Journal of Ecology of Health & Environment
An International Journal

http://dx.doi.org/10.18576/jehe/040301

Iron Regulation of Growth and Heterocyst Formation in the Nitrogen Fixing Cyanobacterium *Nostoc* sp. PCC 7120

Wafaa S. M. Aly[*] *and Simon C. Andrews*

School of Biological Sciences, University of Reading, Whiteknights, Reading, RG6 6AJ, UK.

Received: 13 Jul. 2016, Revised: 19 Jul.2016, Accepted: 22 Jul.2016.
Published online: 1 Sep. 2016.

Abstract: The growth of *Nostoc* sp. PCC 7120 was found to decrease under low iron conditions. A greater degree of iron restriction was achieved when precultures of *Nostoc* sp. PCC 7120 were grown under low iron conditions to deplete iron stores. Iron deficiency was also found to delay the formation of heterocysts in *Nostoc* sp. PCC 7120 grown under nitrogen fixing-conditions. The frequency of heterocysts under high iron was greater than under low iron by up to nearly twofold. These results suggested that iron (as well as nitrogen) regulate heterocyst formation and development in *Nostoc* sp. PCC 7120 under nitrogen-fixing conditions.

Keywords: cyanobacteria; *Nostoc* sp. PCC 7120; iron, nitrogen fixation; heterocyst formation

1 Introduction

Cyanobacteria (blue green algae) are a diverse group of photosynthetic, prokaryotic organisms found in freshwater and marine environments. The origin of these organisms is dated back three or four billion years [1]. Fossil evidence suggests that they were amongst the earliest life forms and may well be responsible for the production of oxygen gas in the early history of Earth's atmosphere [2, 3]. One of the important features of cyanobacteria is the ability of some genera to fix nitrogen, dissolved in water, which enables them to survive with low concentrations of nitrogen. Those genera of cyanobacteria that can fix nitrogen include *Anabaena*, *Aphanizomenon* and *Gloeotrichia* while those that cannot include *Microcystis* and *Coelosphaerium* and others [4].

Cyanobacteria have evolved specific mechanisms for surviving under environmental stress. Under stress conditions, normal biochemical pathways are often altered, as genes responsible for making or modifying different cellular products are up or down regulated. This results in an altered physiology that provides a competitive edge in the changing environment. For instance, in nitrogen fixation processes, cyanobacteria utilize the iron-dependent nitrogenases to fix nitrogen gas into NH_4 [5]. This process is not only important for the availability of nitrogen to the organism itself, but it also acts as an entry point for nitrogen to the aquatic food chain [6].

Iron is an essential element required for the growth of all animals, plants and most microorganisms. It is widely distributed in nature, being the second most abundant metal (after aluminium), and the fourth most abundant element (after oxygen, silcon and aluminium) in the Earth's crust [7]. It plays vital roles in many important biological processes.

Generally, phytoplanktonic cyanobacteria require higher Fe-C quotas than eukaryotic phytoplankton[8]. Also the overall metal quota is much higher in photosynthetic organisms than in non-photosynthetic ones. For instance, the iron content in *Synechocystis* cells is found to be one order of magnitude higher in comparison to *Escherichia coli*[9]. The high iron demand in cyanobacteria is required for both the respiratory redox enzymes as in non-photosynthetic bacteria and also for photosynthetic machinery and nitrogen fixation apparatus [10].

In cyanobacteria many iron-containing molecules are required for necessary biological pathways such as metabolic and catabolic pathways, nitrogen assimilation, electron transport, and chlorophyll *a* production [11]. Iron in cyanobacteria serves as a major component in cytochromes, non-heme iron and in iron-sulphur centres required for the completion of enzymes [12-14]. Iron plays a key role in photosynthetic electron transfer. Photosystem II (PSII) contains two cytochromes and one non-haem iron [15, 16]. The cytochrome b_6f complex has four hemes and one Fe_2-S_2 cluster [17, 18]. Non-haem iron is located between the Q_A and Q_B quinone molecules and it plays a key role in electron transfer between them [19]. However,

[*]Corresponding author e-mail: wsma2002@yahoo.com

the largest contribution for iron in the photosynthetic system is photosystem I (PSI), which contains 12 iron atoms in three Fe_4-S_4 clusters [20]. Recently atomic absorption spectroscopy of isolated thyklakoid membranes of cyanobacteria has revealed that more than 25% of the cellular iron quota is present in PSI [9]. The functional photosynthetic apparatus in all cyanobacteria requires 22-23 Fe atoms, depending on whether cytochrome c_{553} or plastocyanin is used as an electron donor to PSI [21].

Nitrogen fixation is perhaps the most 'iron-expensive' process within phytoplankton, and this Fe requirement forms the basis of arguments regarding the absence of significant populations of nitrogen-fixing phytoplankton in marine systems[22]. Direct evidence in both laboratory and field studies has linked the availability of Fe to N_2-fixation in cyanobacteria[22, 23].Diazotrophy requires increased demand of iron for growth, diazotrophs grown on NO^{3-} or NH^{4+} required less iron in culture media than those grown on N_2. This may be due to the down-regulation of nitrogenase activity[8]. However, the nitrogenase contains 19 iron atoms per heterodimeric protein complex moiety (one [4Fe-4S] cluster in the nitrogenase reductase, and one P cluster ([8Fe-7S]) and one FeMoCo cluster ([7Fe-9S-Mo]) in the nitrogenase), but in comparison to other iron-containing enzymes involved in nitrogen metabolism such as nitrate reductase or nitrite reductase, nitrogenase has a lower iron usage efficiency[24]. The minimal iron cost for the nitrogenase activity was determined to be 10-30 mol Fe per mol fixed N[8].

Iron deficiency results in variety of morphological changes in cyanobacteria. The most obvious effect of iron limitation in cyanobacteria is chlorosis [11]. A decrease in the concentration of chlorophyll *a* per cell as well as phycocyanin due to lack of iron have been seen in *Anacystisnidulans* and *Agmenellumqua druplicatum* [25, 26]. Also, it has been suggested that degradation of cellular phycocyanin may result as a secondary effect of iron limitation [27]. Nitrogen deficiency caused by a reduction in the level of iron-containing enzymes required for nitrogen assimilation may cause the cells to degrade phycocyanin pools and then utilize them as a source of nitrogen [28]. Furthermore, iron starvation of the cyanobacterium *Synechococcus* sp. PCC 7942 results in replacement of the phycobilisome by glycogen containing granules, the disaggregation of most thylakoid membrane stacks and the reduction in the number of carboxysomes [29]. A similar disaggregation of thylakoid membranes was observed with iron starvation in *Acaryochloris marina* [30] and *Nostoc* sp. PCC 7120.

Iron deficiency leads to a number of physical changes in cyanobacteria. A size decrease in *Anacystis nidulans* R2 to between 33 and 50% of the iron satiated cell length has been documented [29]. Similarly a variation in size as well as a pronounced filament coiling was observed in iron-deficient cultures of *Anabaena flos-aquae* [31].

The growth rate (μmax) of some cyanobacteria such as *Synechococcus* sp. PCC 7942, *Synechococcus* sp. PCC 7002, *Anabaena variabilis* and *Oscillatoria tenuis* is decreased when iron availability decreases [28, 32-34]. Furthermore, it has been found that the growth rate of *Oscillatoria tenuis* at an "intermediate" iron concentration was even lower than that at the lowest iron concentration tested [33]. In contrast, the growth rate for *Plectonema boryanum* directly correlates with the iron content of the medium [33].

Here in this work, experiments were performed to investigate the effect of iron restriction on growth and heterocyst formation in the nitrogen fixing cyanobacterium *Nostoc* sp. PCC7120 in order to confirm the importance of iron for this organism, particularly under nitrogen-fixation conditions. The effect of iron-stores on growth under iron-restriction was also investigated.

2 Methodology

2.1 Cyanobacterial strain and growth conditions

The wild-type of *Nostoc* sp. PCC 7120 (= *Anabaena* sp. strain PCC 7120) used in this study was sourced from Pasteur Culture Collection of cyanobacteria (PCC) (France). Liquid cultures of *Nostoc* sp. PCC 7120 were grown in normal BG-11 medium[35]. For nitrogen-fixation conditions, *Nostoc* sp. PCC 7120 cells were grown on BG-11o medium (Low NBG-11) which contained BG-11 medium with 5-10 mM of $NaHCO_3$ in place of $NaNO_3$ and ferric citrate in place of ammonium ferric citrate. Cultures were grown under a continuous fluorescence light of 50 μmol photons m^{-2} s^{-1} of white light at 28 °C with continuous shaking at 100 rpm using a Bibby Stuart SO1 orbital shaker.

2.2 Iron-restricted cyanobacterial growth

For growth under high iron conditions, precultures of *Nostoc* sp. PCC 7120 were prepared by inoculating 50 ml of normal BG-11 medium with cells from an iron sufficient solid BG-11 medium plate and allowing growth for 6 days (OD_{730nm} ~ 1.0). 1-2 ml of the preculture was used to inoculate 50 ml of normal BG-11 medium, and the cell growth was monitored for 11-14 days by measuring the OD_{730nm}. Precultures and cultures were grown under continuous fluorescence white light (50 μmol photons m^{-2} s^{-1}) at 28 °C with continuous shaking at 100 rpm. To achieve iron restriction, the cyanobacterial precultures were grown as before and centrifuged at 4,000 rpm, for 15 min in bench-top centrifuge to pellet the cells. The pellets then resuspended in 50 ml of low-Fe BG-11 medium and re-centrifuged, as before, in order to wash the remaining iron from the cell surface. The pellets were again resuspended in 50 ml low-Fe BG-11 medium and 1-2 ml of the culture was used to inculcate 50 ml of low-Fe medium. The culture was then grown under the same conditions used for normal BG-11 medium. For the low-Fe medium, acid washed

glassware was used and the ferric ammonium citrate present in normal BG-11 medium was replaced with ammonium citrate. 30 µM ferric citrate and 20 µM DTPA were added to low-Fe BG-11 medium to investigate the effect of iron and iron chelator, respectively, on the growth of *Nostoc* sp. PCC 7120.

2.3 Nitrogen-restricted cyanobacterial growth

To achieve nitrogen stress in *Nostoc* sp. PCC 7120, iron replete-precultures were grown as described before in Section 2.2. The precultures were centrifuged at 4,000 rpm, for 15 min in a bench-top centrifuge to pellet the cells. The pellets were then resuspended in 50 ml of low N media (BG-11o media) and re-centrifuged as before in order to wash the remaining nitrogen from the cell surface. The pellets were again resuspended in 50 ml BG-11o medium and 1-2 ml of the pellets was used to inoculate 50 ml of BG-11o medium supplemented with 10 mM sodium bicarbonate. The cultures were then grown for 6-12 days under continuous fluorescence white light (50 µmol photons m^{-2} s^{-1}) at 28 °C with continuous shaking at 100 rpm. For iron stress conditions, acid washed glassware was used and the ferric citrate present in BG-11o medium was replaced with citric acid.

2.4 Heterocyst counting

Heterocyst frequency was determined by counting the number of heterocysts (late proheterocysts/early heterocysts were recognized by their thickened cell wall and pale appearance, and mature heterocysts were recognized by their poles) and vegetative cells that were present along filaments of *Nostoc* sp. PCC 7120. The total number of cells counted was approximately 100 cells per sample. Heterocyst frequency was determined by counting the number of heterocyst per hundred vegetative cells in at least 20-25 healthy and equal length filaments under microscope [36].

3 Results and discussion

3.1 Effect of ferric citrate and iron chelators on the growth of Nostoc sp. PCC 7120

To help to further establish conditions for iron-restriction, the effect of iron (ferric citrate) and the iron chelator, DTPA, on the growth of the *Nostoc* sp. PCC 7120 under consideration was investigated. *Nostoc* sp.PCC7120 cells were precultured in normal BG-11 for 6 days and then inoculated (with 2 ml of the preculture at $OD_{730\ nm}$ 1.6) into four different media (low-Fe BG-11, low-Fe BG-11 with 30 µM ferric citrate, low-Fe BG-11 with 20 µM of DTPA and normal Fe BG-11), and subsequent growth was monitored for 13 days under illumination at 25 °C. The aim was to determine whether the ferric chelator, DTPA, or additional iron (30 µM ferric citrate) had any effect on growth in low-Fe BG-11. The results showed that *Nostoc* sp. PCC 7120 grew similarly in all four media for the first 3 days, but after this its growth was enhanced in the low-Fe BG-11 medium by the presence of 30 µM ferric citrate (Figure 1).Surprisingly, DTPA did not affect growth suggesting that it is not effective at withholding iron from *Nostoc* sp. PCC 7120. Also, the normal Fe BG-11 medium (17 µM ferric ammonium citrate) gave the same growth level as seen for the low-Fe medium (and lower than for the medium with 30 µM ferric citrate). It is difficult to understand why 17 µM iron failed to boost growth of *Nostoc* whereas 30 µM Fe caused enhanced growth. However, this experiment does show that iron-restricted growth can be achieved for *Nostoc* by growth in low-Fe BG-11 medium with/without 30 µM ferric citrate, although the effect obtained is weak (1.4 fold after 13 days).These results are in agreement with many studies showing the effect of iron deficiency on the growth of cyanbacterial species. For example, the growth rate of some of cyanobacteria such as *Synechococcus* sp. PCC 7942, *Synechococcus* sp. PCC 7002, *Anabaena variabilis* and *Oscillatoria tenuis* is decreased under iron starvation [28, 32-34] Also, the growth rate for *Plectonema boryanum* directly correlates with the iron content of the medium [33]. Phytoplanktonic cyanobacteria require higher Fe-C quotas than eukaryotic phytoplankton[8]. Also, the overall metal content is much higher in photosynthetic organisms than in non-photosynthetic ones. For example, the iron content of *Synechocystis* sp. PCC 6803 cells, which was determined by atomic absorption spectroscopy, was found to be one order of magnitude higher in comparison to *E. coli*[9]. The high iron demand in cyanobacteria is caused by the need to supply both the respiratory redox enzymes, as is the case in non-photosynthetic bacteria, and also for the photosynthetic machinery and nitrogen fixation apparatus[10].

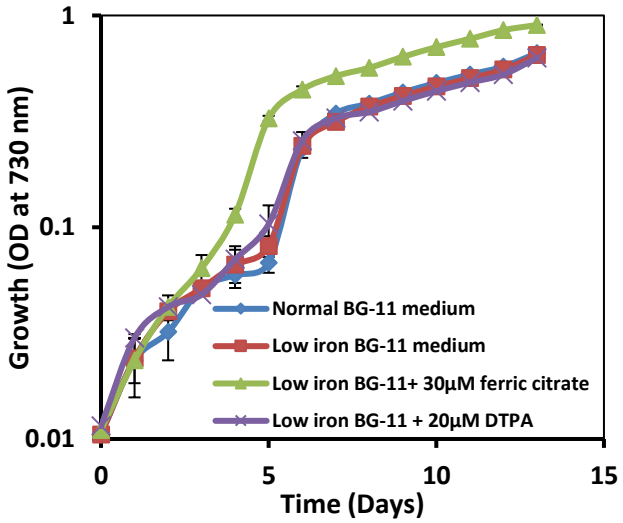

Figure 1: The effect of DTPA and ferric citrate on the growth of *Nostoc* sp 7120. The symbols in the graph represent the growth in normal BG-11 medium (♦), low-Fe BG-11medium (■), low-Fe BG-11 medium + 30 µM ferric citrate (▲) and in Low-Fe BG-11 medium + 20 µM DTPA (×). The values represent the mean ± SD of three independent experiments.

3.2 Effect of iron storage in Nostoc sp. PCC 7120

The relatively small growth difference observed in *Nostoc* sp. PCC 7120 during the early growth stages (1-10 days) under iron-replete and ion-restricted conditions (Figure 1) could be related to iron storage. Intracellular iron stores could compensate for lack of extracellular iron and thus enhance growth under iron deficiency[9]. It is possible that depletion of iron stores could allow a more extreme growth difference under high and low iron conditions. To investigate the effects of iron storage on the growth of cyanobacteria, two 11 day precultures were performed, one on low-Fe BG-11 medium (iron restricted preculture) and the other on normal BG-11 medium (iron sufficient preculture). Then 2 ml of each preculture were inoculated into 50 ml of low-Fe BG-11 medium and subsequent growth was monitored for 12 days. The iron-restricted inoculum hardly grew in the low-Fe BG-11 medium (OD_{730nm} at 12 days was just 0.37), while the iron-sufficient inoculum produced good growth (OD_{730nm} at 12 days was 0.90, ~three-fold higher than that of iron restricted cells) (Figure 2).

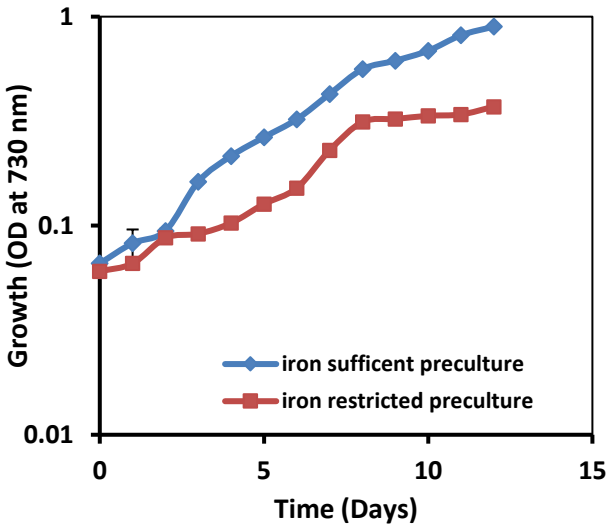

Figure 2: The effect of iron storage on the growth of *Nostoc* sp. PCC 7120. The symbols in the graphs represent the growth of iron sufficient preculture (♦) and the growth of iron restricted preculture (■) on low-Fe BG-11 medium. Error bars represent SD.

The poor growth of the iron-restricted inocula compared to the iron-replete inocula of *Nostoc* sp. PCC 7120 under low iron (low-Fe BG-11 medium) indicates that iron stores were consumed during the first 11 days of the growth of the iron-restricted inocula such that they were unable to grow further without iron. However, the good growth of the iron-sufficient inocula of both species in low-Fe BG-11 medium indicated that they had deposited iron stores that supported subsequent iron restriction growth over the first 11 days of growth under. Thus, the iron-sufficient inocula used their storage iron to grow well under low iron conditions. These results suggest that the failure observed above (Section 3.1) to obtain large growth reductions during iron restriction could be due to utilisation of iron stores (Figure 2).The iron storage in this organism is probably due to the presence of bacterioferritins and ferritins[9].Ferritin homologues have been found in some other cyanobacteria such as *Synechocystis* sp. PCC 6803, *Synechococcus* sp. PCC 7002, *Prochlorococcus marinus* MED4, *Thermosynechoccus elongatus* Bp-1, *Gloeobacter violaceus* PCC 7421 and *Nostoc* sp. PCC 7120[9].

3.3 Effect of iron on heterocyst formation in Nostoc sp. PCC 7120

It is reported that heterocyst formation is influenced by the availability of iron [28, 37]. To confirm whether iron does indeed affect the formation and development of heterocysts in *Nostoc* sp. PCC 7120, *Nostoc* was precultured in normal BG-11 for 7 days (no heterocysts were formed due to high ammonium availability; Figure 3A) and then used to inoculate four different media with high or low amounts of iron or ammonium: normal BG-11, low-Fe BG-11, normal BG-11o and low-Fe BG-11o. Growth was maintained for 7 days under continuous fluorescence white light (50 µmol photons m^{-2} s^{-1}) at 28 °C with shaking at 100 rpm. Nitrogen stress was achieved by culturing the cells in BG11o (BG-11 minus fixed nitrogen) supplemented with 10 mM sodium bicarbonate. The ammonium ferric citrate present in normal BG-11 medium was replaced with citric acid and ferric citrate for low-Fe BG-11o and normal BG-11o, respectively. Light-microscopic images were taken for each culture for 7 days.

It was found that no heterocysts were formed in the cultures grown on normal- and low-Fe BG-11 media for 7 days, while heterocysts were observed in the cultures grown on normal- and low-Fe BG-11o media for the same period of time (Figure 3B). This implies that the heterocysts were formed only under nitrogen stress in order to enable the organism to fix nitrogen. This finding is fully consistent with previous results[38, 39]. Few proheterocysts were seen after 20 h of growth only in the cultures grown on normal BG-11o. However, both the proheterocysts and the mature heterocysts appeared after 2 days of growth on both low-Fe and normal BG-11o, and remained present for the subsequent period of growth (up to 7 days). Heterocyst frequency was determined by counting the number of heterocysts per 100 vegetative cells under the microscope. It was found that the number of heterocysts increased slowly with time under low iron (in Low-Fe BG-11o). No heterocysts were observed after 20 h of growth. Then there was an initial increase in the heterocyst frequency from 2-5 days, giving 4.5 and 9.6% with respect to the vegetative cells, after 2 and 5 days, respectively. Then, not much increase in the heterocyst frequency was observed from 5-7 days, with a frequency of just 10% after 7 days (only a 0.4% increase; Figure 4). However under high iron (in normal BG-11o), the number of heterocysts increased

rapidly with time. Heterocysts were observed after 20 h of growth, giving a frequency of 6%. Then the frequency increased to 12% after 2 days and increasing further to give 15 and 19% after 5 and 7 days, respectively (Figure 4). Thus the frequency of heterocysts under high iron was greater than under low iron by up to nearly twofold.

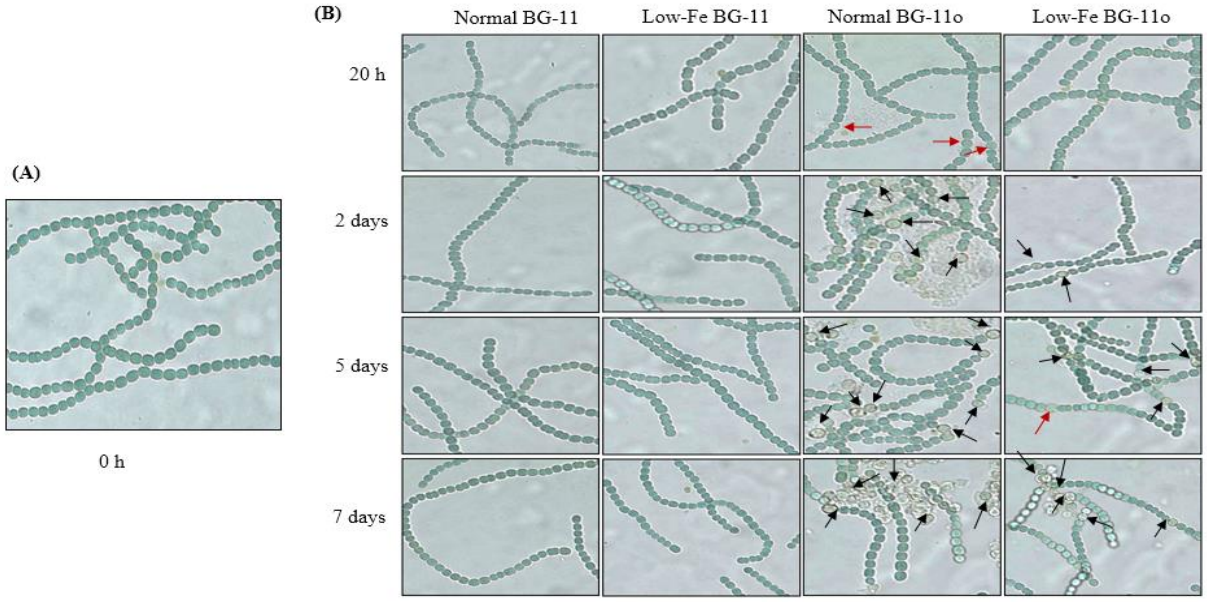

Figure 3: Effect of iron on heterocyst formation in *Nostoc* sp. PCC 7120. Light microscopic photos of A: *Nostoc* precultures grown on normal BG-11 medium after 7 days of inoculation (time 0 h),B: *Nostoc* cultures grown on four different media, normal BG-11, low -Fe BG-11, normal BG11o and low-Fe G11o for 7 days. Black arrows point to the mature heterocysts and red arrows point to the premature heterocysts (proheterocysts).

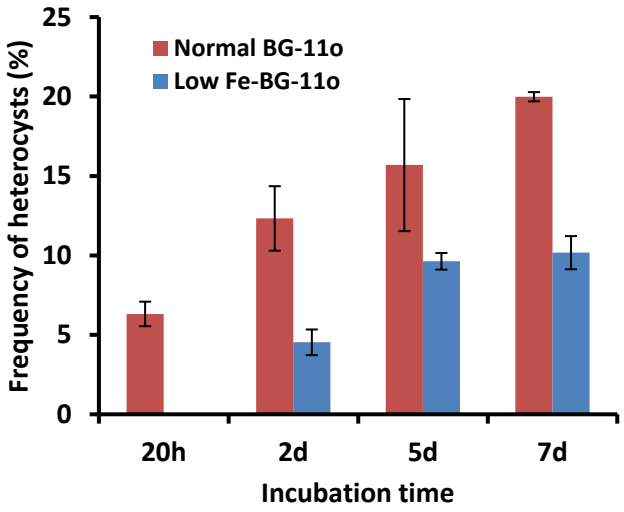

Figure 4: Appearance of heterocysts in *Nostoc* sp. PCC 7120 cells under iron restriction: Heterocyst frequency was determined by counting the number of heterocysts per 100 vegetative cells under the microscope. The frequencies were the average from two counts. Error bars indicate standard deviation (SD).

These results showed that iron deficiency delayed the formation of heterocysts in *Nostoc* sp. PCC 7120 and affected their differentiation. These results are consistent with those reported by Xu *et al*[37] who found that heterocyst differentiation in *Nostoc* sp. PCC 7120 was delayed under moderate iron limitation conditions (as achieved by addition of the iron chelator 2,2′-dipyridyl at <80 μM). However, under severe iron limitation conditions (100 μM 2,2′-dipyridyl) no heterocyst differentiation was observed[37]. These results confirm the importance of iron in heterocyst formation during growth of *Nostoc* sp. PCC 7120 under nitrogen-fixing conditions.

These data indicates that iron (as well as nitrogen) regulates heterocyst formation and development. Also these data suggest that there are certain iron-regulated genes whose function is to control heterocyst development. López-Gomollon *et al*[40] reported a strong activation of P_{furA}, but not P_{furB} and P_{furC}, in proheterocysts and heterocysts of *Nostoc* sp. PCC 7120 due to the binding of NtcA (the principal transcription factor involved in the regulatory network of nitrogen metabolism) to the operators present in the upstream region of *furA*. This finding indicates a direct crosstalk between heterocyst development and iron acquisition. Consistently, the promoter of *furA* possesses several putative NtcA binding sites. The nitrogen status thereby affects *furA* expression[40] and subsequently flavodoxin (*isiB*) synthesis [41]. However, in cyanobacteria many iron–responsive genes are regulated by NtcA, for example *psaL*, *furA*, *furC*, *isiA* and *isiB*[42]. Hence, the coordinated regulation of expression of "photosynthetic genes" by FurA and NtcA offers a regulatory network able to respond to almost all environmental conditions.

4 Conclusions

Iron deficiency was found to decrease the growth of *Nostoc sp*. PCC 7120. A greater degree of iron restriction was achieved when precultures of *Nostoc* sp. PCC 7120 were grown under low iron conditions to deplete iron stores. Furthermore, heterocyst formation and development in *Nostoc* sp. PCC 7120 was found to be regulated by iron. This finding indicates a direct crosstalk between heterocyst development and iron acquisition.

Acknowledgments

This work has been performed at the Animal and Microbial Department, Reading University, UK and sponsored by the Mission Department, Egypt.

References

[1] Schopf JW, Packer BM: Early Archean (3.3 to 3.5 Ga-old) fossil microorganisms from the Warrawoona Group, Western Austrailia. *Science* 1987, 237:70-73.

[2] Adhikary SP: Ecology of freshwater and terrestrial cyanobacteria. *J Scientif Indust* 1996, 55:753-762.

[3] Carmichael WW: Toxins of cyanobacteria. *Sci Am* 1994(270(1)):78-86.

[4] Fogg GE: Survival of algae under adverse conditions. *Symp Soc Exp Biol* 1969, 23:123-142.

[5] Bohme H: Regulation of nitrogen fixation in heterocyst-forming cyanobacteria. *Trends in Plant Science* 1998, 3:346-351.

[6] Rai AN, Borthakur M, Paul D: Symbiotic cyanobacteria: biotechnological applications. *J Sci Indus Res* 1996, 55:742-752.

[7] Zajic JE: *Microbiol Biogeochem Academic Press, New York* 1969:156-168.

[8] Kustka A, Carpenter EJ, Sanudo-Wilhelmy SA: Iron and marine nitrogen fixation: progress and future directions. *Res Microbiol* 2002, 153(5):255-262.

[9] Keren N, Aurora R, Pakrasi HB: Critical roles of bacterioferritins in iron storage and proliferation of cyanobacteria. *Plant Physiol* 2004, 135(3):1666-1673.

[10] Shi T, Sun Y, Falkowski PG: Effects of iron limitation on the expression of metabolic genes in the marine cyanobacterium *Trichodesmium erythraeum* IMS101. *Environ Microbiol* 2007, 9(12):2945-2956.

[11] Boyer GL, Gillam AH, Trick CG: Iron chelation and uptake. In: Fay P, Van Baalen C (eds) The cyanobacteria. *Elsevier, New Yok* 1987:415-436.

[12] Briat JF: Iron assimilation and storage in prokaryotes. *J Gen Microbiol* 1992, 138(12):2475-2483.

[13] Butler A: Acquisition and utilization of transition metal ions by marine organisms. *Science* 1998, 281:207-209.

[14] Geider RJ, Laroche J: The role of iron in phytoplankton photosynthesis, and the potential for iron-limitation of primary production in the sea. *Photosyn Res* 1994, 39:275-301.

[15] Kamiya N, Shen JR: Crystal structure of oxygen-evolving photosystem II from *Thermosynechoccus vulcanus* at 3.7 Å resolution. *Proc Natl Acad Sci USA* 2003, 100:98–103.

[16] Ouzounis C, Sander C: A structure-derived sequence pattern for the detection of type I copper binding domains in distantly related proteins. . *FEBS Lett* 1991, 279:73-78.

[17] Kurisu G, Zhang, H., Smith JL, Cramer WA: Structure of the cytochrome b6f complex of oxygenic photosynthesis: tuning the cavity. *Science* 2003, 302:1009-1014.

[18] Stroebel D, Choquet Y, Popot JL, Picot D: An atypical haem in the cytochrome b6 f complex. *Nature* 2003, 426:413-418.

[19] Shcolnick S, Keren N: Metal homeostasis in cyanobacteria and chloroplasts. Balancing benefits and risks to the photosynthetic apparatus. *Plant Physiol* 2006, 141(3):805-810.

[20] Jordan IK, Makarova KS, Wolf YI, Koonin EV: Gene conversions in genes encoding outer-membrane proteins in *H. pylori* and *C. pneumoniae*. *Trends Genet* 2001, 17:7–10.

[21] Ferreira F, Strauss NA: Iron deprivation in cyanobacteria. *J Appl Phycol* 1994, 6:199-210.

[22] Rueter JG, Ohki K, Fujita Y: The effect of iron nutrition on photosynthesis and nitrogen fixationn in cultures of *Trichodesmium* (Cyanophyceae).*J Phycol* 1990, 126:30-35.

[23] Paerl HW, Prufert-Bebout LE, Guo C: Iron-stimulated N(2) fixation and growth in natural and cultured populations of the planktonic marine cyanobacteria *Trichodesmium* sp. *Appl Environ Microbiol* 1994, 60(3):1044-1047.

[24] Raven JA: The iron and molybdenum use efficiencies of plant growth with different energy, carbon and nitrogen. *New Phytol* 1988, 109:279-287.

[25] Hardie L, P. , Balkwill D, L. , Stevens S, E.: Effects of iron starvation on the physiology of the cyanobacterium *Agmenellum quadruplicatumAppl Environ Microbiol* 1983a, 45:999-1006.

[26] Peschek GA: Nitrate and nitrite reductase and hydrogenase in *Anacystis nidulans* grown in Fe- and Mo-deficient media. *FEMS Microbiol Lett* 1979, 6:371-374.

[27] Boussiba S, Richmond AE: C-phycocyanin as a storage protein in the blue-green alga *Spirulina platensis*. *Arch Microbiol* 1980, 125:143-147.

[28] Wilhelm SW: Ecology of iron-limited cyanobacteria: a review of physiological responses and implications for aquatic systems. *Aquat Microb Ecol* 1995, 9:295-303.

[29] Sherman DM, Sherman LA: Effect of iron deficiency and iron restoration on ultrastructure of *Anacystis nidulans*. *J Bacteriol* 1983, 156:393-401.

[30] Swingley WD, Hohmann-Marriott MF, Le Olson T, Blankenship RE: Effect of iron on growth and ultrastructure of *Acaryochloris marina*. *Appl Environ Microbiol* 2005, 71(12):8606-8610.

[31] Gorham PR, McLachlan JL, Hammer UT, Kim UK: Isolatlon and culture of toxic strains of *A.nabaena flos-*

aquae (Lyngb.). *Verh int Vereln theor angebv Limnol* 1964, 15:796-804.

[32] Brown CM, Trick CG: Response of the cyanobacterium, *Oscillatoria tenuis*, to low iron environments: the effect on growth rate and evidence for siderophore production.*Arch Microbiol* 1992, 157:349-354.

[33] Kerry A, Laudenbach DL, Trick CG: Influence of iron limitation and nitrogen source on growth and siderophore production by cyanobacteria.*J Phycol* 1988, 24:566-571.

[34] Wilhelm SW, Maxwell DP, Trick CG: Growth, iron requirements, and siderophore production in iron-limited *Synechococcus* PCC 7002. *Limnol Oceanogr* 1996, 41:89-97.

[35] Stanier RY, Kunisawa R, Mandel M, Cohen-Bazire G: Purification and properties of unicellular blue-green algae (order Chroococcales). *Bacteriol Rev* 1971, 35(2):171-205.

[36] Shukla MK, Tripathi RD, Sharma N, Dwivedi S, Mishra S, Singh R, Shukla OP, Rai1 UN: Responses of cyanobacterium *Anabaenadoliolum* during nickel stress. *J Environ Biol* 2009, 30:871-876

[37] Xu W-L, Liu Y-D, Zhang C-C: Effect of iron deficiency on heterocyst differentiation and physiology of the filamentous cyanobacterium *Anabaena* sp. PCC 7120. *J Natural Sciences, Wuhan University* 2003, 8:880-884.

[38] Wolk CP: Heterocyst formation in *Anabaena*. *In Y V Brun and L J Shimkets (ed), Prokaryotic development ASM Press, Washington, DC* 2000:83–104.

[39] Wolk CP, Ernst A, Elhai. J: Heterocyst metabolism and development. *In D A Bryant (ed), The molecular biology of cyanobacteria Kluwer Academic Publishers, Dordrecht, The Netherlands* 1994:769–823.

[40] López-Gomollon S, Hernandez JA, Wolk CP, Peleato ML, Fillat MF: Expression of furA is modulated by NtcA and strongly enhanced in heterocysts of *Anabaena* sp. PCC 7120. *Microbiology* 2007a, 153(Pt 1):42-50.

[41] Hernández JA, Peleato ML, Fillat MF, Bes MT: Heme binds to and inhibits the DNA-binding activity of the global regulator FurA from *Anabaena* sp. PCC 7120. *FEBS Lett* 2004, 577:35–41.

[42] López-Gomollon S, Hernandez JA, Pellicer S, Angarica VE, Peleato ML, Fillat MF: Cross-talk between iron and nitrogen regulatory networks in *Anabaena* (*Nostoc*) sp. PCC 7120: identification of overlapping genes in FurA and NtcA regulons. *J Mol Biol* 2007b, 374:267-281.