Taxonomical Characterization of *Fischerella* sp. FS18 collected from paddy-fields of Golestan Province (Iran)

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Abstract

In a multidisciplinary way, taxonomical characterization of the cyanobacterium *Fischerella ambigua* (N?geli) Gomont, collected from paddy-fields of North of Iran have been investigated. Beside morphologies, ecophysiological studies including survivsl, growth and pigment composition at different condition of salinity, temperature and light intensity, and molecular investigations including 16S rRNA gene sequences of the cyanobacterium have been considered. The results indicate the drastic underestimation of the physiological and phylogenetic diversity of these cyanobacteria by the current morphology-based classification and the clear need for new taxa.

Keywords: Cyanobacteria, *Fischerella ambigua*, Iran, Paddy-Fields, Taxonomy

Introduction

It seems that in north paddy fields of Iran, especially Golestan province, some strains of stigonematales especially *Hapalosiphon* spp., and *Fischerella* spp., are common (Shokravi et al., 2001, 2002) but there is no clear report about their morphological characterizations and taxonomic situations. The species of stigonematalean cyanophytes are distributed all over the world but mainly scarcely and strictly in special (extreme) biotopes (Anagnostidis & Komarek, 1990).

Consequently, they are traditionally considered cosmopolitan micro-organisms with remarkable capabilities to acclimatize to broad ranges of environmental conditions (Shokravi et al., 2003; Geitler, 1932). Genera of the stigonematales exhibit the highest degree of morphological complexity and differentiation within the cyanobacteria (Anagnostidis & Komarek, 1990, Castenholz, 2001). Many populations of stigonematales show considerable morphological variation, even at one site (John et al., 2002). The complex variety of forms or developmental stages exhibited by *Fischerella*, which may include primary and secondary trichomes, hormogonia, unicells and amorphous cell aggregates. As we know this variations is not confined to the strains (Castenholz, 2001).

However, morphologybased classification may provide insufficient taxonomic resolution and cyanobacteria with similar or identical morphology may have significantly different physiology. In recent years, the analysis of 16S rRNA gene sequences has demonstrated that morphological groupings of cyanobacteria in some cases correspond to phylogenetically coherent taxa (Shokravi et al., 2007), whereas in others the traditional classification drastically underestimates extant diversity (Gugger et al., 2004). In bacteriology, in particular, the tolerances to and requirements for salt concentrations and temperatures have been recognized as important phenotypic properties correlating with phylogeny (Gugger and Hoffman, 2004). Light is evidently one of the most important factors, which determine the natural distribution of cyanobacteria (Fernandez–Valiente & Leganes, 1989). In addition to light, pH is another factor, which clearly affects the distribution of

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cyanobacteria. Most cyanobacteria grow in environments that are neutral to alkaline and in laboratory cultures the optimal pH ranges from 7.5 to 10. Generally, a wide range of adaptation to pH has been observed not only among different genera but also between different isolates of the same species (Soltani et al., 2006; Poza-carrion et al., 2001).

Besides survival of cyanobacteria in natural environments depends upon their ability to acclimate to the variable conditions of environmental factors. For cyanobacteria with *stigonematalean*-like morphology, uncertainties about the evolutionary coherence of the current generic classification have been expressed sporadically on the basis of analyses of lipid compositions or ultrastructure (Tabatabaii Yazdi et al., 2005).

Fischerella sp. FS18, have been the special strains for our field and laboratory experiments during few years ago until now (Shokravi et al., 2007) We have published some papers about physiology, ecophysiology, pharamaceutical and applied aspects of this strain (Ghasemi et al., 2005; Tabatabaii Yazdi et al., 2006; Soltani et al 2005; Soltani et al.; 2006; Soltani et al., 2007; Shokravi et al., 2007; Shokravi et al., 2008). Unfortunately we had no time for exact concentration on morphology and taxonomy of such a strain. Few researches have been done (Shokravi et al., 2007), but results seem non clear and doubtful. Both for description and identification.

The aim of this research was a new move to characterize the real taxonomical position and possibly determination and almost exact description of this strain. However, a comprehensive comparative study on the physiology and phylogeny of this cyanobacterum has been lacking and, therefore, the diversity within the botanical genus *Fischerella* remains largely unexplored. The question whether morphological counterparts from different environments are related or have undergone convergent evolution is particularly interesting. We have analyzed and compared the 16S rRNA gene sequences, morphologies, halotolerances, temperature requirements, illumination and pH as combined elements, pH as a unique factor, and pigment compositions of cultures of cyanobacterium *Fischerella* sp. FS18 collected from paddy-fields of North of Iran. A phylogenetic pattern emerges which is in part supported by phenotypic characteristics. We propose the reclassification at the species level of this cyanobacterium with tightly branched trichomes from paddy-fields.

Material and Methods

Cyanobacterial strains, cultivation and purification

Soil samples were obtained from paddy fields of different stations of Golestan province (north of Iran and near Caspian sea). A complete description about stations and their geographical and environmental conditions have been reported in Shokravi et al., (2002, 2003). The collected soils were cultured by usual methods (Kaushik, 1987). After colonization and isolation, the cyanobacterium *Fischerella* sp. FS18, was purified and turned to axenic condition (Kaushik, 1987). Identification was done according to John et al., (2002), Anagnostidis and Komarek (1990), Tiffany and Britton (1971), Prescott (1962), Desikachary (1959) and Geitler (1932). Stock cultures were grown in N-free medium. Cultured in solid BG11 medium ($MgSO_4.7H_2O$, 0.3mM; $CaCl_2.2H_2O$, 0.25 mM; $K_2HPO_4.3H_2O$, 0.18 mM; $Na_2MgEDTA$, 0.003 mM, Citrate ferric 0.02 mM; Acid Citric, 0.029 mM; Na_2CO_3 0.188 mM; microelements 1 mll^{-1}). The mixture was bubbled overnight with air to drive excessCO2 out of solution and thus reduce the amounts of carbonate and bicarbonate in the final mixture. The pH was then raised to 8±2 by addition of NaOH and the solution was autoclaved. Axenicity was controlled microscopically.

Illumination and pH

The cultivation was done under different illumination (2, 11, 24, 104, 300$\mu Em^{-2}s^{-1}$) and pHs (5, 6, 7, 8, 9). The temperature was adjusted on 30±1?C. Illumination was supplied with 40W cool white fluorescent tubes. Plates were placed at different distances from the light source to obtain a linear gradient of irradiance. Light measurements were made with a Licor LI-1000 Datalogger equipped with a quantum sensor. Alternatively, other experiments were carried out in batch cultures, using 300 ml of inoculated medium in 500 ml. Erlenmeyer flasks stoppered with cotton plugs. Culture was maintained without aeration or stirring and buffered and illuminated as above. After 48h of culture, when cells were fully adapted to light regime and pH, aliquots were taken and used for determinations.

Salinity Stress

Stock cultures were grown in the BG110. Cells in logarithmic phase of growth were collected from stock cultures and used as inoculate for experiments. BG110 medium of different salinity was made for inoculation of *Fischerella* sp. FS18. The required salinity was obtained by adding sodium chloride. The flasks were maintained for 21 days at 30±1°C under constant illumination of about 60$\mu Em^{-2}s^{-1}$. The cyanobacterium

was treated with different concentrations of NaCl (NaCl-free, 0.5 & 1%).

Determination of temperature requirements

Temperature ranges were determined by visual inspection of growth in test tube cultures with liquid media after incubation for a maximum of 14 days. All of the cultures received constant irradiance of 60 umol photons of white light m^{-2} s^{-1}. Temperatures tested were 4, 10, 15, 20, 25, 35, 40, 45 and 50 °C. Growing cultures were subjected to stepwise temperature shifts of a maximal 6 °C each time.

Growth rate measurements

The strains were grown in deep Petri dishes filled with liquid media. Growth rates were measured by optical density and biomass determinations. For each strain, the correspondence between optical density and biomass (dry wt) was checked (R^2=87%±8; data not shown). Growth was followed in triplicate cultures during periods of one week, so that four to curve doublings during exponential growth could be monitored. Linear regression analysis of the natural logarithms of the biooptical behavior values yielded estimates of growth rates. Means and standard deviations of triplicate measurements have been noticed.

Pigment composition

Chlorophyll content was determined spectrophotometrically at 665 nm according to Marker (1972). Phycobiliproteins were extracted after osmotic shock and measured spectrophotometrically at 652, 615 and 562 nm. (Soltani et al.2006). Carotenoides have been extracted and determinrd using Becker methods (Soltani, 2007)

Stattistical Analysis

All experiments were repeated three times. Data are the means of triplicate tests ± SD. Statistical differences were examined using the ANOVA test.

PCR amplification, cloning and sequence analysis of 16S

For this purpose, DNA content was first extracted from the cyanobacterium and then PCR was applied using two set primers. Sequences were amplified using the primers 5´- GGAATCTTCCGCAATGGG-3´ as forward and 5´-GACTACAGGGGTATCTAATCC -3´ as reverse, which amplify a ~400-bp region of the 16S rRNA gene. To extract of DNA from the *Synechococcus nidulans*, a fresh biomass was obtained by centrifuging at 12000 rpm. After washing of biomass two time with PCR distillated water, it was incubated at 96^0C for 5 min and then centrifuged at 12000 rpm. The supernatant was used as a template for PCR. The applied PCR condition has been described by Nubel et al., (9). PCR products were electrophoresed in a 1% (w/v) agarose gel using TBE buffer containing 1µg/ml ethidium bromide.

A single ~400-bp band of DNA was cut and extracted from the gel using the Core Bio Gel Extraction Kit. The sequence was determined by the CinaGene company with the primers. Sequence similarity searches were done with BLAST through the website of the NCBI.

Morphological observations

Morphological observations were made in liquid as well as on solid media. Thallus growth, filament structure, types of branching, position of the heterocysts, multiplication, in addition of biometrical information were recorded (Gugger & Hoffmann, 2004, Shokravi et al., 2007). Colony formation and cells shapes were evaluated by binocular and light microscope (in addition phase contrast, epifluoresscence, and electron microscopy) each day in two week periods.

Results

Microscopic observations

Usual morphological characteristics, seems true for this strain. Morphological observations with traditional approach strongly emphasize the preliminary identification. Unilateral and one cell layer true branches, intercalary heterocyst with oval to subcylindrical (to cylindrical) forms, mostly one cell layer main axes with portions with more than one layers of cells, and finally the high degree of hormogoniums (Figure 1), will make almost strong evidences for dependency to Fischerella (botanical) or form-genus II (microbial) classification systems (Desikachary, 1959; Gastenholtz, 2001).

Micromorphology of the vegetative cells of *Fischerella* sp. FS18, specially the arrangement of the thylakoids (Figure.2), may clear some doubts about real positions of the strain at the species level (personal communications with L.Hoffman). Circular arrangement of the thylakoids (Figure2), strongly emphasize belonging of the strain to Fischerella groups (Anagnostidis and Komarek, 1990).

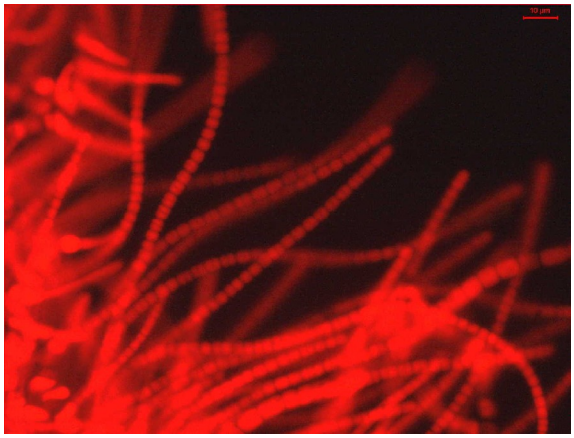

Figure 1: Hormogonia release in *Fischerella sp.* FS18 (fluorescence microscopy)

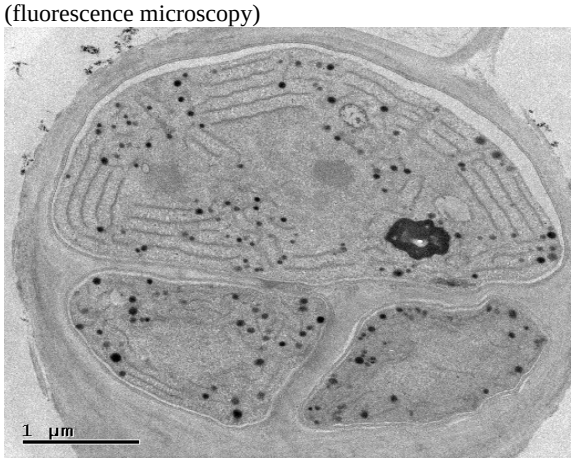

Figure 2: Electron micrograph of a vegetative cell of *Fischerella sp.* FS 18 with special thylakoids arrangement

The high degree occurrence of reserve granules may be related to nitrogen limitation during analysis (Soltani et al., 2006). About the certainty for the species determination, this kind of thylakoids arrangement makes close dependency to *Fischerella ambigua* (personal communications with L. Hoffman). In *F. musicola* and *F. epiphytica* the patterns of thylakoid arrangements differ with such a completely regular pattern (Anagnostidis and Komarek, 1990).

Morphological Variations and Biometrical Analysis

Morphological variations of *Fischerella* sp.FS18 can be related with both acidity and light. Variations were seen in pHs 5 and 9. In low light intensities, there is no hormogonium production in each acidic condition. Biometrical statistical analysis showed that it seems hard to reach a unique pattern in morphological variation analysis in vegetative cells and heterocysts of this strain, especially comparing the pattern of expanding width of main axes with the length and width of branches and even main axes too (Tables 1-4). High light intensity (300 µE m^{-2} s^{-1}), caused noticeable morphological variations especially in pH5. It seemed that in this condition, organism tends seriously to get a new or at least different topological configuration. In other light intensities, this pattern seemed relatively variable and possibly localized. In alkaline condition (pH9), the patterns of growth in low and medium light intensities were completely the same (Tables 1-4).

Table 1: Vegetative cell dimensions at the main axes 4th day after inoculations (um) at different pH and Irradiances

Light µE $m^{-2}s^{-1}$	PH5	PH6	PH7	PH8	PH9
300	17 x 8	13.2 x 7.2	12.5 x 6.5	12.5 x 8	9 x 8
107	13.5 x 8.75	12 x 8	13.5 x 9	11.5 x 8	11 x 5.25
24	14.5 x 9	14 x 6	9.5 x 7.5	13.5 x 6.5	10.5 x 7.75
11	12.75 x 6.5	10.5 x 8.5	10.5 x 8.5	8 x 5	9 x 7.25
2	9.2 x 4	9.6 x 5	9.5 x 6.5	9.25 x 6	10 x 6.5

Table 2: Vegetative cell dimensions at the branches 4th day after inoculations (um) at different pH and Irradiances

Light µE $m^{-2}s^{-1}$	pH5	pH 6	pH 7	pH 8	pH 9
300	16 x 5.6	10.5 x 5.2	12.5 x 4	15.5 x 4	15.5 x 4
107	15 x 4	11.5 x 6	10 x 6	14 x 4.5	17.5 x 5
24	15.5 x 4.5	9.5 x 5	13.5 x 4	15 x 4	13.5 x 4
11	11.5 x 4	10.5 x 5	11.5 x 4.25	8.25 x 4	11.25 x 4
2	10.7 x 4	9.4 x 4	10 x 5	11.75 x 4	11.25 x 4

Table 3: Heterocyst cell dimensions at the main axes 4th day after inoculations (um) at different pH and Irradiances

Light µE $m^{-2}s^{-1}$	pH5	pH 6	pH 7	pH 8	pH 9
300	16 x 6	14.5 x 6.5	14 x 6.5	14 x 6	13.5 x 7
107	12.5 x 9.5	12.5 x 7	14.5 x 8	11 x 6	11.5 x 5
24	16 x 8	11.5 x 7	12.5 x 7	13.5 x 6	12 x 6
11	14 x 6	13.5 x 7.5	12 x 7	11.88 x 5.75	11.5 x 5.75
2	11.75 x 6.8	11.75 x 7.5	11.5 x 6.75	10 x 6.75	10.25 x 6

Table 4: Heterocyst cell dimensions at the branches 4th day after inoculations (um) at different pH and Irradiances

Light µE $m^{-2}s^{-1}$	pH5	pH 6	pH 7	pH 8	pH 9
300	14.5 x 6	13.5 x 5.5	12.5 x 4.5	13.5 x 7	12 x 5
107	13 x 6	14 x 7	11 x 7	13.5 x 5.5	11.5 x 6
24	11 x 6	16 x 5	13 x 4.5	14.5 x 5	11.5 x 6
11	10 x 5	14.5 x 5	13 x 5	11.63 x 4.25	11.5 x 5.75
2	10x4	12 x 5	14 x 4	11.5 x 4.25	11.75 x 5

The presence of sheath seems an important diagnostic feature between different genera of stigonematales. It is not possible to observe any sheath in our strain, but phase contrast photogaraphy seems the existence of such a sheath around main axes and branches (Figure 3.). Unfortunately until now we have not any research about the effects of irradiance and pH on sheath formation at stigonematalean species. However it may be possible an acclimative character or may be constitutive. In all the treatments we were able to see the sheath (even at pH 5). So it may be logical to conclude that this may be a constitutive than an acclimation reflection to environmental conditions (Shokravi, 2007).

Figure 3: Phase- contrast micrograph of of *Fischerella sp.* FS 18.

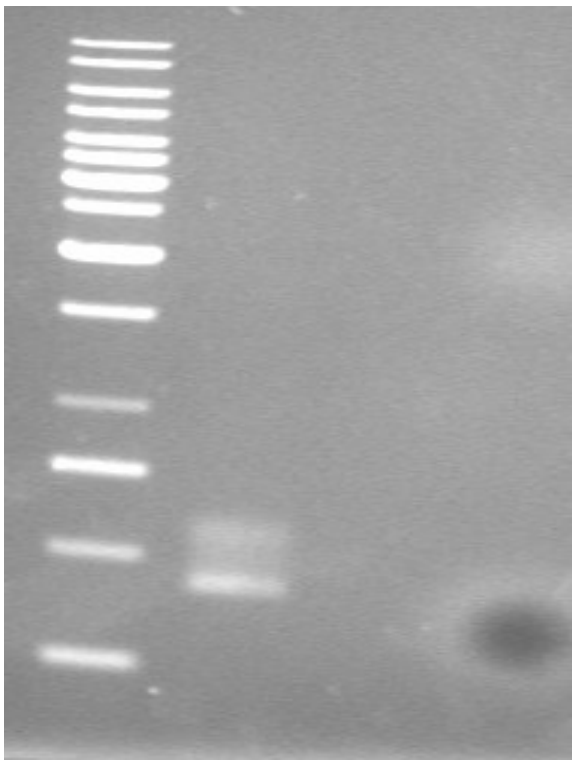

Figure 4: Agarose gel electerophoresis of DNA prepared from *Fischerella sp.* FS 18 which has been purified using PTB reagent.

16S rRNA gene sequences

The partial sequence of the 16S rRNA sequence of the *Fischerella sp.* FS 18 is as follows:

´TGGGGAATTTTcCgAATGGGCGAAAGCCT
GACGGAGCAATACCGCGTGAGGGAGGAAGGC
TCTTGGGTTGTAAACCTCTTTTCTCAGGGAAT
AAGCAAGTGAAGGTACCTGAGGAATCAGCAT
CGGCTAACTCCGTGCCAGCAGCCGCGGTAATA
CGGAGGATGCAAGCGTTATCCGGAATGATTG

GGCGTAAAGCGTCCGTAGGTAGCAGTGTGTGT
CTATTGTTAAAGAGTTTGGCTTAACCAAATAA
AGGCGGTAGAAACTACACAGCTAGAGTGCGT
TCGGGGCAGAGGGAATTCCTGGTGTAGCGGT
GAAATGCGTAGAGATCAGGAAGAACACCGGT
GGCGAAAGCGCTCTGCTAGGCCGCAACTGAC
ACTGAGGGACGaaaGctagGggAGCGAATGGGAT
TAgataCCCCAgTAGTCA3

The sequence of *Fischerella* sp. strain FS 18 was recorded in the NCBI under the accession number EU255584.

Salt requirements

The dependence of growth rates on salinity is illustrated in Figure 5. This strain not show a distinct and narrow salinity optimum, but were able to grow with close to optimum rates in freshwater medium (BG11). Thus, can be termed euryhaline. *Fischerella musicola* (Bornet & Flahault) Gomont tolerated a salinity of 10% but died at 13%. It is the same for *Nostochopsis lobatus*. At this organism, elevated temperature (38°C) resulted in increased growth rates at high salinities and an increased upper salinity limit of growth (20%). This temperature effect on halotolerance had previously been observed for some unicellular, extremely halotolerant cyanobacteria (Garcia-Pichel et al., 1998). We have no seen such ability in our strain.

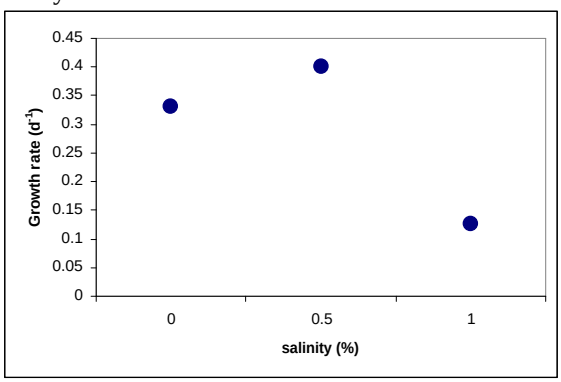

Figure 5: Comparisons of the growth rates of *Fischerella sp.* FS 18 at different salinities

Results revealed that the growth rate was higher in NaCl-free and salinity did not inhibit growth of other treatments. Similar results were seen in the case of other physiological processes. The light-saturated photosynthetic rate was higher in NaCl-free and it decreased with increasing salinity (Results not shown). Also growth and photosynthesis of this strain decrease (but not inhibit) with increasing NaCl in paddy fields. This is not true for *Nostochopsis sp*. (89-45 partial 16S rRNA gene, strain 89-45 AJ544081) () and not been reported until now for *Fischerella musicola* (Personal communication with L.Hoffman).

Temperature requirements

In our experiments, it showed growth between 35 and 45°C and did not grow at 50°C. Natural populations of *Fischerella* ambigua (Bornet & Flahault) Gomont showed an upper temperature limit of 51°C and maximum photosynthesis rates at 45 °C (Castenholz, 1977). The strains that had shown the highest tolerated 40 or 38 °C, respectively, and did not grow at 15 °C and below. Thus they displayed a lower temperature requirement compared to the *Fischerella* ambigua (Bornet & Flahault) Gomont (Castenholz, 2001).

Pigment compositions

Effect of irradiance and pH on chlorophyll concentration can be seen in Table 5. Chlorophyll content at pH 7 was higher than at pH 9 and pH 5 regardless of light intensities. The difference in chlorophyll content between pH 7 and pHs 5 and 9 was significant (ANOVA, $P<0.001$). Also there was higher chl content at 3 µmol photon m^{-2} s^{-1} than 300 µmol photon m^{-2} s^{-1} at pH 7 and 9. Differences were significant in both cases. This feature was not seen at pH 5, since there was no growth of this cyanobacterium at acidic pH and chlorophyll contents were very low at both light intensities (Soltani et al., 2006).

The amount of PBP at pH 7 was higher than in pH 9 and pH 5 (Table 1). Differences were statistically significant (ANOVA, $P<0.001$). By decreasing light intensity, these pigments varied in the same way that chlorophyll, but the differences were less pronounced. The PC and APC contents followed the same trend than total PBP, with higher values at pH 7 and low light intensity (Table 5). PC content at pH 5 was one order of magnitude lower than at the other pHs and APC was below the limit of detection at this acidic pH (Soltani et al., 2006).

The size of phycobilisomes can be usually represented by the ratio (PE (when present) +PC)/APC (Wyman & Fay 1986). At pH 5 it was not possible to calculate since no APC was detectable. The ratio was higher at pH 7 and high irradiance, but differences were not significant (ANOVA, $p<0.001$).

Table 5: Effect of combination of two pH values (7, 9) and two irradiances (3, 300 µmol photon.m^{-2}.s^{-1}) on pigment contents of *Fischerella ambigua* strain FS18 grown under the above conditions. Data are mean values of four experiments±SD

	Culture conditions	Chla	PBP	APC	PC	PE
pH	µmol photon.m-^{2}.s^{-1}			µg.mg dw^{-1}		
5	3	6.81±1.3	9.83±2.3	0	14.99±1.1	0
	300	6.13±0.2	4.00±5.3	0	7.25±9.5	0
7	3	24.40±1.9	111.18±6.7	12.43±1.4	91.02±6.1	7.73±1.0
	300	19.48±1.9	101.36±13.9	10.14±2.55	83.28±11.4	7.94±0.42
9	3	11.99±2.8	79.21±28.3	7.80±9.4	65.68±21.4	5.71±4.9
	300	8.32±0.7	64.66±11.9	6.57±1.5	46.97±11.4	11.12±0.9

The ratios PBP/Chlorophyll or APC/Chlorophyll are used to show the relationship between photosystem II and photosystem I (Poza- Carri?n et al., 2001). The ratio PBP/chlorophyll increased significantly with the pH, but is not affected by light intensity. The APC/chl ratio was also higher at pH 9 than at pH 7 but differences were not significant. This ratio was also not affected by light intensity.

Phylogeny

Comparing 16S rRNA sequences (Figure 6) with the gene bank (Htpp://www.ncbi.nlm.nih.gov/BLAST) showed the highest level of similarities with *Fischerella* sp. partial 16S rRNA gene, strain 1711 AJ544076Int. J. Syst. Evol. Microbiol. 54 (Pt 2), 349-357 (2004) Gugger, M.F. and Hoffmann, L. (99%). So cleaning process have been operated using softwares and at the next time, comparing showed 100% similarity. Considering this high degree of similarity, at the second level of molecular determination, the strain has been identified as:

Fischerella sp. partial 16S rRNA gene, strain 1711 AJ544076Int. J. Syst. Evol. Microbiol. 54 (Pt 2), 349-357 (2004) Gugger,M.F. and Hoffmann,L. Phylogenic trees may be determined taxonomic position of the strain. Drawing phylogenic tree (Fig 4), show that this strain may be narrow borders with the following species:

Nostochopsis sp. 89-45 partial 16S rRNA gene, strain 89-45 AJ544081. Int. J. Syst. Evol. Microbiol. 54 (Pt 2), 349-357 (2004) Gugger, M.F. and Hoffmann, L.

In addition the following strains may be positiones with the strain at the second evoloutionary level:

- *Nostochopsis lobatus* 92.1 partial 16S rRNA gene, strain 92.1. AJ544080. Int. J. Syst. Evol. Microbiol. 54 (Pt 2), 349-357 (2004) Gugger,M.F.R.
- *Fischerella sp.* BB98.1 partial 16S rRNA gene, strain SAG 2027. AJ344560. Mol. Phylogenet. Evol. 23 (1), 82-90 (2002) Friedl, T.

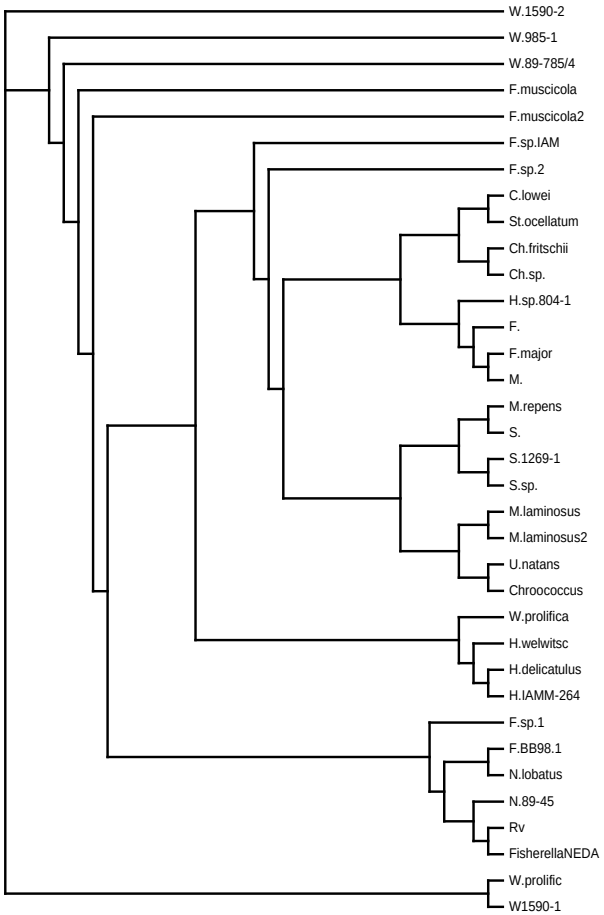

Figure 6: Phylogenetic tree based on 16srDNA sequence of stigonematalean cyanobacterium including *Fischerella sp.* FS18 (Fischerella Neda)

Discussion

Only a few stigonematalean morphotypes have been cultured, and therefore the high variability of morphotypes found in nature is under-represented in culture (Gugger & Hoffmann, 2004). Only two genera have been characterized from axenic culture strains, *Fischerella* (including *Mastigocladus*) and *Hapalosiphon*.

However results could be able to draw a relatively primitive picture of the combination effect of light

intensity and pH in morphological analysis of the organism. This organism showed variable characters from morphological point of view, and this variability was related with both acidity and light. The highest and lowest acidities (pH9 and pH5) showed the points for starting highest variations. On solid medium, all isolates had a creeping growth. This was in agreement with other papers (Gugger & Hoffmann, 2004; Shokravi et al., 2007).

High acidic condition had a remarkable inhibitory effect on the ability of germination. In pH 5, for instance, there was no growth in all irradiances (but slightly in conditions like 300 $\mu E\ m^{-2}\ s^{-1}$). In low light intensities, there was no hormogonium production in each acidity. This was true for both the first and second weeks. It has been emphasized that when mature cultures are inoculated on agar-solidified medium motile hormogonia are readily formed and easily isolated after migration on agar (Castenholz, 2001), but this situation could not be seen in all the acidities and irradiances. It seems that the potential of branch producing (especially main axes) decreased sharply in these conditions. In comparison with growth curves, it seemed that growth of the organism at least in neutral acidity, was not compatible with hormogonium production ability (Shokravi et al., 2007). In 4^{th} day, very low light intensities caused more growth (or at least equal) than high light intensities. It is in agreement with Castenholz (2001), who emphasized that in nonthermophilic strains of *Fischerella*, hormogonia were not always formed under favorite conditions and sometimes the multiseriate axis was rare or lacking.

In the case of cell dimensions; limited light intensities (2 $\mu E\ m^{-2}\ s^{-1}$) could be able to transfer the effects of different pHs on variation of lengths of vegetative cells in the main axes (the same as brnches). These results are in opposite of (Anagnostidis and Komarek, 1990). In 24 $\mu E\ m^{-2}\ s^{-1}$ light intensity, the effect of acidity on variation of cell sizes seemed obviously. The highest lengths in vegetative cells of the main axes (not branches) could be shown in pH 5 as well as liquid medium. Maybe the cells were enlarged but could not divide (Perona et al., 2003; Baftechi et al., 2002).

In the case of heterocysts, it seems that high light intensity (300 $\mu E\ m^{-2}\ s^{-1}$), changed the effects of pH on the width of these cells in the main axes. In branches, very high light intensities changed the effect of acidity on the diameter of heterocyst.

By statistical analysis, it is difficult to reach a unique pattern in morphological variation in vegetative cells of this strain. However, with this exception (cross expanding of the main axis), possibly high light intensity (300 $\mu E\ m^{-2}\ s^{-1}$), caused noticeable morphological variations especially in pH 5. In this condition, *Fischerella* tends to get a different topological configuration. In minimum light intensity (2 $\mu E\ m^{-2}\ s^{-1}$) and pH 5 cross enlargement of the main axis was seen.

In neutral condition (pH 7), the patterns of growth were different. In alkaline condition (pH 9), the pattern of growth in low and medium light intensities were completely the same. But in quantitative point of view is not comparable with neutral conditions. However results showed that this organism can be considered an alkalophilic organism. Optimal growth rates were observed at pH 7, which is nearly equal to acidity than that usually found in the rice fields from which the cyanobacterum was isolated (Soltani & Fernandez- Valiente, unpublished data).

Maximal rates of nitrogenase activity were found at neutral to little alkaline pH (pH 7, 8, Soltani & Fernandez- Valiente, unpublished data). This agrees with the neutral to alkalophilic nature of this cyanobacterium and could probably be related to the higher photosynthetic capacity per unit of dry weight shown by cells cultured at pHs 7-8 (Soltani et al., 2006).

Regarding physiological responses of *Fischerella* sp. FS 18 to NaCl, as shown in figs. 1 & 2, growth rate decreased with increasing in salinity though it continued in NaCl 1%. Maximum photosynthesis rate (P_{max}) is seen in control and confirms the results of short time experiments of photosynthesis. P_{max} decreased in NaCl 0.5 & 1%. Efficiency of photosynthesis reached to maximum in salinity 0.5% but the difference with control was not significant. Taking into account the results, it is concluded that salinity has significant effect on photosynthesis and affect on the usage of minimum light for photosynthesis. This cyanobacterium needs more light to survive in saline environment. This result is in agreement with the result of growth.. Results confirm the variation of chlorophyll content in different salinity. Figure 2 can demonstrate similar growth rate pattern which confirm the role of chlorophyll in cyanobacterial growth and changing of it with varied environmental factors.

Cyanobacteria regulate their relative concentrations of photosynthetic pigments in response to light intensity (Soltani et al., 2006). In general, there is an inverse correlation between light intensity and photopigment contents among cyanobacteria. In cultures of *Fischerella ambigua* strain FS18 we observed that cells grown at high irradiance showed lower values of chlorophyll than cells grown at low irradiance. The effect of light intensity was observed at pH 7 and 9 but not at pH 5 (Table 1). The chl content was higher in pH 7 than in pH 9 at both light

intensities. In *Fischerella ambigua* FS18, PC is the main component of phycobiliproteins, so the changes on total PBP mostly reflect the changes in PC. Total PBP and PC were affected by pH and light intensity. The highest PBP and PC content were observed at pH 7 and the lowest at pH 5. In agreement with other reports (Poza-Carri?n et al., 2001) total PBP and PC were lower at high irradiance, regardless of the pH. Since APC is a component of the core of phycobilisomes, and the core remains constant, a change in APC content reflects a change in the number of phycobilisomes.

Conclusion

Description of *Fischerella* sp. FS 18

Fischerella sp. FS 18 { *Fischerella* ambigua (Bornet & Flahault) Gomont 1895, P.52 }.

A branch and sheated filament with a prostrate portion giving rise to vertically elongate, much narrower, curved or straight branches in which 1 or more hormogonia are formed; cells sub-globose,quadrate,or cylindrical,usually loosely arranged in 1 to several series in the principal filament, in a single series only in the branches; hormogonia with cells closely adjoined and usually increasing in diameter toward the apices; heterocysts globose, barrel shaped, or quadrate; sheath either colorless or brownish, hormogones or lamellated.; plant mass consisting of prostrate mats of interwoven filaments from which vertical fascicles arise; sheaths colorless when young, becoming brownish; cells ovate or subglobular to quadrate in the main axis, rectangular in the branches, 4-6 times longer than wide; in solid medium all isolates had a creeping growth, motile hormogonia are readily formed and easily isolated after migration; circular arrangement of the thylakoids; halotolerant, euryhaline with trichomes coiled into a tight, closed helix, able to grow at salinities between 0 and 1% but not at marine salinitiesies; growth rate decreased with increasing in salinity though it continued in NaCl 1%., show a distinct and narrow salinity optimum, but were able to grow with close to optimum rates in freshwater medium,the highest tolerated 40 or 38 °C, and did not grow at 15 °C and below, Variable characters from morphological point of view, and this variability was related with both acidity and light. High acidic condition had a remarkable inhibitory effect on the ability of germination. In pH below 5, there was no growth in all irradiances (but slightly in conditions like 300 $\mu E\ m^{-2}\ s^{-1}$). In low light intensities, there was no hormogonium production in each acidity, PC is the main component of phycobiliproteins, there was no growth of this cyanobacterium at acidic pH and chlorophyll contents were very low at both light intensities; the amount of PBP at pH 7 was higher than in pH 9 and pH 5, the size of phycobilisomes was higher at pH 7 and high irradiance.

The axenic type strain is JAH FS!8, which was isolated from a microbial mat in a paddy –field near Ramsar, Mazandaran, Iran and has been deposited in the Iranian Microbial Culture Coollection with the code number ITCC1936. The DNA sequence of strain was recorded in the NCBI under the accession number EU255584.

Fischerella sp. partial 16S rRNA gene, strain 1711 AJ544076Int. J. Syst. Evol. Microbiol. 54 (Pt 2), 349-357 (2004) Gugger, M.F. and Hoffmann,L.

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نشان ویژه سازی تاکسونومیک *Fischerella* sp. FS 18 جمع‌آوری شده از شالیزارهای استان گلستان (ایران)

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چکیده

در یک پژوهش چندوجهی، نشان ویژه‌سازی سیانوباکتریوم *Fischerella sp.* FS18 جمع‌آوری شده از شالیزارهای شمال ایران انجام گرفته است. علاوه بر بررسی مورفولوژی، تنوع‌پذیری مورفولوژیک در شرایط توام نور و pH و بررسی‌های اکوفیزیولوژیک شامل بقا، رشد و وضعیت رنگیزه‌ای، در شرایط متفاوت شوری، دما، شدت نور، pH و نیز بررسی مولکولی شامل وضعیت 16srRNA در سیانوباکتریوم انجام شده است. نتایج حاکی از آن است که نشان ویژه‌سازی بر اساس بررسی‌های چند وجهی مبتنی بر مجموعه تنوع‌پذیری مورفولوژیک، فیزیولوژیک و فیلوژنیک، برای شفاف ساختن جایگاه تاکسونومیک این سیانوباکتریوم و معرفی جدیدی از آن می‌تواند برطرف کننده برخی ابهام‌ها باشد. در نهایت نمونه با تلفیقی از صفات چندوجهی تحت نام *Fischerella ambigua* (N?geli) Gomont معرفی شده، توصیفی جدید از نمونه با تکیه بر نتایج بدست آمده ارائه گردیده است.

کلمات کلیدی: ایران، تاکسونومی، سیانوباکتریوم، شالیزار، فیشرلا