Ultra-structural adaptation toward iron deficiency in *Thermosynechococcus elongatus* cells

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Abstract: Iron is considered one of the most essential elements required by all organisms. It involves both photosynthetic and respiratory electron transport chains. Since the biological availability of iron in nature is limited and it is highly required by cyanobacteria, focusing on the adaptation or tolerance mechanism in thermophilic cyanobacteria has a lot of attention. Through the present work, a highlight on the ultrastructural changes of *Thermosynechococcus elongatus* cells due to iron deficiency is investigated. Beside biochemical and spectroscopical analysis, transmitting electron microscope images have been used for description these changes. Results showed remarkable rising in DNA, protein and lipids contents, while reduction in cell size and chlorophyll content. Transmitting electron microscopic images showed reduction in cell diameters, length and width. Moreover, the thylakoid thickness and cytoplasm area have reduced, while the nucleoplasm area was increased. In addition, this work adopts for the first time an effective indicator ratio (A_{280}/A_{440}) that could be used as fast monitor for ultrastructural changes. *Thermosynechococcus elongatus* cells thought to adapt Fe-limitation by decreasing the proteins containing iron and synthesis specific proteins that decrease the rate of photosynthesis. Hence, energy was saved by reduction the cells size and cytoplasm area. Cells produced iron resistant and regulators proteins to achieve the necessary metabolism. Observed changes in cell size, thylakoid membrane thickness and the large nucleoplasm area could be taken as monitor in response to iron-deficiency.
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1.Introduction:

Although iron is existed in high amount on earth, its biological availability is limited **(Frausto da Silva and Williams, 1993, Lippard and Berg, 1994).** Almost all photosynthetic organisms exhibit the same behavior against iron deficiency, where all of them act to decrease the expression of iron containing protein **(Raven 2013).** Phytoplanktons conduct about 40% of global photosynthesis in aquatic environments. Since cyanobacteria contribute remarkably to this fraction, so they require large amount of iron to maintain their Fe-rich photosynthetic apparatus. Biochemical analysis of thylakoid membranes isolated from iron-deficient cells was reported by **Pakrasi *et al.* (1985),** while changing in phycobilins was demonstrated by **Sherman and Sherman (1983).** Structure investigations of IsiA, IdiA and IsiB were identified by **Sandmann, (1985), Falk *et al.* (1995), Ivanov *et al.* (2000), Boekema at al (2001), Cadoret *et al.* (2004), Arteni *et al.* (2005) and Lax *et al.* (2007). Singh *et al.* 2003** used a DNA microarray technology to analyze a full-genome microarray of the cyanobacterium *Synechocystis sp*. PCC 6803 to find regulations of gene transcription. **Watts *et al.* (2003), and Kong *et al.* (2010)** investigated the role of nitrogen monoxide and carbon monoxide in cellular adaptation to iron deficiency.

Responses of iron deficiency effects could be reduction or changing in the molecules structures which directly or indirectly iron-dependent. The role of iron deficiency on cell growth was reported by **Straus (1994),** who summarized these responses into three different categories: acquisition, compensation, and retrenchment. Acquisition is s process by which the cell produces specific iron-chelating molecules **(Dong and Xu, 2009).** This mechanism has been reported in higher plants, fungi, bacteria, and cyanobacteria **(Braun and Winkelmann, 1987).** Compensation is a mechanism by which new proteins are synthesized and replace the ordinary proteins. A prominent example is IsiB **(Sandmann, 1985, Falk *et al.* 1995)**. The changes and the reduction of cellular structures and physiological processes are known as Retrenchment. It is obviously monitored in Anemia. For high plants and green algae lead to chlorosis, a typical iron-deficient symptom and a range of genes related to the iron acquisition are induced **(Henriques *et al.*, 2002, Xie *et al.*, 2012,** Yang ***et al.*, 2012)**

Recently, **Kranzler *et al.* (2013)** reported that cyanobacterial iron requirements exceed 10-fold compared to the non-photosynthetic prokaryotes and are high even among other photosynthetic organisms. As shown, most previous studies were interested in the photosynthetic process and/or involved photosynthetic

protein complexes. This work highlights to the mechanism, including the morphological and ultra-structural changes, in Cyanobacterium *Thermosynechococcus elongatus*, by which it can resist Fe-stress condition. These changes could be a model of prokaryotic oxygenic organism.

2. Material and Methods

Cultivation conditions.

Cultivation of *Thermosynechococcus elongatus* cells were achieved according to **Ivanov *et al.* (2006)** with some modifications. Preculture was prepared by cultivation of cells in liquid BG-11 **(Rippka *et al.*, 1979)** in rod-shaped glass tubes bubbled with 5 % CO_2 in air, 50 °C and continuous illumination of 50 $mmol.m^{-2}.s^{-2}$. Bi-distal water was used to prepare both normal BG-11 medium and that free of iron. Preculture was washed four times with BG-11 free iron media. Inoculums were injected to both normal and BG-11 free iron media to reach OD_{750nm} of 0.2. Both cultures were exposed to the same conditions of preculture. Optical density at 750, 680 and 673 nm was detected after 20, 50, 70 and 90 hours.

Absorption spectral analysis

Absorption spectra of 0.5 ml of cultures samples were measured at different incubation periods using Shimadzu UV-2450. Specific wavelengths (750, 680 and 673 nm) were detected. OD_{680}/OD_{673} ratio (<1) was used to monitor the cell adaptation in case of iron free medium.

Estimation of protein, DNA, total lipids and total chlorophyll contents.

2 µl of culture samples were dropped onto NanoDrop ND-1000 Spectrophotometer. UV-spectral analysis and concentration of protein and DNA were calculated using the instrument program. Total lipids were estimated according to **Bligh and Dyer (1959)** using 3 sequences of chloroform-methanol solution. Total chlorophyll content was estimated using 100% methanol according to **Porra *et al.* (1989).**

Estimation of cell Size and numbers

Beckman Coulter Z Series 9914591-D was used in estimation the cells size and numbers according to manual Beckman Coulter Inc., CA.

Transmitting electron microscope image

30 ml of sample suspensions were precipitated at 5000g, and then fixed by in dry acetone containing 2% glutaraldehyde and 0.1% tannic acid. Following acetone rinses, the samples were incubated in 0.1% uranyl acetate and 1% OsO_4 for 1 h at room temperature. Samples were then washed with dry acetone, infiltrated to increase concentrations over 6 days and polymerized at 60°C. Sections were cut using an Ultracut UCT microtome. Samples were examined and investigated using JEOL 100CX transmitting electron microscope, at the Electron Microscope Unit at the Faculty of Science, Alexandria University, Egypt **(El Shafai *et al.*, 2011).**

3. Results:

Iron is an essential element involves the cell structure in prokaryotic organisms, where it plays an important role in both respiratory and photosynthetic processes. Hence, its starvation effects on cell structure and components were monitored through spectroscopical analysis. Figure 1 shows the growth behavior of *T. elongatus* cells that have been grown in both normal and iron deficiency media. A remarkable reduction in growth rate in case of Fe-deficiency medium was recognized (0.016 A_{750nm}/hours), compared to that of normal media (0.032 A_{750nm}/hours). Since the growth rate is too slow in case of Fe-stress condition, lag and logarithmic phase were not distinguished. In *T. elongatus* cells, the maximum absorbance of chlorophyll content at red region was normally at 680 nm which could be recognized in figure 1A and the ratio of OD_{680}/OD_{673} was always more than 1. In contrast, partial reduction in OD_{680} was detected so the OD_{680}/OD_{673} ratio shifted down to be less than 1. This ratio gradually decreased until the cells dramatically died. UV-Vis absorption spectral analysis of both cultivation conditions exhibited several variations.

As shown in figure 2, the difference in absorption spectra between Fe-deficiency condition and normal condition exhibited interested changes. Absorbance at visible spectra showed remarkable decrease compared to cultivation in normal medium. Negative peaks were detected at 693 nm, 620-650 nm and 500- 540 nm. A positive peak was detected at 673 nm with shoulder at 680 nm. These results were supported by results in table 1, where a remarkable reduction in chlorophyll content was estimated in case of stressed cells to be 0.0083 Chl/ml compared to 0.025 Chl/ml in case of normal cultivated cells. Interested results were also detected at UV-region, where more than double absorbance value was recorded in stressed cells (Figure 3 A,B). Comparison in DNA content of both cells grown in normal and Fe-deficiency media are shown in table 1. More than double amount of DNA (220%) was estimated as a result of Fe-stress. Protein content also exhibited almost the same behavior, since 251% was the rising in protein content in response to iron deficiency. It should be point that A_{260}/A_{280} values were almost constant in both conditions (Figure 3-b). Total lipid contents showed same behaviour results to that of DNA and protein, where enhancement in the lipid content (196%) was observed due to Fe-stress. A_{280}/A_{440} ratio showed interesting difference, where the ratio which is normally less than one, jumped to be 1.91 in case of iron stressed cells. Astonishing results were recorded for cell numbers, where 14 % rising in

cell numbers resulted from Fe-stress. While a remarkable reduction in cell sizes (14 %) were estimated due to Fe-deficiency.

Figure 4 shows transmitting electron microscope images of *T. elongatus* cells cultivated in normal and iron free medium. These images adopted clear pictures for morphological as well as ultra-structural changes. Through longitudinal sections, 14-20 % reductions in cell length and width respectively were calculated in Fe-stressed cells. In addition, 10 % reductions in cell diameter were observed in transverse sections. On the other hand, 26 % reductions in thylakoid membrane thickness were estimated in Fe-stressed cells (Table 2). Compared to normal cultivated cells, the transferred sections of *T. elongatus* grown Fe-limited medium showed remarkable increasing in the nucleoplasm area and reduction in cytoplasm area (Figure 4-C, D, G, H).

It could be concluded that in Fe-stressed cells, remarkable reduction in cell size, diameter, length, width, thylakoid membrane thickness and chlorophyll content were observed. In contrast, rising in protein content, DNA content, lipid content and cell density were recorded as a result of iron deficiency.

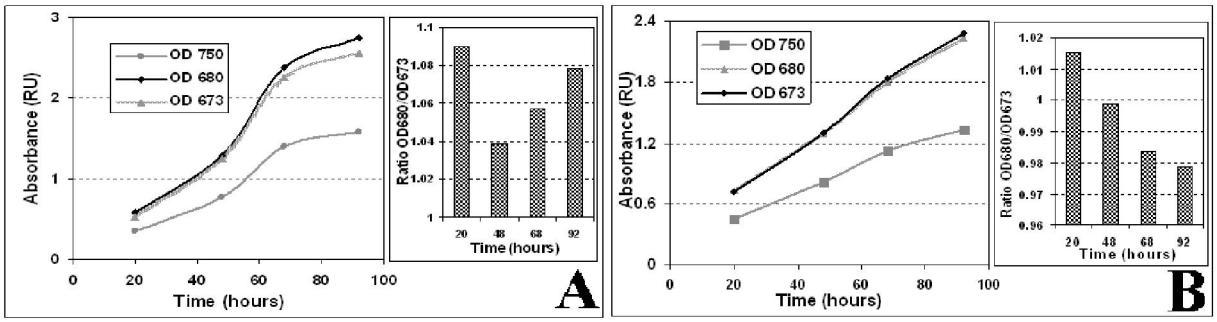

Figure 1 (A, B): *Thermosynechococcus elongatus* growth curve. Cells were grown in normal BG11 medium. Cells were harvested and washed four times with BG11-free iron medium before inoculation to BG-11 media A and BG-11 free iron media B. Cultures were grown in continuous light intensity of 50 mmol.m^{-2}.s^{-2}.

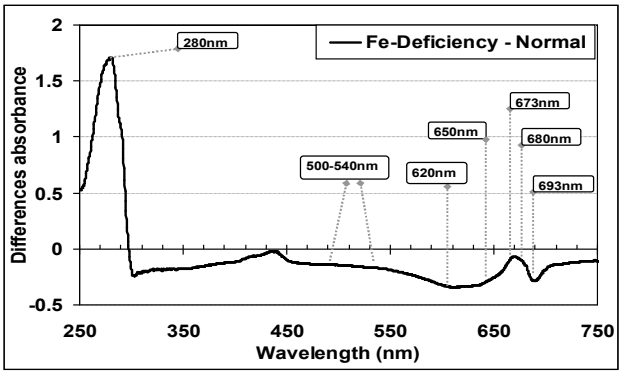

Figure 2: Absorption spectral difference in (Fe deficiency – normal culture) of *T. elongatus* cells grown in normal and iron deficiency media. Both cultures were diluted by HEPES buffer (pH 7.5) to about OD_{440} = 1.5 before measurements.

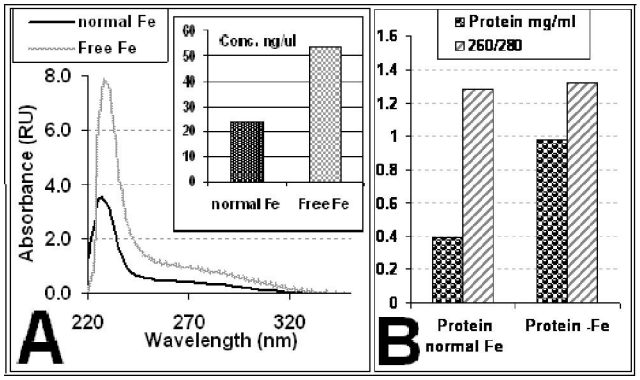

Figure 3: Absorption spectra at UV-region of *T. elongatus* cells grown in normal and iron deficiency media. A) Absorbance and concentration of DNA of both samples were achieved after 92 hours growing period. B) Diagram showing comparison between A280, A260/A280 and protein concentration

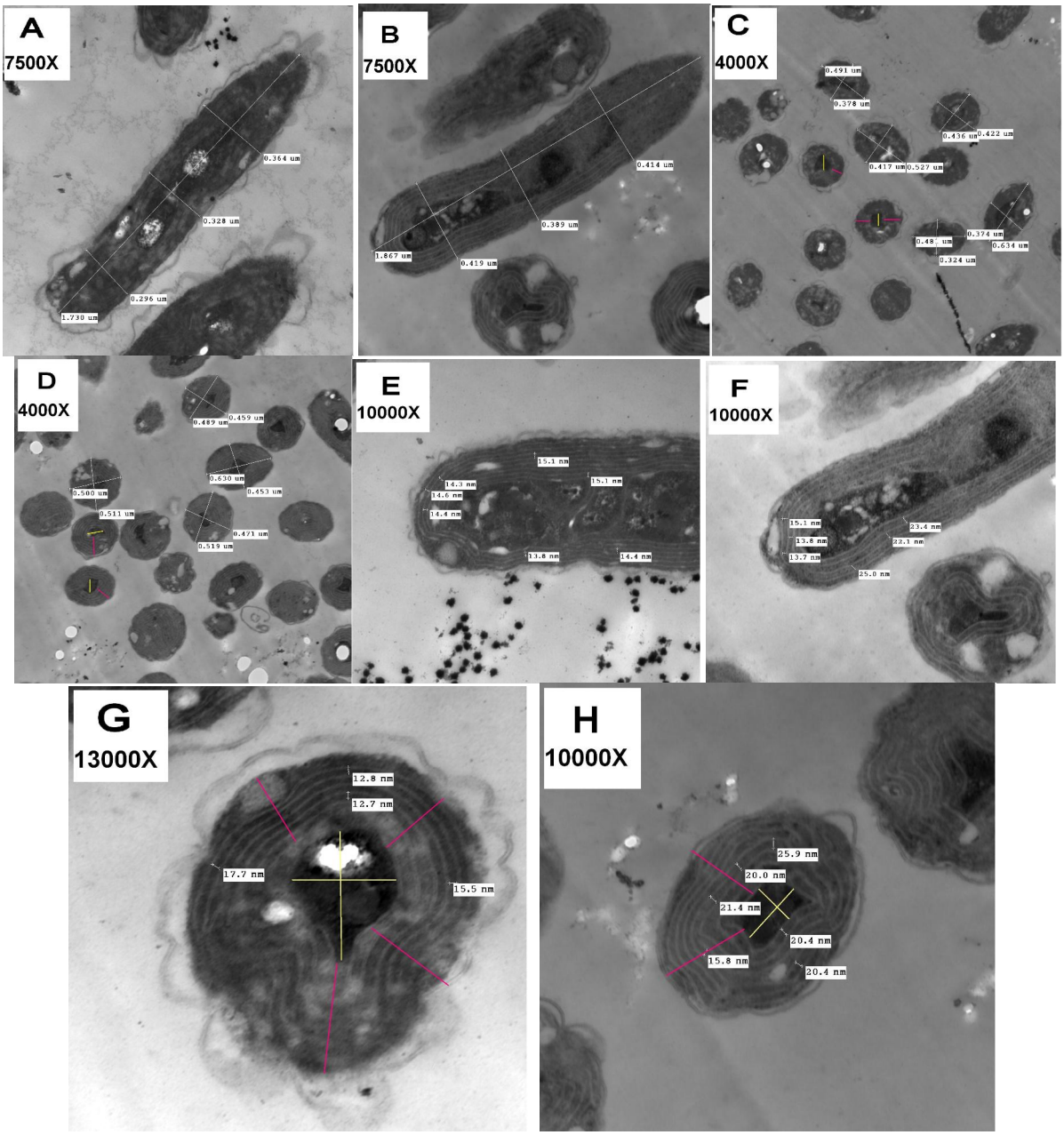

Figure 4: Transmission electron microscope images showing differences between Fe-stressed and normal cultivated *T. elongatus* cells, respectively. A and B show longitudinal sections; C and D are transferred sections; E and F show thylakoid membrane thickness in longitudinal sections; and G and H show thylakoid membrane thickness in transferred sections.

Table 1: Comparison between chlorophyll content, cell no. and cell size of *T. elongatus* cells grown in normal and iron deficiency media.

	Fe Deficiency media	Normal media	Changing %
Chl_{mg}/ml	0.0083	0.025	33.2%
DNA content (ng/ml)	53.64	24.13	220 %
Protein content (mg/ml)	0.98	0.39	251%
Lipids content (mg/ml)	0.51	0.26	196%
A_{280}/A_{440} ratio	1.95	0.85	228%
Cell no. /ml	$1.91E^{+08}$	$1.68E^{+08}$	114%
Cell size	2.152 µm	2.511 µm	86%

Table 2: Comparison between *T. elongatus* cells grown in normal and iron free medium of cell according to: length, width, diameter and thickness of thylakoid membrane. These data are based on electron microscope measurements.

	Fe Deficiency media	Normal media	Changing %
Length (µm)	1.803 ± 0.055	2.093 ± 0.118	86%
Width (µm)	0.358 ± 0.034	0.449 ± 0.05	80%
Diameter (µm)	0.452 ± 0.052	0.504 ± 0.053	90%
Thylakoid membrane Thickness (nm)	14.58 ± 1.2995	19.75 ± 4.053	74%

4. Discussion:

Distribution and metabolism of living organisms are strongly affected by availability of nutrients. In spite of high existence of iron in nature, its biological availability is very limited. The abundant of *Thermosynechococcus elongatus* was limited at low Fe- availability **(Suzuki *et al.*, 2005)**. Beside its role in all photosynthetic electron transport, iron is required essentially as Co-factors in most enzymatic systems especially those of respiration **(Singh *et al.* 2002).** Since iron is estimated in high quantities within the structure of thylakoid membrane in cyanobacteria and other photosynthetic organisms, the rate of growth exhibited slow behavior, where *T. elongatus* cells switched their metabolic priority to overcome or adapt the stress. The genomes of cyanobacterial species code for a multitude of iron transporters, iron storage complexes and iron-responsive elements involved in maintaining homeostasis in a highly variable environment **(Kranzler *et al.* 2013)** Combination of cell size reduction with low growth rate seemed to be an effective way for controlling the metabolic machinery of the cells. Also, it obviously explained high DNA content. Increase DNA amount was accompanied with high protein content. The ordinary proteins were expressed in low amount under Fe stress (e.g. trimeric photosystem 1 and phycobilins, see Figure 2), while several new responding proteins were expressed to adapt this stressed condition**. Dong and Xu (2009)** detected five newly synthesized proteins at outer membrane of *Anabaena sp* under iron-deficient conditions. They suggested that this protein could enhance the uptake of iron. Also, **Nield *et al.* 2003** pointed to expression of new proteins as a signal for iron limitations or high hydrogen peroxide. Several publications cited the adaptation of the multiprotein complexes PSII and PSI to iron starvation is a sequential process, which is characterized by the enhanced expression of two major iron-regulated proteins, IdiA (iron deficiency induced protein A) and IsiA (iron stress induced protein A). These proteins thought to be energy quencher protective for photosystems against oxidative stress under conditions of mild iron limitation **(Nield *et al.* 2003, Ivanov *et al.* 2006, Van der Weij-de *et al.* 2007)**, replace phycobilins **(Pakrasi *et al.* 1985, Burnap *et al.* 1993)** or chlorophyll storage protein, which can provide chlorophylls for the synthesis of chlorophyll-binding proteins during recovery from iron stress **(Burnap *et al.* 1993).** Although expression of IsiA is accompanied with Chlorophyll *a* **(Van der Weij-de *et al.* 2007),** present results exhibited large depletion in chlorophyll content as response of Fe-stress. These results could be the art fact of reduction in thylakoid membrane, cytoplasm area and cell size. Here, a new effective parameter (A_{280}/A_{440} ratio) was adopted and suggested as sensor for monitoring the cell structural changes. Less than one is the A_{280}/A_{440} ratio that indicates the balance between chlorophyll content and protein content under normal condition. The rising of this ratio gives strong evidence to cell modifications or adaptations.

Lipids are the most effective source of storage energy and have important role in tolerance to several physiological stressors in all organisms including cyanobacteria. The enhancement of lipid content could be another adaptation way in *T. elongatus*. Iron starvation in *C. reinhardtii* leads to formation of lipid droplets and accumulation of TAGs and enhancement the amount of total and saturated fatty acid that thought resulting in remodeling of lipid membranes as suggested for *T. elongatus* (**Ivanov *et al.*, 2000**) and for *C. Reinhardtii* **(Urzica *et al.*, 2013).** On the other hand, lipid oxidation is problematic as enzymes do not control many oxidative chemical reactions and some of the products of the attack are highly reactive species that modify proteins and DNA **(Singh *et al.* 2002).** The electron microscope images saved clear explanations of other results. The reduction in size and thickness of thylakoid membrane gave strong evidence of reduction in membranous photosynthetic apparatuses containing iron (e.g. PS1, PS2 and Cytb6f), so Chl *a* content and phycobilins were consequently reduced. In contrast, increase nucleoplasm area per cell volume came in agreement with total DNA content, which indicated that *T. elongatus* cells switch the prior metabolic activity. *T. elongatus* cells adapted Fe-limitation by reduction the proteins containing iron even photosynthetic complexes. Moreover, they

produced specific proteins that decrease the rate of photosynthesis (IsiA and IdiA). For this reason, the available energy was consequently reduced; hence cells may save energy by reduction the cells size and thylakoid membrane. Cells produced iron resistant and regulators proteins to achieve the necessary metabolism. The high lipid and protein content thought to be the mechanism by which nutrients and metabolites movements are controlled. It could be concluded that several ultrastructural changes were observed as responses to iron-deficiency. The reductions in cell size and cytoplasm area are considered an effective mechanism for saving energy and monitoring iron deficiency in cyanobacteria. (A_{280}/A_{440} ratio) is considered a new effective parameter by which the cell structural changes could be monitored.

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References:

Arteni, A., Nowaczyk, M., Lax, J., Kouril, R., Rogner, M. and Boekema, E. J. 2005. Single particle electron microscopy in combination with mass spectrometry to investigate novel complexes of membrane proteins. *J Struct Biol*. **149** (3):325-31.

Bligh, E .G. and Dyer, W. J. 1959. A rapid method for total lipid extraction and purification. *Can.J.Biochem.Physiol.* **37:**911-917.

Boekema, E. J., Hifney, A., Yakushevska, A. E., Piotrowski, M., Keegstra, W., Berry, S., Michel, K. P., Pistorius, E. K. and Kruip, J. 2001. A giant chlorophyll-protein complex induced by iron deficiency in cyanobacteria. *Nature*. **412** (6848):745-748.

Braun, V. and Winkelmann, G. 1987. Microbiol iron transport: Structure and function of siderophores. *Prog Clin Biochem Med*. **5:**67-69.

Burnap, R. L., Troyan, T. and Sherman, L. A. 1993. The highly abundant chlorophyll-protein complex of iron-deficient *Synechococcus* sp. PCC 7942 (CP43') is encoded by the *isiA* gene. *Plant Physiol*. **103** (3):893-902.

Cadoret, J. C., Demoulie`re, R., Lavaud, J., van Gorkom, H. J., Houmard, J., Etienne, A. L. 2004. Dissipation of excess energy triggered by blue light in cyanobacteria with **CP43#** (isiA). *Biochim Biophys Acta* **1659:**100–104.

Dong, Y., Xu, X. 2009. Outer membrane proteins induced by iron deficiency in *Anabaena sp.* PCC 7120. *Progress in Natural Science* **19**(10-11), 1477-1483.

El Shafai, A., Zohdy, N., El-Mulla, K., Hassan, M., Morad, N. 2011. Light and electron microscopic study of the toxic effect of prolonged lead exposure on the seminiferous tubules of albino rats and the possible protective effect of ascorbic acid. *Food and chemical toxicology* **49** (4), p.734-743.

Falk, S., Samson, G., Bruce, D. and Huner, N. P. A. 1995. Functional analysis of the iron-stress induced CP430 polypeptide of PS II in the cyanobacterium *Synechococcus sp.* PCC 7942. *Photosynth. Res.* **45**, 51–60.

Frausto da Silva, J. J. R. and Williams, R. J. P. 1993. The Biological Chemistry of the Elements. The Inorganic Chemistry of Life, Clarendon Press, Oxford, pp.370-387.

Henriques, R., Ja´sik J., Klein, M., Martinoia, E., Feller, U., Schell, J., Pais, M. S. and Koncz, C. 2002. Knock-out of *Arabidopsis* metal transporter gene IRT1 results in iron deficiency accompanied by cell differentiation defects. *Plant Mol Biol* **50**, 587–597.

Ivanov, A., Krol, M., Sveshnikov, D., Selstam, A., Sandström, S., Koochek, M., Park, Y., Vasilev, S., Bruce, D., Öquist, G., and Huner, N. 2006. Iron Deficiency in Cyanobacteria Causes Monomerization of Photosystem I Trimers and Reduces the Capacity for State Transitions and the Effective Absorption Cross Section of Photosystem I in Vivo. *Plant Physiology*, **141**, 1436–1445.

Ivanov, A., Park, Y., Miskiewicz, E., Raven, A., Huner, N. P. and Oquist, G. 2000. Iron stress restricts photosynthetic intersystem electron transport in *Synechococcus sp.* PCC 7942. *FEBS Lett*. **485** (2-3), 173-177.

Kong, W. W., Zhang, L. P., Guo, K., Liu, Z. P. and Yang, Z. M. 2010. Carbon monoxide improves adaptation of Arabidopsis to iron deficiency. *Plant Biotechnol J* **8,** 88– 99.

Kranzler, C., Rudolf, M., Keren, N. and Schleiff, E. 2013. Iron in Cyanobacteria. *Advances in Botanical Research*, **65,** 57-105, Jan 2013

Lax, J., Arteni, A., Boekema, E., Pistorius, E., Michel, K. and Rögner, M. 2007. Structural response of Photosystem 2 to iron deficiency: Characterization of a new Photosystem 2–IdiA complex from the cyanobacterium

Thermosynechococcus elongates BP-1 *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, **1767** (6), 528-534.

Lippard, S. J. and Berg, J. M. 1994. Control and Utilization of Metal-Ion Concentrations in Cells. In: Principles of Bioorganic Chemistry. University Science Books, Mill Valley, California

Nield, J., Morris, E., Bibby, T. and Barber, J. 2003. Structural analysis of the photosystem I supercomplex of cyanobacteria induced by iron deficiency *Biochemistry*, **42** (11), p.3180-3188.

Pakrasi, H. B., Riethman, H. C. and Sherman, L. A. 1985. Organization of pigment proteins in the photosystem II complex of the cyanobacterium *Anacystis nidulans* R2. *Proc Natl Acad Sci USA*. **82,** 6903-6907.

Porra, R. J., Thompson, W. A., Kriedemann, P. E. 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophyll a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochim. Biophys. Act.* **975**, 384-394.

Raven, J. A. 2013. Iron acquisition and allocation in stramenopile algae**.** *J Exp Bot* 64(8):2119-27.

Rippka, R., Deruelles, J., Waterbury, M., Herdman and R. Stanier 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J Gen Microbiol* **111**, 1-61.

Sandmann, G. 1985. Consequences of iron deficiency on photosynthetic and respiratory electron transport in blue–green algae. *Photosynth. Res*. **6,** 261–271.

Sherman, D. M. and Sherman, L. A. 1983. Effect of iron deficiency and iron restoration on ultrastructure of *Anacystis nidulans*. *J Bacteriol*. **156** (1), 393-401.

Singh, S., Sinha, R. and Häder, D. 2002. Role of Lipids and Fatty Acids in Stress Tolerance in Cyanobacteria. *Acta Protozool*. **41,** 297 – 308.

Singh, A. K., McIntyre, L. M. and Sherman, L. A. 2003. Microarray analysis of the genome-wide response to iron deficiency and iron reconstitution in the cyanobacterium *Synechocystis sp*. PCC. 6803. *Plant Physiol*. **132,** 1825–1839.

Straus, N. A. 1994. Iron deprivation: Physiology and gene regulation. In: Bryant, D. A. (ed.) Advances in Photosynthesis. Vol. 1: The Molecular Biology of Cyanobacteria. Kluwer Academic Publishers, Dordrecht, Boston, London, pp. 731-50.

Suzuki, K., Hinuma, A., Saito, H., Kiyosawa, H., Liu, H., Saino, T. and Tsuda, A. 2005. Responses of phytoplankton and heterotrophic bacteria in the northwest subarctic Pacific to in situ iron fertilization as estimated by HPLC pigment analysis and flow cytometry *Progress in Oceanography*, **64** (2-4), p.167-187.

Urzica, E., Vieler, A., Hong-Hermesdorf, A., Page, D., Casero, D., Gallaher, S., Kropat, J., Matteo, P., Christoph, M. and Merchant, S. 2013. Remodeling of Membrane Lipids in Iron-starved *Chlamydomonas*. *The Journal of biological chemistry*, **288** (42), 30246-30258.

Watts, R. N., Ponka, P. and Richardson, D. R. 2003. Effects of nitrogen monoxide and carbon monoxide on molecular and cellular iron metabolism: mirror-image effector molecules that target iron. *Biochem J* **369,** 429–440.

Van der Weij-de, W., Chantal D., Ihalainen, A., van de Vijver, E., D'Haene, S., Matthijs, H., van Grondelle, R. and Dekker, J. 2007. Fluorescence quenching of IsiA in early stage of iron deficiency and at cryogenic temperatures *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, **1767** (12), 1393-1400.

Xie, Y. J., Mao, Y., Lai, D. W., Zhang, W. and Shen, W. B. 2012. H_2 Enhances *Arabidopsis* Salt Tolerance by Manipulating ZAT10/12-Mediated Antioxidant Defense and Controlling Sodium Exclusion. *PLoS ONE* **7**(11), e49800.

Yang, L., Liu, T. Y., Li, B., Sui, Y., Chen, J. S., Shi, J., Wing, R., Chen, M. 2012. Comparative Sequence Analysis of the Ghd7 Orthologous Regions Revealed Movement of Ghd7 in the Grass Genomes. *PLoS ONE* 7(11), e50236.