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RESEARCH ARTICLE

Physiological and biochemical modulations in the thermophilic cyanobacterium *Westiellopsis* sp. TPR-29 under high sulfur supplementations

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Abstract

Cyanobacteria demonstrate versatile utilization of inorganic sulfur sources, contributing to the global sulfur cycle. Their resilience is evident in adapting to diverse sulfur bioavailability in different ecosystems. However, the impact of high sulfur concentrations on cyanobacterial physiology is a concern. This study focused on *Westiellopsis* sp. TPR-29, a heterocytous branched cyanobacterium inhabiting a sulfur-rich hot spring. The cyanobacterium was exposed to different sulfate concentrations (50 mM, 500 mM, and 650 mM), along with a control group (0.3 mM). After a 7-day incubation period, physiological and biochemical assessments were conducted. The results showed decreased cyanobacterial growth in the presence of 500 mM and 650 mM sulfur concentrations, while 50 mM sulfate stimulated growth. Similar trends were observed for photosynthetic efficiency, protein, and carbohydrate content. Moreover, elevated levels of H2O2 and MDA at 500 mM and 650 mM indicated oxidative stress in cyanobacterial cells. These findings suggest that high sulfur concentrations, particularly 500 mM and 650 mM, are toxic to cyanobacteria, impairing their physiological processes and leading to reduced growth. Conversely, 50 mM sulfur had a positive effect on cyanobacterial physiology, enhancing growth. This study highlights the detrimental effects of high sulfur concentrations on cyanobacteria and provides insights into the elaborate mechanisms of stress tolerance in these prokaryotic photoautotrophs

Keywords: Cyanobacteria; Sulfur; Thermophile; Hotspring; Oxidative stress.

1. Introduction

Being a pivotal component in cellular and redox metabolites, the importance of sulfur in life processes is unquestionable (Giordano et al., 2005). Most of the organisms acquire sulfur from surrounding as sulfate, however, due to periodic change in the level of sulfate, modulations in the mechanisms of sulfur acquisitions are observed in different life forms. In today's scenario marine ecosystem have a concentration of 28 mM of sulfate (Ksionzek et al., 2016) whereas the fresh water ecosystem sustains only 10 – 50 µM sulfate (Bochenek et al., 2013), conferring the former as the major reservoir of sulfur. In spite of the varying concentration of sulfur in different habitat, the organic form of sulfur is predominant in the environment. However, presence of organic sulfur compounds do not display any significant impact on living biota, as they generally utilize thiosulfates, cysteine, methionine, and glutathione to attend the cellular demand along with sulfate, the major source of sulfur (Fernandez-Gonzalez et al., 2019).

Sulfur acquisition, assimilation, reduction into cysteine, and catabolism into sulfur secondary metabolites are the sequential processes which was catalysed by specific enzymes. The metabolism of sulfur and its homeostasis at cellular level requires the regulation of transporters and enzymes which were involved in the assimilatory process and their transcriptional regulation through various regulatory proteins. For example, in *Escherichia coli*, *Salmonella typhimurium*, and *Synechococcus,* there are some Lys R type transcriptional regulators that control the expression of the Cys operon named Cys B, Cys M, and Cys R respectively. Likewise, SAC1/SAC3 are involved in green algae *Chlamydomonas reinhardtii* and SLIM1 in *Arabidopsis thaliana*. Studies reviewed that the assimilation and regulation of sulfur and their roles along with this how this process impact other cellular processes either directly or indirectly in cyanobacteria. In cyanobacteria, cysteine synthesis is a major regulator of various reactions. So far, many studies are going on sulfur metabolism of photoautotrophs out of which maximum studies were on physiology, biochemistry, and transcript analysis. Physiological studies and cyanobacterial gene expression databases have showed that the genes and enzymes that were involved in sulfate assimilation are also expressed in heterotrophic organisms along with photosynthetic autotrophic organisms (Zhang et al., 2008; Ludwig and Bryant, 2012; Kolesinski et al., 2017; Kumaresan et al., 2017; Hughes et al., 2018; Kharwar et al., 2021). By using DNA array technique, the transcriptomic analysis of *Synechocystis* have been performed (Zhang et al., 2008). Similar kind of works have also been done in *Arthrospira platensis* (Kumaresan et al., 2017). Limitation of sulfur affects the ultrastructure, cell growth and differentiation, survival, photopigments, protein profile as well as nitrogen, carbon, and sulfolipid metabolism. These changes cause several sulfur stress related events including photosynthetic damage, degradation of pigments, inactivation of proteins/enzymes, reduced protein synthesis, changes in redox homeostasis, and signal transduction. On the other hand, the stress induced upon higher sulfur concentrations are less studied and the responses of the organisms are yet to be elucidated.

High sulfur concentration which can modulate growth and other physiology of the organisms are found in the most thermal springs. To understand such physiological alterations endorsed by high sulfur concentration, a thermophilic filamentous-branched cyanobacterium strain *Westiellopsis* sp. TPR-29, a natural isolate of Tatapani sulfur-lake has been selected for this study because of its high sulfur tolerance ability. Here, we performed sequential analysis of physiological and biochemical modulation in the cyanobacterium under high sulfur concentration. This study will aid to our understanding on how these organisms tolerate high sulfur by modulating the fundamental metabolisms.

2. Materials and methods

2.1. Organism and growth condition

The cyanobacterium, *Westiellopsis* sp. TPR-29, used in the present study was isolated from Tatapani Lake, Chhattisgarh. This cyanobacterial strain is thermophilic and filamentous with true branching. The strain was axenically cultured in the 250 mL flask containing BG-11 medium (pH 7.4) under fluorescent cool light (50–55 µmol m^{-2} s^{-1}) at 28 ± 2 °C in a photoperiod of 12/12 h light/dark cycle. The culture was purified and maintained in the laboratory of Prof. S.S. Singh, Department of Botany, Banaras Hindu University, Varanasi, India.

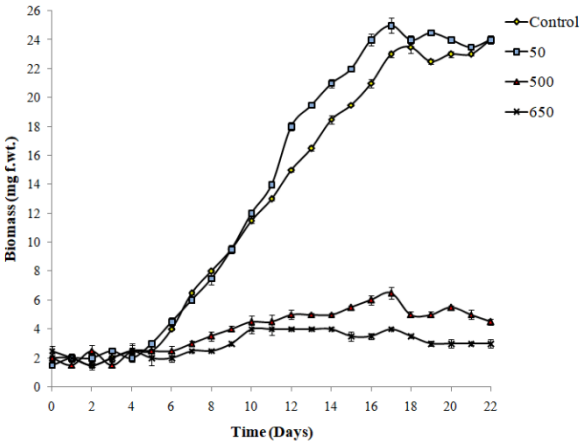

Figure 1. Growth behaviour (fresh weight biomass in mg) of *Westiellopsis* sp. TPR-29 at different sulfate concentrations

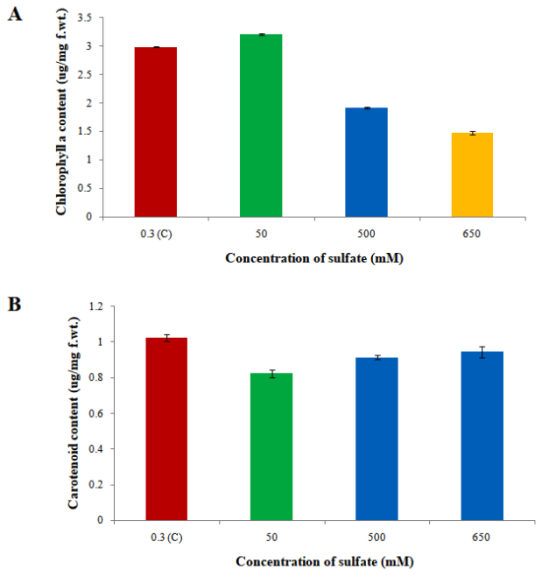

Figure 2. Chlorophyll (A) and carotenoid (B) content at different concentrations of sulfate supplementations.

2.2. Experiment design

For determining the toxic effects of high supplementation of sulfate concentrations on physiology of cyanobacterium *Westiellopsis* sp. TPR-29, the graded sulfate treatments with higher concentrations of $MgSO_4$, prime sulfate source in BG-11 medium (Kharwar and Mishra, 2020), were used. Initially, the viability of cyanobacterium was checked on a wide range of $MgSO_4$ concentrations [0.3 (C), 0.5, 1, 10, 25, 50, 100, 150, 250, 500, 650, 750, 1000, 1500 and 2000 mM] by adding requisite quantity to the stock solution. Later, on the basis of growth pattern observed for 22 days on each individual treatment the lethal concentrations i.e., 1000, 1500 and 2000 mM were eliminated along with lower doses i.e., 0.5, 1, 10 and 25 mM $MgSO_4$ which apparently displayed no visible alterations in the growth pattern. Concentrations like 100 and 150 mM $MgSO_4$ were also removed since they appeared similar to 50 mM treatment, while 250 mM $MgSO_4$ treatment appeared similar to 500 mM was also disregarded. Finally, three treatment concentrations i.e., 50, 500 and 650 mM $MgSO_4$, in addition to the control were selected for further experiments. Exponentially grown *Westiellopsis* sp. TPR-29 was cultured in three sets of treatment having different sulfate concentrations along with basal BG11 media i.e., Control [Contain basal BG11 medium with 0.075 g/L (0.3mM) $MgSO_4.7H_2O$), T_1 (12.5 g/L (50 mM) $MgSO_4.7H_2O$), T_2 (125 g/L (500 mM) $MgSO_4.7H_2O$) and T_3 (162.5 g/L (650 mM) $MgSO_4.7H_2O$)]. All the physiological assessments were performed on 7th day that displayed initiation of log phase in control.

2.3. Growth estimation

Cyanobacterial growth under different sulfate regimes were analyzed by obtaining the fresh weight biomass of each culture until stationary phase was attained in the control. Each sample was harvested by centrifuging at 10,000 rpm for 10 minutes. The pellets were air dried on blotting paper until the entire water adherent to the culture biomass was removed. Further, the fresh weight of these pellets was taken and recorded in mg. The experiment was performed in triplicates.

2.4. Chlorophyll estimation

To determine the change in primary photosynthetic pigment content of *Westiellopsis* sp. TPR-29 in four different concentrations of sulfate ($MgSO_4$) i.e., Control(C), 50, 500 and 650 mM, chlorophyll 'a' estimation was done (Mackinney, 1941). For that, 1mL of cyanobacterial culture was harvested and centrifuged at 15,000 rpm for 7 minutes. The supernatant was thoroughly discarded and the pellet containing cyanobacterial cells was further treated with 1mL precooled methanol. Each of the samples was homogenized by vortexing at 2,000 rpm for 4 seconds. The samples were covered with aluminum foil and incubated at 40°C for 20 minutes. This allows chlorophyll to get extracted in methanol. The procedure was repeated till the cell become bluish purple in colour. The optical density of each of the samples was taken at 665 nm and 720 nm. The chlorophyll 'a' content per mL solution was determined by-

$$\text{Chlorophyll a } (\mu g/ml) = 12.9447\ (A_{665}-A_{720})$$

2.5. Extraction and estimation of accessory Pigments

Determination of carotenoid follows same protocol as for chlorophyll extraction (Mackinney, 1941). 1 mL of cyanobacterial culture was harvested and centrifuged at 15,000 rpm for 7 minutes. The pellet containing the cyanobacterial cells were further treated with 1 mL precooled methanol and homogenized by vortexing at 2,000 rpm for 4 seconds. The samples were covered with aluminium foil and incubated at 4°C for 20 minutes. This allows the carotenoid to get extracted in methanol. The procedure was repeated till the pellet become bluish purple. The optical density was determined at 470 nm, using methanol as blank. The concentration of carotenoid was calculated using the formula given below:

$$\text{Carotenoid } (\mu g/ml) = [1000\ (A_{470}-A_{720}) - 2.86\ (\text{Chlorophyll a in } \mu g/ml)]/221$$

The phycobiliproteins of the cyanobacterium *Westiellopsis* sp. TPR-29 was estimated as per the protocol of Moreno et al (1995). In this method, 1mL of cyanobacterial culture was harvested and centrifuged at 15,000 rpm for 7 minutes. The supernatant was thoroughly discarded and the pellet containing cyanobacterial cells was further treated with 1mL phosphate buffer followed by heat and cold shock treatment until the cell burst after that centrifuge the sample. The homogenized sample was further treated with 5% EDTA and 1% PMSF to avoid protein degradation. Finally, the optical density was recorded at 562, 615 and 652 nm. The phycocyanin (PC), phycoerythrin (PE) and allophycocyanin (APC) content were measured using the formula given below:

$$\text{PC } [\mu g/ml] = (A_{615}-0.474*A_{652})/5.34 \quad \text{APC } [\mu g/ml] = (A_{652}-0.208*A_{615})/5.09$$
$$\text{PE } [\mu g/ml] = (A_{562}-2.41*\text{PC}-0.849*\text{APC})/9.62$$

2.6. Photosynthetic Efficiency

Fluorescence of chlorophyll in each of the treatments and control were measured using a pulse- amplitude modulated (PAM) 2000 (Walz GmbH, Effeltrich, Germany) photometry. The

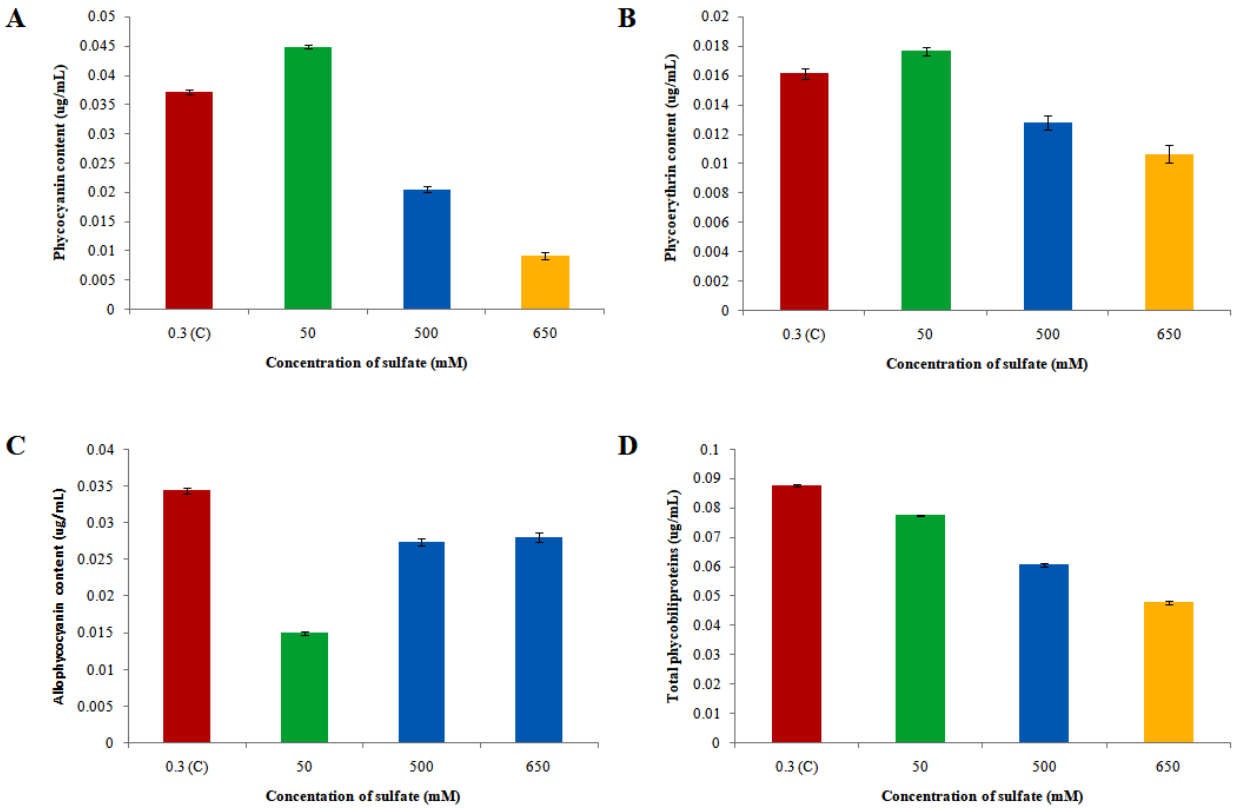

Figure 3. Changes in the phycobillins upon graded sulfate treatment. Phycocyanin (A), phycoerthrin (B), allophycocyanin (APC) and total phycobilliproteins (D) content at differential sulfate supplies.

cyanobacterial cell suspension was dark-adapted for 20 min before fluorescence measurements at room temperature (Demmig et al., 1987). Photosynthetic efficiency of cyanobacterium *Westiellopsis* sp. TPR-29 was further measured in terms of Fv/Fm and Y(NO). Fv/Fm represents the quantum yield of PSII and photochemical quenching with corresponds to photosynthesis, whereas, Y(NO) represents energy dissipation in the form of heat from PSII, which corresponds to the damages in PSII structure and inability to channelize energy to reduce $NADP^+$.

2.7. *Protein and carbohydrate content*

The estimation of protein content in cyanobacterium *Westiellopsis* sp. TPR-29 was done according to the method described by Lowry et al (1951). 1mL culture of *Westiellopsis* sp. TPR- 29 was homogenized in 0.5 mL 1N NaOH and incubated at 65°C for 10 minutes. After that, 2.5 mL of reagent C was added and further the mixture was incubated at room temperature for 15 minutes. Finally, 0.5 mL of Folin Reagent was added and again incubated at room temperature for 15 minutes. Optical density was then recorded at 650 nm against a blank made up of only reagent A, B and C with Folin reagent. Protein content was calculated using a standard curve of BSA (bovine serum albumin).

Carbohydrate estimation was done by Phenol- Sulfuric acid method (Dubois et al., 1951). In this method about 1mL *Westiellopsis* sp. TPR-29 culture was hydrolyzed in boiling water bath with 2.5 N H_2SO_4 for 3 hours. This treatment hydrolyze the total soluble compounds in to monomer like glucose, fructose etc. in the cell. Carbohydrate got extracted into the supernatant after prolong acid hydrolysis. 1mL of supernatant was then treated with 1 mL 5% Phenol and 5 mL (96%) H_2SO_4. The reaction mixture was shaken well and incubated in water bath for 10 minutes. Absorbance was recorded at 490 nm against a blank containing only distilled water, 5% phenol and 96% sulfuric acid. Carbohydrate content was calculated using a standard curve of glucose.

2.8. *Oxidative stress markers*

For estimation of H_2O_2 the protocol proposed by Velikova et al (2000) was used. 1 mL of cyanobacterial culture was taken and crushed in 0.1% TCA and further centrifuged at 12,000 rpm for 15 minutes. 200 µL supernatant was mixed with 10 mM Phosphate buffer (pH 7) and 1 M (400 µL) KI. The mixture was kept in dark for 30 minutes and then the absorbance was recorded at 390 nm. The H_2O_2 content was calculated using a standard curve of H_2O_2.

Estimation of MDA follows the method described by Heath and Packer (1968). According to this method 10 mL of cyanobacterial culture was homogenized in 10% TCA. The homogenate was centrifuged at 10,000 rpm for 20 minutes and then 2 mL of supernatant reacted with 0.6% 2-thiobarbituric acid. The absorbance was obtained at 532 and 600 nm respectively. The amount of MDA was calculated by the formula:

MDA (µg/mL) = $(A_{600}- A_{532}*1.75)/A_{600}$

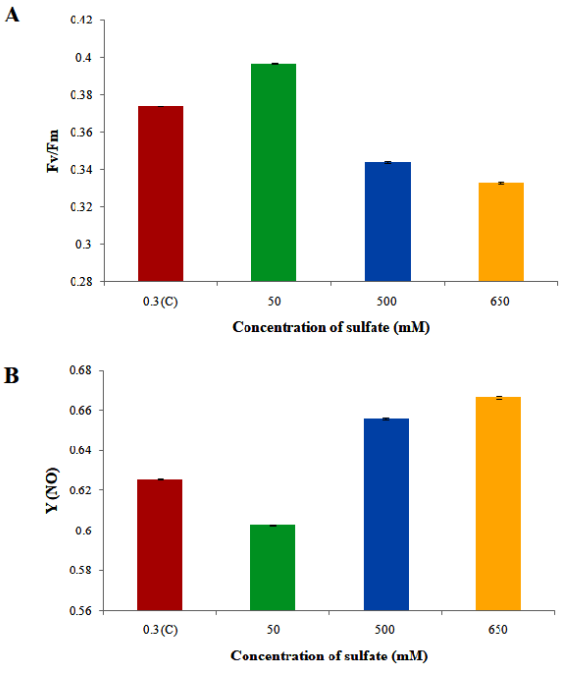

Figure 4. Alteration in photosynthetic efficacy at graded sulfate treatments. Fv/Fm (A) and Y (NO) (B) changed at different sulfate treatments.

2.9. Statistical analysis

Significance of difference among the treatments and control were determined by One-Way ANOVA ($p<0.05$) using both Tukey and Waller-Duncan post hoc test. The significantly different values for a particular parameter were denoted by colour codes in the graph. The results were represented as mean ± standard error. All the experiments were performed in triplicates. Statistical analysis was done using SAS university edition server.

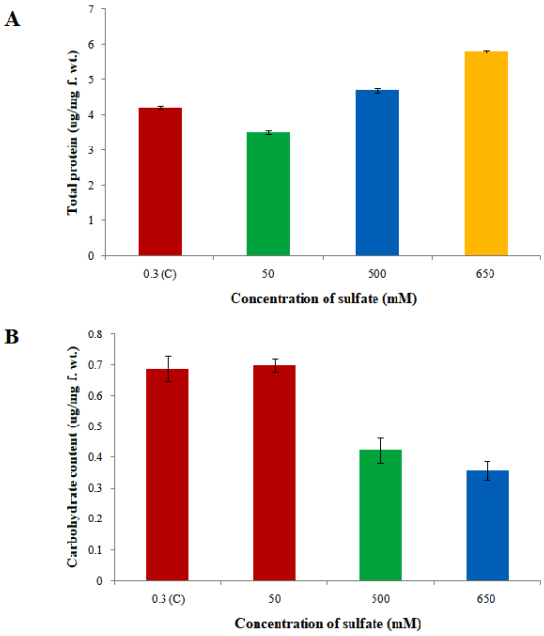

Figure 5. Changes in total cellular proteins (A) and carbohydrate (B) under different sulfate treatments.

3. Results

3.1. Analysis of growth, lethality and viability

The higher concentrations of sulfate i.e., 500 and 650 mM affected the growth of cyanobacterium negatively. It was observed that after an incubation of 7 days the growth of cyanobacterium was largely inhibited in these two concentrations. Nevertheless, the 50 mM concentration of exogenous sulfate supplementation was observed to be stimulatory after 10 days of incubation. In this concentration, the cyanobacterial growth was luxuriant with highest of 114% biomass increment with respect to control observed on 12th day. Viability of cells in these concentrations were also analyzed which showed lower survival at 650 mM followed by 500 mM sulfate concentrations. Whereas, highest viability was achieved by cells treated with 50 mM sulfate throughout its log phase with respect to control. Since, 7th day displayed the onset of exponential phase in the control therefore, all the experiments were performed on this day. On 7th day, the growth in 500 and 650 mM sulfate were reduced to 62.5 and 50% respectively, while it increased in 50 mM sulfate concentration by 112% than control. The growth curve for each treatment was depicted in Figure 1.

3.2. Effect of graded sulfate concentrations on chlorophyll content

After being treated with different concentrations of $MgSO_4$ (control, 50, 500 and 650 mM) cyanobacterium *Westiellopsis* sp. TPR-29 exhibited different content of chlorophyll in each treatment. It has been observed that, at 50 mM the chlorophyll a content was found to be 7.54% higher than the control. However, the percent reductions in the chlorophyll a were 35.81 and 50.49 % with respect to the control at 500 mM and 650 mM concentrations, respectively (Figure 2A). All the changes in chlorophyll content were observed to be significant ($p<0.05$).

3.3. Effect of graded sulfate on carotenoids

It has been observed that from all the applied concentrations of $MgSO_4$ there was a decrease in carotenoid content. The carotenoid content at 50, 500 and 650 mM were significantly ($p<0.05$) reduced by 19.49, 9.54, and 8.60% than the control, respectively (Figure 2B). While, all the reductions were significant with respect to control, the alterations in carotenoid at 500 and 650 mM were not significantly different respective to one another and hence, depicted by same colour code.

3.4. Effect of graded sulfate on phycobilliproteins

The phycocyanin content of test cyanobacterium was significantly increase by 20.88% in 50 mM sulfate supplementation. However, significant ($p<0.05$) rapid decrement of phycocyanin was observed in 500 and 650 Mm sulfate concentrations i.e., 44.67%, 75.07%, respectively, compared to the control (Figure 3A). The pattern of changes observed in phycoerythrin was similar to phycocyanin with significant ($p<0.05$) increment of 9.50% at 50 mM, while reduction of 20.65 and 33.78% were observed at 500 and 650 mM than control (Figure 3B). Conversely, allophycocyanin was found lowest in 50 mM with significant ($p<0.05$) 56.39% decrease, whereas, 20.36 and 18.35% of significant ($p<0.05$) reduction were observed at 500 and 650 mM exogenous sulfate supplementations respectively than control (Figure 3C). Interestingly, the total cellular phycobilins showed gradual decrease with increasing exogenous sulfates (Figure 3D).

3.5. Effect of graded sulfate on photosynthetic efficiency

Effect of high sulfate on the photosynthetic efficacy for cyanobacterium, in terms of quantum yield of PSII, was measured by PAM fluorometry. Photochemical quenching displayed by Fv/Fm ratio increased by 6.14% in 50 mM sulfate treatment, while it progressively reduced by 8.02 and 10.96% at 500 and 650 mM sulfate concentrations respectively than control. Inversely, the heat dissipation denoted by Y(NO) was observed lowest at 50 mM sulfate while, it was significantly ($p<0.05$) higher at 500 and 650 mM sulfate by 4.79 and 6.54% respectively than control (Figure 4).

3.6. Effect of graded sulfate on carbohydrate and protein

A significant ($p<0.05$) decrease in the total protein content by 11.90% was observed in the cyanobacterium, at 50 mM exogenous sulfate supplementation. However, the protein content in 500 and 650 mM sulfates were significantly ($p<0.05$) increased by 11.90% and 38.09%, respectively than control (Figure 5A). For carbohydrate the total content were significantly ($p<0.05$) reduced by 35.5% and 45.6% in 500 and 650 mM sulfate supply. However, the increase observed in 50 mM sulfate treatment was found insignificant to the control (Figure 5B).

3.7. Effect of graded sulfate concentration on oxidative stress markers

Rapid increase in the H_2O_2 concentration was observed with increasing concentrations of exogenous sulfate. The percent increase in the H_2O_2 was 176, 400 and 444% with respect to the control value at 50, 500, and 650 mM respectively (Figure 6A). Like H_2O_2, MDA also increase rapidly with increasing sulfate concentration. The increase in MDA concentration was much more drastic than the former. The percent increase in the MDA content was 679.40, 1499.23 and 1900.59% than control value at 50, 500 and 650 mM, respectively (Figure 6B).

4. Discussion

Cellular responses of the cyanobacterium *Westiellopsis* sp. TPR-29 towards high sulfate supplementation, relies on combination of several key mechanisms functioning together with various components to achieve cellular homeostasis. Sulfate is required for several key cellular functions (Shoveller et al., 2005) but as evident experimentally, can be lethal in high doses. In cyanobacterium, *Westiellopsis* sp. TPR-29, the growth reduces with increasing concentrations of sulfate in the media. It is also evident that, the photosynthetic pigments i.e., chlorophyll, carotenoids, phycobilliproteins (PBPs), comprises of phycoerythrin (PE), phycocyanin (PC) and allophycocyanin (APC), constantly degraded at higher sulfate supplementations. Further, the quantum yields of PSII also reduced significantly at higher concentrations of sulfate resulting in lower photosynthetic efficacy and thus, yield. Therefore, reduction in the carbohydrate content was also observed in cells treated with higher concentration of sulfate. Additionally, rapid increase in lipid peroxidation, depicted by progressive increment in the MDA content and very high intracellular concentration of hydrogen peroxide was also observed with increasing exogenous sulfate supplementations. Therefore, it can

be precluded that the growth inhibition at higher sulfate concentrations is due to the production of prodigious amount of reactive oxygen species (ROS), resulting in subsequent, degradation of pigment proteins and other sub-cellular components. The low rate of photosynthesis due to pigment damage leads to lower photosynthate assimilation (Sultana et al., 1999) and thus less energy is available to the cell for metabolic activities. Possibly, this results in lower growth rate of cyanobacterium at higher exogenous sulfate concentration.

The reduction of photosynthetic efficiency of cyanobacterium *Westiellopsis* sp. TPR-29 with increasing concentration of sulfate was probably due to the reduction in the photosynthetic pigments i.e., chlorophyll, carotenoids, phycobilliproteins content. Reduction in carotenoid content indicated that lesser resonance transfer of light energy to the reaction center in the stressed cells. Moreover, cyanobacteria specific accessory pigment, phycobilliproteins (PBPs) comprises of phycocyanin (PC), phycoerythrin (PE), and allophycocyanin (APC) also depleted significantly, and subsequently lowering the amount of light energy harvests. Nevertheless, graded sulfate induced generation of free radicals may also damage the thylakoid (Alscher et al., 1987) and thus, results in the reduced photosynthetic efficiency. However, chlorophyll fluorescence measurement reflected a decrease in the photochemical quenching that may have occurred due to PSII damage. The analysis of maximum photosynthetic quantum yields (Fv/Fm) of PSII severely reduced along the increasing sulfate gradient, whereas, increasing value of non-regulated Y(NO) photochemical quenching was well evident. This lower photosynthetic efficiency may occur due to the accumulation of electrons in the transportation chain because of impaired reaction centers (Guidi et al., 2002). It was also clear with an increase in Y (NO) that photosynthetic apparatus, especially, the components of PS II is being impaired, increasing non-regulated heat dissipation (Kharwar and Mishra, 2020).

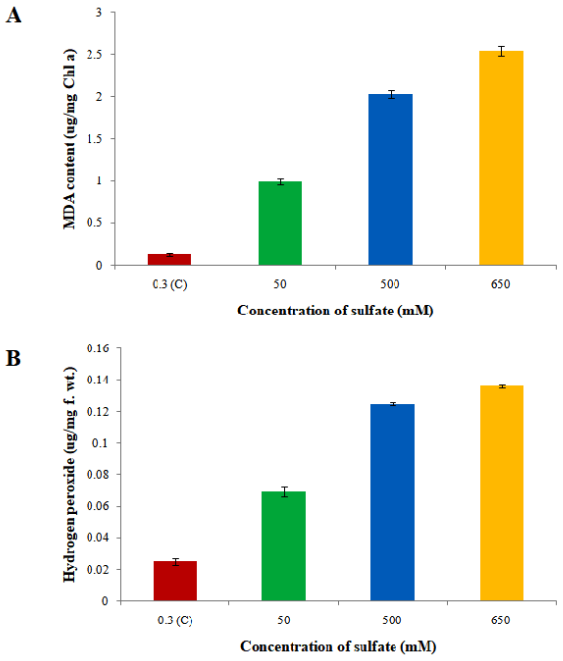

Figure 6. MDA content (A) and intracellular hydrogen peroxide (B) concentrations at different exogenously applied sulfates.

The progressive decrease in the total carbohydrate content is probably due to more carbohydrate catabolism for energy to overcome stress and lesser anabolism due to decrease in the photosynthetic rate and efficiency as decrease in the cellular pigment content (Pressman et al., 1993). Because of this reason the total carbohydrate gradually reduces with increasing graded sulfate concentration in the medium. Unlike carbohydrates, there is a sharp increase in the total cellular protein content on moving above 50 mM concentration and excessively increased at 650 mM concentration in the media. The rise in protein content is probably due to the rapid and *de novo* synthesis of stress proteins, heat shock proteins and increased activity of enzymes that are required by cell to counter oxidative stress (Pacifici et al., 1991) The rapid increment further with increasing concentration of graded sulfate probably because at this level of stress the cell started expressing stress related m-RNA which will tries to cope with the stress and prevent the cell from dying (Ahmed, 2010). Any significant energy to synthesis stress related proteins, and uses available proteins as a catabolic source to derive energy. The sharp increase in the MDA content, with increasing concentration of graded sulfate ion is most likely because of accelerated production of ROS and subsequent peroxidation of membrane lipids. Free oxygen radical per oxidation of membrane lipid and production of MDA under stress condition has been observed in *Westiellopsis* sp. TPR-29. Osmotic balance of the cell is necessary for proper cell metabolisms as it is required for the maintenance of turgor pressure and pH of the cell. This osmotic balance in the cell is maintained by the ion homeostasis. The maintenance of the ion homeostasis in the cell is possible due to the selective permeability of the plasma membrane. The lipid layer, being hydrophobic, resists the free ion movement across the cell and thus maintains the requisite homeostasis.

5. Conclusions

In essence, high sulfur has deleterious impact on the cyanobacterial physiology and thus, reduces growth. The metabolic aberrations observed here are the genuine outcome of rapid oxidative burst that have occurred in the cell due to putative accumulation of sulfate to toxic level. This accumulation of sulfate may have occurred due to saturation of sulfate pools and downstream metabolism operating in the organism. Although, it seemed arbitrary, yet, the genetic engineering of various enzymes in the pathway may have revealed the stress by faster utilization of the accumulated sulfate by increasing their Km (concentration of substrate which may allow the enzyme to achieve 50% of the Vmax). Additionally, increasing the transcript number or the rate of mRNA synthesis may also have considerable effect on metabolic channelizing of accumulated sulfates in the cell (Lawry and Jensen, 1979; Schmidt, 1988). Such modifications can be carried out for the production of value-added metabolites like exopolysaccharides (EPS) (Di Pippo et al., 2013; Chug and Mathur, 2013). Nevertheless, the 50 mM concentration of exogenous sulfate was observed to have stimulatory role in cyanobacterial growth. It is probably because of proper metabolism of sulfate inside the cell upon uptake, resulting in more efficient cellular functionalities like protein synthesis, photosynthesis, and redox stability etc. However, the accumulation of ROS, in the form of hydrogen peroxide, was being observed along with significantly high lipid peroxidation causing membrane damage was also evident in this concentration. Therefore, in this concentration occurrence of firm antioxidative defense has certainly occurred, that ultimately stabilized the cellular redox status and allowed proliferation. Such stimulatory concentrations of sulfate may also be used to optimize the medium composition in several cases of industrial production of metabolites, pigments, EPS, etc. using cyanobacteria (Pereira et al., 2013; Pereira et al., 2015).

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Authors' contributions

AP- Conceptualisation, experimentation, manuscript writing
SB- Conceptualisation, experimentation, manuscript writing
PS- Illustrations, figures and referencing
AKM- Conceptualisation, manuscript editing

Conflict of interests

All the author's declared no potential conflict of interest.

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