Study of Photosynthetic System Fluidity and Long-term Growth Caused by Salinity in Cyanobacterium *Fischerella* sp. FS 18

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Received: 2020-07-11 Revised and accepted: 2020-09-09

Abstract

The present study examined the responses of different parts of the photosynthetic system in *Fischerella* sp. FS 18 based on different concentrations (17, 80, and 160 mM) of sodium chloride and short time at regular intervals of 20, 40, and 60 min. The results revealed that salinity application (80 and 160 mM) after 20 minutes of inoculation significantly increased the yield of phycobilisome system. Increasing the time up to 40 minutes after inoculation could restore all parts of the photosynthetic system. Then, cyanobacteria can rearrange and activate photosystem II, phycobilisome, and light-collecting complex. However, the behavior of cyanobacteria at salinity of 160 and 80 mM were opposite at 20 and 60 min. Compared to the untreated sample, pretreatment application within less than one hour changed in terms of growth rate and attenuation at time intervals of 24 and 96 hours. The sample was capable of moderating the destructive effects of 160 mM for 20 min and 80 mM in 60 min treatments over 24 hours, which is incomplete. The growth rates up to 96 h in 80 mM for 20 min and 160 mM for 60 min treatments were higher than those without salinity. While the system changed its pattern after 24 hours, the initial pattern remained unaffected by time and salinity levels after this time. In general, simple salinity pretreatments and very short times increased the efficiency of energy transfer in photosystems and produced short and long-term energy and reduction, which could be considered as a major advantage for biotechnology of mass crops.

Keywords: Ecophysiology, Pretreatment, Cyanobacteria, Salinity, *Fischerella* sp. FS 18

Introduction

Cyanobacteria appeared in Precambrian (Schirrmeister et al., 2015; Gérard et al., 2018; Mloszewska et al., 2018). Precambrian can be considered chaotic in terms of environmental conditions with two characteristics: a) the unpredictable interaction including environmental factors in which the intertwined change network is formed, and b) the role of very short times in the formation of this change network. The life span of cyanobacteria, which is measured by human

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time, is short. Therefore, these organisms should complete their growth and reproduction within this short time frame. If the time is generalized to a whole chaotic period like Precambrian (i.e., when things were radically changing at any given moment), the importance of short times would become clearer (Abbasi et al., 2019; Andrault et al., 2018; Shokravi et al., 2014). Seymour et al. (2005) studied marine cyanobacteria and maintained that time in seconds and minutes could cause a complete change in the ecological behavior of these organisms. It is currently not possible to simulate a network of complex changes during Precambrian. Factors such as salinity, alkalinity, acidity, temperature, and light were introduced as environmental shocks (Amirlatifi et al., 2018; Shokravi et al., 2010). To the best of our knowledge, no study has been performed on stegonmatical cyanobacteria in the world for less than an hour. The only studies performed in this field were Amirlatifi et al. (2018) and Abbasi et al. (2019), which were related to cyanobacterum *Calothrix* sp. FS 65 and *Fischerella* sp. FS 18 and performed at 24 and 96 hours. Although they used two 24 and 96 h time periods, the results revealed that the photosynthetic system in these cyanobacteria could be affected by shorter time periods. The overlapping absorption spectra represented that the photosystem yield (especially Photosystem II and phycobilisome) significantly changed under 24-hour intervals. However, there were noticeable changes in fluorescence spectra and photosystem ratios in 24 h periods, which were particularly pronounced in combination with alkalinity (Abbasi et al., 2019). Accordingly, the present study aims to analyze the effects of time and environmental factors since pretreatments can change cyanobacteria acclimation and compatibility conditions. Nowadays, under 30-min pretreatments are often used to change the compatibility of bacteria such as Salmonella (Shigenobu et al., 2004). In the present study, the effect of pretreatment on growth behaviors of *Fischerella* sp. FS 18 and a model is considered as the first step in examining very less than 60 minutes times. The hypothesis is that 'pretreatments are recorded in the cyanobacterial memory system'. Hence, the results can be applied for changing growth behaviors and mass cultures (Amirlatifi et al., 2013, 2018; Fraser et al., 2013; Wang et al., 2011).

Materials and method

Fischerella sp. FS 18 was obtained from algal museum of Islamic Azad University of Gorgan Branch. The information on collection site and techniques was provided by Soltani et al. (2012). The identification and purity were determined using light and fluorescence microscopy based on some researchers (Soltani et al., 2009; Amirlatifi et al., 2018; Desikachary, 1959; Prescott, 1962; John et al., 2003). The species was cultured on BG0-11 solid and liquid media at 60 µmol photon.m^{-2}.s^{-1}, 28 °C, and pH 7.8 (Soltani et al., 2009). After initial growth, the isolated samples were placed under 2 µmol photon.m^{-2}.s^{-1} and at different concentrations (17, 80, and 160 mM NaCl). After 20, 40, and 60

minutes, *Fischerella* sp. FS 18 was removed from the media, centrifuged, and washed. The absorption spectra were analyzed in the visible range as an overlap by CECIL spectrophotometer model 740 CE. Extraction and full intact sample methods were used for analyzing in vivo overlap. Acetone, methanol, and ethanol were used as solvent, where the results of the primary absorption spectra confirmed the effectiveness of acetone. In order to increase the accuracy of the results, protein adsorption was measured and subtracted from total absorption, and the result was divided into protein adsorption (Tang and Wincent, 1999). The growth rate and attenuation time were measured based on Poza-Carrion et al. (2001) and Swapnil et al. (2018) by turbidity meter. Following Amirlatifi et al. (2018) and Abbasi et al. (2019), the statistical analysis was performed using SPSS (Version 11) for standard data based on Poza-Carrion et al. (2001) and RSP (Version 10) (Ghobadian et al., 2015).

Results

The absorption rates were compared at different times and salinity of 80 mM (Fig. 1). The short-term salinity (20 minutes) and medium-term salinity (40 minutes) in the photosystem II, phycobilomes, and light-collecting antennas had a stimulating effect on long-term salinity (60 minutes). No significant difference was observed the interval between 20 and 40 minutes after inoculation. However, the state of the photosynthetic system was modified 40 minutes after inoculation in 80 mM salinity. In other words, cyanobacteria are sensitive to the combination of salinity and time and alter their state of photosynthesis system 40-60 minutes after the effect of salinity. It is noteworthy that chlorophyll uptake was not abnormal in cyanobacteria at the range of 660-670 nm (Cai et al., 2015).

In addition, the 160 mM salinity increasing during two times makes the situation quite different mainly at 20 and 60 minutes after inoculation (Fig. 2). It seems that 160 mM

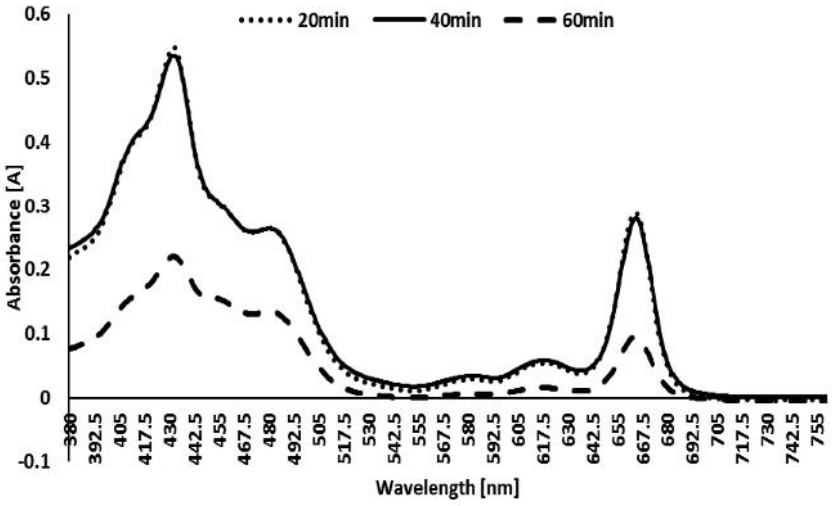

Fig. 1. Comparative study of absorption rate in acetone extract at 80 mM salinity and different times after inoculation

in a short time (20 min) can prohibit all parts of the photosynthetic system, particularly when phycobilisome is lost. Furthermore, increasing the time up to 40 minutes after inoculation can restore all parts of the photosynthetic system. After this period, cyanobacteria are able to rearrange and activate the photosystem II, phycobilisome, and light-collecting complex. However, increasing the time up to 60 minutes has a negative effect on carotenoid and a positive effect on the red area of chlorophyll. Phycobilisome and photo-systems are generally less sensitive to time fluctuations.

Thus, the photosynthetic system in the cyanobacteria was affected by the combination of time and salinity. Thus, changes depending on both variables (particularly 20 and 60 min time intervals) were affected by 80 and 160 mM salinity. The 40-minute time requires some of these two effects and brings the patterns of behavior closer together.

Table 1 shows the state of the progressive phase of growth under pre-treatment conditions. As can be seen, 60 min pretreatment at 160 mM salinity increases the production of live material by approximately 10%, which is a significant practical achievement. In addition, this property is affected by time before it is caused by salinity. When the pretreatment time decreases from 60 to 20 minutes, a decrease occurs in the pretreatment in the progressive phase of growth (Table 1). The pretreatment condition is similar to 80 mM salinity. It seems that increasing time could increase the progressive phase and the reproductive rate. The effect of time is significant at high salinity pretreatment. When the time decreases from 60 to 20 minutes, the effect is completely reversed and the highest intensity of the progressive phase reduces to the lowest level.

To study the effect of different salinity concentrations, the impact of 17 mM NaCl on growth rate was investigated and 24 (short term) and 96 hours (long term) were determined. The comparison of pre- and non-pretreatment samples is presented at two 24-hour times (Table 1) and at 96 hours (Table 2), respectively.

Table 1. The effect of different short-term pretreatments on growth rate and attenuation time after 24 hour (The number in brackets represents treatment for minutes)

Salinity (mM)	Time (min)	Growth rate (μ)	Generation time (G) (Day)
17		0.11±0.01	6.27
80	20	0.31±0.09 (0.38)	2.22 (1.81)
160		0.08±0.02	8.62
17		0.11±0.01	6.27
80	60	0.04 ± 0.01	17
160		0.28±0.06 (0.31)	2.43 (2.22)

Table 2. The effect of different short-term pretreatments on growth rate and attenuation time after 96 hours (The number in brackets represents treatment for 40 minutes)

Salinity (mM)	Time (min)	Growth rate (μ)	Generation time (G) (Day)
17	20	0.14±0.02	4.93
80		0.14 ±0.09 (0.24)	4.93 (2.87)
160		0.11±0.01	6.27
17	60	0.15±0.02	4.6
80		0.11 ± 0.01	6.27
160		0.16 ± 0.04 (0.22)	4.31 (3.14)

The results of the growth rates at two time periods (Figs 3 and 4) reveal that under non-saline condition, the growth rate is 0.11 after 24 hours then increases to 0.14 at 96 hours. Treatments Under salinity 80 mM, at 20 min for up to 24 hours revealed growth rate 0.31. Over 24 hours, growth rate reached to 0.14. Under 80 mM salinity, treatments at 60 minutes demonstrated growth rate of 0.04 at 24 hours and increased after 96 hours up to 0.11. Treatments exposed to 160 mM salinity for 20 minutes showed growth rate 0.08 after 24 hours and when the time increased to 96 hours changes to about 0.11. Under 160 mM salinity, treatments at 60 minutes growth rate was 0.28 up to 24 h and by time increasing to 96 hours was reduced to 0.16.

Based on RSP analyses (Figs. 5 and 6) and the layering pattern of distribution, time and salinity in combination affect the specific growth rate. The distribution pattern at 24 hours (Fig. 5) has a regular pattern and diversifies at 96-hour treatment (Fig.6). The transition from 20 to 30 minutes and 50 to 60 minutes at 96 hours causes a critical increase/decrease in growth rate and matter production (opposite of 24 hours). While the acclimation of *Fischerella* sp. FS 18 at 96 hours reduces the growth rate, the sensitivity to the time increases and exhibits more severe reactions to time variations. Finally, interstitial salinity levels are critical at short time periods and more effective.

Discussion

Several studies investigated the impact of salinity on cyanobacteria while neglecting short time effects (Bajwa et al., 2015; Shamim et al., 2017; Swapnil et al., 2018; Li et al., 2019). Although this hypothesis was not examined on cyanobacteria, it is suggested that the application of short-term pretreatment times can stimulate the development of new features in the cyanobacterial photosynthetic system (Affenzeller et al., 2009; Hamilton et al., 2018).

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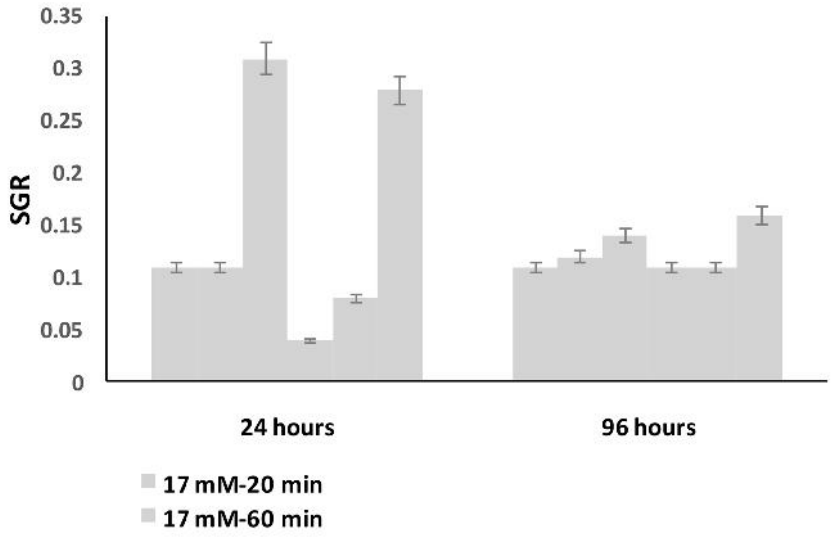

Fig. 3. Comparison of 17, 80, and 160 mM salinities on growth rate, at short-term pretreatments (20 and 60 min), after 24 and 96 hours inoculation.

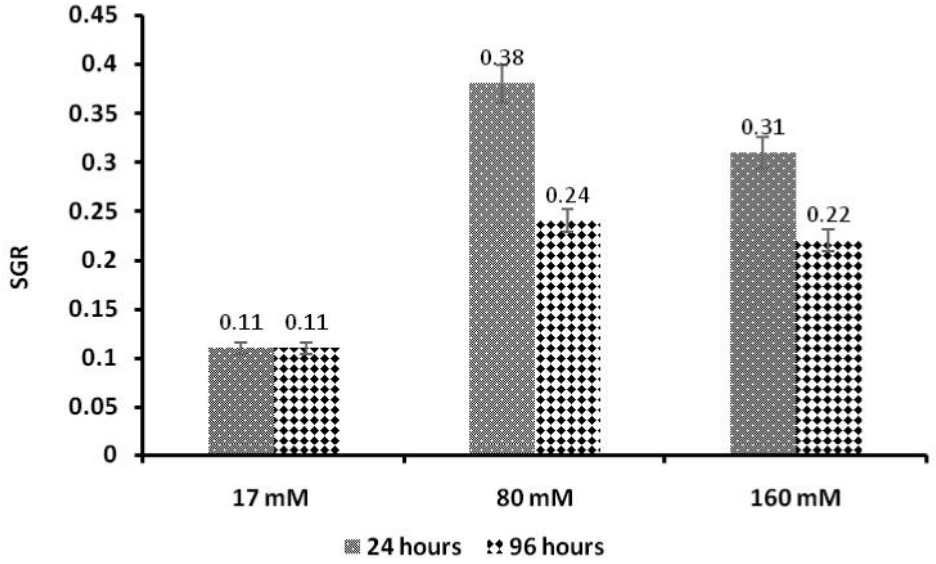

Fig. 4. Comparison the effect of 40 min pretreatments in 17, 80 and 160 mM salinities on growth rate, after 24 and 96 hours inoculation.

phycobilisome dynamics at 160 mM salinity indicated that (2x) affects the passage of time in phycobilisome and photosynthetic systems. Akulinkina et al. (2015) compared *Synechocystis* phycobilisome absorption rate at different times and found that the mutant had twice the amount of phycobilisome in comparison to the wild type. The effects of time duration were altered by increasing salinity. Furthermore, the yield was poor in the first 20 minutes, while the yield increased significantly after 20 minutes and up to 40 minutes in light-collecting antennas, phycobilisome, and photosystem II although it was unchanged at 60 minutes. However, the effect was diminished (for up to 40 minutes) in the light-collecting antennas and slightly increased in the red chlorophyll area. The phycobilisome was

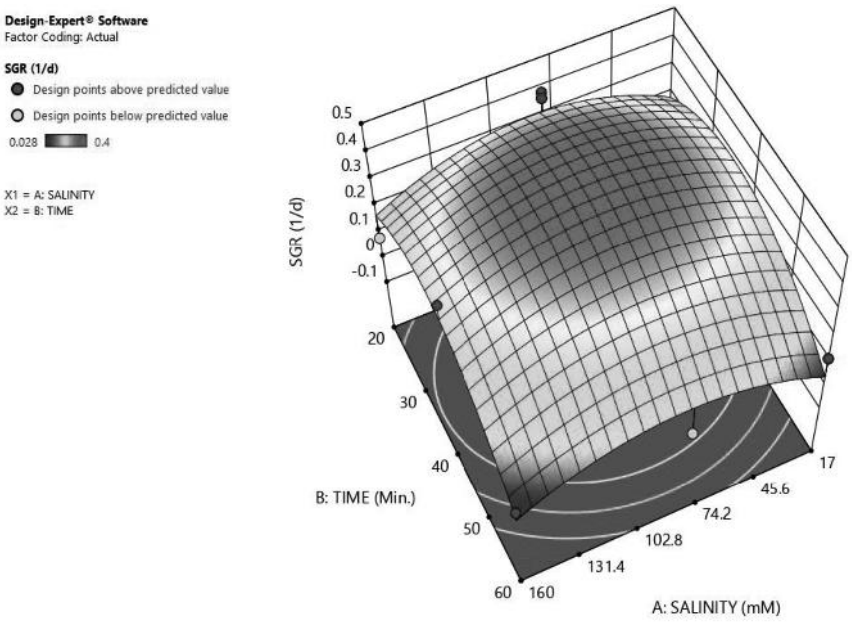

Fig. 5. RSP analysis of the growth rate at different pretreatment time and salinity after 24 hours.

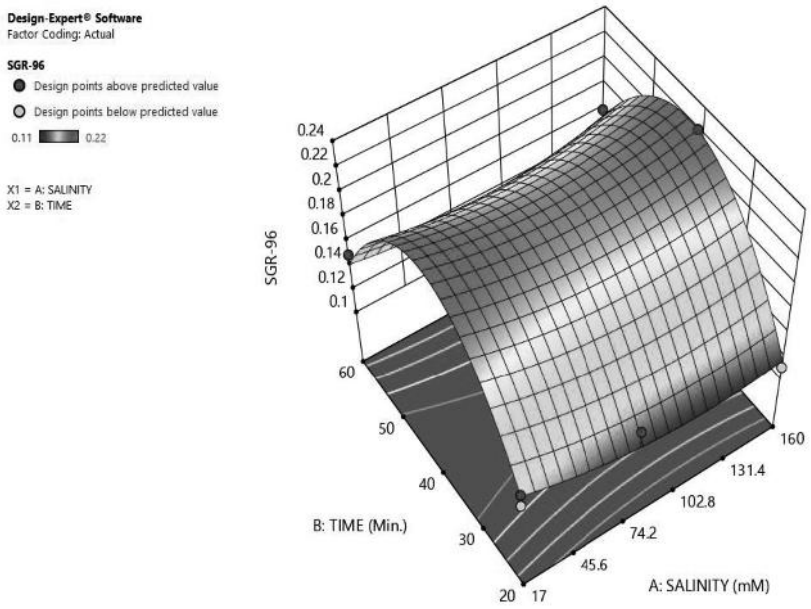

Fig. 6. RSP analysis of the growth rate in different pretreatment time and salinity after 96 hours in *Fischerella* sp. FS18.

similarly affected in 40 minutes and 60 minutes. Therefore, salinity increases at 20 minutes decreased while the yield decreased up to 40 minutes. The rearrangement is somewhat consistent in *Synechococcus* (Lefort-Tran et al., 1988). However, the phycobilisome part did not change by increasing 20 minutes to 60 minutes, and there was a slight change in the light collecting antennas in photosystem II. In general, phycobilisome, photosystem II,

and light-collecting antennas were damaged at 160 mM in the first 20 minutes. Further, photosynthesis happened by reducing the number of phycobilisomes per cell originating from reducing the level of photosynthetic thylakoids and a transformation in the internal structure of phycobilisome (Six et al., 2011). However, Six et al. (2011) studied the light problem and found that the effects on the light-collecting antennas decreased over the time and the damages were repaired. Furthermore, 160 mM salinity concentration was time dependent, especially in the first and second twenty-two minutes when the effect decreased while increased at the third. Based on the results, 40 minutes is the optimum time in 80 and 160 mM salinity concentration for photosynthesis efficacy. It seems that the behavior of cyanobacteria at salinity levels of 160 and 80 mM are different at 20 and 60 min. Further, 60 min photosynthesis is stimulated at 80, 20, and 160 mM salinity levels. The obtained results from the initial stages of the experiment were generally promising. According to Amirlatifi et al. (2018) and Abbasi et al. (2019), there is a strong reason for developing the practical use of this achievement in cyanobacteria and replacing the simple pretreatment method rather than biologics or at least in parallel should not be repeated. When less than one hour is applied compared with 24 and 96 hours, it leads to some changes in cyanobacterial photosynthesis system, which is time-, energy- and cost-effective.

The present study focused on answering the question whether changes are maintained over time or cyanobacteria was reacted based on 17 mM concentration. Further, acclimatization or adaptation was physiologically encountered. Based on the results, the destructive and stimulant effect of 160 mM for 20 and 60 min on photosynthesis system was maintained for up to 24 hours and then disappeared after 96 hours. However, the destructive effect maintained at 80 mM for 60 minutes to 24 hours did not continue after 96 hours. In fact, the stimulant effect of 80 mM for 20 minutes was maintained up to 24 hours not more than 96 hours.

Thus, *Fischerella* sp. FS 18 could modulate the destructive effect of the two salinity treatments (160 mM at 20 min and 80 mM at 60 min) after 24 hours. However, the growth rates up to 96 h were higher in the samples treated with 20 min salinity at 80 mM and 60 min salinity at 160 mM than 17 mM. In other words, the system changed its pattern over time although the initial pattern remained unaffected by time and salinity conditions until after 24 hours. Obviously, the results showed the effect of combined changes of salinity and short time on the photosynthetic system, which is consistent with those of Iranshahi et al. (2014) and Moisander et al. (2014). More specifically, some of these mechanisms were unknown to researchers (Shokravi, 2017). The findings of this study could be used in mass crop technology and applied phycology (Fraser et al., 2013). Therefore, cyanobacterial species can tolerate environmental changes and provide sustainable and economically viable biomass throughout the years (Bravo-Fritz et al., 2016; Pathak et al., 2018).

Acknowledgments

The authors would like to appreciate Professor Neda Soltani from Shahid Beheshti University, (Tehran) as well as Mr. Mohammad Aiineh and Ms. Malliheh Rasaie from Islamic Azad University (Gorgan) for their kind collaboration in theoretical and laboratory studies.

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