Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 5, No. 2, p. 77-84, 2014
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Morphological variability survey of cyanobacterium *Anabaena* sp. ISC55 in liquid mixotrophe treatment

Ehteram Deylami[1], Taher Najadsattari[1*], Younes Ghasemi[2], Shadman Shokravi[3]

[1] *Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran*

[2] *Pharmaceutical Sciences Research Center, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran*

[3] *Department of Biology, Gorgan Branch, Islamic Azad University, Gorgan, Iran*

Article published on August 02, 2014

Key words: *Anabaena* sp. ISC55, Cyanobacteria, 16S rRNA, Mixotrophe, Phenotype.

Abstract

The variety of phenotypes of cyanobacteria is accompanied by morphological plasticity changing accordingly to different environmental or culture conditions. In this research, the effect of different treatments of liquid mixotrophe on morphological responses, structural changes and biometrical of *Anabaena* sp. ISC55 were studied. This investigation was performed in time periods of weekly and daily. Due to the morphological variability of cyanobacteria in different conditions, molecular and genetics studies should be performed for identify of this cyanobacterium. In this study identification of *Anabaena* sp. ISC55 was performed based on diacritical morphological such as cell shape, cell size, presence or absence of sheath and 16S rRNA gene sequencing. The results indicated that shape and size of vegetative cells and heterocytes in this species have changed in different treatments of liquid mixotrophe compared with control treatment; also this species in glucose treatment has produced more biomass.

***Corresponding Author:** Taher Najadsattari ✉ nejadsattarit@gmail.com

Introduction

Cyanobacteria are one of the prokaryotic organisms groups that growth forms range from uni-to multicellular. These various growth forms have enabled cyanobacteria to inhabit almost every terrestrial and aquatic habitat on Earth (Schirrmeister *et al.*, 2012). Based on fossil records, Cyanobacteria have occurred as long ago as 2,600 to 3,500 million years (Myr),(Brocks *et al.,* 1999) . These earliest cyanobacteria are believed to have been played an important role in producing an oxygen-rich atmosphere on earth about 2,300 Myr ago (Blankenship, 1992). Cyanobacteria are oxygenic photoautotrophic bacteria with morphologically very diverse and inhabiting a multiplicity of environments worldwide (Knoll, 2008). These organisms are identified according to their morphological characters such as morphology of vegetative cells, akinetes and heterocytes (Rippka *et al.*, 1979, Rajaniemi *et al.*, 2005). The variety of phenotypes of cyanobacteria is accompanied by morphological plasticity changing accordingly to different environmental or culture conditions. This can result in misidentifications when using morphological analysis alone (Lyra *et al.*, 2001).

Several properties of the 16S rRNA gene, such as evolutionary properties and ubiquity, have allowed it to become the most commonly used molecular marker to distinguish and establish relationships between cyanobacterial genera and species (Case *et al.*, 2007). The environmental factors are playing an important role in the production of Photosynthetic compounds and pigments (Bennet and Bogorad, 1973, Grossman *et al.*, 2001, Tandeau De Marsac, 2003). Cyanobacteria and microalgae are highly adaptive and can grow in autotrophic, heterotrophic and mixotrophic conditions. Mixotrophy, the combination of phototrophy and heterotrophy enables some algal species to use organic nutrient pools, augment photosynthetic energy, and function at multiple trophic levels (Sanders *et al.*, 1990, Cloern and Dufford, 2005). Thus, it can lend a competitive advantage over strict phototrophs and heterotrophs (Bockstahler and Coats, 1993). Type of culture medium can achieve high cell densities and synthesize light-induced products such as photosynthetic pigments and was especially suitable for the production of high value bioactive compounds (Ducat *et al.*, 2011). The aim of this investigation was to study the morphological responses and structural changes *Anabaena* sp. ISC55 to treatment of liquid mixotrophe.

Materials and methods

Isolation of strain

Anabaena sp. ISC55 was isolated from soils in the Masjed Soleiman region (x=0339545 y=3535959) of Iran. Isolation and purification was made by ordinary methods (Andersen, 2005). Following achievement of axenic culture, cyanobacterium was cultivated in liquid and solid BG-11_0 medium. Preliminary identification of this species was performed by (John *et al.* 2002, Anagnostidis and Komarek, 1990, Prescott, 1962, Desikachary, 1959, Geitler, 1932).

Culture conditions

Stock cultures were grown in the *BG-11_0 solid* medium. Temperature was maintained at 30±1°C and cultures were bubbled with air under a constant light intensity of 60 µmol photon m^{-2} s^{-1} supplied by three white fluorescent tubes. Cells in logarithmic phase of growth were collected from stock cultures and used as inoculate for experiments.

Three sets of *BG-11_0 liquid* medium were prepared depending on 0.05% of sugars solutions (glucose, fructose and sucrose) and then mixotrophe *BG-11_0 liquid* mediumes containing *Anabaena* sp. ISC55 were placed culture room and then morphological changes with the control sample (*BG-11_0 liquid* medium containing *Anabaena* sp. ISC55) were compared.

Morphological studies

For morphological studies, semi permanent slides were prepared every day (for two weeks). These studies were performed by light (Labomed, X400)

and florescence microscopes. Factors such as type meetings, fluidity filament, biometry, status and color of trichome, the size of the vegetative and heterocyte cells, morphology of the terminal cells and presence or absence of sheath were evaluated (Rippka *et al.* 1979, Rajaniemi *et al.* 2005). More detailed morphological studies were carried out by scanning electron microscope (SEM).

PCR amplification, cloning, and sequence analysis of 16S rRNA

To extract DNA from the *Anabaena* sp. ISC55 a fresh biomass was prepared by centrifuging at 12000 rpm and using Fermentas kit (k0512). The applied PCR condition has been described by Nübel *et al.* (2000). PCR amplification, cloning and sequence analysis of 16S DNA content was first extracted from the cyanobactrium and then PCR was applied with using two set of primers (Nübel *et al.* 1999). Sequences were amplified using the primers PA (5´-CGGACGGGTGAGTAACGCGTGA -3´) as forward and PH (5´- GACTACAGGGGTATCTAATCCCTTT -3´) as reverse PCR products were obtained by electrophoresis in a 1% (w/v) agarose gel using TBE buffer containing DNA set stain. The sequence was determined by the Cina Gene Company. The sequence data was analyzed using a similarity search by using the BLAST through the website of the NCBI.

Statistical analysis

Data are the means and standard deviation of at least three replicates. Statistical differences were examined by ANOVA test using software SPSS ver. 18.

Results and discussion

The classification of cyanobacteria has normally relied on morphological specifications which are not always reliable, as they may show variation on form depending on culturing and environmental conditions (Nayak *et al.*, 2007). These problems of usual morphological classification, together with the deficiency of molecular data, pose serious hindrances for taxonomy of cyanobacteria (Hayes *et al.*, 2007, Komárek, 2010). In this study morphological observations showed that in *Anabaena* sp. ISC55, communities are brown and diffused; Right trichome and sometimes curved; Vegetative cells are Rectangular – foursquare; intercalary heterocysts, ellipsoid or spherical; apical cell domical or spherical; There are around of the trichomes mucilage sheath that is very thin, amorphous.

Morphological studies *Anabaena* sp. ISC55 in *BG-11₀ liquid* medium (control) was performed by photos provided with optical, SEM and fluorescence microscopes that life cycle of *Anabaena* sp. ISC55 and results were seen in figs. 1 and 2.

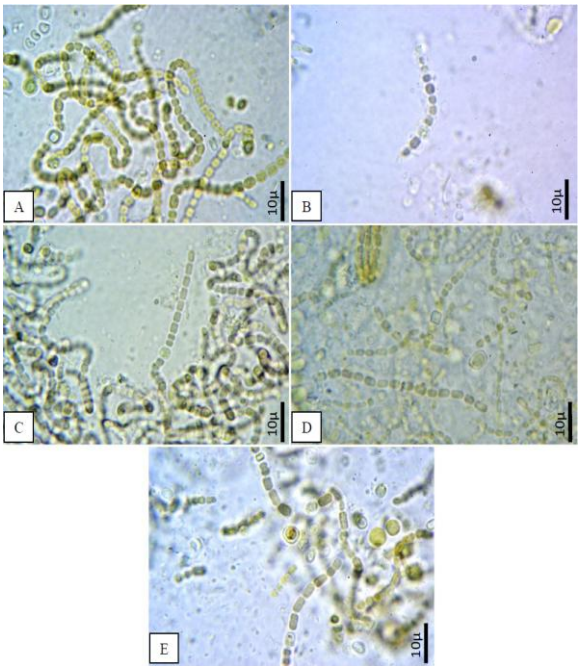

Fig 1. life cycle of *Anabaena* sp. ISC55 in BG-11₀ liquid medium (control) by light microscope. (A) First day, (B) Third day, (C) Fifth day, (D) Seventh day, (E) Ninth day.

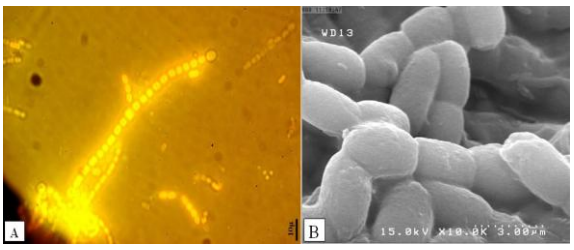

Fig 2. *Anabaena* sp. ISC55 in BG-11₀ liquid medium (controle). (A) fluorescence, (B) SEM.

Morphological studies by light microscope in treatments of liquid mixotrophe were shown that in glucose, sucrose and fructose treatments, shape, color and size of vegetative and heterocyte cells on every day and in every treatment were different. vegetative cells were divided in glucose, sucrose and fructose treatments on third, fifth, seventh and ninth days and most cell divisions were done in glucose treatment while cell divisions in control treatment were done on seventh and ninth days and intensity of division was much less. Also there was the maximum of akinets in fructose medium on ninth day that this akinets were divided. (fig.3, 4and 5). With increase of cell divisions were produced more biomass in liquid mixotrophe and especially in glucose treatment. Biomass changes on third, fifth, seventh and ninth days compared with control have significant difference (ANOVA, $p<0.05$) (Fig. 6).

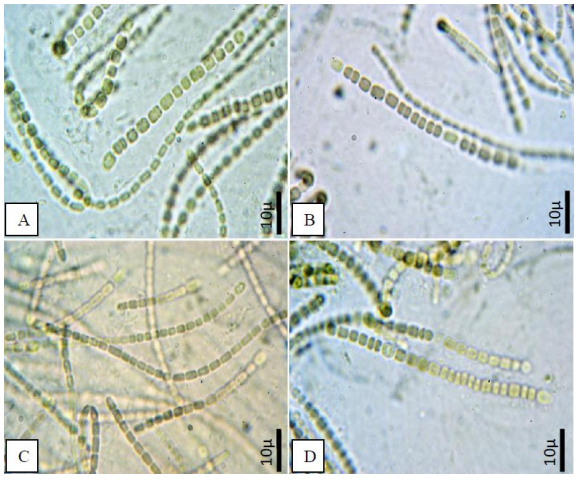

Fig 3. *Anabaena* sp. ISC55 in glucose medium by light microscope. (A) Third day, (B) Fifth day, (C) Seventh day, (D) Ninth day

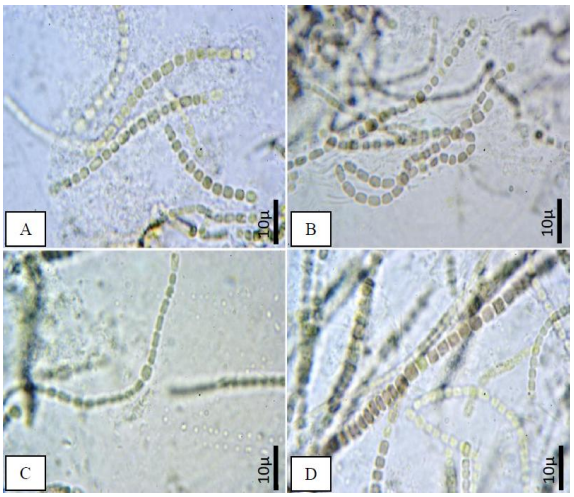

Fig 4. *Anabaena* sp. ISC55 in sucrose medium by light microscope. (A)Third day, (B) Fifth day, (C) Seventh day, (D) Ninth day.

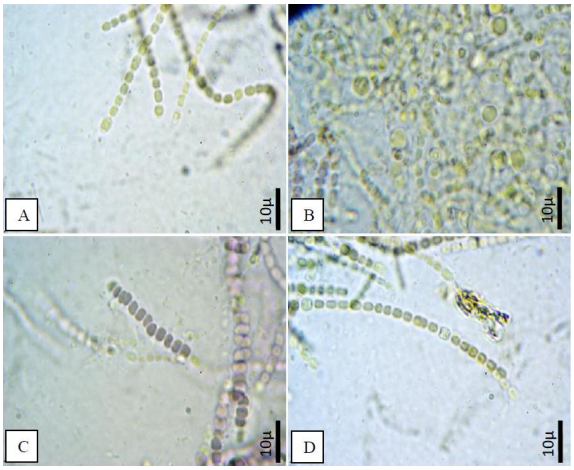

Fig 5. *Anabaena* sp. ISC55 in fructose medium by light microscope. (A) Third day, (B) Fifth day, (C) Seventh day, (D) Ninth day.

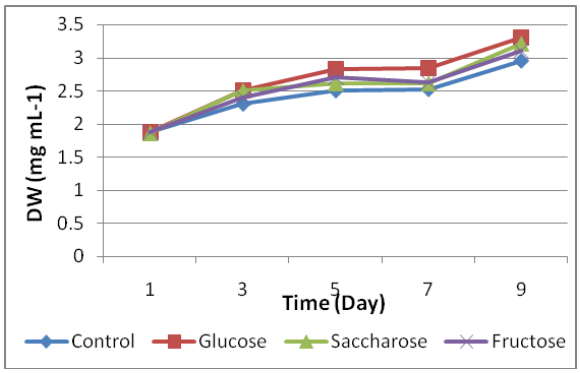

Fig 6. Growth curve comparison of *Anabaena* sp. ISC55.

Biometrical studies were shown that the pattern of changes in average length of vegetative cells on third, fifth, seventh and ninth days and in average diameter of vegetative cells and average length and diameter of heterocytes on fifth, seventh and ninth days have significant difference in glucose, sucrose and fructose mediums compared with control (ANOVA, $p<0.05$). (Fig. 7, 8, 9 and 10).

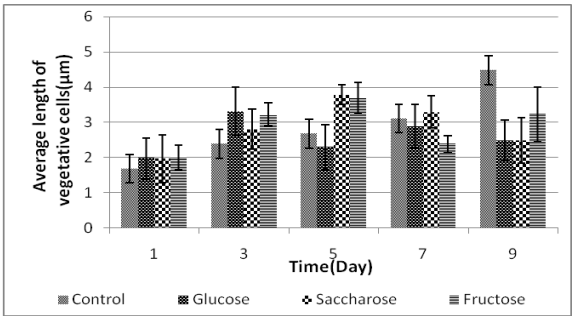

Fig 7. Comparison of the average length of vegetative cells in BG-11$_0$ liquid medium and liquid mixotrophe treatment.

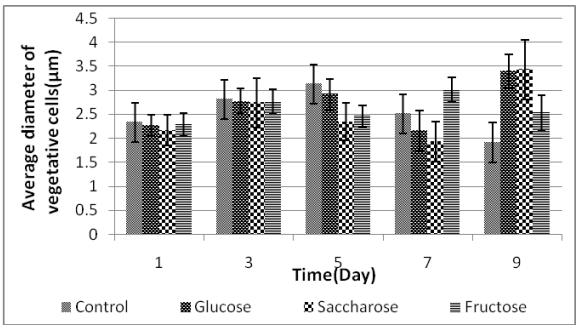

Fig 8. Comparison of the average diameter of vegetative cells in BG-11$_0$ liquid medium and liquid mixotrophe treatment.

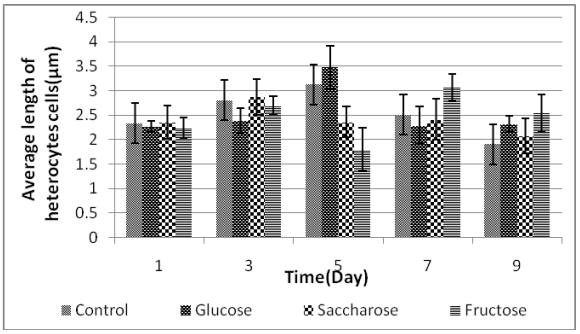

Fig 9. Comparison of the average length of heterocytes in BG-11$_0$ liquid medium and liquid mixotrophe treatment.

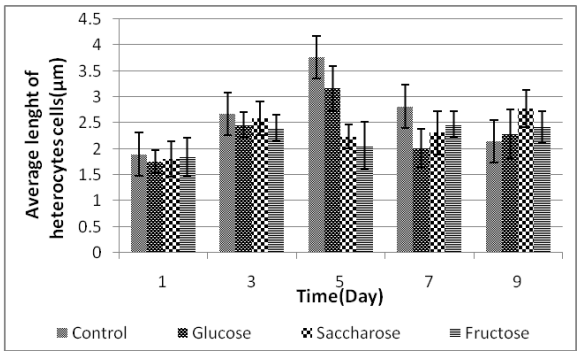

Fig 10. Comparison of the average diameter of heterocytes in BG-11$_0$ liquid medium and liquid mixotrophe treatment.

Algal cultures are influenced by a variety of environmental factors and they play a significant role in the production and composition of the photosynthetic pigments (Bennet and Bogorad, 1973, Grossman *et al.*, 2001, Tandeau De Marsac, 2003). This study was shown that morphology, size and division of cells are different in liquid mixotrophe treatment. That cause of them can be changes in photosynthetic products. (Vigaya *et al.* 2009, Ostroff *et al.*, 1980, Latała and Misiewicz, 2000). Also Mixotrophy may increase trophic efficiency (Sanders, 1991) and is an important pathway for nutrient cycling in some biosystems (Bird and Kalff, 1987, Maranger *et al.*, 1998, Stibor and Sommer, 2003).

Morphological and biometrical studies in different environmental conditions indicate that there are morphological diversity and high adaptability in *Anabaena* sp. ISC55. Therefore molecular studies such as sequence analysis of 16S rRNA are essential for accurate identification of this species. Scientists have also confirmed this topic that besides morphological studies, it is currently accepted that characterization and taxonomy of cyanobacteria must combine multidisciplinary approaches (Gillis *et al.*, 2005, Hayes *et al.*, 2007, Komárek, 2010) This so-called polyphasic methodology (including phenotypic, chemotaxonomic and genotypic data) has been increasingly followed by many cyanobacteriologists worldwide, e.g., Nayak *et al.* (2007), Li *et al.* (2008), Saker *et al.* (2009) and Schleifer (2009). Among the

molecular methods, the analysis of the 16S rRNA gene sequences has proved to be a useful tool for exploring phylogenetic relationships among cyanobacteria (Willame *et al.*, 2006, Pan *et al.*, 2008, Han *et al.*, 2009, Zapomeˇlova´ *et al.*, 2010). Molecular studies in *Anabaena* sp. ISC55 on the conserved region of 16S rRNA was performed by using specific primers for cyanobacteria. This region is a highly variable region that enables precise molecular diagnosis of genus. Comparison of gene sequences of sample studied in Gene Bank (http://www.nbci.nlm.nih.gov/BLAST) NBCI due to blasting operations (BLAST) showed that *Anabaena* sp. ISC55 with number GU584196 on NBCI database is accessible.

References

Anagnostidis K, Komarek J. 1990. Modern approaches to the classification of Cianobacteria. Stigonematales. Archiv für Hydrobiologie, **14(8)**, 224-286.

Andersen RA. 2005. Algal culturing techniques. USA: Academic press**.**

Bennet A, Bogorad L. 1973. Complimentary chromatic adaptation in a filamentous blue-green alga. Journal of Cell Biology **58,** 419–425.

Bird DF, Kalff J. 1987. Algal phagotrophy: regulating factors and importance relative to photosynthesis in *Dinobryon* (Chrysophyceae). Limnol. Oceanogr **32,** 277–284.

Blankenship, RE. 1992. Origin and early evolution of photosynthesis. Photosynthesis Research **33,** 91-111.

Bockstahler KR, Coats DW. 1993. Grazing of the mixotrophic dinoflagellate Gymnodinium sanguineum on ciliate populations of Chesapeake Bay. Marine Biology **116,** 477– 487.

Brocks, JJ, Logan GA, Buick R, Summons RE. 1999. Archean Molecular Fossils and the Early Rise of Eukaryotes. Science **285**, 1033-1036

Case RJ, Boucher Y, Dahllof I, Holmstrom C, Doolittle WF, Kjelleberg S. 2007. Use of 16S rRNA and *rpoB* genes as molecular markers for microbial ecology studies. Applied and Environmental Microbiology **73,** 278–288.

Cloern JE, Dufford R. 2005. Phytoplankton community ecology: principles applied to San Francisco Bay. Marine Ecology Progress Series, **285,** 11–28.

Desikachary TV. 1959. Cyanophyta. Indian council of agricultural research. Monographs on Algae, New Delhi, India.

Ducat DC, Way JC, Silver PA. 2011. Engineering cyanobacteria to generate high-value products. Trends in Biotechnology **29(2),** 95-103.

Geitler L. 1932. Cyanophyceae von Europa Kryptogamen flora Akademiche Verlagsgesellschaft. Leipzig

Gillis M, Vandamme P, De Vos P, Swings J, Kersters K. 2005. Polyphasic taxon-omy. In DJ Brenner, NR Krieg, JT Staley, eds, Bergey's Manual of System-atic Bacteriology. The Proteobacteria, Vol. 2A, 2nd ed. Springer-Verlag, New York, USA, 43-48 P.

Grossman AR, Bhaya D, He Q. 2001. Tracking the light environment by cyanobacteria and the dynamic nature of light harvesting. Journal of Biological Chemistry 276, 11449–11452.

Han D, Fan Y, Hu Z. 2009. An evaluation of four phylogenetic markers in *Nostoc*: Implications for cyanobacterial phylogenetic studies at the intrageneric level. Current Microbiology 58, 170–176.

Hayes PK, El Semary NA, Sa´nchez-Baracaldo P. 2007. In Brodie, J. & J. Lewis (eds), Unravelling The Algae: The Past, Present, and Future of Algal Systematics. *CRC Press/Taylor & Francis Group, Boca Raton*, 93–101.

John DM, Whitton BW, Brook AJ. 2002 The freshwater algae of the British Isles, Cambridge University Press.

Knoll AH. 2008. Cyanobacteria and earth history. In Hererro A & Flores E (Eds.) The Cyanobacteria: Molecular Biology, Genomics and Evolution. Cais- ter Academic Press, Norfolk, UK, 1-20 P.

Komárek J. 2010. Modern taxonomic revision of planktic nostocacean cyanobacteria: a short review of genera. Hydrobiologia **639**, 231–243.

Latała A, Misiewicz S. 2000. Effects of light, temperature and salinity on the growth and chlorophyll-a content of Baltic cyanobacterium *Phormidium* sp. Arch. Hydrobiol. 136, Algological Studies **100,** 157–180.

Li R, Wilhelm SW, Carmichael WW, Watanabe MM. 2008. Polyphasic characterization of water bloom forming *Raphidiopsis* species (cyanobacteria) from central China. Harmful Algae **7,** 146–153.

Lyra C, Suomalainen S, Gugger M, Vezie C, Sundman P, Paulin L, Sivonen K. 2001. Molecular characterization of planktic cyanobacteria of Anabaena, Aphanizomenon, Microcystis and Planktothrix genera. International Journal of Systematic and Evolutionary Microbiology **51,** 513-526.

Maranger R, Bird DF, Price NM, 1998. Iron acquisition by photosynthetic marine phytoplankton from ingested bacteria. Nature **396,** 248–251.

Nayak S, Prasanna R, Prasanna BM, Sahoo DB. 2007. Analysing diversity among Indian isolates of *Anabaena* (Nostocales, Cyanophyta) using morphological, physiological and biochemical characters. World Journal of Microbiology and Biotechnology 23,1575–1584.

Nübel U, Garcia-Pichel F, Kühl M, Muyzer G. 1999. Quantifying microbial diversity: Morphotypes, 16S rRNA genes, and carotenoids of oxygenic phototrophs in microbial mats. Applied and Environmental Microbiology, 422-430.

Nübel U, Garcia-Pichel F, Clavero E, Muyzer G. 2000. Matching molecular diversity and ecophysiology of benthic cyanobacteria and diatoms in communities along a salinity gradient. Environmental Microbiology **2,** 217-26.

Ostroff CR, Karlander EP, Van Valkenberg SD. 1980. Growth rates of *Pseudopendinella pyriformis* (Chrysophyceae) in response to 75 combinations of light, temperature, and salinity, Journal of Phycology **16,**421–423.

Pan X, Chang F, Kang L, Li G, Li D, Liu Y, Shen Y, Wei Z. 2008. Morphological characteristics and phylogenetic relationship of *Anabaena* species from Lakes Dianchi and Erhai, China. Hydrobiologia **614,** 353-362.

Prescott GW.1962. Algae of the western Great Lakes area, W.C. Brown Company publishers.

Rajaniemi P, Hrouzek P, Kastovská K, Willame R, Rantala A, Hoffmann L, Komárek J, Sivonen K. 2005. Phylogenetic and morphological evaluation of the genera Anabaena, Aphanizomenon, Trichormus and Nostoc (Nostocales, Cyanobacteria). International Journal of Systematic and Evolutionary Microbiology **55,** 11–26.

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY. 1979. Generic assignments, strain histories and properties of pure cultures of Cyanobacteria. Journal of General Microbiology 111, 1–61.

Saker M, Moreira C, Martins J, Neilan B, Vasconcelos VM. 2009. DNA profiling of complex

bacterial populations: toxic cyanobacterial blooms. Applied Microbiology and Biotechnology 85, 237–252.

Sanders RW, Porter KG, Caron DA. 1990. Relationship between phototrophy and phagotrophy in the mixotrophic chrysophyte Poterioochromonas malhamensis. Microbial Ecology **19,** 97–109.

Sanders RW. 1991. Mixotrophic protists in marine and freshwater ecosystems. Journal of Protozoology. **38,** 76-81.

Schirrmeister BE, Dalquen DA, Anisimova M, Bagheri HC. 2012 Gene copy number variation and its significance in cyanobacterial phylogeny. BMC Microbiolgy **12(1),** 177.

Schleifer KH. 2009. Classification of *Bacteria* and *Archaea*: past, present and future. Systematic & Applied Microbiology 32, 533–542.

Stibor H, Sommer U. 2003. Mixotrophy of a photosynthetic flagellate viewed from an optimal foraging perspective. Protist **154,** 91–98.

Tandeau de Marsac N. 2003. Phycobiliproteins and phycobilisomes: the early observations. Photosynthesis Research 76, 197–205.

Vigaya V, Narayanaswamy A. 2009. Blue light enhance the pigment synthesis in cyanobacterium *Anabaena ambigua* rao(Nostacales). ARPN Journal of Science and Technology **4(3),** 36-43.

Willame R, Boutte C, Grubisic S, Wilmotte A, Hoffmann L. Komárek J. 2006. Morphological and molecular characterization of planktonic cyanobacteria from Belgium and Luxembourg. Journal of Phycology **42,** 1312–1332.

Zapomeˇlova´ E, Rehakova K, Jezberova J, Komarkova J. 2010. Polyphasic characterization of eight planktonic *Anabaena* strains (Cyanobacteria) with reference to the variability of 61 *Anabaena* populations observed in the field. Hydrobiologia **639,** 99-113.