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The Role of Photosynthetic Pigments in Mitigating Thermal Stress in Presence of Sodium Sulfide in Mesophilic Cyanobacterium Westiellopsis Prolifica

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Abstract

Thermophilic cyanabacteria thrive in sulphidic environments, such as hot water springs containing sulfide. The presence of sulfide positively affects the growth of bacteria and cyanobacteria. However, temperature stress impedes the photosynthetic system in mesophilic cyanobacteria, leading to oxidative damage, decreased efficiency, and cellular metabolism changes. Photosynthetic pigments like carotenoids and chlorophylls are crucial for cyanobacteria survival under heat stress and potential adaptation mechanisms may be employed to maintain photosynthetic activity. The growth of cyanobacterial cultures depends upon the amount of photosynthetic pigments during thermal stress. The current study sought to investigate if mesophilic cyanocateria can withstand high temperatures in the presence of sulphide. This study investigates the role of these pigments. Thus, amounts of Chl a, carotenoids and phycobiliproteins in the test organisms under thermal stress and in presence of sulphide were determined.

1. Introduction

Cyanobacteria, or blue-green algae, are gram-negative prokaryotes that feed themselves. These species initially emerged some 3.5 billion years ago, during the Precambrian period (Kulasooriya, 2011; Pandey and Pandey, 2013; Singh et al., 2013; Chaurasia, 2015). Cyanobacteria were instrumental in converting Earth's essentially anaerobic atmosphere into an oxygen-rich one via oxygenic photosynthesis. This process cleared the path for the formation of oxygen-dependent species, which subsequently expanded and dominated modern global life (Rasmussen et al., 2008; Vincent, 2009; Kulasooriya, 2011).

1.1. Cyanobacteria as Highly Adaptive Prokaryotes

Throughout their lengthy evolutionary history, cyanobacteria have showed extraordinary survivability in a variety of ecological circumstances, adjusting to geochemical, climatic, and anthropogenic changes (Pearl and Otten, 2013). Cyanobacteria have the most diverse habitats of any creature on the planet and are regarded as cosmopolitan. They can be found in freshwater, marine, and terrestrial ecosystems, as well as severe conditions as hot springs, hypersaline zones, freezing habitats, and arid deserts (Nakatsubo and Ino, 1987; Kashyap et al., 1991; Whitton and Potts, 2000; Kulasooriya, 2011). Cyanobacteria are notable for their capacity to flourish over a wide temperature range (Kulasooriya, 2011; Chaurasia, 2015). Cyanobacteria use a variety of survival tactics to live in extremely competitive and severe

conditions. These tactics include the development of adaptive systems, such as freeze-thaw protection, which allows photosynthesis and other physiological activities to resume within minutes to hours of thawing. They have also developed adaptations to protect themselves from UV radiation, desiccation, bright light, and excessive salinity. Furthermore, cyanobacteria can enter a latent state with little metabolic activity, use a CO2 concentrating mechanism, and create specific proteins such as cold-shock and antifreeze proteins. Other protective chemicals include glycine betaine, exopolysaccharides, and an increase in unsaturated fatty acids to keep the membrane fluid (Whitton and Potts, 2000; Scandalios, 2005; Makhalanyane et al., 2015). Furthermore, cyanobacteria have enzymatic defense mechanisms against reactive oxygen species that occur in that arise in harsh conditions. Cyanobacteria have an important role in carbon and nitrogen cycling, phosphorus buildup, and, as primary colonizers, contribute to soil structural integrity, moisture retention, and fertility (Scandalios, 2005; Makhalanyane et al., 2015). Cyanobacteria are classed as psychrophilic, mesophilic, or thermophilic depending on their optimal temperature range for growth. Psychrophilic cyanobacteria grow in Arctic and Antarctic lakes at temperatures ranging from 0°C to 10°C (Alexander et al., 1978; Basilier et al., 1978; Davey, 1983; Smith, 1984; Nakatsubo and Ino, 1987; Kashyap et al., 1991; Skulberg, 1996). Mesophilic cyanobacteria grow best at temperatures ranging from 25°C to 37°C, whereas thermophilic cyanobacteria thrive at temperatures ranging from 45°C to 60°C. Some thermophilic species, which can grow at temperatures ranging from 73°C to 90°C, have developed unique adaptations to withstand intense heat (Castenholz, 1977; Apte, 2011).

1.2. Cyanobacteria in Sulfidic Environments

Sulfur springs are important environments for bacteria, including cyanobacteria, which can develop in the presence of hydrogen sulfide, proving their ability to tolerate anoxic conditions. The temperature of sulfide-rich hot springs normally ranges between 42°C and 85°C (Castenholz, 1976, 1981). Adams (1994) examined the metabolic diversity of sulfur-dependent, hyperthermophilic bacteria that grow at or above 90°C. There are currently 20 genera of these microbes, known as Archaea. Sulfur is commonly found in ecosystems because it occurs in source waters and/or through biological sulfate reduction. Hypersaline and marine sediments frequently include cyanobacterial mat communities (Oren et al., 1979). Like higher plants, cyanobacteria have two photosystems (PS-I and PS-II) and use water as an electron donor during photosynthesis, creating oxygen as a byproduct. Several cyanobacteria species can also perform anoxygenic photosynthesis using only PS-I and other electron donors such as hydrogen sulfide (Madigan et al., 2003). Research on thermophilic cyanobacteria's usage of sulfide for photoautotrophic growth has been reported (Miller and Bebout, 2004; Bhusare and Wakte, 2011; Lukavský et al., 2011; Giampaoli et al., 2013; Bilyj et al., 2014; Mongra, 2014). These findings indicate that Oscillatoria limnetica can employ sulfide as an electron donor for CO2 assimilation, implying that facultative anoxygenic photosynthesis provides a selection advantage for survival in situations with high sulfide levels. Similarly Oscillatoria amphigranulata, isolated from sulfide-rich hot springs in New Zealand (56°C), has been demonstrated to perform anoxygenic and oxygenic photosynthesis in the presence of sulfide (Castenholz and Utkilen, 1984). Several strains of Microcoleus chthonoplastes from throughout the world have also shown the ability to undertake both types of photosynthesis in the presence of high sulfide concentrations (Cohen et al., 1986). Cyanobacteria live in microbial mats, which is a significant ecological feature. In sulfide-rich settings, microbial mats are susceptible to extreme variations in physical and chemical gradients such as light, oxygen, and sulfide, resulting in cyanobacteria experiencing high oxygen levels during the day and anaerobically at night. During this process, cyanobacteria may be exposed to high sulfide levels at night. Some species can perform sulfide-dependent anoxygenic photosynthesis, whereas others boost their rates of oxygenic photosynthesis in the presence of sulfide and have detoxification and toxicity prevention mechanisms. When the environment is anoxic at night, mat-forming cyanobacteria convert to fermentative metabolism. Sulfide can be produced from elemental sulfur by a variety of species. Anaerobic dark energy metabolism is a feature of

cyanobacteria that have been isolated from these settings (Stal, 1995). Sulfide tolerance varies among cyanobacteria. While strains from sulfide-rich environments usually have one or more adaptations to sustain their photoautotrophic metabolism in such circumstances (Castenholz, 1976; Cohen et al., 1975; Cohen et al., 1986), this compound inhibits strains that are typically sensitive to sulfide (Castenholz, 1977; Garlick et al., 1977; Oren et al., 1979; Cohen et al., 1986).

1.3. Thermal stress

Changes in immediate surrounding temperature occur more swiftly than changes in stress factors such as drought, salinity and nutrient imbalances. Moreover, extreme temperature or thermal stress aggravates the adverse effects of other stresses, including drought and salinity on plants physiological processes (Ashraf and Foolad, 2007). Thermal stress is one of the major factor to which organisms are susceptible. Therefore, plants must be protected from temperature-induced oxidative stress so that they can survive under high temperature (Qu et al., 2013). When mesophilic cyanobacteria are subjected to temperature beyond their optimum growth range, they experience thermal stress (Apte, 2011). Exposure to thermal stress is followed by excessive generation of ROS which ultimately leads to oxidative stress (Demmig-Adams and Adams, 2006; Martin et al., 2007; Hasanuzzaman et al., 2013). A rise in temperature intensfies the metabolic rate of organisms which results in an increase in oxygen consumption and can lead to an oxidative stress condition in cells. In algal cells exposed to up-shift of growth temperature, higher production of superoxide radicals (O2−) and hydrogen peroxide (H2O2) was reported (Rady et al., 1995). Under harsh temperature conditions cyanobacterial flora such as Aphanocapsa, Aphanothece, Chroococcus, Tolypothrix, Gleocapsa, Gleothece, Lyngbya, Microcoleus and Scytonema are spotted on building tops, barks of trees in tropics and are subjected to high temperature stress due to fluctuations in day temperature from 20 °C to 50-70 °C and face frequent wetting and drying in Indian continent (Vaishampayan et al., 2001; Kesheri et al., 2011; Kapoor et al., 2013). carotenoid levels or modifying the chlorophyll-to-carotenoid ratio. This improves light harvesting efficiency and reduces photodamage

1.4. Protective role of photosynthetic pigments under high temperature stress

Cyanobacteria have developed defenses against heat and water stress. According to Seyed et al. (2012), some of the processes include the selective expression of tolerance genes, the regulation of ion fluxes for osmotic adjustment, and the utilization of new stress proteins to repair oxidative damage brought on by an excess of free radicals. Cyanobacteria are widely scattered microbes that perform oxygenic photosynthesis which is required for the global nitrogen and carbon cycles to function. Environmental stressors, particularly temperature changes, have a significant impact on their growth and photosynthetic efficiency. Higher temperatures can cause protein denaturation, reactive oxygen species (ROS) accumulation, and disruption of the photosynthetic electron transport chain (PETC), all of which have an impact on cellular activity. Carotenoids and other photosynthetic pigments, like as chlorophyll, act as important molecular buffers against heat stress. This study investigates how these pigments protect cyanobacteria from high temperatures and help them become more thermally resistant. Thermal stress affects the photosynthetic apparatus in a variety of ways, including thylakoid membrane instability, protein function alterations, and the balance of light absorption and energy dissipation. The photochemical functions of photosystem I (PSI) and PSII rely on the principal photosynthetic pigments of cyanobacteria, carotenoids and chlorophylls. These processes become less effective during heat stress, which increases ROS production and may cause damage to cellular constituents. Thermal stress causes PSII inactivation, which frequently results in damage to the oxygen-evolving complex (OEC) and D1 protein degradation. This leads to decreased electron transport, reduced ATP synthesis, and increased ROS generation. Chlorophylls, particularly chlorophyll a, absorb light energy but can get excited under heat stress, resulting in the generation of triplet states and subsequent ROS formation. Carotenoids, which are related with PSII, operate as antioxidants by quenching these excited states and scavenging ROS. Thermal stress in cyanobacteria causes significant oxidative stress. ROS such as superoxide ($O_2^{\bullet -}$), hydrogen

peroxide (H_2O_2), and hydroxyl radicals (OH•) can harm biological macromolecules such lipids, proteins, and DNA. Photosynthetic pigments like carotenoids (e.g., β-carotene and zeaxanthin) operate as antioxidants and neutralize ROS before they affect cellular structures. Chlorophylls are the principal pigments that absorb light energy. Chlorophyll molecules are photosensitive under temperature stress. However, the creation of heat-shock proteins (HSPs) and other protective processes can aid in the stability of chlorophyll molecules under stress. Some cyanobacterial species' chlorophyll a level may remain steady under modest heat stress. Carotenoids, such as β-carotene, zeaxanthin, and astaxanthin, can protect cyanobacteria from temperature stress. These pigments work in several ways: Carotenoids play a photoprotective role by dissipating surplus light energy as heat through non-photochemical quenching (NPQ), minimizing photodamage to photosynthetic machinery. Carotenoids have antioxidant activity by scavenging ROS and reducing oxidative damage. For example, zeaxanthin has been found to protect against thermal-induced oxidative stress by neutralizing singlet oxygen and other free radicals. Certain cyanobacteria boost carotenoid synthesis in response to heat stress, potentially improving tolerance to oxidative stress. Cyanobacteria have light-harvesting pigments called phycobiliproteins, which include phycocyanin and allophycocyanin. While their primary job is to capture light energy, they also play a role in protecting the organism during stress conditions. Heat stress can degrade phycobiliproteins, while particular cyanobacteria increase their synthesis to maintain photosynthetic effectiveness under variable temperatures. Complex gene networks and signaling pathways regulate pigment manufacture in response to heat stress. Heat shock factors (HSFs) are important in cyanobacteria for regulating the transcription of genes involved in pigment production and stress response. Under heat stress, the expression of genes involved in chlorophyll production may be increased or down regulated according to the intensity of stress. Temperature regulates the carotenoid production pathway. Heat stress can activate enzymes like phytoene synthase (psy), resulting in the buildup of carotenoids like β-carotene, zeaxanthin, and astaxanthin. This buildup not only improves antioxidant defense, but also helps to maintain cellular homeostasis in high-temperature environments. Cyanobacteria use a variety of adaptation techniques to deal with temperature stress, the majority of which include changes in pigment composition and photosynthetic machinery. These approaches include: Certain cyanobacteria species adapt to heat stress by changing their pigment makeup, either by raising carotenoid levels or modifying the chlorophyll-to-carotenoid ratio. This improves light harvesting efficiency and reduces photodamage. There are few studies on growth of mesophilic cyanobacteria in the presence of sulfide. Singh (1978) studied nitrogen fixation by Nostoc calcicola by varying pH levels in the presence of sulfide. The effect of three sulfide compounds such as cysteine, sodium sulfide and sodium dithionite on growth of Synechococcus cedrorum has been studied (Gupta and Talpasayi, 1984). The present work was undertaken to know whether mesophilic cyanobacteria can tolerate temperature beyond their optimum range in the presence of sulphide? If yes, whether any photosynthetic pigment system is triggered to counter thermal induced oxidative stress in mesophilic cyanobacteria. This can be a basis for the enrichment of fields by cyanobacteria a t42°C to 50°C under sunlight in the presence of sulphide, a condition which otherwise does not support the growth of mesophilic cyanobacteria.

2. Materials and Methods

2.1. Westiellopsis prolific

Selection of Test Organism

The growth of random mesophilic cyanobacteria from our cyanobacterial culture collection was studied for 6 d at four temperatures i.e. 37°C, 42°C, 45°C and 50°C. Westiellopsis prolific exhibited growth at 42°C and was selected for the present study. The cyanobacterium Westiellopsis prolifica, an isolate from Patiala, Punjab, India, is a filamentous, heterocystous bacterium with a thin sheath, trichome 8-12 µm broad, elongate cylindrical cells, pale-yellowish heterocysts, and subspherical to oval akinetes (Figure 1). chain (PETC), all of which have an impact on cellular activity. Carotenoids and other photosynthetic pigments, like as chlorophyll, act as important molecular buffers against heat stress. This study investigates how these pigments protect cyanobacteria from high temperatures and help them become more thermally resistant.

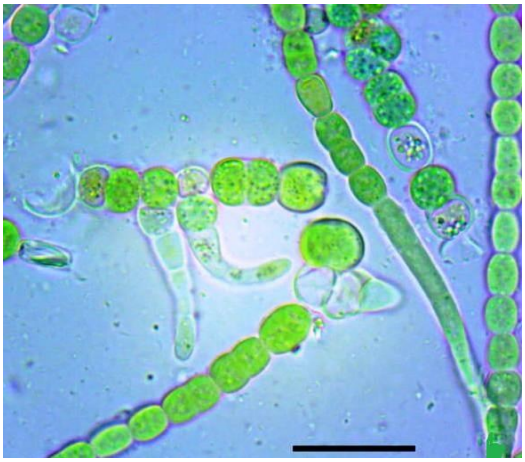

Figure 1 Photomicrographs of Selected Test Organisms.: Westiellopsis Prolifica
Scale bar= 10 µm

2.2. Composition of The Nutrient Medium and Propagation of Cultures

The test organism was grown in slightly modified Chu-10 medium (Safferman and Morris, 1964) in which CaNO3 was replaced with equivalent amount of CaCl2 and micronutrients (Allen and Arnon, 1955) were added. The culture was propagated in basal medium and maintained in a room at 28±2 oC. They were illuminated for 14 hours a day with 1800 lux light. To maintain exponential growth, the cultures were transferred to fresh media every 6-7 days and hand shaken at least twice a day.

2.3. Absorbance and Dry Weight Biomass

The absorbance of cultures was measured using a spectrophotometer (Spectronic 20D+, Thermo Scientific,USA). To determine the dry weight of the biomass, known volumes were harvested, washed three times with double distilled water, and dried overnight at 60°C. The dry biomass weight was expressed in mg mL-1 cultures.

2.4. Protein estimation

Protein content in cultures was determined using Lowry et al.'s 1951 method. Cultures were harvested, centrifuged, and a protein extract was prepared by hydrolyzing cells with 1N NaOH in boiling water for 10 minutes. The contents were cooled, centrifuged, and the supernatant was used for protein estimation.

2.5. Photosynthetic Pigments Estimation

2.5.1. Acetone Soluble Pigments

A known volume of homogeneous algal suspensions were withdrawn, centrifuged and washed with distilled water. Pellet was suspended in a known volume of acetone. After shaking vigorously, the mixture was kept at room temperature for 12 h. The contents were centrifuged at 5,000 g and absorbance of supernatant was measured at 665 nm and 450 nm. The total amount of Chl a and Car was quantified as per method of Myers and Kratz (1955).

2.5.2. Water Soluble Pigments

The amounts of phycobiliproteins, (Phycocyanin; PC, allophycocyanin; APC and Phycoerythrin; PE), was estimated following the method of Bennett and Bogorad (1973). A known volume of cell suspension of the test organisms was centrifuged at 5,000 g for 10 min. The pellet was suspended in double distilled water and vigorously shaken. The contents were subjected to many freeze-thaw cycles until all the pigments were released from the cells. The contents were then centrifuged at 5,000 g for 5 min Absorbance of the supernatant was recorded at 565, 615 and 652 nm and quantified (µg mg-1 dry weight biomass).

2.5.3. Thermal Stress

The study used BOD incubators with thermostats and day light fluorescent tubes to determine temperature tolerance of mesophilic cyanobacterial organisms, as the optimal temperature range is 28°C to 37°C. The light intensity was 1800 lux for 14 hours per day.

3. Statistical Analysis

The data is the average of three independent experiments, analyzed using one-way analysis of variance and Tukey's honest significance difference test.

4. Results and Discussion

Growth of test organism was measured in terms of increase in absorbance, protein content and dry biomass weight of the cultue.

4.1. Response of Westiellopsis prolifica to Varied Doses of Sulphide

In early investigations, the growth of nine mesophilic cyanobacteria for 6 days was evaluated at 37°C, 42°C, 45°C, and 50°C in terms of absorbance at 720nm. The findings indicated that none of the examined mesophilic cyanobacteria survived at 45°C, and the cells lysed. However, only Westiellopsis prolifica was able to grow at 42°C (Table 1).Thus, this organism was chosen for the current investigation, and its growth was compared at 28°C, 37°C, and 45°C. It was

discovered that after 12 days, Westiellopsis prolifica growth decreased by 10% at 37°C and 38.0% at 42°C as compared to the growth of the test organism at 28°C (Fig. 2). When the development of the test organism was analyzed at 42°C in the absence and presence of varying doses of sulphide, it was discovered that sulphide up to 2.5 mM in the medium promoted increased growth of the test organism at 42°C in a concentration-dependent way (Fig. 3). The absorbance of cultures at 28°C rose from 0.06 on day zero to 0.54 on day twelve.The increase in absorbance of cultures in the presence of 0.5 mM, 1.0 mM, 2.0 mM, and 2.5 mM at 42°C was 8.8%, 11.6%, 13.5%, and 24.7%, respectively. The organism did not survive in the presence of 5.0 mM sulphide at 42°C, indicating that this concentration was harmful to it. Sulphide is toxic because it binds to metalloproteins and inhibits electron transport in the photosynthetic electron chain. The addition of 2.5mM sulphide in the media had a favorable influence on the organism's growth at 42°C, thus this concentration was chosen for further tests. (Figure 3)

4.2. Growth Under High Temperature Stress

Since the ideal temperature range for the growth of mesophilic cyanobacteria is between 28°C and 37°C, 42°C was chosen as higher temperature to produce thermal stress to the cultures. Thus the development of the organism was investigated at 28°C and 42°C in absence and presence of sodium sulphide as an increase in absorbance at 720 nm. To evaluate the organism's development at 42°C, cultures were grown in a BOD incubator equipped with fluorescent tube lights. Figure 2 Growth of Westiellopsis Prolifica in Basal Medium

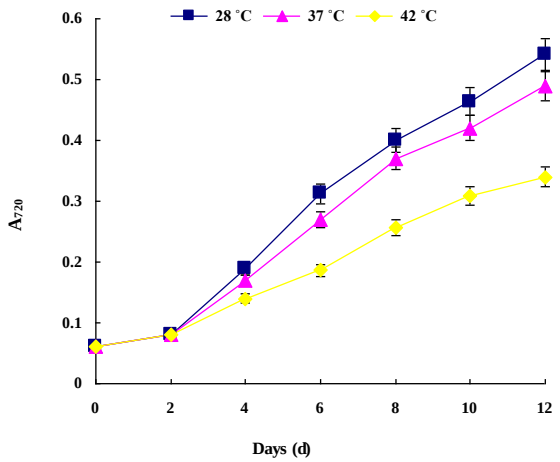

Figure 2 Growth of Westiellopsis Prolifica in Basal Medium

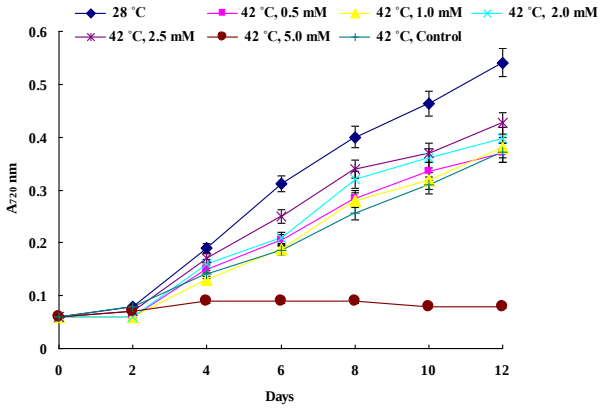

Figure 3 Growth of Westiellopsis Prolifica in Absence of Sulphide at 28ºc, 42ºc and Presence of Sulphide at 42ºc

Table 1 Effect of Thermal Stress on Growth of Selected Test Organisms Grown in Basal Medium Supplemented with Sulphide

Organism	Culture conditions	Absorbance (720 nm)	Protein content (µg mL^{-1})	Biomass dry weight (mg mL^{-1})
Westiellopsis prolifica	28 °C	0.54±0.02	237.3±11.86	5.34±0.05
	42 °C, Control	0.34±0.01 (37)↓	123.3±6.16 (48)↓	2.64±0.13 (50.5)↓
	42 °C, Sulphide	0.42 ±0.02 (23.5)	204.0±10.2 (65.4)	4.24±0.21 (60.6)

4.3. Effect of Thermal Stress on Photosynthetic Pigments

The effect of sulphide on organic solvent soluble as well as water soluble photosynthetic pigments of the all selected test organisms was studied. presence of 5.0 mM sulphide at 42°C

4.4. Chlorophyll (Chl) a content

The effect of thermal stress on Chl a content of Westiellopsis prolifica grown in basal medium is shown in Fig. 10. On 2 d, the Chl a content of

cultures grown at 28 oC, 42 oC and in presence of sulphide was almost same. On 4 d and 6 d, the Chl a content of cultures grown at 42 oC decreased by 26.4% and 35.3 % respectively, which increased in sulphide containing cultures, reaching almost to the level of Chl a content of 28 oC grown cultures (Fig.4). Four types of adaptations for survival of cyanobacteria in sulphidic conditions are reported (i) sulphide-resistant oxygenic photosynthesis (ii) sulphide-sensitive oxygenic photosynthesis (iii) sulphide insensitive oxygenic photosynthesis concurrent with sulphide dependent anoxygenic photosynthesis (iv) sulphide-sensitive oxygenic photosynthesis replaced by sulphide-dependent anoxygenic photosynthesis (Cohen 1985; Abed et al., 2006). The level of tolerance to sulphide vary in different cyanobacteria. This variation may be due to inherent capacity of the organism to adapt to sulphidic conditions and time of exposure to which a particular organism is subjected (Predmore et al., 2012; Qian et al., 2013). It is apparent from our results that there was 26.4% and 35.3 % decrease in Chl a content of Westiellopsis prolifica cultures grown at 42 oC on 4 d and 6 d, respectively. The loss of Chl a content in control cultures of test organisms under the influence of thermal stress could be due to the peroxidation of thylakoid membrane or increased production of free radicals (Latifi et al., 2009). It has been shown that Chl-a content of M. aeruginosa subjected to temperature stress at 35°C was significantly lower than Chl a content of cultures at 25°C (Han et al., 2015). Figure 4 Shows Chl a Content in Westiellopsis Prolifica When Grown at 28ºC and 42ºC in Absence of Sulphide and at 42ºC in Presence of Sulphide

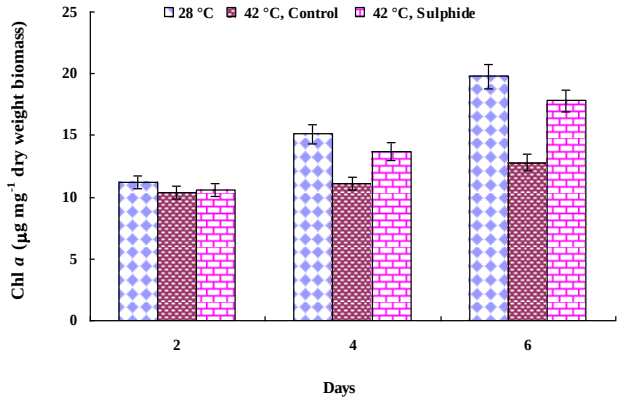

Figure 4 Chl a Content in Westiellopsis Prolifica When Grown at 28ºC and 42ºC in Absence of Sulphide and at 42ºC in Presence of Sulphide

4.5. Carotenoid (Car) Content

The effect of thermal stress on Car content of Westiellopsis prolifica was similar to effect on Chl a content (Fig.5). The Car content of control cultures at 28 oC increased with increase in growth of the cultures. The Car content of cultures grown at 28 oC increased from 16.1 µg mg-1 biomass dry weight on zero d to 20.1, 24.8 and 36.1 µg mg-1 biomass dry weight on 2 d, 4 d and 6 d, respectively. When the cultures were grown at 42 oC, the Car content decreased by 10.9%, 21.3% and 33.2% on 2 d, 4 d and 6 d, respectively. In presence of sulphide the Car content increased to the level of Car content of cultures at 28 oC. Carotenoids are important to study as they play vital role in photosynthesis by participating in the process of energy-transfer to photosystem(s). These pigments may also protect the reaction centers from photoauto-oxidation (Armstrong and Hearst, 1996). It was observed during the present study, thermal stress led to a decrease in carotenoids in control cultures, but in presence of sulphide the content of carotenoids was more than the control cultures of test organisms. This indicated protective role of sulphide. It has been reported that most of the carotenoid synthesizing enzymes are membrane bound, thus the reduction in carotenoid biosynthesis under thermal stress might be due to the effect of high temperature on membrane structural integrity (Michelangeli et al., 1990; Mohapatra et al., 2003; Latifi et al., 2009). High amounts of carotenoid in sulphide grown cultures indicated the role of sulphide in the survival of these test organisms under thermal stress (Figure 5)

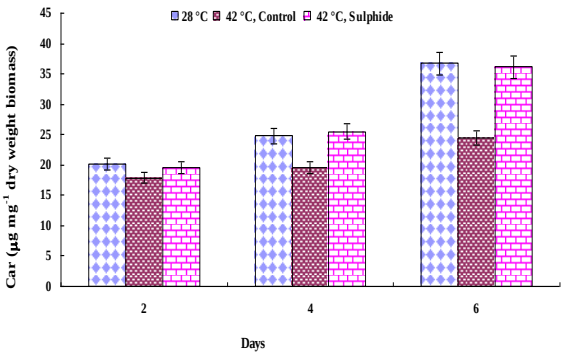

Figure 5 Carotenoid Content in Westiellopsis Prolifica When Grown at 28ºC and 42ºC in

Absence of Sulphide and at 42ºC in Presence of Sulphide

5. Water Soluble Photosynthetic Pigments

5.1. Phycocyanin (PC) Content

The effect of thermal stress on PC content of Westiellopsis prolifica grown in basal medium is shown in Fig. 6. On 2 d and 4 d, the PC content of control cultures grown under thermal stress was almost same. On 6 d, the PC content of control cultures under thermal stress decreased by 21.6% and in presence of sulphide increased by 27.3%. (Figure 6)

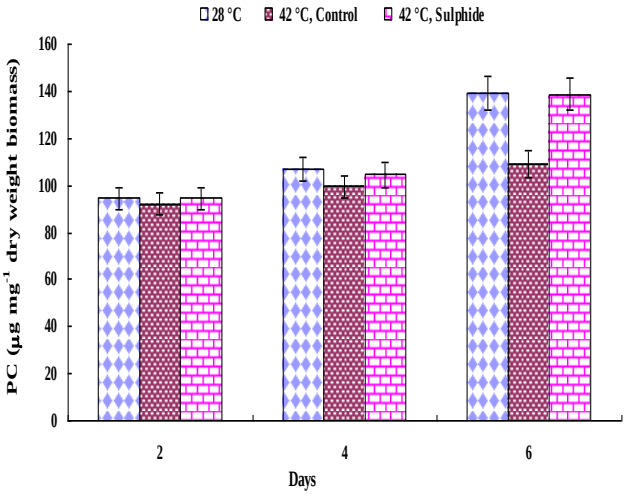

Figure 6 Phycocyanin Content in Westiellopsis Prolifica When Grown at 28ºc and 42ºc in Absence of Sulphide and at 42ºc in Presence of Sulphide

5.2. Allo-phycocyanin (APC) Content

The effect of thermal stress on APC content of Westiellopsis prolifica is shown in Fig. 7. When the cultures were grown at 42 oC, the APC content decreased by 18.6%, 12.5% and 29.5% on 2d, 4d and 6d. In presence of sulphide the APC content increased almost to the level of APC content of cultures at 28 oC. (Figure 7)

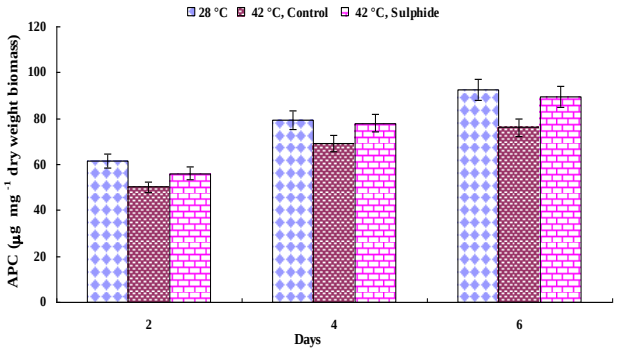

Figure 7 Allo-phycocyanin Content in Westiellopsis Prolifica When Grown at 28ºC and 42ºC in Absence of Sulphide and at 42ºC in Presence of Sulphide

5.3. Total Phycobiliproteins (PBPs) Content

When the cultures of Westiellopsis prolifica were grown at 42 oC, total PBPs decreased by 7.7%, 19.1% and 24.1% on 2 d, 4 d and 6 d, respectively compared to cultures grown at 28 oC. In presence of sulphide the total PBPs content increased by 4.9%,10% and 15.7%, respectively, over the control cultures grown at 42 oC (Figure 8).

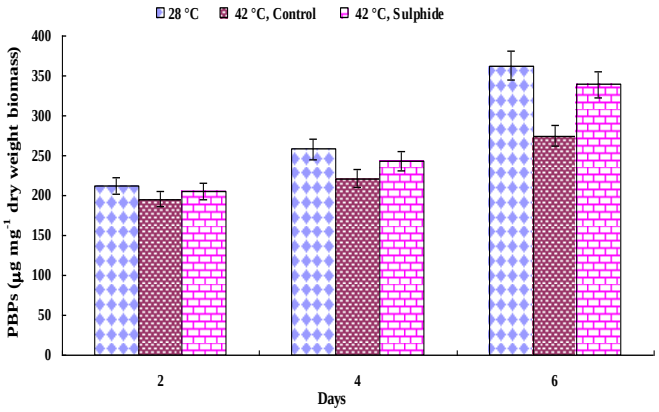

Figure 8 Total Phycobiliprotein Content in Westiellopsis Prolifica When Grown at 28ºC and 42ºC in Absence of Sulphide and at 42ºC in Presence of Sulphide

Table 2 Effect of Thermal Stress on Photosynthetic Pigments of Selected Test Organisms

Organism	Culture conditions	Chl a content	Car content	PC content	PE content	APC content	Total PBPs content
Westiellops is prolifica	28 °C	19.8±0.99	36.7±1.83	149.2±7.46	121±6.05	92.4±4.62	326.6±16.33
	42 °C	12.8±0.64	24.5±1.22	123.3±6.16	100.2±5.00	86.7±4.33	310.2±15.51
	2.5 mM	17.8±0.89 (39.0)↑	36.1±1.80 (47.3)↑	138.8±6.94 (27.3)↑	110.7±5.53 (21.3)↑	89.5±4.47 (17.4)↑	339±16.95 (15.7)↑

*Figures in parenthesis with (↑) symbol show maximum percent increase in sulphide treated cultures over control cultures at respective time interval in the above microorganisms

**Chlorophyll (Chl a), Carotenoid (Car), Phycocyanin (PC), Allo-phycocyanin (APC),

Phycoerythrin(PE), Phycoerythrin (PE) content, Total phycobiliproteins (PBPs) content (µg mg-1biomass dry weight)

6. Discussion

Phycobiliproteins are important light-harvesting pigments located at the outer surface of the thylakoid membranes in the form of phycobilisomes, are attached to thylakoids through 75-kDa linker proteins and transfer light energy to reaction centres (Glazer, 1985; Rodrigo and Robaina, 1997). The number of phycobilisomes on thylakoid membrane depends on environmental conditions (Grossman et al., 1993). Thus, the effect of high temperature on phycocyanin (PC), allophycocyanin (APC), phycoerythrin (PE) and total phycobiliproteins (PBPs) content of test microorganisms was studied (Table 2). Though thermal stress led to a decrease in PC, APC, PE and total PBPs content, their significant increase in presence of sulphide was responsible for the growth of test organism under thermal stress. The pigments decreased in control cultures at high temperature but in presence of sulphide their content enhanced thus supporting growth of test organisms. Proteinaceous nature and exterior localization of phycobiliproteins on thylakoid membranes could be one of the reasons for severe damaging effect of elevated temperature on PBPs of control cultures of test organisms. Also there may be disorganization of phycobilisome assembly or partial degradation at elevated temperature which resulted in the decrease of total PBPs content (Bhattacharyya et al., 2011). Further, these water-soluble pigments have been reported to degrade at a faster rate than those of Chl a and Carotenoids (Kumar et al., 2012). There are very few reports regarding study of photosynthetic pigments in presence of sulphide during thermal stress. In one such study, sulphide resistant strain of Microcystis aeruginosa, a mesophilic freshwater organism, was observed to have higher levels of phycoerythrin and phycocyanin than the wild type, therefore, S r strain was able to survive in a sulphide medium (Forte et al., 2016). It is reported that H2S under sulphidic conditions regulates the expression of photosynthetic genes which code for RuBisCO and ferredoxin, this in turn, induces an increase in the chlorophyll content and possibly affects photosystem stoichiometry (PSI:PSII ratio) (Chen et al., 2011). In view of above reports and our results, this can be suggested that thermal stress on affected the photosynthetic pigments negatively and presence of sulphide in the cultures contributed in a positive way. The cyanobacterium Microcystis aeruginosa exhibited increase in chlorophyll a content during thermal stress along with adjusting compatible solutes (soluble protein and sugar) (Han et al., 2015) but our results showed that chlorophyll a content along with other pigments increased, even higher than control cultures, during thermal stress in presence of sulphide. Role of hydrogen sulphide in sulphidic conditions is an area which needs to be explored extensively. Since long, hydrogen sulphide has been known only for its toxicity but recently its pivotal role in anaerobic and aerobic organisms has gained more attention (Llyod, 2013). H2S has been recognized as a member of gasotransmitters or gasomolecule along with the carbon monoxide and nitric oxide. These endogenous transmitters command a very fine regulatory and modulatory role over various cellular functions by exerting their influence on intracellular signaling process. Sulphur induced resistance against biotic stresses has been put forward by many workers. Elemental sulphur and other sulphur containing defense compounds such as phytochelatins, glutathione, and sulphur rich proteins play an important role in plants under both biotic and abiotic stress (Bloem et al, 2004). The role of H2S as a new antioxidant signal molecule has been suggested although molecular mechanism of its role is not well understood (Li et al., 2011; Fu et al., 2013). Exposure to high temperature causes water deficit like conditions (Seyed et al., 2012). Under such conditions, damage to PSII is induced but these effect were curtailed by NaHS (H2S donor), as H2S decreased the production of ROS by increasing the activities of SOD and CAT in higher plants (Li et al, 2015). Comparing growth and photosynthetic pigments in cultures of test organisms under thermal stress in presence of sulphide, it was observed that sulphide supported these parameters maximally in Westiellopsis prolifica. It was observed that enhancement in photosynthetic pigments in presence of sulphide got initiated on 4 d. Presence of sulphide in cultures protected the photosynthetic machinery and photosynthetic pigments from damaging effects of thermal stress.

Conclusion

Data obtained in the present study suggested that

during thermal stress ROS were produced which affected photosynthetic pigments of test organisms. This ultimately led to slow growth of test organisms under thermal stress. Test organisms under thermal stress responded by activating more induction of photosynthetic pigments which protected the organisms from lethality. This can confer on an organism a selective competitive advantage to grow in nature in high sulphide concentration. Photosynthetic pigments protect cyanobacteria from thermal stress by regulating light absorption, energy dissipation, and antioxidant activity. Carotenoids, in particular, are critical for dissipating excess energy and neutralising ROS, hence reducing oxidative damage. The ability of cyanobacteria to change pigment composition and enhance pigment synthesis in response to heat stress is critical to their survival in environments with variable temperatures. Understanding the molecular processes that drive these reactions will aid in the development of strategies for increasing cyanobacteria temperature tolerance, which may have consequences for biotechnology applications such as biofuel production and environmental monitoring.

Competing Interests

Authors have declared that no competing interests exist.

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