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Protective role of photosynthetic pigments in mesophilic cyanobacterium under thermal stress in the presence of sodium sulphide.

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ABSTRACT :

It is widely recognized that sulfur-rich thermal springs are ideal for the growth of thermophilic cyanobacteria. Mesophilic cyanobacteria grow best in temperatures between 28 and 37°C. The purpose for this research was to find out whether mesophilic cyanobacteria could grow at high temperatures when sulphide was present, as well as what potential function sulfide would have in reducing thermal stress in these organisms. An increase in temperature causes an increase in an organism's metabolic rate, which in turn causes an increase in the use of oxygen and the development of an oxidative stress condition in cells. The photosynthetic process of algae cells may be harmed by this (Rady and associates, 1995). However, the current study found that the presence of sulfide enhanced the amount of photosynthetic pigments. Therefore, it has been proposed that the increase in photosynthetic pigments limited the negative effect of heat stress on the test organism's photosynthetic activity.

Keywords: Photosynthetic pigments; mesophilic cyanobacteria; sulphide; high temperature stress.

Objectives: Thermal springs rich in sulfur are an ideal environment for thermophilic cyanobacteria. The temperature range that mesophilic cyanobacteria prefer is 28 to 37°C. The objective of this study was to ascertain whether sulphide could enable mesophilic cyanobacteria to grow at high temperatures and what potential function sulfide might have in reducing thermal stress in these organisms.

Research Methodology: The investigation focused on the growth of *Nostoc ellipososporum* , a mesophilic cyanobacterium, under thermal stress conditions, including its ideal growth temperature and the pre sence or absence of sulphide.The amount of photosynthetic pigments was measured in the same conditions.

Out of nine mesophilic cyanobacterial organisms tested, *Nostoc ellipososporum* was one that grew at 42°C, though at a slower rate than at 28°C. This organism's tolerance threshold for Sulphide was determined by growing it in a range of sodium sulphide concentrations (0.5 to 5 mM).The deleterious effect of thermal stress was eliminated by adding 2.5 mM sulphide to the cell cultures at 42°C. Quantitative analysis of photosynthetic (Chllorophyll a, Carotenoids, Phycocyanin (PC), Allo-phycocyanin (APC), Phycoerythrin (PE) and Total phycobiliproteins (PBPs) in the test organism was done under temperature stress and in the presence of sulphide.

Keywords: Antioxidants; mesophilic cyanobacteria; oxidative stress; sulphide; high temperature stress.

1. Introduction

Blue-green algae, also referred to as cyanobacteria, are gram-negative organisms that are autotrophic. During the Precambrian epoch, approximately 3.5 billion years ago, these species first appeared (Kulasooriya, 2011; Pandey and Pandey, 2013; Singh et al., 2013; Chaurasia, 2015). These species have contributed significantly to the gradual transformation of the Earth's anaerobic atmosphere into an aerobic one because of oxygenic photosynthesis. As a result, oxygen-requiring species began to evolve and subsequently expanded in diversity, eventually taking center stage in the diversity of life on Earth as we know it today. (Vincent, 2009; Rasmussen et al., 2008; Kulasooriya, 2011).

Cyanobacteria's evolutionary history demonstrates that these organisms have flourished in a variety of ecological settings by adjusting to turbulences caused by climatic, geochemical, and human factors. Otten and Pearl, 2013).Cyanobacteria are said to be cosmopolitan since they have the most varied habitats of any living thing on Earth. Per Potts and Whitton (2000). They can be found in severe environments including hot springs, hypersaline regions, freezing temperatures, and dry deserts, but they are most frequently found in freshwater, marine, and terrestrial habitats.(Kashyapet al., 1991; Whitton and Potts, 2000; Kulasooriya, 2011; Nakatsubo and Ino, 1987).A notable feature of cyanobacteria is their ability to survive in a broad range of temperatures. (Chaurasia, 2015; Kulasooriya, 2011).

Psychrophilic, mesophilic, and thermophilic cyanobacteria are categorized according to the range of temperatures in which they thrive optimally. Lakes in the Arctic and Antarctic with mean temperatures between 0°C and 10°C have been reported to harbor psychophilic cyanobacteria (Alexander et al., 1978; Basilier et al., 1978; Davey, 1983; Smith, 1984; Nakatsubo and Ino, 1987; Kashyap et al., 1991; Skulberg, 1996). The ideal temperature range for mesophilic plants is between 25 and 37°C. The ideal temperature range for thermophilic cyanobacteria to flourish is between 45 and 60 oC. Because they can survive at temperatures between 73 and 90°C, cyanobacteria have evolved special adaptations to withstand high temperatures (Castenholz, 1977; Apte, 2011).

Microorganisms have a vital home in sulfur springs. Due to its presence in source water and/or biological sulphate reduction, sulfur is either continuously or sporadically present in many ecosystems. The ability of cyanobacteria to survive in anoxic conditions is demonstrated by their ability to flourish in the presence of hydrogen sulfide. According to Castenholz (1976, 1981), the hot springs that contain sulfur dioxide range in temperature from 42 to 85°C.

The metabolic diversity of hyperthermophilic, sulfur-dependent bacteria that grow at temperatures above 90°C was studied by Adams (1994).

Similar to higher plants, cyanobacteria use water as an electron donor during photosynthesis, which produces oxygen. They have two photosystems, PS-I and PS-II.

Certain cyanobacteria can also perform anoxygenic photosynthesis using only PS-I when electron donors such as hydrogen sulfide are available (Madigan et al., 2003).

Studies on thermophilic cyanobacteria that use sulfur for photoautotrophic growth can be found in the literature. (Bhusare and Wakte, 2011; Giampaoliet al., 2013; Bilyjet al., 2014; Mongra, 2014; Miller and Bebout, 2004).Oscillatorialimnetica was able to employ sulfide as an electron donor for CO_2 assimilation in these studies, suggesting that facultative anoxygenic photosynthesis provides an advantage to organisms that grow in high sulphide concentrations in nature. It was demonstrated that *Oscillatoria amphigranulata*, an isolate from sulfide-

containing hot springs (56°C) in New Zealand, could perform both oxygenic and anoxygenic photosynthesis when sulfide was present (Castenholz and Utkilen, 1984). Similar to this, it has been demonstrated that different isolates of *Microcoleus chthonoplastes* from all over the world are capable of performing both anoxygenic and oxygenic photosynthesis at high sulphide concentrations. (Cohen et al., 1986).

Cyanobacteria, however, differ in their ability to withstand sulfur.

This substance poisons strains that are typically sensitive. According to Castenholz (1977), Garlick et al. (1977), Oren et al. (1979), and Cohen et al. (1986). Those from sulfide habitats, on the other hand, show one or more adaptations to keep their photoautotrophic metabolism going in these circumstances (Castenholz, 1976; Cohen et al., 1975; Cohen et al.,1986).

According to reports, hot springs with temperatures ranging from 42 to 85°C are typically high in sulfur (Castenholz, 1976, 1981). According to Garcia-Pichel and Castenholz (1990), there is a favorable correlation between the proliferation of bacteria and cyanobacteria in hot springs and the amount of sulfur.

It was shown that when the concentration of sulfide increased, the growth of every test organism decreased. Thus, for the sake of this investigation, sodium sulfide was selected as the source of sulfide.

The objective of the present investigation was to ascertain if mesophilic cyanobacteria can tolerate temperatures that above their ideal range when exposed to sulfur dioxide. If true, how does sulfur contribute to the ability to withstand thermal stress? Fields that would not normally sustain the growth of mesophilic cyanobacteria could be enhanced with cyanobacteria at 42 to 50°C using sulfur dioxide.

There are four types of reported adaptations for cyanobacteria to survive in sulphidic conditions: (i) sulphide-resistant oxygenic photosynthesis; (ii) sulphide-sensitive oxygenic photosynthesis; (iii) sulphide insensitive oxygenic photosynthesis concurrent with sulphide-dependent anoxygenic photosynthesis; and (iv) substitution of sulphide-dependent anoxygenic photosynthesis for sulphide-sensitive oxygenic photosynthesis (Cohen 1985; Abed et al., 2006). Different cyanobacteria exhibit varying degrees of sulfide tolerance. This difference might result from an organism's innate ability to adapt to sulfuric conditions and the length of exposure that a specific

organism experiences (Predmore et al., 2012; Qian et al., 2013). The effect of heat stress in the presence of sulphide on the pigments involved in photosynthetic reactions, photosynthesis, and respiration of test microorganisms was examined while keeping in mind the previously mentioned reports.

2. MATERIALS AND METHODS

2.1 Selection of test organism

Eight mesophilic cyanobacteria *Anabaena naviculoides, Nostoc muscorum, Nostoc calcicola,Plectonem aboryanum,Phormidium molle, Nostoc ellipososporum Nostoc punctiforme, Lyngbya faveolarum* from our collection of cyanobacterial cultures were grown for six days at four different temperatures: 37°C, 42°C, 45°C, and 50°C. For this investigation, *Nostoc ellipososporum* was chosen since it showed growth at 42°C (Fig.1)

2.2 Microorganism

The cyanobacterium *Nostoc ellipososporum* is isolate was obtained from Patiala, Punjab (India) rice fields and was kept in the laboratory at Punjabi University Patiala

2.2 Methods

2.2.1 Culture conditions

Chu-10 media was employed in the organisms' cultivation. A pH of 7.8 was chosen for the medium. The stock and experimental cultures, unless otherwise noted, were maintained at 28°C and exposed to 45 µE of light for 14 hours per day. Six-day-old test organism cultures that were growing exponentially were used in all investigations.

2.2.2 Sulphide endurance of the organism

Growing the test organisms in graded doses of sodium sulphide was used to assess its endurance threshold (0.5 m M, 1.0 mM, 2.0 mM, 2.5 mM and 5.0 mM). In 250 mL Erlenmeyer flasks, 100 mL sterilised Chu -10 medium without or with the desired amount of sodium sulphide was added to each flask, along with enough washed inoculum of exponentially developing stock cultures to achieve 0.06 initial absorbance of the cultures at 720 nm. 15 mL samples were taken every 2 days for up to 12 days, and absorbance was measured with a Spectronic 20D+ spectrophotometer (Fig. 2)

2.2.3 Growth under thermal stress

Because the optimal temperature range for the growth of mesophilic cyanobacteria is between 28°C and 37°C, a h igher temperature of 42°C was chosen to cause thermal stress in the cultures.As a result, the organism's growth w as measured as an increase in absorbance at 720 nm at 28°C and 42°C in the absence and presence of sodium sulp hide. Cultures were incubated in a BOD incubator with fluorescent tube lights to study the organism's growth at 4 2°C.

3. Photosynthetic Pigments Estimation:

3 .1 Acetone soluble pigments:

Myers and Kratz's approach (1955) was used to measure the total amount of Chl a

3.2 Water soluble pigments:

The amounts of phycobiliproteins, or phycocyanin (PC), allophycocyanin (APC), and phycoerythrin (PE), were determined using the method proposed by Bennett and Bogorad (1973).

4. RESULTS AND DISCUSSION

4.1Tolerance Level of *Nostoc ellipososporum* to Sulphide

The test organism was grown at varying sodium sulfide concentrations (0.5 mM, 1.0 mM, 2.0 mM, 2.5 mM, and 5.0 mM) in order to determine its endurance threshold. For a maximum of twelve days, 15 mL samples were collected every two days, and a Spectronic 20D+ spectrophotometer was used to quantify absorbance to estimate growth.

4.2 Growth under thermal stress

42°C was selected as the higher temperature to induce thermal stress in the cultures since the ideal temperature range for mesophilic cyanobacteria growth is between 28°C and 37°C. Increases in absorbance at 720 nm at 28°C and 42°C in the presence and absence of sodium sulfide were therefore used to gauge the organism's growth. To observe the growth of the organisms at 42°C spectrophotometer, cultures were kept in a BOD incubator under fluorescent tube lamps.

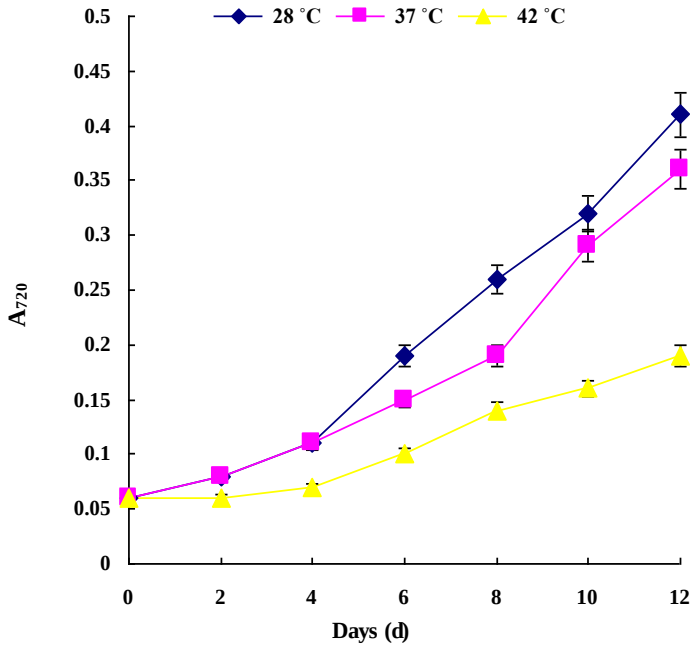

Fig. 1. Growth of *Nostoc ellipososporum* in basal medium.

This was because sulphide is toxic and targets PSII and irreversibly blocks it. Sulphide toxicity is due to its binding with metalloproteins and inhibition in the electron flow in the photosynthetic electron transfer chain Because of its interaction with the electron chain, sulphide is hazardous .

However, cyanobacteria are reported to survive in sulphidic conditions which definitely require acquisitions of certain adaptations by these organisms.

2.5 mM sulphide in medium had a beneficial influence on organism development at 42 °C, 2.5 mM was chosen for further experimentation. It has been suggested that the toxicity and the type of adaptation to sulphide depend on the time and intensity of exposure to sulphide (Cohen 1985; Abed et al., 2006). It is well known that sulphur is required for the growth of all living organisms as it is an essential constituent of amino acids such as cysteine and methionineand some coenzymes. Sulphur is available to microorganisms in the form of sulfate (SO_4^{2-}), thiosulfate ($S_2O_3^{2-}$) and sulphide (S^{2-}). Sulphide is soluble in water at alkaline and neutral pH.Sulphide is important in living systems as it plays structural, regulatory and catalytic functions in the context of proteins, and acts as a major cellular redox buffer in the form of the tripeptide glutathione and certain proteins such as thioredoxin, glutaredoxin and protein disulphideisomerase (Jamal et al., 2010).

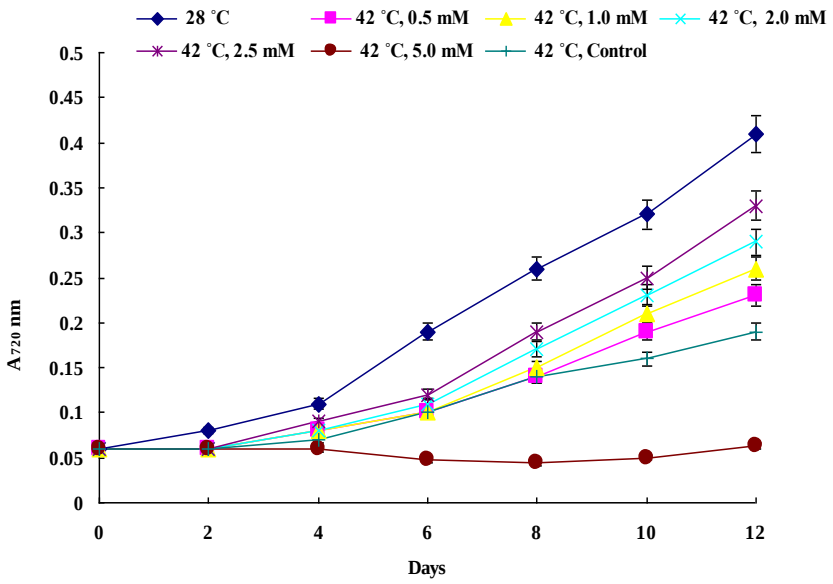

Fig. 2. Growth of *Nostoc ellipososporum* in absence of sulphide at 28 ºC, 42 ºC and presence of sulphide at 42 ºC.

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.

4.3.1 Chlorophyll (Chl) *a* content

On 4 and 6 days, the Chl a content of *Nostoc ellipososporum* cultures maintained at 42 ºC declined by 22.7 and 15.8 %, respectively. Chl a content increased by 9.8% and 15.8% on 4 and 6 days in the presence of sulphide, respectively, when compared to control cultures at 42 ºC.(Fig. 3).

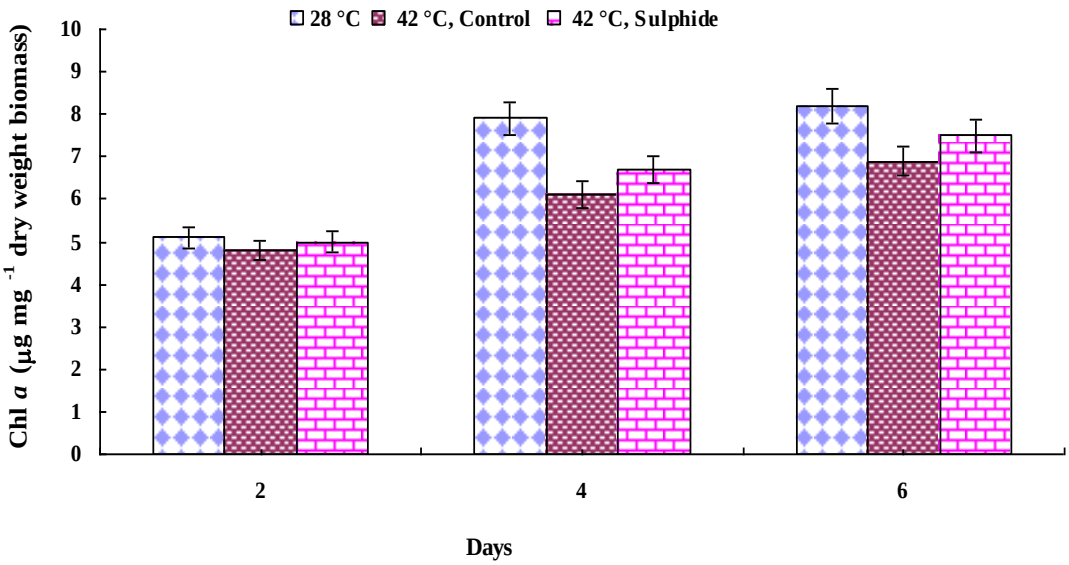

Fig. 3. Chl*a* content in *Nostoc ellipososporum* when grown at 28 °C and 42 °C in absence of sulphide and at 42 °C in presence of sulphide.

4.3.2 Carotenoid (Car) content

The Car content of cultures of *Nostoc ellipsosporum* grown at 28°C increased from 14.0 µg mg^{-1} biomass dry weight on zero d to 17, 19 and 29 µg mg^{-1} biomass dry weight on 2 d, 4 d and 6 d, respectively. Cultures grown at 42 °C exhibited decrease in Car content by 11.1%, 10.5% and 13.7% on 2 d 4 d and 6 d, respectively. In presence of sulphide, the Car content increased to almost same level of content of cultures at 28 °C (Fig. 4).

High temperature lowered the content of organic as well as water soluble pigments of the control cultures of test organisms. The Chl a content of *Nostoc ellipososporum* decreased by 22.7% and15.8% on 4 d and 6 d, respectively While in presence of sulphide the Chl a content of all selected organisms got enhanced significantly. 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 productionof free radicals (Latifi *et al*., 2009). It has been shown that Chla content of *M. aeruginosa* subjected to temperature stress at 35°C was significantly lower than Chl a content of cultures at25°C (Han *et al*., 2015). 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). Thus, the effect of thermal stress on carotenoids of test organisms was studied.

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 Chl a and carotenoids was more than the control cultures of test organisms. This indicated protective role of sulphide.

In *Nostoc ellipososporum* carotenoids leve ldecreased by 13.7% on 6 d under thermal stress at 42 °C It was observed that in presence of sulphide carotenoid content of *Nostoc ellipososporum* (29 µg mg $^{-1}$ dry weight biomass) nearly reached to the carotenoid levels of respective controls. 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.

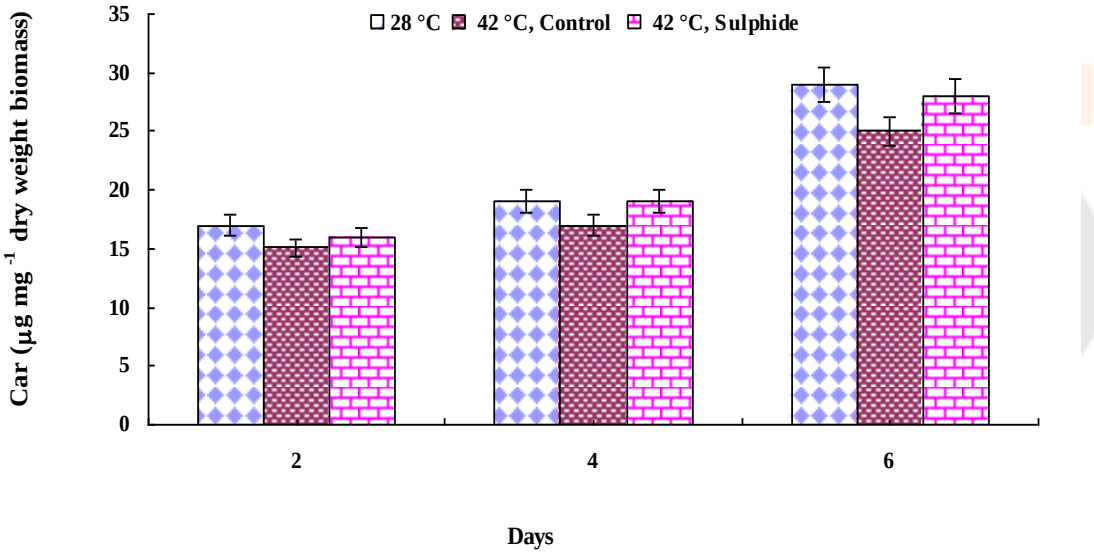

Fig. 4. Carotenoid content in *Nostoc ellipososporum* when grown at 28 °C and 42 °C in absence of sulphide and at 42 °C in presence of sulphide.

4.3.3 Water soluble photosynthetic pigments

4.3.3.1 Phycocyanin (PC) content

When the cultures of *Nostoc ellipososporum* were grown at 42 °C, the PC content decreased by 5.7%, 14.2% and 11.1% on 2 d, 4 d and 6 d, respectively as compared to PC content of cultures at 28 °C. But in presence of sulphide, PC content increased to the level of PC content of cultures at 28 °C (Fig. 5).

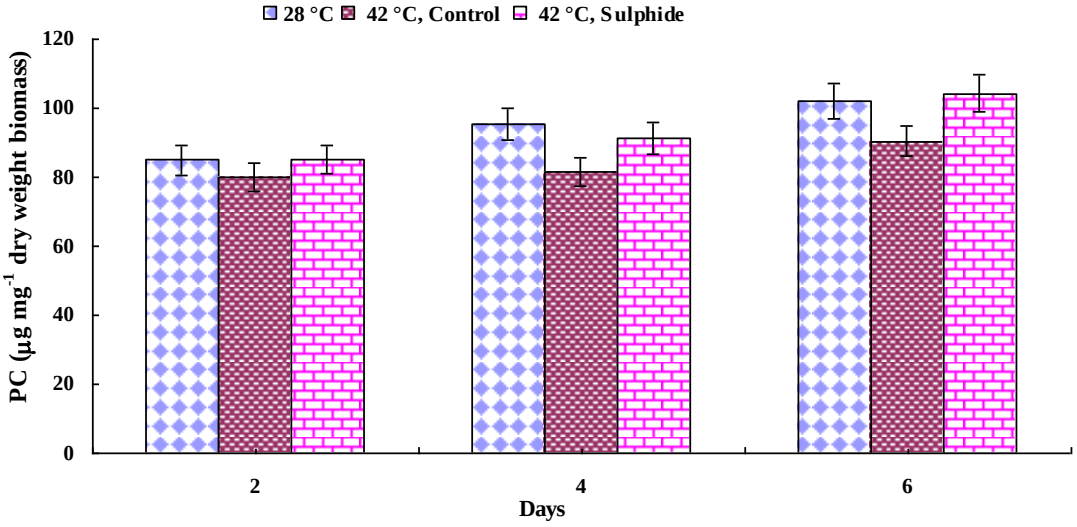

Fig. 5. Phycocyanin content in *Nostoc ellipososporum* when grown at 28 °C and 42 °C in absence of sulphide and at 42 °C in presence of sulphide.

4.3.3.2. Allo-phycocyanin (APC) content

When the cultures of *Nostoc ellipososporum* were grown at 42 °C, the APC content decreased by 8.9%, 10.2% and 16.6% on 2 d, 4 d and 6 d, respectively. In presence of sulphide the APC content increased to the level of APC content of cultures at 28 °C (Fig. 6).

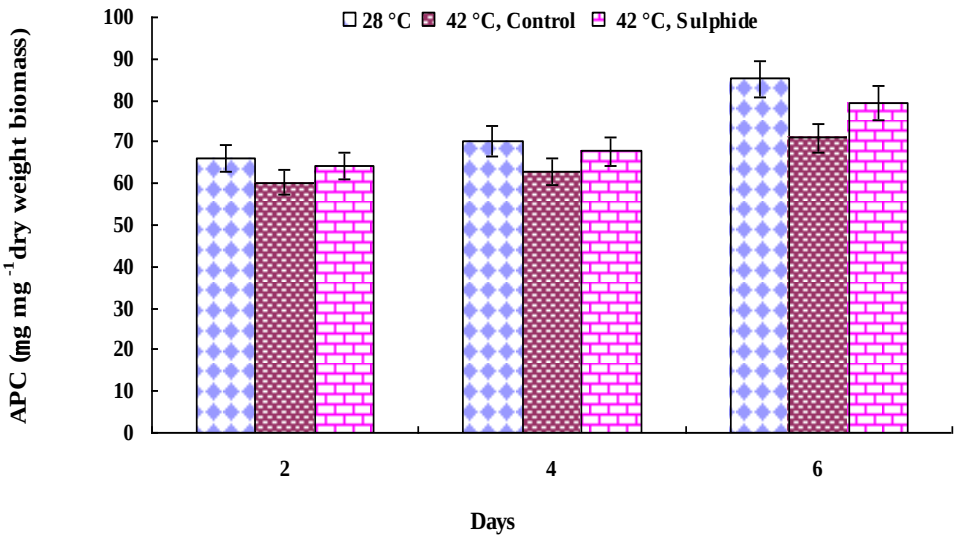

Fig.6. Allo-phycocyanin content in*Nostoc ellipososporum* when grown at 28 °C and 42 °C in absence of sulphide and at 42 °C in presence of sulphide.

4.3.3.3 Phycoerythrin (PE) content

The effect of thermal stress on PE content of all the four test microorganisms in absence and presence of sulphide was similar to that of PC and APC content (Fig. 7).

PE content of *Nostoc ellipososporum* cultures grown at 28 °C increased from 57.9µg mg^{-1} biomass dry weight on zero d to 71.9, 78.5 and 97.4 mg^{-1} biomass dry weight on 2 d, 4 d and 6 d, respectively. The cultures grown at 42 °C in absence and presence of sulphide exhibited almost same PE content.

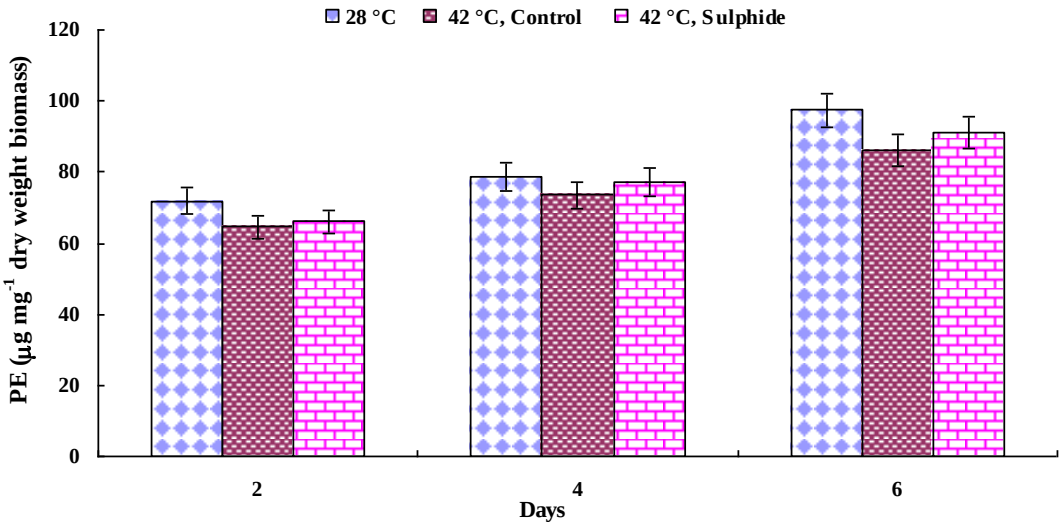

Fig. 7. Phycoerythrin content in *Nostoc ellipososporum* when grown at 28 °C and 42 °C in absence of sulphide and at 42 °C in presence of sulphide.

4.3.3.4 Total phycobiliproteins (PBPs) content

The effect of thermal stress ontotal PBPs content of *Nostoc ellipososporum* is shown in Fig. 31. When the cultures were grown at 42 ^{o}C, total PBPs content decreased by 7.2% on 2 d and 4 d while on 6 d decrease in PBPs content was 12.1%. In presence of sulphide the total PBPs increased to the level of total PBPs of cultures at 28 ^{o}C (Fig. 8).

Phycobiliproteins are important light-harvesting pigments located at the outer surface ofthe 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 onphycocyanin (PC), allophycocyanin (APC), phycoerythrin (PE) and total phycobiliproteins (PBPs) content of test microorganisms was studied. It was observed that high temperature affected these in a time dependent manner. When cultures of test organisms were grown at high temperature there PC contentdecreased. PC content in *Nostoc ellipososporum*, PC content of sulphide cultures at 42 °Cincreased to the level of PCcontent of cultures at 28 °C. Though thermal stress led to a decrease in PC content, theirsignificant increase in presence of sulphide was responsible for the growth of test organismunder thermal stress.

Similar results were obtained for APC and PE content of test organisms. Both the pigments decreased in control cultures at high temperature but in presence of sulphide their content enhanced thus supporting growth of test organisms. When the cultures of test organisms were grown at elevated temperature a decrease of12.1% to 24.1% in PBPs content was noted on 6 d. But when the cultures were grown in presence of sulphide, there was an increase of 18.4% in PBPs Proteinaceous nature and exterior localization of phycobiliproteins on thylakoid membranes could be one of the reasons for severe damaging effect of elevated temperature onPBPs 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 beenreported to degrade at a faster rate than those of Chla and Carotenoids (Kumar et al., 2012).

It was observed that enhancement in photosynthetic pigments in presence of sulphide at elevated temperature was initiated on 4 d but photosynthesis and respiration were affected on 2d.There are very few reports regarding

study of photosynthetic pigments in presence ofsulphide during thermal stress. In one such study, sulphide resistant strain of *Microcystis aeruginosa*, a mesophilic freshwater organism, was observed to have higher levels ofphycoerythrin 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 H_2S under sulphidic conditions regulatesthe 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. This effect was true for both mesophilic and thermophilic cyanobacteria studied presently. 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.

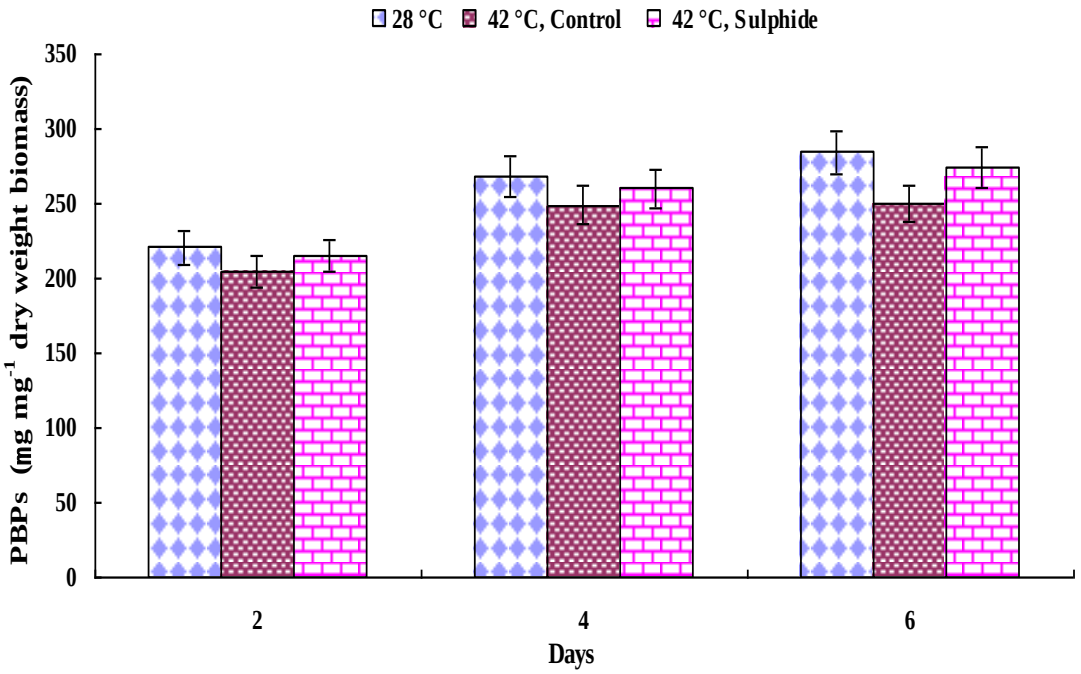

Fig. 8. Total phycobili protein content in *Nostoc ellipososporum* when grown at 28 °C and 42 °C in absence of sulphide and at 42 °C in presence of sulphide.

It has been known since long that few cyanobacteria can switch between anoxygenic andoxygenic photosynthesis which depends upon (i) the H_2S concentration which is connected to the toxicity of H_2S for PSII (ii) concentration-dependent activity of enzyme sulphide: quinine oxidoreductase (SQR), which catalyzes H_2S oxidation in anoxygenic photosynthesis (iii) the duration of exposure of microorganisms to sulphide and light because de novo synthesis of SQR

has to be induced (Klatt, 2016). It has been reported that SQR catalyzes sulphide oxidation during sulphide-dependent chemo-and phototrophic growth of bacteria (Chan et al., 2009).Cyanobacteria which are resistant to sulphide have copies of sqr (Miller and Bebout, 2004).

Cyanobacteria such as *Anabaena* PCC 7120 cannot survive in sulphidic conditions as they are not able to perform anoxygenic photosynthesis (Miller and Bebout, 2004). On the other hand, *Geitlerinema* sp. PCC 9228 was reported to perform anoxygenic photosynthesis as they possess gene for sulphide quinine reductase (SQR) (Grimand Dick, 2016; Klatt et al., 2016). Induction of anoxygenic photosynthesis in somecyanobacteria is reported to occur during 2 to 10 h induction period in sulphidic conditions (PostandArieli, 1997). Sulphide-dependent anoxygenic photosynthesis is reported in *Aphanoth ecehalophytica* and *Synechococcu*s 6311 (Oren et al., 1979). *Synechocystis* sp. strain PCC 6803 is reported to exhibit sulphide-based alternative photosynthesis due to activation of specific genesin the presence of sulphide that encode a light-dependent type-I sulphide:quinine oxidoreductase (Nagy et al., 2014). *Oscillatoria amphigranulata*, an isolate from sulphide enriched hot springs of New Zealand, was shown to perform anoxygenic and oxygenic photosynthesis in presence of sulphide (Castenholz 1976; Castenholz and Utikelen, 1984). Similarly, several isolates of *Micro coleus cthonoplastes* have been shown to possess the ability of simultaneously performing anoxygenic and oxygenic photosynthesis under relatively high sulphide concentrations (Cohen et al., 1986).Sulphide is known to support anoxygenic photosynthesis and stimulate H_2 production in N_2 -fixing filaments of *Nostoc muscorum* (Luo and Mitsui, 1996). *Pseudanabaena* strain FS39 was reported to perform anoxygenic photosynthesis after 2 h exposure to very high concentration ssulphide of (10 mM). *Geitlerinema* sp. PCC 9228 (formerly *Oscillatoria limnetica*) can perform anoxygenic photosynthesis in presence of sulphide (Klatt et al., 2015).

Oscillatoria strains WHS-4 and DV-00-7, *Arthrospira* strain PCC 8005, *Lyngbya* strain PCC 7419, *Cyanothece* strain PCC 7418, *Pseudanabaena* strain CCMEE 5435, *Geitlerinema*

strain B33, *Leptolyngbya* strain PCC 7375 and *Synechococcus* strain PCC 7335 attained theability to utilize sulphide as an electron donor in photosynthesis during the course of evolutionand are thus capable of surviving in sulphidic habitats (Miller and Bebout, 2004).Resistance of PSII to sulphide toxicity, allowing oxygenic photosynthesis in presence ofsulphide, seems to operate in test organisms. Reports on cyanobacteria performing oxygenicphotosynthesis in presence of sulphide are available. *Geitlerinema*, *Leptolyngbya* and*Oscillatoria*, isolated from widely varying regions, were able to tolerate sulphide-rich environment and performed oxygenic photosynthesis in the presence of sulphide (Myers andRichardson, 2009). *Geitlerinema* performed oxygenic

photosynthesis in the presence of sulphideeven when this strain was maintained under aerobic (sulphide-free) conditions for 5 years beforethe experiments were conducted (Richardson and Kuta, 2003). *Pseudanabaena* was reported to perform oxygenic photosynthesis at a much lower rate in the presence of sulphide (Dodds and Castenholz.1990). Hydrogen sulphide in sulphidic environments can enhance oxygenic photosynthesis in cyanobacteria as H_2S up to 210 µM accelerated the recovery of photosynthesis after exposure to darkness and the higher photosynthetic activity was accompanied by higher respiratory rates (Klatt et al., 2015).Toxicity of sulphide to microorganisms is pH dependent (Hawsley and Pearson, 1979). In *Dactylococcopsis salina*, oxygenic photosynthesis was observed at pH range from 6.5 to 9.2 (Rijin and Cohen, 1983). During present study, the pH of culture medium was maintained as 7.8.

Thus, this pH can also be one of the reasons that oxygenic photosynthesis was operating in testorganisms. On the basis of our results and above discussion it can be suggested that sulphideresistant oxyenic photosynthesis is responsible for the growth of test organisms in presence ofsulphide. According to Cerveny et al. (2015), cyanobacteria are not able to tolerate thermal stresswhen their growth ceases, photosynthetic and respiratory activities decline and, all pigments aredestroyed. But our results suggested that all these parameters were enhanced in presence ofsulphide. Therefore, growth of selected organisms was better under the thermal stress in presenceof sulphide.

5. Discussion

Four types of adaptations for survival of cyanobacteria in sulphidic conditions arereported (i) sulphide-resistant oxygenic photosynthesis (ii) sulphide-sensitive oxygenicphotosynthesis (iii) sulphide insensitive oxygenic photosynthesis concurrent with sulphidedependentanoxygenic photosynthesis (iv) sulphide-sensitive oxygenic photosynthesis replacedbysulphide-dependent anoxygenic photosynthesis (Cohen 1985; Abed et al., 2006). The level oftolerance to sulphide vary in different cyanobacteria. This variation may be due to inherentcapacity of the organism to adapt to sulphidic conditions and time of exposure to which aparticular organism is subjected (Predmore et al., 2012; Qian et al., 2013). Keeping in view theabove reports, the effect of thermal stress, in presence of sulphide, on photosynthetic pigments,photosynthesis and respiration of test microorganism was studied.

6. Conclusions

At 42°C, the mesophillic cyanobacterium *Nostoc elliposorum* grew slowly.When basal media was supplemented with 2.5 mM sulphide, the test organism grew at a faster rate than control cultures at 42°C. When the mechanism of survival at high temperatures

was analysed in context with the photosynthetic pigments system under thermal stress, it was discovered that at sulphide prompted photosynthetic pigments (Chllorophyll a, Carotenoids, Phycocyanin (PC), Allo-phycocyanin (APC), Phycoerythrin (PE) and Total phycobiliproteins (PBPs) content that defended the organism from thermal stress.

COMPETING INTERESTS

The authors have declared that there are no competing interests.

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