



Growth and photosynthesis acclimated response of the cyanobacterium *Fischerella* sp. FS 18 exposed to extreme conditions: alkaline pH, limited irradiance, and carbon dioxide concentration

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Abstract

The true-branching heterocystous cyanobacterium *Fischerella* sp. FS 18 is widely distributed in paddy fields (North) and petroleum polluted soils (South) in Iran. This investigation tested the hypothesis that the cyanobacterium can acclimatize under the combined effect of extreme environmental conditions. Here, we analysed the physiological response of the cyanobacterium under extremely limited irradiance ($2 \mu\text{mol photon m}^{-2} \text{s}^{-1}$); limited carbon dioxide concentration (no aeration) at alkaline pHs (9 and 11) for up to 96 h. When the cyanobacterium was exposed to these extreme conditions at pH 11, we observed a decline in growth, oxygen liberation, photosystems ratio, chlorophyll a, and phycobilisomes activity compared to pH 9 after 24 h. Besides, we registered a significant decrease in maximum photochemical efficiency and activity of photosystem II at pH 11. The comparative single-cell study revealed that pH 9 caused higher efficiency of photosystem II and I, while increasing alkalinity pH 11 led to disturbed phycobilisomes activity after 24 h. This strain was able to recover its structures after 96 h. In addition, spectroscopy analyses revealed the presence of the Mycosporine-like amino acid at pH 9.

Keywords Alkaline pH · Cyanobacteria · Extreme condition · *Fischerella* sp. FS 18 · Photosynthesis

Abbreviations

Chla	Chlorophyll a
CCM	Carbon dioxide concentrating mechanism
DIC	Dissolved inorganic carbon
PBS	Phycobilisome
PSI, PSII	Photosystems I and II
CLSM	Confocal laser scanning microscopy

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Introduction

Cyanobacteria are oxygenic photosynthetic microorganisms widely distributed across terrestrial environments and play a fundamental role in the soil biological cycle, oxygen production, and the Nitrogen cycle with tremendous applied capabilities (Mareš et al. 2014; Chittora et al. 2020; Munagamage et al. 2020). They can thrive with limited growth requirements, and efficiently use the available carbon dioxide, light, and inorganic nutrients, even under environmental stress conditions (Singh et al. 2016).

In paddy fields, under natural conditions, cyanobacteria are exposed to the combined influence of diverse factors, such as pH, irradiance, salinity, temperature, and dissolved inorganic carbon fluctuations, which vary both during the day and over the crop cycle (Chris et al. 2006; Bouazzara et al. 2020). Environmental stresses, such as low irradiance or shade (extremely limited irradiance), increase during rice ripening (Shokravi and Soltani 2011). Cyanobacteria can adapt through complex acclimatization strategies to variations in light intensity by modulating their photosynthetic apparatus and physiological activities, such as

dinitrogen-fixation and photosynthesis rate (Burns et al. 2005). Waterlogging limits carbon dioxide diffusion ability and causes high bicarbonate production in alkaline soils (Amirlatifi et al. 2018). Cyanobacteria for survival under stress conditions can induce a powerful Carbon-dioxide concentration mechanism (CCM) to actively concentrate sparse environmental C_i into the cell using photosynthesis (Müller et al. 1993; Mackenzie et al. 2004); this mechanism described in paddy-fields cyanobacteria in *Hapalosiphon* sp. (Shokravi and Soltani 2011), *Nostoc* sp. UAM 205 (Fernandez Valiente and Leganes 1990) and *Nostoc* sp. UAM 206 (Poza-Carrión et al. 2001).

Petroleum exploration and production lead to oil pollution in the environment and catastrophic damages to the soil (Ahmed and Fakhruddin 2018). Oil pollutions cause limited microorganisms' survival in the local ecosystems (Das and Chandran 2011). From a practical point of view, terrestrial cyanobacteria improve the soil structure by oxidizing oil components and adding organic matter (Issa et al. 2014). Therefore, due to the importance of agriculture and petroleum extraction in Iran, it is essential to evaluate the potential viability of diazotrophic cyanobacteria in response to extreme environmental fluctuations.

To characterize the acclimation and adaptation behaviors of cyanobacteria at extremely limited irradiance, carbon dioxide concentration and alkaline pH is of particular interest from several points of view. The solar irradiance is significantly reduced in both agricultural and oil-polluted areas, which urges the finding of a potent efficient photosynthesis system to adapt to limited-light conditions. Additionally, carbon dioxide limitation at pH fluctuations is a severe problem in Iranian soil (Amirlatifi et al. 2013; Abbasi et al. 2019). In the present study, among free-living cyanobacteria, we have selected one of the most abundant native cyanobacterium in agricultural and oil-polluted lands: *Fischerella* sp. FS 18. Besides, the strain has been well characterized at the morphological, taxonomical, ecological, and ecophysiological levels (Soltani et al. 2010). Here, we investigated whether the simultaneous environmental stress exposure could play a key role in adjusting and controlling the growth, biomass production, and photosynthesis of this strain, and consider its applicability to future large-scale cultivation and economic programs.

Material and methods

Cell culture and growth

Fischerella sp. FS 18 was obtained from the algae museum of the Institute of Sciences of Shahid Beheshti University, Tehran-Iran, and has been described previously in detail (Soltani et al. 2010). The true-branching, Nitrogen-fixing,

heterocystous cyanobacterium was initially isolated from an edaphic and epilithic of Khark Island (South of Iran) and Golestan paddy fields (North of Iran). The stock culture was grown in Nitrogen-free, BG-011 liquid medium buffered 10 mM BTP (Bis-Tris Propane) and adjusted to the desired pH (9 and 11) with KOH (Shokravi and Soltani 2011). The temperature and irradiance were maintained at 30 ± 2 °C and $2 \mu\text{mol photon m}^{-2} \text{s}^{-1}$, respectively. Cells were examined in the exponential phase when adapted to light regime and pHs. Measurements were performed at 24 and 96 h after inoculation.

Growth and spectral characteristics

The chlorophyll production, chlorophyll contents per cell, chlorophyll absorption peak, and PBS production were analyzed under limited irradiances ($2 \mu\text{mol photon m}^{-2} \text{s}^{-1}$) and limited carbon dioxide at different alkaline pHs (9 and 11) using spectral characteristics according to Fraser et al. 2013 and Abbasi et al. 2019. Growth rates (μ) were calculated according to Li et al. 2014 and Khazi et al. 2018.

Photosynthesis measurement

Oxygen evolution was measured using a Hansatech oxygen electrode. Cells cultured at temperature 30 ± 2 °C and in constant illumination of $2 \mu\text{mol photon m}^{-2} \text{s}^{-1}$. Photosynthesis-Irradiance (P-I) curves and parameters were calculated by measuring oxygen evaluation rates (Inoue-Kashino et al. 2005). The amount of liberated oxygen was normalized by chlorophyll according to Poza-Carrión et al. 2001 and Soltani et al. 2006.

Spectroscopic measurements

The in vivo absorbance spectra were measured from 230 to 760 nm using Synergy HTX (Multi-Mode Microplate Reader, USA). The absorbance spectra were normalized to biomass (OD 750) according to Tang and Vincent 1999. The operation of photosystems and phycobilisomes characteristics were measured and analyzed spectrofluorimetrically (including confocal spectra) according to Inoue-Kashino et al. 2005; Vermaas et al. 2008 and Zorz et al. 2015. PSI:PSII ratio analysis was done according to Gan et al. 2014, and Amirlatifi et al. 2018. Fluorescence emission spectra were recorded following Tiwari and Mohanty 1996 and Fraser et al. 2013. Emission and excitation spectra were recorded at λ_{ex} : 440 and 550 nm and λ_{em} 610–760 nm. The fluorescence intensity of single-cell was measured using λ scan of confocal laser microscope system (Leica TCS-SP5 CLSM -Leica Microsystems Heidelberg GmbH, Mannheim, Germany). CLSM enabled us to study different physiological processes, including the intensity

of fluorescence emitted (as spectral unmixing) from single in-vivo cyanobacteria cells (Grigoryeva and Chistyakova 2020). Photosynthetic pigment excitation was carried out with an argon laser at 405 nm. The fluorescence emission spectrum was collected by detecting wavelengths between 415 and 760 (Ramírez et al. 2011; Sugiura and Itoh 2012). Analysis of the lambda scan data was carried out using the Leica Confocal Software.

Statistical analysis

Data were analyzed using one-way Analysis of Variance (ANOVA) with the SPSS-24 155 software. The ANOVA showed a significant difference between treatments with $p < 0.05$. All the experiments were carried out in three replicates, and data are presented as mean values of three independent replicates.

Results

Spectral characteristics

Ln 750 (light scatter at 750 nm, optical density analysis) showed that alkaline conditions (pH 9 and 11) were favorable to growth which maintained exponential growth over 96 h (Table 1). The accumulation of chlorophyll (A680–A750) decreased significantly at pH 11 compared to pH 9, with a steady ratio of chlorophyll per cell over the first 96 h. In contrast, the accumulation of chlorophyll at pH 11 was slower; therefore, the chlorophyll per cell

Table 1 The effect of alkaline pHs (9 and 11) on spectral characteristics of *Fischerella* sp. FS 18 under different pHs and DIC limitation at irradiance $2 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ after 24 and 96 h from inoculation

	Time (hours)	pH 9	pH 11
Ln (A 750)	24	–2.22	–2.59
	96	–1.45	–1.79
Ln (A680–A750)	24	–3.34	–3.87
	96	–2.12	–2.79
(A680–A750)/A750	24	0.34	0.19
	96	0.33	0.22
Chlorophyll λ_{max} (nm)	24	676	680
	96	680	674
(A630–A750)/(A680–A750)	24	1.11	0.72
	96	1.13	0.78

In A750 to track optical scattering, Ln (A680–A750) to track chlorophyll content of the cultures, (A680–A750)/(A750) to track chlorophyll per cell, the wavelength for the chlorophyll absorbance peak, an optical measure of the accumulation of chlorophyll bound to IsiA and (A630–A750)/(A680–A750) to track phycobilisome absorbance normalized to chlorophyll absorbance. Values are means of three independent biological replicates \pm standard deviation

and chlorophyll absorbance peak was declining over the 96 h. We observed, under these conditions, chlorophyll absorbance peak and chlorophyll-binding proteins shifted to shorter wavelengths and PSI, respectively. In addition, the content of phycobilisome pigment increased about 20% after 24 h and 10% after 96 h at pH 9 in contrast to pH 11. Collectively, *Fischerella* sp. FS 18 was able to survive and grow at extreme alkaline conditions (pH 11), although we observed a significant decrease in growth, chlorophyll content, and chlorophyll content per cell (Table 1).

Photosynthesis

The results of photosynthesis activity of *Fischerella* sp. FS 18—to obtain information about the activity of photosynthetic and respiratory electron transport chains—showed that oxygen liberation was higher at pH 9 compared to pH 11, in particular after 96 h (Table 2). The amount of liberation significantly decreased at pH 11 over 24 h. While the cyanobacterium could recover the inhibitory effect of photosynthesis ability about 40% after 96 h. Photosynthesis-Irradiance (P-I) curves and characteristics at different alkalinities and times were summarized in Table 3. Maximum light-saturated photosynthesis activity (Pmax) and the quantum efficiency of photosynthesis (α) significantly increased at pH 9, while further increasing the pH resulted in a reduction of both parameters (Table 3). A decline in the quantum efficiency of photosynthesis led to the strain acclimating to limited light intensity under extremely DIC limitation at pH 11. Extreme alkaline pH (pH 11) increased the saturating irradiance (I_k) in contrast to pH 9 at both time points (24 and 96 h). These results confirm that pH 11 led to decreased photosynthesis activity, oxygen liberation, and the capability to acclimatize with limited light conditions, while energy consumption was increased (results not shown). Interestingly, we observed that *Fischerella* sp. FS 18 was not susceptible to photoinhibition (up to $1000 \mu\text{mol photon m}^{-2} \text{s}^{-1}$) at pH 11 compared to pH 9 after 24 h (result not shown).

Table 2 The effect of alkaline pHs (9 and 11) on photosynthetic oxygen liberation ($\text{nmol O}_2 \text{ mg chl}^{-1} \text{h}^{-1}$) of *Fischerella* sp. FS 18 under DIC limitation at irradiance $2 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ after 24 and 96 h

pH	Time (hours)	Photosynthesis
9	24	214.74 ± 15.07
11	24	148.38 ± 13.68
9	96	268.53 ± 13.68
11	96	168.12 ± 12.18

Values are means of three independent biological replicates \pm standard deviation

Table 3 The effect of alkaline pHs (9 and 11) on Photosynthetic- Irradiance curve parameters of *Fischerella* sp. FS 18 under DIC limitation at irradiance of $2 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ after 24 and 96 h

Time (hours)	pH	The maximum photosynthetic rate (Pmax) ($\mu\text{mol O}_2 \text{ mg chl}^{-1} \text{ h}^{-1}$)	Light harvesting efficiency (α) ($\mu\text{mol O}_2 \text{ mg chl}^{-1} \text{ h}^{-1}$)/ ($\mu\text{mol photon m}^{-2} \text{ s}^{-1}$)	Light saturation point (Ik) ($\mu\text{mquanta. m}^{-2} \text{ s}^{-1}$)
96	9	268.53 ± 13.68	3.8 ± 0.23	87.24
	11	168.12 ± 12.18	1.8 ± 0.13	156.44

Values are means of three independent biological replicates \pm standard deviation

Table 4 The effect of alkaline pHs (9 and 11) on the photosystems ratio in *Fischerella* sp. FS 18 at $2 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ and DIC limitation

Time (hours)	pH	PSI:PSII
24	9	1.42 ± 0.14
96	9	1.88 ± 0.28
24	11	0.88 ± 0.17
96	11	1.15 ± 0.16

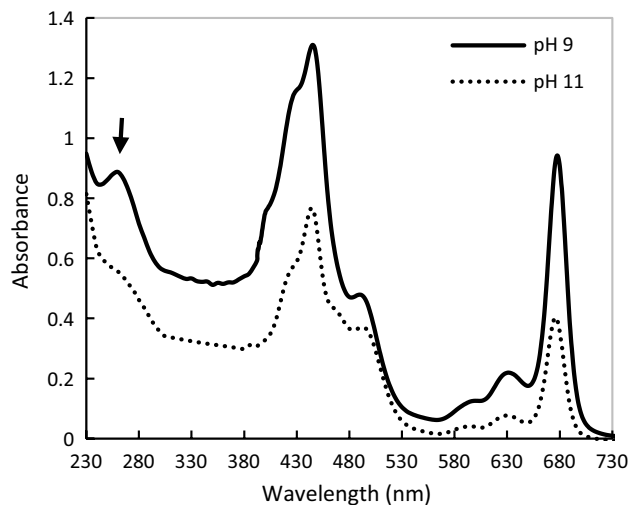
Values are means of three independent biological replicates \pm standard deviation

PSI:PSII ratio

The high PSI:PSII ratio inherent to cyanobacteria helps maximize electron transport away from PSII as there are multiple PSI complexes to carry electrons away from the plastoquinone pool for each PSII (Fraser et al. 2013). Extreme alkaline conditions pH 11 caused the PSI:PSII ratio declined sharply compared to pH 9 after 24 h (Table 4). So, in these extreme conditions, *Fischerella* sp. had multiple PSI for every PSII in the cells which led to better energy transfer (spillover) after 96 h at pH 11.

Absorption spectra

Comparison of the in-vivo absorption spectra revealed that in both alkaline conditions the main peaks corresponded to $\sim 265 \text{ nm}$ (Mycosporine-like amino acids (MAAs)), $\sim 445 \text{ nm}$ (chlorophyll blue), $\sim 490 \text{ nm}$ (carotenoids), $\sim 630 \text{ nm}$ (phycocyanin), $\sim 680 \text{ nm}$ (chlorophyll red) (Fig. 1). Our results revealed that the UV absorbing, the PS II, and phycobilisomes pigments significantly increase in all parts of the photosynthesis apparatus at pH 9 in comparison to pH 11, after 96 h. The pigment contents decreased at pH 11 and the shift of the red chlorophyll ($\sim 680 \text{ nm}$) peak region was significant. The stability of phycocyanin ($\sim 630 \text{ nm}$) and chlorophyll (related to PSII) significantly decreased at pH 11 after 24 h, although they maintained their structure at 96 h. Noticeably, we found UV-absorbing pigment, Mycosporine-like amino acids (MAAs) under combined extreme DIC and light intensity limitations at pH 9. MAAs play the principal role as natural sunscreens to protect their producers against harmful solar irradiation and

**Fig. 1** The effect of different alkaline pHs and DIC limitation at $2 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ on the in vivo absorbance of *Fischerella* sp. FS 18 normalized to optical density (OD 750) after 96 h of inoculation. Arrow shows the distribution of Mycosporine-like amino acids (MAAs)

other stress factors, cellular antioxidants, and osmoprotectants (Katoch et al. 2016; Hu et al. 2018).

Spectrofluorimetry

We study the energy distribution between phycobiliproteins and photosystem II under varying environmental conditions by excitation of 550 nm 440 nm , respectively (Fig. 2). The fluorescence emission analysis showed that the PBS activity significantly increased at pH 9 compared to pH 11 (Fig. 2). While it is considerable that variation in the level of the PBS activity at pH 11 depends on pigment composition which led to decrease their activity. Similar trend was observed for PSII operation (Ex: 440 nm). Chl fluorescence presented the efficiency and effectiveness of PSII activity increased at pH 9 in contrast to pH 11 (Fig. 2).

Spectroscopic study of single cell by CLSM

Investigation of single-cell reveals maximum fluorescent of pigment-protein which enables us to monitor the

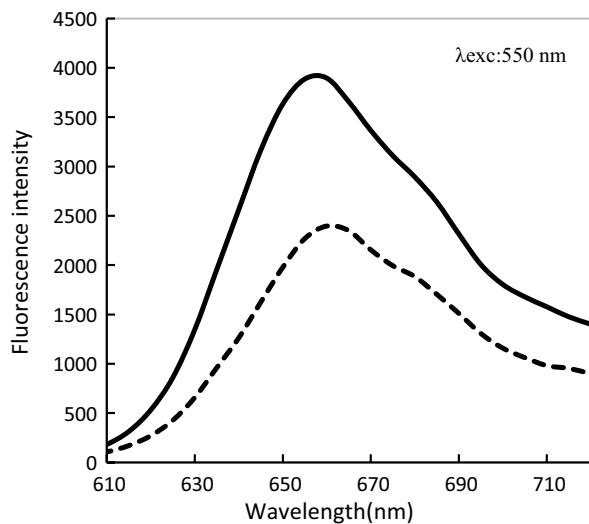
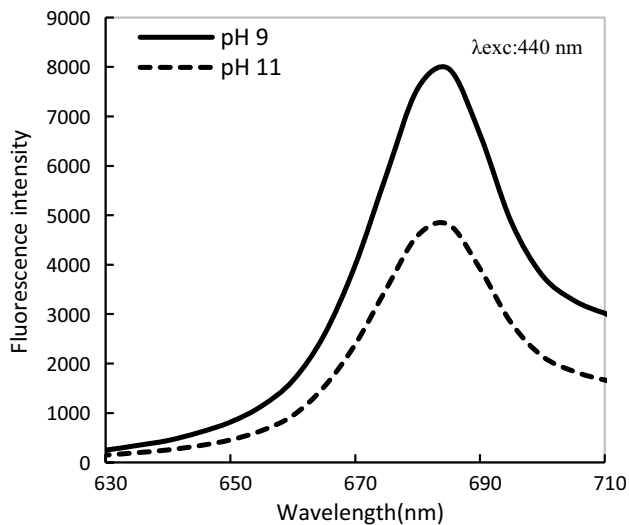


Fig. 2 Comparison of the fluorescence intensity of *Fischerella* sp. FS 18 at different pHs and DIC limitation at irradiance of $2 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ after 96 h

dynamic processes of the chosen cells as spectral unmixing and all steps of the energy transfer chain (Grigoryeva and Chistyakova 2020) (Fig. 3). In addition, study of the physiological state of single-cell illustrated the viability of the whole culture under these extreme conditions. The lambda scan results confirmed the growth, *in-vivo* absorption spectra, and spectrofluorimetry analysis (Tables 2, 3 and Figs. 1 & 2). In the single-cell study of *Fischerella* sp. FS 18 observed photosystems and PBS activity were significantly higher at pH 9 compared to pH 11. Increasing pH (pH 11) led to a significant decline in PSI and PSII activity and demolished their structure after 24 h. While by passing time (96 h) cyanobacterium recovered their structure and increased photosystems activity.

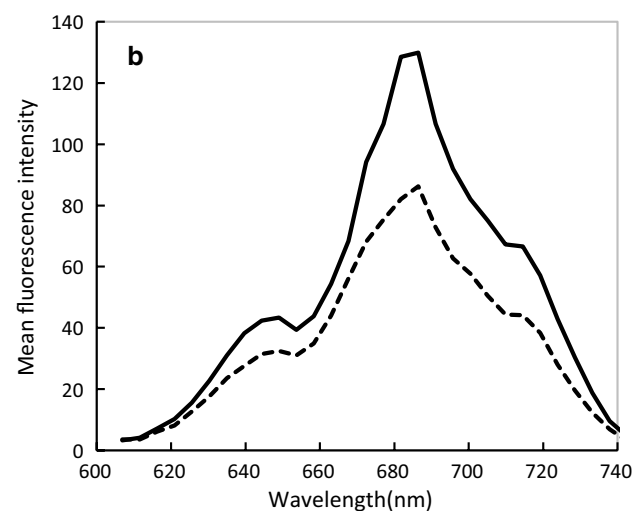
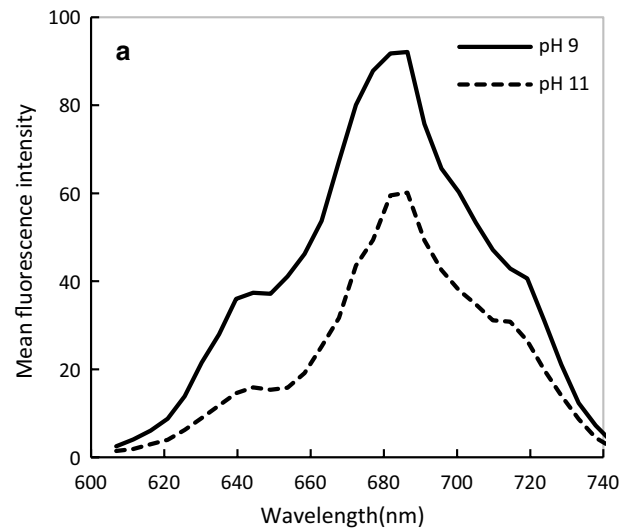


Fig. 3 Fluorescence spectra of individual cell (Lambda scan) of *Fischerella* sp. FS 18 under different alkaline pHs and DIC limitation at irradiance $2 \mu\text{mol photon m}^{-2} \text{s}^{-1}$. $\lambda_{\text{exc}}=405 \text{ nm}$. **a** after 24 h; **b** after 96 h of inoculation

Discussion

Our hypothesis revealed that combine multiple stress factors are affecting the acclimatization of the *Fischerella* sp. FS 18. The previous study of *Fischerella* sp. FS 18 shown that regardless of irradiance, the maximum biomass production and growth rate improves at pH 9 and relatively DIC limitation (Soltani et al. 2006, 2010). Our studies indicate that the interaction between alkalinity, DIC, and irradiance limitation in *Fischerella* sp. FS 18 led to resistance to the wide range of extreme conditions-to survival and growth- due to remaining active in photosynthetic apparatus. This result is in agreement with Alcorta et al. 2019 who reported that *Fischerella thermalis* could adapt to various environmental conditions,

with its photosynthetic apparatus remaining active in a temperature range from 15 to 58 °C.

Increasing pH (11) caused a decline in growth rate and biomass production (dry weight and chlorophyll-results not shown) compared to pH 9 after 24 h. In contrast, growth and biomass production increased at pH 11, when incubation time is extended to 96 h. Time is an essential factor in the resistance and growth in harsh conditions (Alcorta et al. 2019). Zhao and Brand 1989 observed that *Fischerella thermalis* cultures subjected to temperatures between 60 and 65 °C for a few minutes decreased the absorption of their phycobilisomes while chlorophyll and carotenoids were not affected. Radway et al. 1992 found that phycobilisomes and chlorophyll of *Fischerella thermalis* reduced by 20% when cultures are exposed for at least 1 h to temperatures between 60 and 65 °C (Alcorta et al. 2019). However, it should be noted that growth rate and biomass can differ depending on the environmental conditions. Ahmed et al. 2021 found that exposure *Fischerella* sp. strain HKAR-14 to UVR caused a decline in growth and Chl a, while total carotene content increased after 60 and 72 h, while a slight increase up to 72 h.

The carbon dioxide concentration mechanism (CCM) is the critical process that enables cyanobacteria to adapt to alkaline conditions (Klanchui et al. 2017). The operation of CCM requires high amount of energy and nutritional resources (Giordano et al. 2005) and naturally need a high operation of photosynthesis (Raven et al. 2014; Mangan and Brenner 2014). Besides, Beardall (1991) reported that in most autotrophs photosynthesis, cells exhibit a lower affinity for CO₂ when grown at limited light as the down-regulation of CCM. Regarding the highest growth, chlorophyll production, and photosynthesis at pH 9, *Fischerella* sp. FS 18 has a powerful concentration mechanism to induce at these extreme conditions. As known, cyanobacteria can acclimate to different external DIC concentrations during growth intracellular accumulation of inorganic carbon occur against a concentration gradient and is activated and energized by light through light photosynthesis (Badger and Price 1992; Young and Beardall 2005). Mackenzie et al. 2004 reported that *Synechococcus elongatus* cells grown under bubbled air (approximately 370 mmol CO₂ mol) induced a high-affinity CCM, which maintained growth rates as high as cells grown with 50,000 mmol CO₂ mol 21 air. They hypothesized that the induced CCMs in low-Ci cells would constrain the rate and amplitude of light acclimation. There are no obvious mechanistic interpretations of the occurrence of CCMs algae adapted to low light (Raven et al. 2014).

Growth measuring reflects the balance between photosynthesis and respiration (Nygård and Dring 2008). The analysis of oxygen liberation (a marker of PSII activity) confirmed the growth results, which reached a maximum at combined extreme DIC and irradiance limitation at pH 9, compared to

pH 11. Previous analysis of *Fischerella* sp. FS 18 showed significant variability in the irradiance conditions (Soltani et al. 2006). We observed increasing alkalinity (pH 11) causes a decrease in maximum photosynthesis, in contrast to higher quantum yield and shade-adapted capacity after 24 h at pH 9. The low P_{max} can be attributed to the low efficiency of water oxidation in PSII under extremes DIC and irradiance limitation conditions. Briand et al. 2004 concluded that I_k is the most reliable parameter for assessing and comparing the variable-light requirement in ten strains of *Cylindrospermum raciborskii*, ranging from 15 to 26 mmol photons $m^{-2} s^{-1}$. The high I_k value of *Fischerella* sp. FS 18 can be attributed to the extreme limitation conditions used, implying an increase in energy for growth. Inoue-Kashino et al. 2005 reported in *Synechococcus* 6803 that the increase of α value usually depends on the increase in the relative content of PS II and the antenna size of PS II, which is in agreement with our findings. *Fischerella* sp. FS 18 is not resistant to photoinhibition at pH 11, which depends on DIC limitation. Prior studies using the same strain observed that acclimation to non-limited and relatively limited Carbon dioxide concentration causes resistance to photoinhibition (Soltani et al. 2006, 2010).

PSI:PSII ratio of cyanobacteria has shown PSI rate increases under stress conditions (Inoue-Kashino et al. 2005; Amirlatif et al. 2018). We observe that the stability of the PSII:PSI ratio decreased at pH 11. Furthermore, the energy transfer was higher at pH 9, which led to increased photosystem operation and wasted energy (Fv'/Fm' and NPQ results not shown). Singh et al. 2016 reported that photosynthetic activity and whole chain electron transport of *Nostoc calicicola* increased in the presence of 500 mM NaCl compared to control inhibition of PS II activity, could be due to loss of D1 protein, the interaction of salt with the oxygen-evolving complex, electron carriers as well as reducing sites of PS II and the reaction center itself.

The dynamism of the photosystems and phycobilisomes (as the main parts of energy and matter production) (Watanabe et al. 2014) enhances under extreme irradiances and DIC limitation after 24 h at pH 9 compared to pH 11, and is associated with a more stable structure and better coupling energy by PBS. In other words, cyanobacterium in response to irradiance and DIC limitation within 96 h at the alkaline condition change phycobilisomes conformation to increase strength and flexibility. When the cyanobacterium is exposed to pH 11 for 24 h, PBS conformation gradually changes and decreases their activity by destroying the rode part. These results are in agreement with Alcorta et al. 2019 who reported that the function and structure of protein complexes involved in the photosynthetic process of *Fischerella thermalis* showed high stability and tendency in temperatures range of ~45–55 °C.

In cyanobacteria, phycobilisome antenna conform a flexible system that can deal with a wide range of environmental conditions to provide the optimal energy flux to photosystems activities (Adir et al. 2020). Whereas Kumar et al. 2020 reported that low light intensity and variable photoperiods play enhancing role in PC content in heterocystous cyanobacteria, they found that *Fischerella* sp. HKAR-5 showed a decline in PC content after exposure to UV for 24 h against the control. The single-cell study revealed that the maximum activities of photosystems occur at pH 9 within 96 h compared to pH 11. In parallel, PS activity significantly decreased at pH 11 which was time-dependent. Moreover, the single-cell study confirmed that exposure at pH 11 for 24 h led to a noticeable decrease in all components of the phycobilisome, chlorophyll (and protein) production, and PSII activity, but without loss of its structure.

How MAA production occurs in cyanobacteria is still not answered (Hu et al. 2015 & 2018). Historically, the production of MAAs has related to a photo-protective strategy against the harmful effects of solar UV irradiation and other stress factors (Hu et al. 2018). Katoch et al. 2016 reported that the success of cyanobacteria life—photosynthetic microbes—which have persisted on Earth from the Precambrian Era might depend on the ubiquity and diversity of MAAs. It is interesting that spectroscopy analyses of *Fischerella* sp. FS 18 reveals the occurrence of MAAs under extreme DIC and irradiance limited condition at pH 9 after 24 h. Ahmed et al. 2021 observed that induction of MAA synthesis of *Fischerella* sp. strain HKAR-14 is time-dependent. With increasing exposure to PAB (up to 72 h), the Mycosporine-like amino acid content in the cells increased compared to the control.

These results suggest *Fischerella* sp. FS 18 has a strong defense mechanism specifically for biomass production, chlorophyll content, phycobilisome activity, and photosystems operation that is able to survive and grow under multiple stress conditions. The combination of 2 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$, extremely limited DIC, and alkaline pHs created a synergy that favored the viability of the strain in comparison to single individual effects. Therefore, here we tried to combine multiple factors under laboratory conditions to better simulate what happens in natural ecosystems. Interestingly, this strain was rich in pigment MAAs under these conditions. From an applied point of view, the present data reveal that the strain could significantly contribute to agricultural and oil-polluted land as multi-purpose biotechnological applications.

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