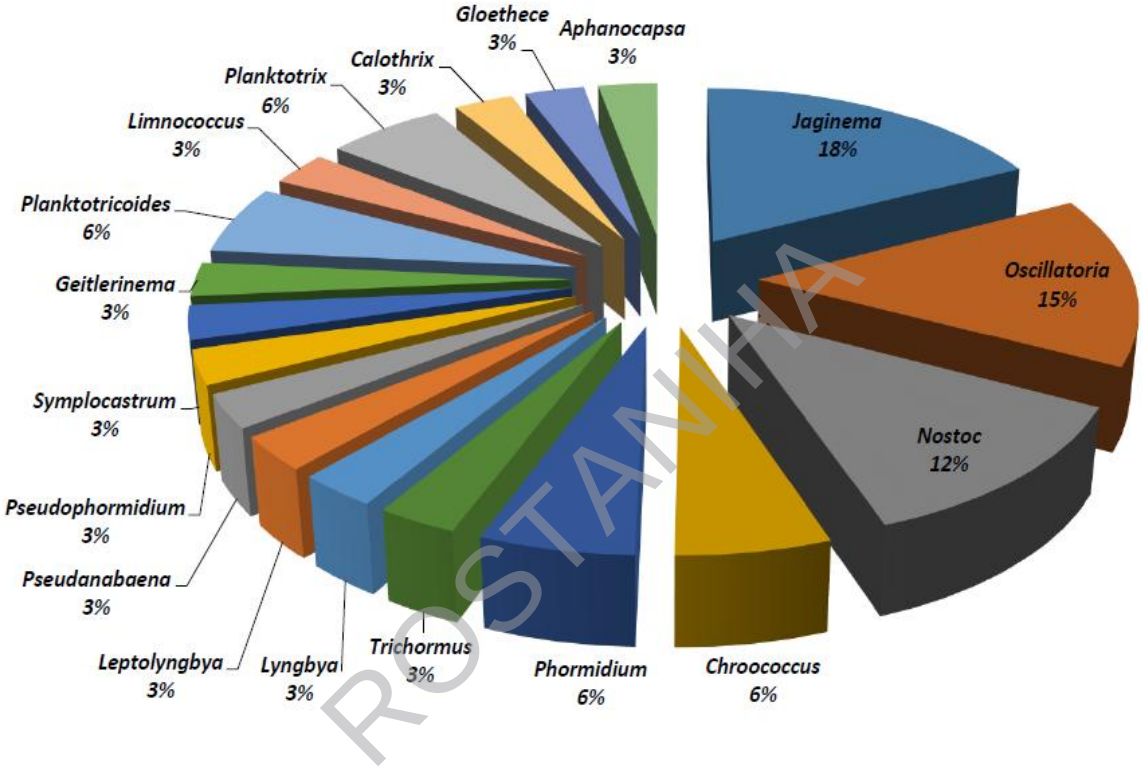

Fig. 6. Frequency percentage of cyanobacterial genera in the soil of the studied stations.

Among the various molecular markers for phylogenetic study of cyanobacteria, 16S rRNA gene sequences is the most appropriate marker for phylogenetic analyses (Korelusová 2005), because the sequence of this region accepts the least impact of horizontal displacement gene and is a more or less stable area for molecular studies.

In the present study, the 16S rRNA gene sequences were not suitable for separation of some complex taxa, but it seems that, this marker can be acceptable in higher taxonomic ranks such as genus and family. Cyanobacteria's responses to environmental conditions create complexity in this group of prokaryotic microorganisms, especially in complex taxa. Complex cyanobacteria are defined as microorganisms which have not been well-defined yet, and further investigations for new characters are required to clearly define these taxa (Palinska *et al.* 2011).

In this study, phylogenetic tree and the cluster analyses partly confirm each other (Figs 7 & 8). In the resulting phylogenetic tree, several specimens of *Pseudoanabaena* and *Leptolyngbya* were isolated from the other genera (Fig. 8). It should be noted

that, this genetic similarity is supported by the morphological similarity of taxa (Premanandh *et al*. 2009). Among the most significant differences between these two genera, characteristics such as slight differences in sheath, compression at the cross walls and the width of trichome can be emphasized. On the other hand, recent phylogenetic studies of species within the *Leptolyngbya* genus have demonstrated that, this genus is a polyphyletic assemblage and its molecular sequences are dispersed throughout the phylogenetic tree (Lopes *et al.* 2011, Perkerson *et al.* 2011), as some strains fitting in the current circumscription of *Leptolyngbya* are genetically and phylogenetically distinct from *Leptolyngbya*. Members of this clade have both morphological synapomorphy and shared 16S-23S internal transcribed spacer (ITS) secondary structures, resulting in the diagnosis of the new cyanobacterial genus *Nodosilinea* (Perkerson *et al.* 2011). Moreover, in the other sub-clade, heterocystous taxa were separated from non-heterocystous specimens. Those belonging to *Nostoc* genus are placed in one group and taxa belonging to *Planktothricoides*, *Phormidium*, and *Geitlerinema* genera were located in the other one. It should be noted that, phylogenetic analyses based on the 16S rRNA gene sequences often show orders such as *Oscillatoriales* and *Chroococcales* to be polyphletic and taxa belonging to *Nostocales* order as being monophyletic (Korelusová 2008). The phylogenetic analyses of present study also confirmed monophyly of *Nostocales* as well as polyphyly of *Oscillatoriales*.

Placement of taxa belonging to some genera such as *Planktothricoides*, *Phormidium*, and *Geitlernema* in one clade indicates their genetic similarity as compared to the others. The genetic similarity of the studied taxa was confirmed by morphological characteristics especially for *Phormidium* and *Planktothricoides* as these similarities are far greater and quite distinct (Hašler *et al*. 2012). Some features of the filaments such as being non-branched, less than 6 µm in diameter, and indent in the cross walls; are considered as the main similar characteristic. The main differences between the two genera are granulated cells and relatively narrow end of *Planktothricoides* genus.

Finally, this study showed that, 16sRNA sequences phylogenetic analyses can approve morphological characteristics clustering analyses of some filamentous cyanobacteria. Many of these taxa have locations changing in similar genera. For example, *Pseudanabaena frigida* species can be mentioned in this regard which is today grouped in *Stenomitos* genus and as *Stenomitos frigidus* (http://www.algaebase.org).

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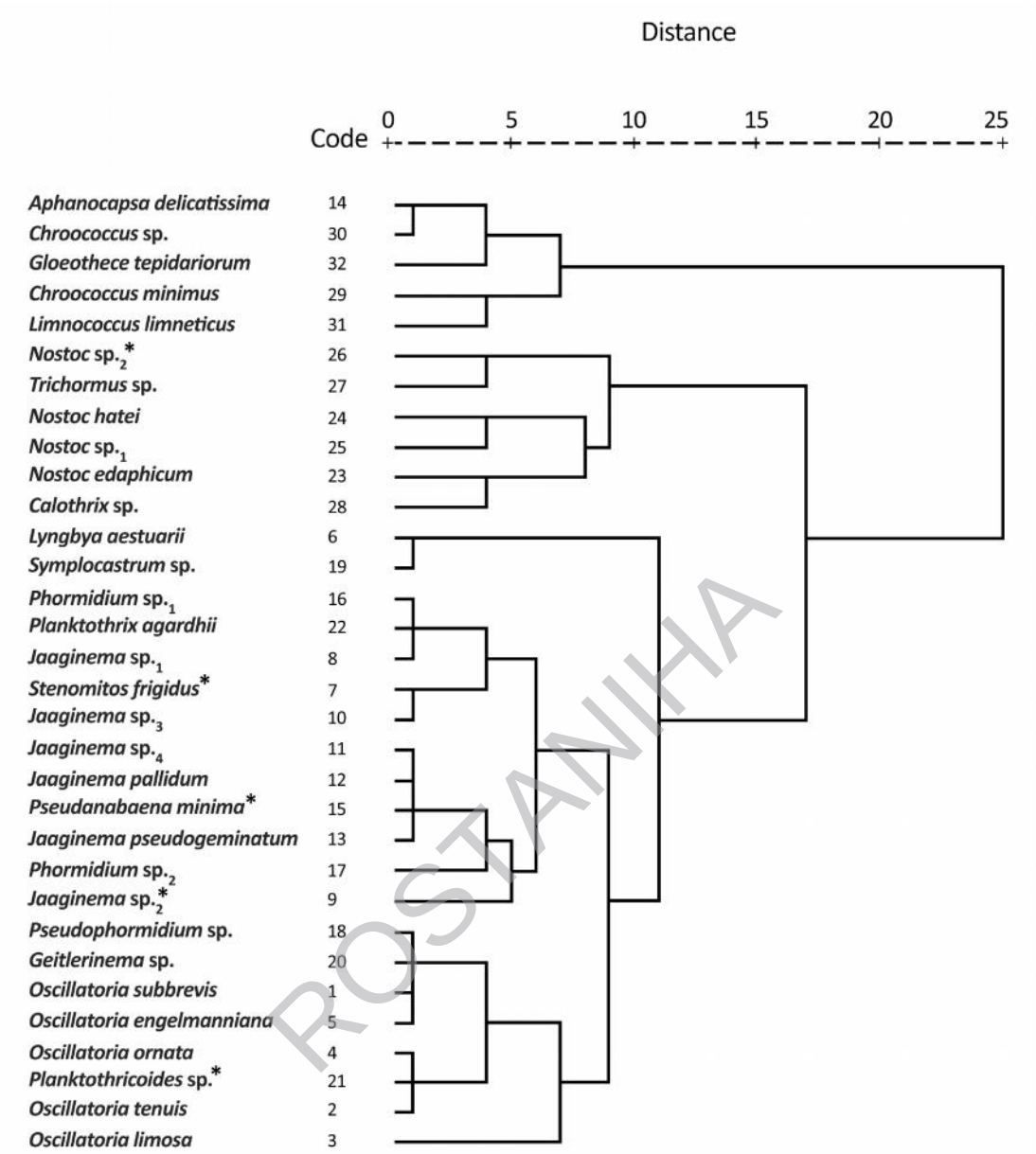

Fig. 7. Hierarchical cluster analysis dendrogram of cyanobacterial taxa based on morphological characters using UPGMA method. *Species studied in phylogenetic tree.

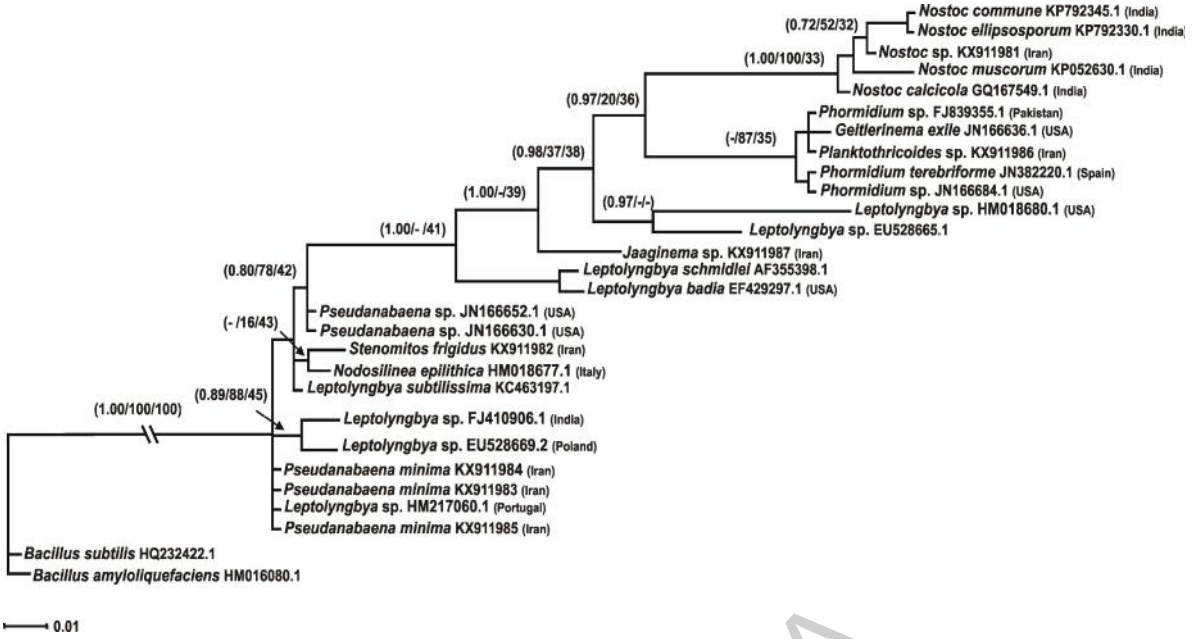

Fig. 8. Fifty percent majority rule consensus tree resulted from Bayesian analysis of the 16S rRNA dataset. Numbers above branches are posterior probability and likelihood as well as parsimony bootstrap values, respectively. Values <50 % were not shown.

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