Three New Records of Oscillatorian Cyanophyta for the Paddy –Fields Algal Flora of Iran

Siahbalaie, R[1]., Afsharzadeh, H.[1]., [*]Shokravi, Sh[2]

1. Dep. Biology, Faculty of Science, Isfahan Univ., Isfahan, Iran
2. Dep. Biology, Islamic Azad Univ-Branch Gorgan, Gorgan, Iran

Abstract

In this study, three Cyanophyceae species new for Iran are reported. These specimens are *Oscillatoria okeni* Agardh ex.Gomont, *O.earlei* Gardner and *O.bornetii* Fritsch. They were determined to be epidaphic and endaphic which were found at the paddy-field of Golestan province north of Iran and near the Caspian Sea.

Key Words: Cyanophyta, Iran, Paddy-Field, Taxonomy

Introduction

The species of Oscillatorean cyanophytes are distributed all over the world (Anagnostidis & Komarek, 1990). Many populations of Oscillatoriacen cyanophyta show considerable morphological variation (John et al., 2002). However a combination of traditional and modern taxonomy (in addition of physiology and biochemistry); need to determine the real place of this genus and oscillatorean cyanophyta as a whole. Although it has been emphasized that the taxonomy, however, based still on morphological characters (Anagnostidis & Komarek, 1990).

It seems that in north paddy fields of Iran, especially Golestan province, some strains of Oscillatorian cyanophyta especially are common (Shokravi et al., 2002, 2003) but there is no clear report about their morphological characterizations and taxonomic situations. Morphological variability, degree of polymorphism and geographical variation in form of the *Oscillatoria, lyngbya* and *plankthothrix* make some problems in studying of this organism. Our personal experiments have shown that using famous common manuals for determination of this genus like the other oscilatoriales in our country have no useful results and it is seriously need revision of these manuals or even identification keys with regard to special morphological variations of specimens with emphasize on local conditions (Shokravi et. al 2002).

Material and Methods

Soil samples were obtained from paddy fields of different stations of Golestan province (north of Iran and near Caspian sea–Fig.1). A complete description about stations and their geographical and environmental conditions have been reported in Shokravi et al. (2002). The collected soils were cultured by usual methods (Kaushik, 1987). After colonization and isolation, the cyanobacterium *Fischerella* sp., 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 ($NaNO_3$, 17.65 mM; $MgSO_4.7H_2O$, 0.3 mM; $CaCl_2.2H_2O$, 0.25 mM; $K_2HPO_4.3H_2O$, 0.18 mM; $Na_2MgEDTA$, 0.003 mM, Citrate ferric ammonium, 0.02 mM; Acid Citric, 0.029 mM; Na_2CO_3 0.188 mM; microelements 1 ml l^{-1}). The cultivation was done under different illumination (2, 11, 24, 104, 300µE m^{-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

[*]*e.mail: sshokravi@yahoo.com*

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.

Morphological observations were made in liquid as well as on solid media. Thallus growth, filament structure, in addition of biometrical information were recorded (Gugger & Hoffmann, 2004). Colony formation and cells shapes were evaluated by binocular and light microscope (in addition phase contrast and epifluoresscence, microscopy) each day in two week's periods. The growth curves were attained via measurement of chlorophyll daily by Jensen method (1978). Statistical analysis was done with software SPSS ver.10.

This spiciments show the high distribution, that it depends on physical and chemical charecteries of the soil, specially pH(Table1).

Fig1. Goles tan Province map. Stations have been shown with*

Table1. Distrobotion of blue-green algae Oscillatoria in the paddy field of Golestan province

Genus	Species	spring					summer					autumn					winter				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Oscillatoria	earlei	F	R	R	R	F	-	-	-	-	R	-	-	-	-	-	R	-	R	-	-
	okeni	-	-	-	-	-	-	-	-	-	-	-	-	-	R	-	-	-	-	-	-
	bornetii	R	R	F	F	R	R	R	R	F	F	R	F	F	R	R	R	F	R	R	R

D= Dominant (75-100%), A= Abundence (50-75%), F= Frequence (25-50%), R= Rare (< 25%).
1- Aliabad ,2-Kordkoy ,3-Minodasht ,4- Azadshahr,5-Gorgan

Results

In this study 17 species from blue-green algae Oscillatoria at the paddy field of Golestan province was identified.only new records listed in this paper.the taxonomy of this species is as follows.

Divisoin:Cyanobacteria
Classis:Cyanophyceae
Order:Oscillatoriales
Familia:Oscillatoriaceae
Genus:Oscillatoria

***Oscillatoria okeni* Agardh ex.Gomont**

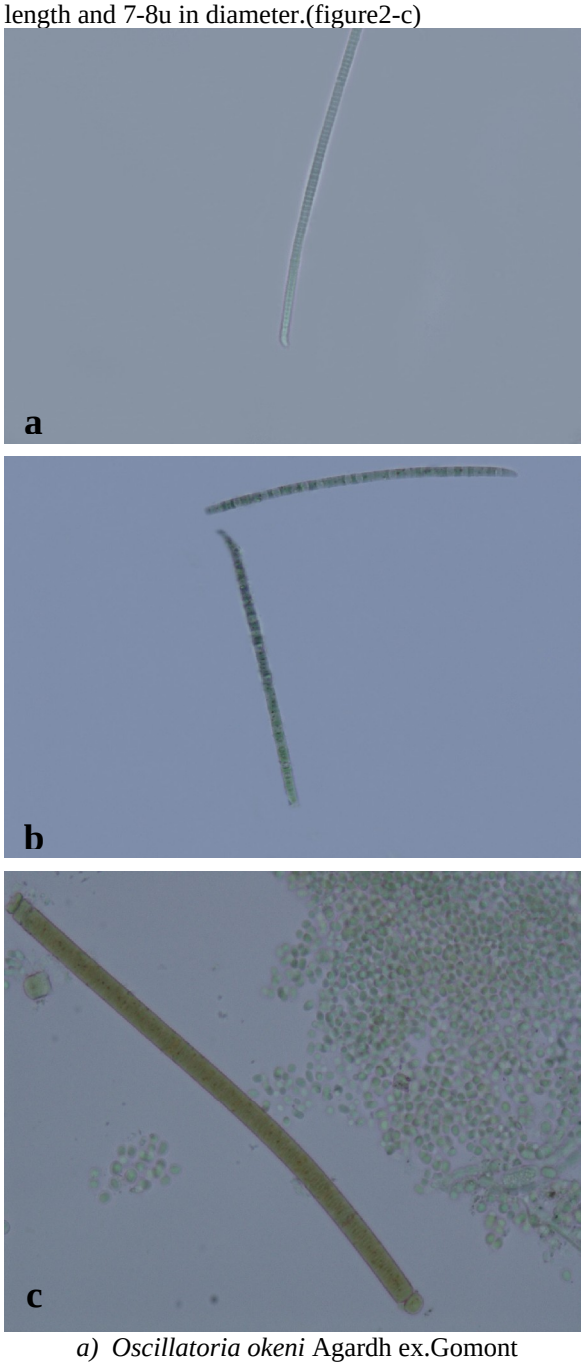
Trichome green, more or less curved toward the ends; apical cell without calyptra, obtuse; cells ungranulate, 3u in length and 4u in diameter (Fig. 2-a).

***O.earlei* Gardner**

Trichome green, relatively erect, not constricted, usually short and nearly curved toward the apical cell; apical cell at light microscopy seems conspicuously tapered, not capitate and not calyptrate; cells 1.3u in length and 3-3.2u in diameter (Fig 2-b).

***O.bornetii* Zukal**

Trichome more or less erect, slightly curved, not tapering or even decreasing in diameter through the end; apical cell rounded, not capitate, not calyptrate; cells granulated, not constricted at the cross wall, 3u in length and 7-8u in diameter.(figure2-c)



a) Oscillatoria okeni Agardh ex.Gomont
b) O.earlei Gardner
c) O.bornetii Zukal

Figure2. Three new records of Oscillatorian Cyanophyta for the paddyfields Algal Flora of Iran

Discussion

Cyanobacterian researches is a new matter in Golestan province and Iran as a whole. So only a few oscillatorian morphotypes have been cultured, and therefore the high variability of morphotypes found in nature is under-represented in culture (Shokravi et al.2002). Before this, only some genera of stigonematalean cyanophyta have been characterized from axenic culture strains, including some strains of *Fischerella* and *Nostoc* (Soltani et al 2006).

However results could be able to draw a relatively primitive picture of the morphological and taxonomical situation of oscillatorian cyanophyta in paddy-Fields of North of Iran. These organisms showed relatively variable characters from morphological point of view. It seems that pH fluctuations caused noticeable changes in the morphology of the organism. 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 (Perrona et al., 2004).

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 μE m^{-2} s^{-1}), caused noticeable morphological variations especially in pH 5. In this condition, *O.okeni* tends to get a different topological configuration. In minimum light intensity (2 μE m^{-2} s^{-1}) and pH 5 cross enlargement of the trichome of *O.bornetti* was seen. However results showed that these organisms can be considered an alkalophilic organism. Optimal growth rates were observed at pH 7 for *O.okeni and O.bornetti*, but pH 8.4 for *O.earlei* 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).

Acknowledgments

The authors would like to appreciate Professor Elvira Perona, Mrs. Ladan Baftechi for their kind collaboration in laboratory studies and Dr. Youness Ghasemi for the molecular identification confirmation of cyanobacterial strains.

Refrences

Anagnostidis, K. and Komarek, J. (1990) Modern approaches to the classification of cyanobacteria. Stigonematales. Archieves for hydrobiology 14, 224-286.

Anand, N.L., Radha, R.S., Hopper, G.R., Subramanian, T.D. (1990) Blue-green algae as biofertilizers: Certain view points on the choice of suitable isolates. Perspective in phycology, International symposium of phycology at university of Madras, Today and Tomorrow's Publishers. New Delhi, India.

Poza-Carrion, C., Fernandez-Valiente, E., Pinas, F., Leganes, F. (2001) Acclimation of photosynthetic pigments and photosynthesis of the cyanobacterium *Nostoc* sp. Strain UAM 206 to combined fluctuations of irradiance, pH, and inorganic carbon availability. Journal of Plant Physiology 158, 1455-1461.

Desikachary, T.V. (1959) Cyanophyta. Indian council of agricultural research monographs on Algae New Delhi, India.

Falch, BS., Konig, G.M., Wright, AD., Sticher, O., Ruegger, H., Bernardinelli, G. (1993) Ambigol a and B - New Biologically Active Polychlorinated Aromatic Compounds from the Terrestrial Blue-Green Alga *Fischerella ambigua*. Journal of Organic Chemistry 58, 6570-6575.

Geitler, L. (1932) Cyanophyceae von Europa Kryptogamen flora Akademiche Verlagsgesellschaft.- Leipzig

Gross, EM., Wolk, CP., Juttner, F. (1991) Fischerellin, a New Allelochemical from the Fresh-Water Cyanobacterium Fischerella-Muscicola. Journal of Phycology 27, 686-692.

Gugger, MF., Hoffmann, L. (2004) Polyphly of true branching cyanobacteria (Stigonematales). International Journal Of Systematic and Evolutionary Microbiology 54, 349-357.

Jensen, A. (1978) Chlorophylls and carotenoides, In: Handbook of Phycological Methods, Physiological and Biochemical Methods. eds. Hellebust, J.A. & Craigie, J.S., Cambridge University Press.

John, D.M., Whitton, B.W., Brook, A.J. (2002) The Freshwater Algal Flora of The British Isles - Cambridge University Press.

Kaushik, B.D. (1987) Laboratory methods for blue-green algae. Associated Publishing Company, New Delhi, India.

Baftechi, L., Nejad Sattari, T., Ebrahimzadeh, H., Shokravi, Sh. (2002) The effects of light intensity and duration on growth and heterocyst frequency of the cyanobacterium *Fischerella sp*.- M.Sc. thesis, Faculty of Science, Tehran University.

Boussiba, S. (1988) *Anabaena azollae* as biofertilizer. In: Algal biotechnology, eds, Stadler, T., J., Millon, M.C.Verdus, Y. Karamanos,H.Morvan and D. Christiaen, Elsevier applied science.

Castenholz, R.W. (2001) Class I: "Chloroflexi". In: Bergey's Manual of Systematic Bacteriology, eds. Boone, D.R.; Castenholz, R. W. and Garrity, G.M., New York, Springer-Verlag.

Perona, E., Abol, M., Bonilla, I., Mateol, P. (2003) Cyanobacterial diversity in Spanish river determined by means of isolation cultures. Morphological variability of isolates in relation to natural populations. Algological Studies 109 Cyanobacterial research 4, 475 486.

Prescott, G.W. (1962) Algae of the western great lake area. W.M.C. Brown Company Pub.

Shokravi, Sh., Tabatabaei, M., Ghasemi, Y., Baftechi, L., Soltani, N. (2003) The effects of light intensities and duration on antibacterial production abilities, morphological variations and ammonium liberations of *Fischerella* sp. collected from Paddy-fields of Iran. Proceeding of the 11th International symposium on phototrophic prokaryotes, Tokyo, Japan.

Shokravi, S., Soltani, N., Baftechi, L. (2002) Cyanobacteria as biofertilizer in paddy fields.- National Research Council of Islamic Republic of Iran,Grant no. NRCI 489-66.

Shokravi, Sh., Soltani, N., Baftechi, L. (2001) Applied research management of cyanobacteria in Iran: problems and solutions. The first Iranian Congress on Applied Biology , Islamic Azad university, Mashhad, Iran.

Soltani, N., Khavari-Nejad, R., Tabatabaei, M., Shokravi, Sh., Fernandez-Valiente, E. (2006) Variation of nitrogenase activity, photosynthesis and pigmentation of cyanobacterium *Fischerella ambigua* strain FS18 under different irradiance and pH. World journal of microbiology and biotechnology 22(6), 571-576.

Tiffany, L.H., Britton, M. (1971) The algae of the Illinois. New York McGrow Hill.

Valiente, E.F., Leganes, L. (1989) Regulatory effect of pH and Incident Irradiance on the levels of Nitrogenase activity in the cyanobacterium UAM205 Journal of Plant Physiology 135, 623-627.

گزارش سه گونه جدید اسیلاتوریا برای فلور شالیزارهای ایران

رقیه سیاه‌بالایی[۱]، حمید افشارزاده[۱]، شادمان شکروی[۲]

۱. گروه زیست‌شناسی، دانشکده علوم، دانشگاه اصفهان

۲. گروه زیست‌شناسی، دانشگاه آزاد اسلامی، واحد گرگان

چکیده

در این پژوهش، سه گونه جدید سیانوفیت برای نخستین بار از ایران گزارش شده است. این گونه‌ها عبارتند از: *Oscillatoria okeni* Agardh ex.Gomont, *O.earlei* Gardner and *O.bornetii* Fritsch. گونه‌های مذکور به صورت اپی دافیک و اندافیک از شالیزارهای استان گلستان، مجاور دریای خزر معرفی گردیده است.

کلمات کلیدی: ایران، تاکسونومی، سیانوفیتا، شالیزار