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Sulphide Ameliorates Thermal Induced Oxidative Stress in a Mesophilic Cyanobacterium *Westiellopsis prolifica*

Jasvirinder Singh Khattar[1*], Manpreet Kaur[1] and Davinder Pal Singh[1]

[1]Department of Botany, Punjabi University, Patiala-147002, India.

Authors' contributions

This work was carried out in collaboration between all authors. Authors JSK and DPS conceived and designed the experiments. Author MK performed the experiments and prepared the initial draft of the manuscript. All authors read and approved the manuscript for publication.

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Original Research Article

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ABSTRACT

Aims: Thermophilic cyanobacteria generally grow in sulphur containing thermal springs. Mesophilic cyanobacteria have optimal temperature in the range of 28℃ to 37℃. The aim of the study was to see whether mesophilic cyanobacteria can grow at elevated temperature in the presence of sulphide? If yes, what is the role of sulphide in combating thermal stress in the mesophilic cyanobacteria?
Study Design: Growth of mesophilic cyanobacterium *Westiellopsis prolifica* was studied at its optimum growth temperature and in presence and absence of sulphide under thermal stress. Simultaneously, level of enzymatic and non-enzymatic antioxidants was studied under above conditions.
Place and Duration of Study: Department of Botany, Punjabi University, Patiala-147002, India, from November 2014 to December, 2015.
Methodology: Out of nine mesophilic cyanobacterial organisms screened, only *Westiellopsis prolifica* exhibited growth at 42℃, though less than at 28℃. T he tolerance level of this organism to

**Corresponding author: E-mail: jisk_pbi@rediffmail.com;*

sulphide was determined by growing it in graded concentrations (0.5 to 5 mM) of sodium sulphide. Supplementation of 2.5 mM sulphide in the cultures at 42℃ negated the negative effect of thermal stress. Enzymatic (SOD, POD, and CAT) and non-enzymatic (glutathione, proline, and ascorbic acid) antioxidants in the test organism grown under thermal stress and in the presence of sulphide were determined.

Results: It was observed that level of enzymatic and non-enzymatic antioxidants (glutathione) increased while the content of ascorbic acid decreased in the presence of sulphide.

Conclusion: The organism exhibited less growth with a simultaneous increase of antioxidants such as SOD,POD, and CAT. This is the first report on the role of sulphide in ameliorating thermal stress in mesophilic cyanobacterium *Westiellopsis prolifica* by triggering enzymatic and non-enzymatic antioxidant defense system. This indicated that under thermal stress reactive oxygen species were produced which were scavenged by these antioxidants. Whether this is true for mesophilic cyanobacteria, in general, can be tested by studying some more strains.

Keywords: Antioxidants; mesophilic cyanobacteria; oxidative stress; sulphide; thermal stress.

1. INTRODUCTION

One of the most remarkable features of cyanobacteria is their ability to survive in an extreme range of habitats. On one hand, they are widespread in freshwater, marine, and terrestrial ecosystems, on the other hand, these are found in extreme habitats such as hot springs, hyper saline localities, freezing environments and arid deserts [1-4]. Strategies employed by cyanobacteria to survive in such extreme environments include the development of certain adaptive mechanisms such as protection of cells from freeze–thaw damage, ultra-violet radiation, desiccation, alkalinity and production of a diverse array of secondary metabolites that contribute to survival in various competitive ecological environments [3,5,6]. In context with their optimal range of temperature for growth, cyanobacteria have been classified as psychrophilic, mesophilic and thermophilic. Psychrophilic cyanobacteria are known to occur in Arctic and Antarctic lakes [1,2,7,8] with a mean temperature range between 0℃ and 10℃ for growth. The mesophilic cyanobacteria have optimum growth temperatures between 25℃ and 37℃. The thermophilic ones have higher temperature optima ranging from 45℃ to 60℃ [9]. Cyanobacterial strains such as *Phormidium tenue*, *Mastigocoleus laminosus* and *Synechococcus elongatus* var. *amphigranulatus* are among the common species which have the ability to grow at temperature as high as 69℃ [10]. The temperature range in the most of the sulphide containing hot springs is reported to be between 42℃ to 85℃ [11]. Adams [12] reviewed the biochemical diversity of sulphur dependent, hyperthermophilic microorganisms that grow at or above 90℃. These currently comprise 20 genera which all are classified as Archea [4].

Sulphide is either permanently or periodically present in many ecosystems where cyanobacteria are found as a result of its presence in source water and/or biological sulphate reduction [13].Cyanobacterial strains that typically are not exposed to sulphide in natural environments get irreversibly poisoned when exposed to an even low concentration of this chemical [14,15]. In contrast, strains from sulphide habitats such as hot springs exhibit one or more adaptations for maintaining their photoautotrophic metabolism active under such conditions [16,17]. Several cyanobacteria inhabiting sulphur rich habitats also perform oxygenic photosynthesis using the only photosystem-I if electron donors such as hydrogen sulphide are present [18-19].

It is well known that thermophilic cyanobacteria grow at higher temperatures in the presence of sulphide. Mesophiles grow optimally at a temperature range of 28℃ to 35℃. But when mesophilic organisms are subjected to high temperature, it induces oxidative stress leading to slow or no growth. Higher temperature creates drought or water stress along with thermal stress. Water stress occurs by the loss of water from the cells in spite of its availability [20]. Water stress creates an imbalance between light capture and its utilization, and excess light energy is dissipated in the photosynthetic apparatus resulting in the generation of ROS. Thus removal of free radicals generated during abnormal and regular processes is essential [21].

Cyanobacteria have evolved mechanisms to confront water and heat stress. Some of the

mechanisms involve regulation of ion fluxes for osmotic adjustment, selective expression of tolerance genes and novel stress proteins to repair oxidative damage caused by an excess of free radicals [9]. In response to oxidative stress, activation of radical scavenging compounds such as ascorbate, glutathione and superoxide dismutase, peroxidase and catalase are known to occur.

The organisms generally respond to the oxidative stress by the activation of the expression of genes responsible for the production of enzymatic or non-enzymatic antioxidants [22]. The level of antioxidative protection is proportional to tolerance and adaptation of organisms to stress conditions. It is the efficiency of the antioxidative defense system which determines the fate of organisms during the harsh conditions [23].

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 antioxidative defense system is triggered to counter thermal induced oxidative stress in mesophilic cyanobacteria. This can be a basis for the enrichment of fields by cyanobacteria at 42℃ to 50℃ 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 Materials

2.1.1 Selection of test organism

The growth of nine mesophilic cyanobacteria from our cyanobacterial culture collection was studied for 6 d at four temperatures i.e. 37℃, 42℃, 45℃ and 50℃. Only *Westiellopsis prolifica* exhibited growth at 42℃ and was selected for the present study.

2.1.2 Microorganism

Westiellopsis prolifica, is an isolate of our laboratory from rice fields of Patiala (30° 19' N and 76°24' E), Punjab, India.

2.2 Methods

2.2.1 Culture conditions

The organism was propagated in Chu-10 medium [24]. The medium contained 0.232 g $CaCl_2.2H_2O$, 0.025 g $MgSO_4.7H_2O$, 0.02 g Na_2CO_3, 0.044 g $Na_2SiO_3.5H_2O$, 0.01 g K_2HPO_4, 0.0035 g each of citric acid and ferric citrate and 1g KNO_3 per liter. The pH of the medium was adjusted to 7.8. The stock and experimental cultures were maintained at 28±2℃ unless otherwise stated and illuminated for 14 h daily with a light intensity of 45 µE. Exponentially growing cultures (6 d old) of the test organism were used for all experiments.

2.2.2 Tolerance of the organism to sulphide

The tolerance level of the test organism was determined by growing it in graded concentrations of sodium sulphide (0.5 mM, 1.0 mM, 2.0 mM, 2.5 mM and 5.0 mM). Total 100 mL sterilized Chu-10 medium without or with the desired amount of sodium sulphide was taken in 250 ml Erlenmeyer flasks and sufficient volume of washed inoculum of exponentially growing stock cultures was added to each flask to obtain 0.06 initial absorbance of the cultures at 720 nm. After every 2 d, up to 12 d, 15 mL samples were withdrawn and absorbance was noted with Spectronic 20D+ spectrophotometer.

2.2.3 Growth under thermal stress

Since the optimal temperature range for the growth of mesophilic cyanobacteria is between 28℃ and 37℃, 42℃ was chosen as higher temperature to induce thermal stress to the cultures. Thus the growth of the organism was studied at 28℃ and 42℃ in absence and presence of sodium sulphide as an increase in absorbance at 720 nm. To study growth of the organism at 42℃, cultures were incubated in a BOD incubator fitted with fluorescent tube lights.

2.2.4 Antioxidant enzymes

The activity of Superoxide dismutase (SOD) was determined as photochemical inhibition of reduction of nitroblue tetrazolium chloride (NBT) following Beauchamp and Fridovich [25]. A known volume of experimental cultures was harvested at desired time by centrifugation at 5000 g, washed twice with double distilled water, resuspended in 4 ml of 100 mM phosphate buffer (pH 7.8) and disintegrated with a sonicator (Soniprep 150, Sanyo, UK).The supernatant obtained after centrifugation 15,000 g (4℃) for 20 min was used as an enzyme extract. The cocktail mixture for SOD contained 27 ml of 100 mM phosphate buffer (pH 7.8), 1 mL of NBT (2.25 mM), 1.5 mL of methionine (200 mM), 1 mL of Na_2CO_3 (1M) and 1.5 mL of EDTA (3 mM). To 2.7 mL of the reaction mixture taken from cocktail solution, 0.2 mL enzyme extract was added and

the reaction was started by the addition of 0.2 mL of riboflavin (60 µM). The reaction mixture was illuminated for 10 min with daylight fluorescent tubes (45 µE). The reaction was terminated by transferring test tubes containing reaction mixture to the dark. Identical reaction mixture kept in dark served as blank. The test tube containing reaction mixture with enzyme extract kept in dark served as blank and control tube without enzyme extract kept in light served as a control for 100% colour development. The absorbance of control test tube containing reaction mixture without enzyme and test tube with enzyme extract with their respective blank was measured at 560 nm. The percent inhibition of NBT reduction was calculated as:

$$Y=\frac{(\text{Absorbance of A - Absorbance of B})}{\text{Absorbance of A}} \times 100$$

where A=Control tube without enzyme kept in light, B=Test tube with enzyme extract kept in light.

One unit of SOD activity is defined as the amount of protein (mg) required to cause 50% inhibition in the reduction of NBT under the light.

Peroxidase (POD) activity was measured following Gahgen et al. [26]. The assay mixture contained 2.0 mL double distilled water, 0.35 mL phosphate buffer (100 mM, pH 6.8), 0.2 mL of 0.5% H_2O_2, 0.3 mL of 5% pyrogallol and 0.2 mL of enzyme extract. The increase in absorbance of the assay mixture was recorded at 420 nm for 5 min against blank. The amount of purpurogallin formed was determined by using 2.64 as the millimolar extinction coefficient for purpurogallin at 420 nm. One unit of enzyme is defined as µmol of purpurogallin formed μg^{-1} protein min^{-1}.

Catalase (CAT) was measured by measuring the amount of O_2 released from the dissociation of H_2O_2 in dark following the method of Egarshira et al. [27]. A known volume of experimental cultures was centrifuged at 5,000 g, washed twice with double distilled water and resuspended in phosphate buffer (50 mM, pH 7.0). The cell suspension (2 mL) was kept in the reaction vessel fitted to oxygen analyzer (YSI, USA). One mL of H_2O_2 (100 mM prepared in 50 mM phosphate buffer) was added to the cell suspension contained in the reaction vessel and kept in the dark. The amount of oxygen released due to enzymatic dissociation of H_2O_2 was measured as dissolved oxygen in the analyzer. One unit of CAT is defined as nmol O_2 released from H_2O_2 mg^{-1} protein min^{-1}.

2.2.5 Level of non-enzymatic antioxidants

Cellular glutathione (GSH) content was determined following the method of Ellman [28]. Sufficient volume of experimental cultures was harvested, washed with distilled water and sonicated in 5 mL of 0.2 M phosphate buffer (pH 8.0) to obtain one mg protein per ml of the extract. The supernatant obtained after centrifugation at 20,000 g (4℃) for 20 min was used for the estimation of GSH. The assay mixture was prepared by mixing 0.2 mL of crude cell extract, 0.5 mL of 3-3'-dithiol(6-nitrobenzoic acid), (0.02% in 0.1% sodium citrate) and 3 mL of 0.2 M phosphate buffer (pH 8.0). The absorbance of yellow colour developed was measured at 412 nm. GSH content was expressed in µg mg^{-1} protein. Reduced glutathione served as standard.

Cellular Proline content was measured following the method of Bates et al. [29]. Crude cell extract of experimental cultures was prepared in 3% sulphosalicylic acid by sonication and centrifuged at 20,000 g for 20 min at 4℃. Ninhydrin solution was prepared by dissolving 1.25 g ninhydrin in 30 mL glacial acetic acid and to this were added 20 mL of 6 M phosphoric acid with continuous agitation. The assay mixture was prepared by mixing 2 mL of cell-free extract, 2 ml each of glacial acetic acid and freshly prepared ninhydrin solution. The contents were incubated in a water bath at 100℃ for 1 h. The reaction was terminated by transferring the test tubes to an ice-bath. Then, 4 mL of toluene were added to the assay mixture and stirred for 20–30 s. The toluene layer was separated and the absorbance of red colour was measured at 520 nm. Proline content was expressed in µg mg^{-1} protein. Proline served as standard.

The cellular ascorbic acid content was estimated following the method of Roe and Keuther [30]. Washed experimental cultures were sonicated in 3 ml of 4% TCA and to the supernatant, obtained after centrifugation at 2000 g for 10 min, a pinch of activated charcoal was added, shaken and kept at room temperature for 5 min. Contents were again centrifuged to remove the charcoal particles and cell-free supernatant was used for the estimation of ascorbic acid. To the 2 ml of the extract obtained above, 0.5 ml DNPH (2, 4-dinitrophenyl hydrazine reagent, 2% in 9 N H_2SO_4), 2.5 ml of TCA (4%) and 0.1 ml thiourea (10%) solution were added. The contents were incubated at 37℃ for three hours resulting in the formation of osazone crystals. Orange coloured

osazone crystals were dissolved in 85% sulphuric acid. The absorbance of the orange coloured solution thus obtained was recorded at 540 nm. Ascorbic acid content was expressed in µg mg^{-1} protein.

Protein content in the crude enzyme cell extract was determined following Lowry et al. [31].

2.3 Statistical Analysis

All the data are the average of three independent experiments ± Standard Deviation (SD). Data were statistically analyzed by applying one-way analysis of variance and Tukey's honest significance difference test. All statistical analyses were tested against the probability value at 95% confidence level ($p < 0.05$) using GraphPad Prism 6.0 version 6.0 (www.graphpad.com).

2.4 Chemicals

All chemicals used in media preparation and enzymatic assays were obtained from SD- FINE, India.

3. RESULTS AND DISCUSSION

3.1 Tolerance Level of *Westiellopsis prolifica* to Sulphide

In preliminary experiments, growth of nine mesophilic cyanobacteria for 6 d was studied at 37℃, 42℃, 45℃ and 50℃ in terms of increase in absorbance at 720 nm. Results revealed that none of the tested mesophilic cyanobacteria survived at temperature 45℃ and the cells lysed. However, only *Westiellopsis prolifica* was able to grow at 42℃ (Table 1).Thus this organism was selected for the present study and its growth at 28℃, 37℃ and 45℃ was compared. It was observed that on 12 d there was 10% decrease in growth at 37℃ and 38.0% decrease in the growth of *Westiellopsis prolifica* at 42℃ as compared to the growth of test organism at 28℃ (Fig. 1). When the growth of the test organism was studied at 42℃ in absence and presence of a graded concentrations of sulphide, it was observed that sulphide up to 2.5 mM in the medium supported more growth of test organism at 42℃ in a concentration-dependent manner (Fig. 2). The absorbance of cultures at 28℃ increased from 0.06 on zero day to 0.54 on 12 day. The increase in absorbance of cultures in presence of 0.5 mM, 1.0 mM and 2.0 mM and 2.5 mM at 42℃ 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℃ which indicated that this concentration of sulphide was toxic to the organism. Toxicity of sulphide is known due to its binding with metalloproteins and inhibition in the electron flow in the photosynthetic electron chain [16,19,32]. Since the presence of 2.5 mM sulphide in the medium exerted a positive effect on the growth of the organism at 42℃, this concentration of sulphide was selected for further experiments.

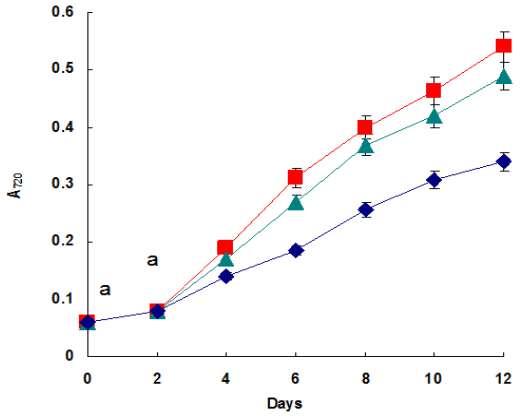

Fig. 1. Growth of *Westiellopsis prolifica* at 28℃ (■), 37℃ (▲) and 42℃ (◆ in basa l medium

Data with same small alphabets are not significantly different from each other at 95% confidence level ($p < 0.05$)

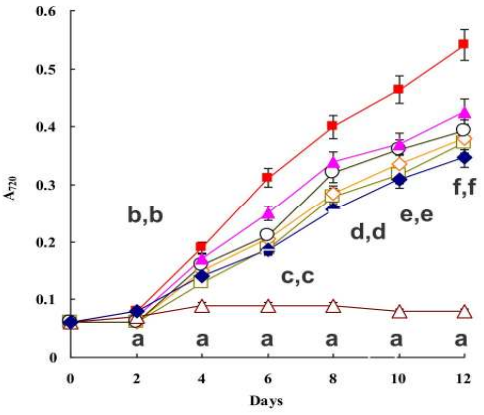

Fig. 2. Growth of *Westiellopsis prolifica* in absence of sulphide at 28℃ (■), 42℃ (◆ and presence of sulphide at 42℃. 0.5 mM (□), 1.0 mM (◇), 2.0 mM (⬡), 2.5 mM (▲), 5.0 mM (△) sulphide

Data with same small alphabets are not significantly different from each other at 95% confidence level ($p < 0.05$)

Table 1. Growth of mesophilic cyanobacteria at different temperatures

Organism	Temperature (°C)			
	37	42	45	50
Anabaena naviculoides RFJ1	+	-	-	-
Nostoc muscorum PUPCCC104.10	+	-	-	-
Nostoc calcicola RFKP2	+	-	-	-
Plectonema boryanum SR28	+	-	-	-
Phormidium molle SR14	+	-	-	-
Anabaena sp.2 RFP32	+	-	-	-
Westiellopsis prolifica PUPCCC514.1	+	+	-	-
Nostoc punctiforme RFKP2	+	-	-	-
Lyngbya faveolarum PWH6	+	-	-	-

3.2 Antioxidant Enzymes

Temperature stress in mesophilic organisms may lead to the generation of free radicals. During the process of evolution, microorganisms have acquired mechanisms to defend reactive oxygen species. Such a system includes antioxidative enzymes such as superoxide dismutase, catalase, ascorbate, peroxidase and glutathione reductase [33]. Differences in survival rates among species or ecotypes in a wide range of taxa are determined by the greater and more refined capability to scavenge ROS in comparison to their sensitive or less tolerant counterparts [34]. The responses of microorganisms to changes in abiotic conditions are generally very quick leading to the acquisition of acclimation by the organisms within a few hours of exposure [35]. Thus, the level of antioxidant enzymes in the test organism at 42℃ was measured after 48, 96 and 144 h after inoculation.

The activity of SOD in control cultures after 48 h of inoculation incubated at 42℃ was 36.6% more as compared to cultures at 28℃. In the presence of sulphide, SOD activity of the cultures at 48 h was 86.0% more than the control cultures at 42℃. At 96 and 144 h though the SOD activity slightly decreased even then, it was 44.4% and 24.1% more, respectively, over control cultures at 42℃ (Fig. 3). SOD is considered as the first line of defense against superoxide anion and is known to catalyze the conversion of two superoxide anions $O_2^{\cdot -}$ into a molecule of H_2O_2 and O_2. Catalase and/or peroxidase act on H_2O_2 to form water and oxygen and thus the reaction initiated by SOD to neutralize superoxide anions is completed [23,33,36]. Wen-yan et al. [37] reported that survival of *Spartina alterniflora* in the high sulfur environment was attributed to its enzymatic antioxidant defensive activities of SOD and ascorbate peroxidase which increased when the organism was exposed to high levels of Na_2SO_4.

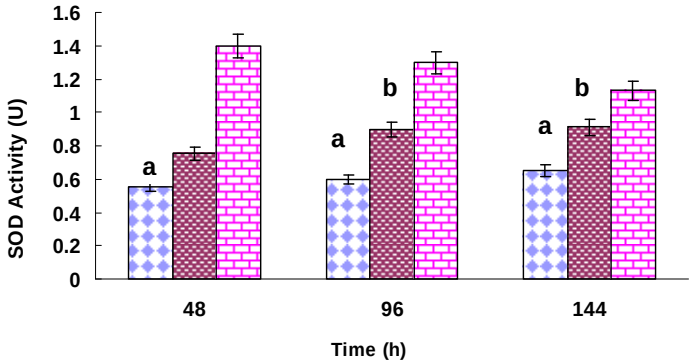

Fig. 3. Activity of SOD in *Westiellopsis prolifica* when grown in absence of sulphide at 28℃ ( ) at 42℃ ( ) and in presence of sulphide at 42℃ ( )

SOD activity at zero h: 0.58 U

Data with same small alphabets are not significantly different from each other at 95% confidence level ($p < 0.05$)

When POD level in cultures was determined it was observed that the activity of POD was maximum at 96 h with 50% increase in the presence of sulphide at 42℃ compared to cultures without sulphide. After 96 h, the decline in POD activity in sulphide containing cultures at 42℃ was observed but the decrease was comparatively less than the decrease in SOD (Fig. 4). Catalase provides an energy efficient detoxification mechanism as the enzyme removes H_2O_2 without using reducing equivalents such as NADPH [22]. CAT and POD act upon the H_2O_2 before it starts diffusing to other cellular parts [5,23]. It was observed that up to 48 h CAT activity was almost same in control and sulphide containing cultures at 42℃, but at 144 h an increase of 19.7% was observed in the presence of sulphide over control cultures at 42℃ (Fig. 5).

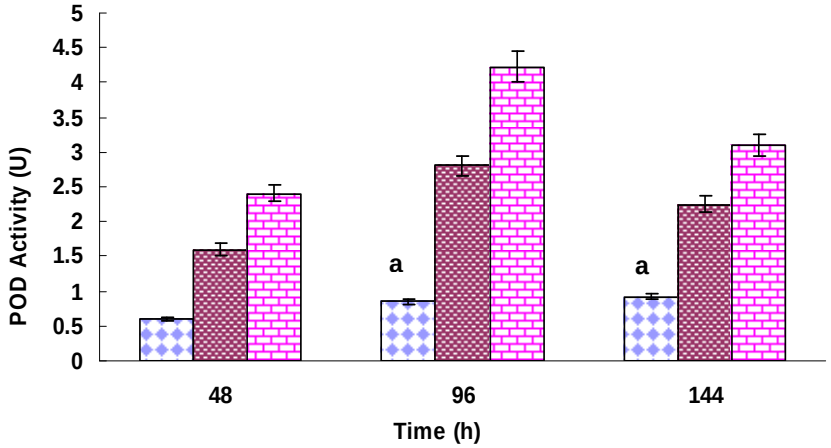

Fig. 4. Activity of POD in *Westiellopsis prolifica* when grown in absence of sulphide at 28℃ (▨) at 42℃ (▩) and in presence of sulphide at 42℃ (▨)

POD activity at zero h: 0.51 U.

Data with same small alphabets are not significantly different from each other at 95% confidence level ($p < 0.05$)

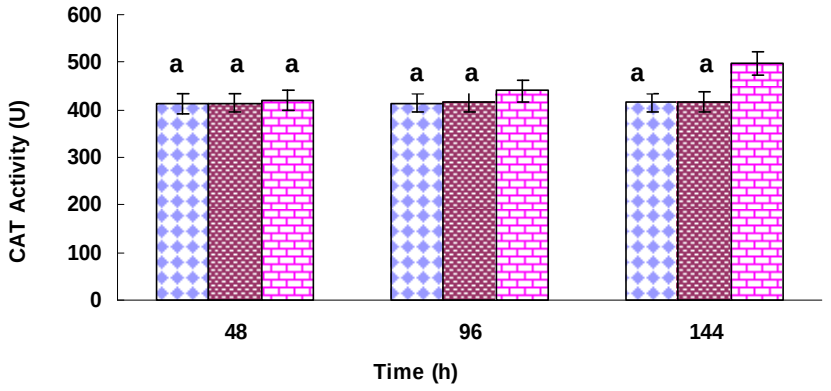

Fig. 5. Activity of CAT in *Westiellopsis prolifica* when grown in absence of sulphide at 28℃ (▨) at 42℃ (▩) and in presence of sulphide at 42℃ (▨)

CAT activity at zero h: 411.5 U.

Data with same small alphabets are not significantly different from each other at 95% confidence level ($p < 0.05$)

These results revealed that level of antioxidant enzymes was triggered in the presence of sulphide at elevated temperature. The increased activities of SOD, POD and CAT indicated that thermal stress may have led to the generation of reactive oxygen species in this organism. These ROS were scavenged by the high levels of these enzymes which helped the organism to tide over the stress caused by elevated temperature.

3.3 Non-enzymatic Antioxidants

The presence of sulphide in cultures at 42℃ affected the level of non-enzymatic sulphur containing antioxidative agent glutathione. GSH increased significantly by 61.9% and 52.3% over the control cultures at 42℃ after 48 and 96 h of inoculation, respectively (Fig. 6). Reduced form of glutathione scavenges radical $O_2^{\cdot -}$, H_2O_2 and $HO^{\cdot}$ by donating hydrogen atom. When GSH reacts with ROS, it gets oxidized and in turn, is reduced by NADPH-dependent reductase and is regenerated [33].

Only negligible amounts of cellular proline were observed in control cultures which slightly increased in presence of sulphide at 42℃, but this increase in proline content ceased with the passage of time and contents were almost same after 96 h and 144 h (Fig. 7). Proline level in the seedlings of rice cultivars under drought conditions was found to be high [38]. Proline acts in osmoregulation, enzyme protection, the stability of proteins and inhibits lipid peroxidation [33]. It is supposed that proline provides a defence against reactive oxygen and $HO^{\cdot}$ [21]. It appears that proline did not play a significant role in this organism.

It was observed that sulphide at elevated temperature affected the ascorbate content of test microorganism in a time-dependent manner. At 42℃ ascorbic acid content was low in sulphide containing cultures than control cultures (Fig. 8). A constant decline in ascorbic acid level in seedlings of rice cultivars was observed with an increase in intensity and duration of exposure to water deficit conditions [38]. By participating in ascorbate–glutathione cycle, ascorbic acid may indirectly help in the removal of H_2O_2 [36,39].

Sulphur induced resistance against biotic stresses in various organisms has been shown by many workers [40-43]. Elemental sulphur and sulphur containing compounds such as phytochelatins, glutathione, and sulphur-rich proteins play an important role in plants under both biotic and abiotic stresses [40-41]. The role of H_2S in alleviating oxidative damage under heavy metal stress has been demonstrated. It has been observed that H_2S promoted germination of wheat seeds, confronted the loss of chlorophyll, reduced oxidative damage due to osmotic stress in sweet potato and can promote the embryonic root length of *Pisum sativum* [42]. It is also believed that H_2S may be a signaling molecule to abiotic stresses such as drought, salinity, and temperature in plants. The induction of resistance to such abiotic stresses in plants was was due to the enhancement of the level of antioxidant enzymes such as SOD, POD, CAT when H_2S was exogenously applied [43].

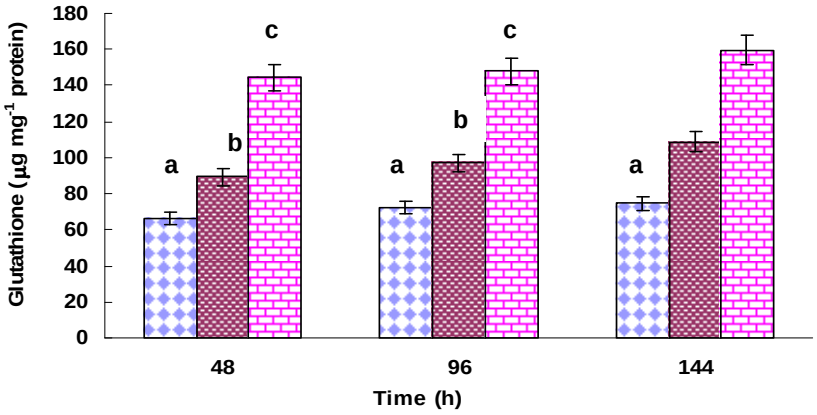

Fig. 6. Content of GSH in *Westiellopsis prolifica* when grown in absence of sulphide at 28℃ ( ) at 42℃ ( ) and in presence of sulp hide at 42℃ ( )

GSH content at zero h: 65.0 µg mg^{-1} protein.

Data with same small alphabets are not significantly different from each other at 95% confidence level ($p < 0.05$)

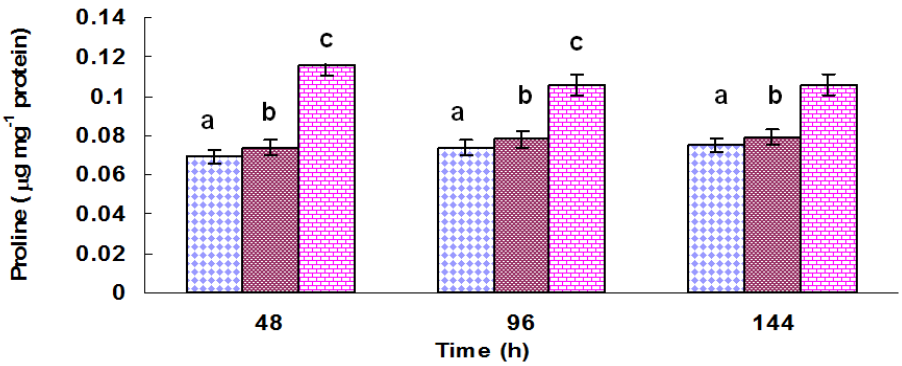

Fig. 7. Content of proline in *Westiellopsis prolifica* when grown in absence of sulphide at 28℃ ( ) at 42℃ ( ) and in presence of sulp hide at 42℃ ( )

Proline content at zero h: 0.069 µg mg^{-1} protein.

Data with same small alphabets are not significantly different from each other at 95% confidence level ($p < 0.05$)

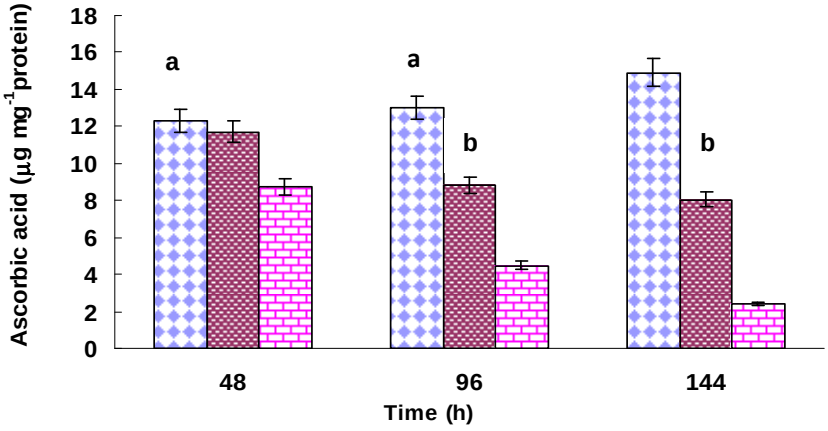

Fig. 8. The content of ascorbic acid in *Westiellopsis prolifica* when grown in the absence of sulphide at 28℃ ( ) at 42℃ ( ) and in the pr esence of sulphide ( ) at 42℃

Ascorbic acid content at zero h: 12.0 µg mg^{-1} protein

Data with same small alphabets are not significantly different from each other at 95% confidence level ($p < 0.05$)

Predmore et al. [44] have also put forward H_2S as a powerful antioxidant and free radical scavenger via up-regulation of antioxidant enzymes. Spraying of NaHS (one of the hydrogen sulfide, donating agent) promoted the activities of superoxide dismutase, catalase, ascorbate, peroxidase and decreased the concentrations of hydrogen peroxide [45]. The above results indicated that sulphide triggers enzymatic and non-enzymatic defense system of the test organism and provides protection against thermal stress. This is the first report on the role of sulphide in ameliorating thermal stress in mesophilic cyanobacteria by triggering enzymatic and non-enzymatic antioxidant defense system.

4. CONCLUSIONS

The mesophilic cyanobacterium *Westiellopsis prolifica* exhibited slow growth at 42℃. Supplementation of basal medium with 2.5 mM sulphide supported more growth of the test organism as compared to control cultures at 42℃. When the mechanism of survival at elevated temperature was studied in context with antioxidant defense system under thermal stress, it was observed that sulphide triggered enzymatic antioxidants (SOD, POD, and CAT) and non-enzymatic antioxidant (GSH) agents which protected the organism from thermal stress.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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