



Plant Archives

Journal homepage: <http://www.plantarchives.org>
DOI Url : <https://doi.org/10.51470/PLANTARCHIVES.2023.v23.no1.020>

ELEVATION IN ENZYMATIC ANTIOXIDANT ACTIVITY IN *NOSTOC ELLIPOSOSPORUM* UNDER THERMAL STRESS IN THE PRESENCE OF SODIUM SULPHIDE

Manpreet^{1*}, Punita¹, Nancy Mandal¹, Mandeep Kaur¹ and Jasvirinder Singh Khattar²

¹Department of Botany, Sri Guru Teg Bahadur Khalsa College, Sri Anandpur Sahib, Ropar (Punjab) –14011, India

²Department of Botany, Punjabi University, Patiala-147002, India.

*Corresponding author Email : manpreet.botany@gmail.com

(Date of Receiving : 01-11-2022; Date of Acceptance : 05-01-2023)

ABSTRACT

Thermophilic cyanobacteria thrive in sulphur-rich thermal springs. Mesophilic cyanobacteria thrive at temperatures between 28°C and 37 °C. This research was aimed at determining whether mesophilic cyanobacteria could thrive at high temperatures in the presence of sulphide and what could be the possible role of sulphide in lessening thermal stress in mesophilic cyanobacterial organisms. The growth of the mesophilic cyanobacterium *Nostoc elliposporum* was examined under thermal stress at its optimal growth temperature and in the presence and absence of sulphide. Under the same settings, the amount of enzymatic antioxidant was determined. Out of nine mesophilic cyanobacterial organisms tested, *Nostoc elliposporum* 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. Enzymatic antioxidants (SOD, POD, and CAT) in the test organism were estimated under temperature stress and in the presence of sulphide. In the presence of sulphide, the levels of enzymatic antioxidants was evaluated. With an increase in antioxidants such as SOD, POD, and CAT the organism does exhibited growth with a slower rate at high temperature stress conditions. This is the first study to look into the role of sulphide in thermal stress relief by stimulating enzymatic reactions in the mesophilic cyanobacterium *Nostoc elliposporum*. This revealed that reactive oxygen species were produced in response to heat stress but these antioxidants scavenged the free radicals that were generated.

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

Introduction

Cyanobacteria's capacity to thrive in a wide range of environmental conditions is among their most impressive characteristics. On the one hand, they may be found in a diversity of freshwater, marine, and terrestrial ecosystems; on the other hand, they can also be found in extreme settings such as hot springs, hyper saline habitats, freezing environments, and arid deserts (Nakatsubo *et al.*, 1987; Kashyap *et al.*, 1991, Whitton and Potts 1991 and Kulasooriya 2011).

To survive in such hostile climates, cyanobacteria use a variety of adaptive mechanisms, including protection from freeze-thaw damage, ultraviolet radiation, desiccation, alkalinity, and the production of a diverse range of secondary metabolites that allow them to survive in a variety of competitive ecological environments. (Whitton and Potts 2000, Scandalios 2005 and Makhalanyane *et al.*, 2015).

Cyanobacteria are classified as psychrophilic, mesophilic, or thermophilic depending on their optimal range of temperature for growth. Psychrophilic cyanobacteria have been found in Arctic and Antarctic lakes (Nakatsubo *et al.*, 1987; Kashyap *et al.*, 1991, Smith 1984 and Skulberg 1996), where the average temperature for growth is between 0°C and 10°C. Temperatures between 25°C and 37°C are

optimum for the growth of mesophilic cyanobacteria. Temperature optima for thermophilic species range from 45°C to 60°C (Apte, 2011). *Phormidium tenue*, *Mastigocoleus laminosus* and *Synechococcus elongatus* var. *amphigranulatus* are some of the most prominent cyanobacterial strains that can thrive at temperatures as high as 69°C (Thajuddin and Subramanian 2005). Most sulphide-containing hot springs are known to have temperatures ranging from 42°C to 85°C (Castenholz 1981). Adams (1994) investigated the metabolic diversity of sulphur-dependent hyperthermophilic bacteria that grow at or above 90°C Because of its presence in source water and/or biological sulphate reduction, sulphide is either constantly or seasonally present in many environments where cyanobacteria are abundant (Oren *et al.*, 1979). When cyanobacterial strains that are not generally exposed to sulphide in natural habitats are exposed to even modest concentrations of this chemical, they get killed (Garlick *et al.*, 1977). Strains from sulphide ecosystems, such as hot springs, on the other hand, have one or more adaptations for keeping their photoautotrophic metabolism active in such environments (Castenholz 1977). If electron donors such as hydrogen sulphide are present, numerous cyanobacteria that dwell in sulphur-rich environments can perform oxygenic photosynthesis employing only photosystem-I (Madigan

2002, Miller and Bebout 2003 and B, Chen *et al.*, 2012). The presence of sulphide promotes thermophilic cyanobacteria to thrive at greater temperatures. Mesophiles like a temperature range of 28°C to 35°C for best growth. When mesophilic organisms are subjected to heat, oxidative stress occurs, resulting in delayed or no growth. Drought or water stress, along with thermal stress, is caused by rising temperatures. Water stress is defined as the loss of water from cells despite the availability of water (Foyer and Noctor 2000). An imbalance is created by water stress. Excess light energy is dissipated in the photosynthetic system, resulting in the production of reactive oxygen species (ROS). As a result, neutralizing free radicals produced during both anomalous and typical activities is critical (Seyed *et al.*, 2012).

Cyanobacteria have evolved methods to deal with water and heat stress. Ion flux modulation for osmotic adaptation, selective expression of tolerance genes, and novel stress proteins are among the strategies employed to repair oxidative damage induced by an excess of free radicals (Apte 2011).

In reaction to oxidative stress, radical scavenging molecules such as ascorbate, glutathione, superoxide dismutase, peroxidase, and catalase are activated. In response to oxidative stress, the expression of genes involved in the activation of enzymatic or non-enzymatic antioxidants is routinely stimulated (Gill and Tuteja 2010). Tolerance of organisms is proportional to antioxidant protection and stress adaptation.

The ability of the antioxidative defence mechanism influences how organisms deal in stressful environments (Liang *et al.*, 2014). The study sought to determine whether mesophilic cyanobacteria can withstand temperatures over their optimum range in the presence of sulphide.

If so, whether antioxidative defence systems in mesophilic cyanobacteria are engaged to counter act thermally induced oxidative damage.

This might be used to enrich fields with cyanobacteria cultivated at 42°C to 50°C in the presence of sulphide, which would ordinarily inhibit the growth of mesophilic cyanobacteria.

Materials and Methods

Materials

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°C, 42°C, 45°C and 50°C. Only *Nostoc elliposporum* exhibited growth at 42°C and was selected for the present study.

Microorganism

The cyanobacterium *Nostoc elliposporum* is an isolate of laboratory at Punjabi University Patiala, collected from rice fields of Patiala, Punjab (India).

Methods

Culture conditions

Chu-10 medium was used to culture the organism (24). The medium's pH was set to 7.8.

Unless otherwise specified, the stock and experimental cultures were kept at 28°C and lit for 14 hours daily with a light level of 45 µE. For all studies, exponentially growing cultures of the test organism (6 days old) were used.

Sulphide endurance of the organism Growing the test organism in graded doses of sodium sulphide was used to assess its endurance threshold (0.5 mM, 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.

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

Antioxidant enzymes Following Beauchamp and Fridovich (1971), the activity of Superoxide dismutase (SOD) was measured as photochemical reduction of nitroblue tetrazolium chloride (NBT) reduction. 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.*, One unit of enzyme is defined as µmol of purpurogallin formed µg⁻¹proteinmin⁻¹.

Catalase (CAT) was determined using the method of Egarshira *et al.*, which involved measuring the quantity of O₂ produced from the dissociation of H₂O₂ in the dark.

One unit of CAT is defined as nmol O₂ released from H₂O₂ mg⁻¹ protein min⁻¹. Protein content in the crude enzyme cell extract was determined following Lowry *et al.*

Statistical Analysis

All of the data is the average of three independent studies with a standard deviation of 5%. (SD). One-way analysis of variance and Tukey's honest significance difference test were used to statistically examine the data. GraphPad Prism 6.0 version 6.0 was used to test all statistical analyses against the probability value at the 95 percent confidence level (p 0.05). (www.graphpad.com).

Chemicals

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

Results and Discussion

Tolerance Level of *Nostoc elliposporum* to Sulphide

In early studies, the development of nine mesophilic cyanobacteria was assessed in terms of increase in absorbance at 720 nm over 6 days at 37°C, 42°C, 45°C, and 50°C.

According to the findings, the cells dissolved and none of the tested mesophilic cyanobacteria survived at 45°C (Table 1.)

Nostoc elliposporum, on the other hand, thrived at 42°C (Table 1).

As a result, this organism was chosen for the current investigation.

A comparison of its growth at 37°C, 42°C, and 45°C was done, and it was discovered that after 12 days, the organism's growth at 37°C and 42°C decreased.

In addition, the growth of test organisms was investigated in the presence and absence of sulphide at various temperatures (Fig.2).

Sulphide doses of 0.5 mM, 1.0 mM, 2.0 mM, and 2.5 mM were tested. The absorbance of the cultures at 42 °C in presence of 0.5, 1.0, 2.0 and 2.5 mM sulphide was 21%, 36.8%, 52.5% and 73.6% more compared to absorbance of control cultures at 42°C. In 5.0 mM sulphide the organism survived but did not exhibit growth. This indicated that 5.0 mM was toxic to organism at 42 °C.

Table 1 : Growth of mesophilic cyanobacteria at different temperatures

Organism	Temperature (°C)			
	37	42	45	50
<i>Anabaena naviculoides</i>	+	-	-	-
<i>Nostoc muscorum</i>	+	-	-	-
<i>Nostoc calcicola</i>	+	-	-	-
<i>Plectonema boryanum</i>	+	-	-	-
<i>Phormidium molleS</i>	+	-	-	-
<i>Nostoc elliposporum</i>	-	-	-	-
<i>Lyngbya faveolarum</i>	+	+	-	-
	+	-	-	-

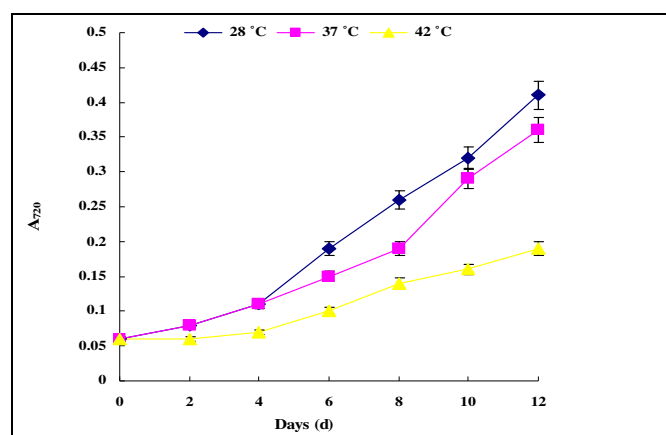


Fig. 1 : Growth of *Nostoc elliposporum* in basal medium.

Because of its interaction with the electron chain, sulphide is hazardous (Garlick *et al.*, 1977, Oren *et al.*, 1979 and Abed *et al.*, 2006). Because 2.5 mM sulphide in medium had a beneficial influence on organism development at 42 °C, 2.5 mM was chosen for further experimentation.

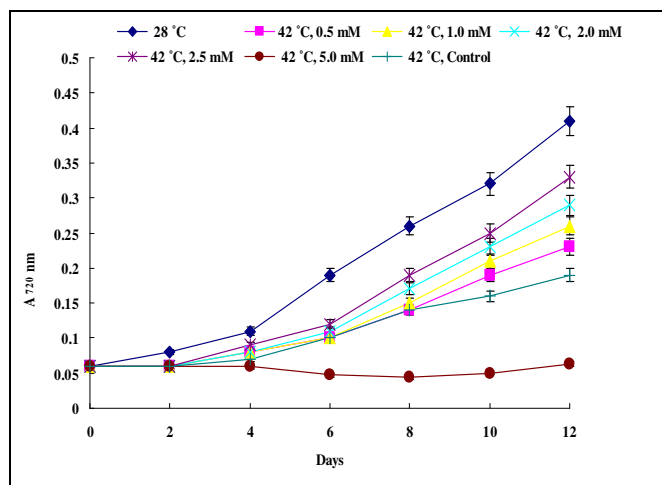


Fig. 2 : Growth of *Nostoc elliposporum* in absence of sulphide at 28 °C, 42 °C and presence of sulphide at 42 °C.

Antioxidant Enzymes

In mesophilic species, temperature stress may result in the formation of free radicals. Microorganisms have evolved mechanisms to protect themselves from reactive oxygen species as a result of evolution. Superoxide dismutase, catalase, ascorbate, peroxidase, and glutathione reductase are antioxidant enzymes found in this system (Gupta *et al.*, 2015). Survival rates differ between species or ecotypes in a wide range of taxa due to their stronger and more refined ability to scavenge ROS in comparison to their sensitive or less tolerant counterparts. (Sulmon *et al.*, 2015).

Microorganisms' responses to changes in abiotic settings are frequently quick, resulting in organism acclimatisation within a few hours of exposure (Singh *et al.*, 2005). The level of antioxidant enzymes in the test organism at 42°C was evaluated 48, 96, and 144 hours after inoculation. The activity of superoxide dismutase (SOD) in *Nostoc elliposporum* cells grown at 28 °C did not significantly increase. On the other hand, control cultures of organisms grown at 42 °C had 41.4% higher SOD activity after two days than cultures cultivated at 28 °C.

The maximum SOD activity in the test organism was obtained after 2 days in 2.5 mM sulphide supplemented cultures (Fig. 3).

SOD activity was 41.4% percent higher in these cultures at 42 °C than in the control cultures.

After 4 and 6 days at 42 °C, sulphur supplemented cultures had 42% and 31 % more SOD activity than control cultures.

As a result, the presence of sulphide in the cultures had a significant impact on the growth rate. SOD is considered the first line of defence against superoxide anion and is known to catalyse the conversion of two superoxide anions O₂ into a molecule of H₂O₂ and O₂. Catalase and/or peroxidase react with H₂O₂ to generate water and oxygen, completing the reaction began by SOD to neutralise superoxide anions (Liang *et al.*, 2014, Gupta *et al.*, 2015 and Christoul *et al.*, 2013). Wen-yan *et al.* revealed that the survival of *Spartina alterniflora* in high sulphur environments was attributed to its enzymatic antioxidant defensive activities of SOD and ascorbate peroxidase, which increased when the organism was exposed to high levels of Na₂SO₄. POD activity of *Nostoc elliposporum* grown at 28

°C was identical for up to 6 days (Fig. 4). At 42 °C, POD activity control cultures of the test organism increased after 2 days and remained nearly constant for the next 6 days.

POD activity increased by 52.6 percent on the second day when 2.5 mM sulphide was present, but remained virtually unchanged for the next four days.

POD activity in sulphide containing cultures declined after 6 days, although it was remained 13.5 percent higher than in control cultures at 42 °C.

Control cultures of *Nostoc elliposporum* grown at 28 °C revealed the same level of CAT after 2 days (Fig. 5).

CAT levels in sulphide cultures increased by about 12.7 percent and 6 percent after 4 and 6 days, respectively, when compared to their respective controls at 42 °C.

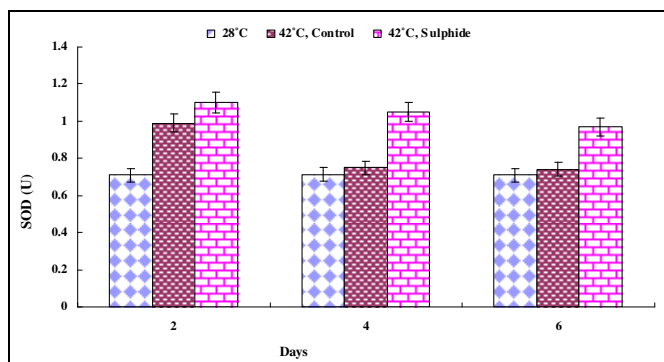


Fig. 3 : SOD activity in *Nostoc elliposporum* when grown at 28 °C and 42 °C in absence of sulphide and at 42 °C in presence of sulphide.

SOD activity at zero h: 0.70 U

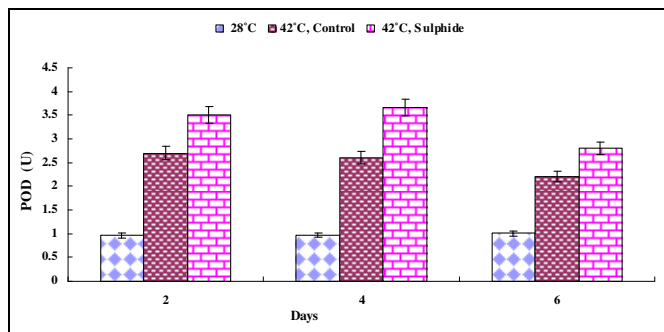


Fig. 4 : POD activity in *Nostoc elliposporum* when grown at 28 °C and 42 °C in absence of sulphide and at 42 °C in presence of sulphide.

POD activity at zero h: 0.6 U

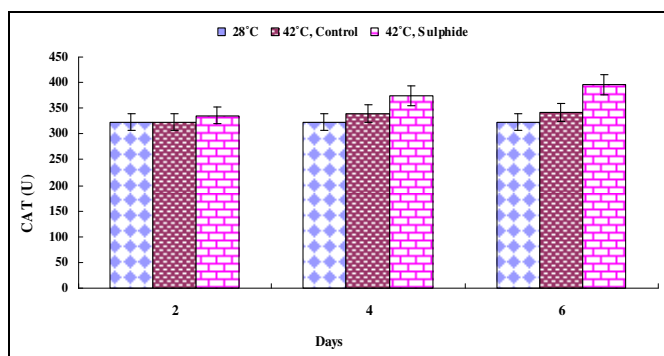


Fig. 5. : CAT activity in *Nostoc elliposporum* when grown at 28 °C and 42 °C in absence of sulphide and at 42 °C in presence of sulphide.

CAT activity at zero h: 323.0 U

As an enzyme, it has a detoxifying mechanism. H_2O_2 is removed without the need of reducing equivalents like NADPH. Before H_2O_2 starts diffusing, CAT and POD act on other cellular components (Gill and Tuteja 2010 and Liang *et al.*, 2014).

These studies indicated that antioxidant enzymes were active when sulphide was present at high temperatures.

Thermal stress may have caused reactive oxygen species to develop in this organism, as demonstrated by increased SOD, POD, and CAT activity.

These enzymes scavenged the ROS, allowing the organism to deal with the stress imposed by the increasing temperature.

H_2S has also been proposed as a potent antioxidant and free radical scavenger by Predmore *et al.* (2012) through up-regulation of antioxidant enzymes. The Sulphide triggers, according to the findings defensive system of enzymatic of the organism being tested, as well as providing protection against the effects of heat. This is the first of a series of reports on the significance of sulphide in thermal stress relief by inducing enzymatic defense system mesophilic cyanobacteria. Many researchers have demonstrated sulphur induced tolerance to biotic stressors in a variety of species (Bloem *et al.*, 2007 and Fu 2012). Under both biotic and abiotic stressors, elemental sulphur and sulphur containing compounds such as phytochelatins, glutathione, and sulphur rich proteins play a significant role in plants (Bloem *et al.*, 2007 and Atmaca 2004). The importance of H_2S in reducing oxidative damage caused by heavy metals has been established.

H_2S has been shown to improve wheat seed germination, combat chlorophyll loss, minimise oxidative damage caused by osmotic stress in sweet potatoes, and promote the embryonic root length of *Pisum sativum* (Atmaca 2004). Plants may also use H_2S as a signalling molecule in response to abiotic stressors like drought, salinity, and temperature. When H_2S was exogenously administered, the amount of antioxidant enzymes such as SOD, POD, and CAT increased, inducing resistance to such abiotic stressors in plants (Fu 2012).

The above findings show that sulphide activates the test organism's enzymatic defence systems and protects it against temperature stress.

Conclusions

At 42°C, the mesophilic cyanobacterium *Nostoc elliposporum* 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 explored in conjunction with the antioxidant defence system under thermal stress, it was discovered that sulphide prompted enzymatic antioxidants (SOD, POD, and CAT) that defended the organism from thermal stress.

Competing Interests

The authors have declared that there are no competing interests.

Acknowledgements

We are grateful to the Department of Botany, Punjabi University Patiala, PG Department of Botany Sri Guru Teg Bahadur Khalsa College Sri Anandpur Sahib and DBT scheme for providing necessary facilities for the completion of this manuscript.

References

- Abed, R. M., Polerecky, L., Al Najjar, M., & De Beer, D. (2006). Effect of temperature on photosynthesis, oxygen consumption and sulfide production in an extremely hypersaline cyanobacterial mat. *Aquatic Microbial Ecology*, 44(1), 21-30.
- Adams, M.W. (1994). Biochemical diversity among sulphur dependent, hyperthermophilic microorganisms. *FEMS Microbiol Rev.*:15:261-277
- Apte, S.K. (2011). Coping with salinity/water stress: cyanobacteria show the way. *Proc Indian NatnSci Acad.*, 5(67): 285-310.
- Atmaca, G. (2004). Antioxidant effects of sulfur-containing amino acids. *J. Yonsai Medi.*, 45(45): 776-88.
- Bates, B.; Waldern, R.P. and Teare, I.D. (1973). Rapid determination of free proline for water stress studies. *Plant Soil*, 39(1): 205-207.
- Beauchamp, C. and Fridovich, I. (1971). Superoxide dismutase improved assay and an assay applicable to acrylamide gels. *Analyt, Biochem.*, 44(1): 276-287.
- Bloem, E.; Haneklaus, S.; Salac, I.; Wickenhä, P. and Schnug, E. (2007). Facts and fiction about sulfur metabolism in relation to plantpatho-gen interactions. *Plant Biol.*, 9: 596-607.
- Braga, LF.; Sousa, M.P. and Ferreira, L.C. (2009). Delachiave MEA, Cataneo AC, Braga JF. Proline level and amylase and ascorbate peroxidase activity in the germination of *Plantagoovata* forsk (Plantaginaceae) seeds. *J Agri. Biol Sci.*: 4(6): 49-54.
- Castenholz, R.W. (1977). The effect of sulfide on the blue-green algae of hot springs II. Yellowstone National park. *Microb Ecol.*, 3: 79-105.
- Castenholz, R.W. (1981). Isolation and cultivation of thermophilic cyanobacteria. In: Starr MP, Stulp H, Truper HG, Baloiva A, Schlegel HG, editors. *The Prokaryotes*. Berlin Heidelberg, New York: Springer, 1.
- Chen, J.; Wang, W.H.; Wu, F.H.; You, C.Y.; Liu, T.W.; Dong, X.J.; He, J.X. and Zheng, H.L. (2012). Hydrogen sulfide alleviates aluminum toxicity in barley seedlings. *Plant Soil*, 362: 301-318.
- Choen, Y.; Jorgensen, B.B.; Pandan, E. and Shilo, N. (1975). Sulfide dependent anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica*. *Nature*, 257: 489-491.
- Choen, Y.; Jorgensen, B.B.; Revschbech, N.P. and Poplawasky, R. (1986). Adaptation to hydrogen sulphide of oxygenic and an oxygenic photosynthesis among cyanobacteria. *Appl Environ Microbiol.*, 51: 398-407.
- Christoul, A.; Manganaris, G.A.; Papadopoulos, I. and Fotopoulos, V. (2013). Hydrogen sulfide induces systemic tolerance to salinity and non-ionic osmotic stress in strawberry plants through modification of reactive species biosynthesis and transcriptional regulation of multiple defence pathways. *J Exp Bot.*: 64(7):1953-1966.
- Egashira, T.; Takahama, U. and Nakamura, K. (1989). A reduced activity of catalase as a basis for light dependent methionine sensitivity of a *Chlamydomonas reinhardtii* mutant. *Plant Cell Physiol.*, 30: 1171-1175
- Ellman, G.L. (1959). Tissue sulfhydryl groups. *Arch Biochem and Biophys.*, 82: 70-77.
- Fu, M.; Zhang, W.; Wu, L.; Yang, G.; Li, H. and Wang, R. (2012). Hydrogen sulfide (H₂S) metabolism in mitochondria and its regulatory role in energy production. *Cell Biology*, 109(8): 205-212.
- Foyer, C.F. and Noctor, G. (2000). Oxygen processing in photosynthesis regulation and signaling. *New Phytol.*, 146(3): 359-388.
- Gahagen, H.E.; Holm, E. and Abeles, F.B. (1968). Effect of ethylene on peroxidase activity. *Physiol. Plantarum.*, 21: 1270-1279.
- Garlick, S.; Oren, A. and Padan, E. (1977). Occurrence of facultative anoxygenic photosynthesis among filamentous and unicellular cyanobacteria. *J Bacteriol.*, 129: 623-629.
- Gill, S.S. and Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol Biochem.*, 48: 909-930.
- Gupta, S.; Singh, S. and Sharma, S. (2015). Tolerance against heavy metal toxicity in cyanobacteria: Role of antioxidant defense system. *Int J Pharm Sci.*, 7(2): 9-16.
- Kashyap, A.K.; Pandey, K.D. and Gupta, R.K. (1991). Nitrogenase activity of Antarctic cyanobacteria *Nostoc commune* influence of temperature. *Folia Microbiol.*, 36: 557-560.
- Kulasooriya, S.A. (2011). Cyanobacteria: Pioneers of planet earth. *Ceylon J Sci. Biol Sci.*, 40(2): 71-88.
- Lowry, O.H.; Rosebrough, N.J.; Farr, A.L. and Randall, R.J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 193(1): 265-275.
- Liang, W.; Wang, L.; Shi, J.; Lei, X.; Yang, J.; Wu, S. and Chen, W. (2014). Differential expression of antioxidant proteins in the drought-tolerant cyanobacterium *Nostoc flagella forme* under desiccation. *Plant Omics J.*:7(4): 205- 212.
- Madigan, M.T. (2002). Anoxygenic phototrophic bacteria from extreme. *Photosyn Res.*, 76: 57-171.
- Makhalanyane, P.T.; Valverde, A.; Vela'zquez, D.; Eoin, G.; Van, G.M.W.; Quesada, A. and Cowan, D.A. (2015). Ecology and biogeochemistry of cyanobacteria in soils, permafrost, aquatic and cryptic polar habitats. *Biodivers Conserv.*, 24: 819-840.
- Miller, S.R. and Bebout, B.M. (2003). Variation in sulfide tolerance of photosystem II in phylogenetically diverse cyanobacteria from sulfidic habitats. *Appl Environ Microbiol.*, 70(2): 736-734.
- Nakatsubo, T. and Ino, Y. (1987). Nitrogen cycling in an Antarctic ecosystem. Estimation of the amount of nitrogen fixation in a mass community of east Ongulisland. *Ecol Res.*, 2: 31.
- Oren, A.; Pandan, E. and Malkin, S. (1979). Sulfide inhibition of photosystem II in cyanobacteria (blue-green algae) and tobacco chloroplast. *Biochim Biophys Acta.*, 546: 270-279.
- Predmore, B.L.; Lefer, D.J. and Gojon, G. (2012).

- Hydrogen sulfide in biochemistry and medicine. *Forum Rev Article.*, 17: 119-140.
- Pyngrope, S.; Bhoomika, K. and Dubey, R.S. (2012). Reactive oxygen species, ascorbate-glutathione pool, and enzymes of their metabolism in drought-sensitive and tolerant indica rice (*Oryza sativa* L.) seedlings subjected to progressing levels of water deficit. *Protoplasma.*, 250(2): 585–600.
- Roe, J.H. and Keuther, C.A. (1943). The determination of ascorbic acid in whole blood and urine through 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. *J Bio Chem.*, 147: 399-407.
- Safferan, R.S. and Morris, M.E. (1964). Growth characteristics of the blue-green algal virus. LPP-I. *J Bacteriol.*, 88(3):771–775.
- Scandalios, J.G. (2005). Oxidative stress: Molecular perception and transduction of signals triggering antioxidant gene defense. *Braz J Med Biol Res.*: 38(7): 995-1014.
- Seyed, Y.S.; Lisar, R.M.; Mosharraf, M.H. and Ismail, M.M.R. (2012). In: Rahman IMM, editor. Water stress in plants: Causes, effects, and responses. Croatia: Intech: ISBN: 978-953-307-963-9.
- Singh, A.P.; Asthana, R.K.; Kayastha, A.M. and Singh, S.P. (2005). A comparison of proline, thiol levels and GAPDH activity in cyanobacteria of different origins facing temperature-stress. *World J. Microbiol. Biotechnol.*, 21:1-9.
- Smith, V.R. (1984). Effect of abiotic factors on acetylene reduction by cyanobacteria epiphytic on moss at a sub-Antarctic island. *App Environ Microbiol.*, 48: 594-600.
- Skulberg, O.M. (1996). Terrestrial and limnic algae and cyanobacteria. Elvebakk A, Prestrud P, editors: Skrifter.
- Sulmon, C.; Baaren, J.V.; Cabello-Hurtado, F.; Gouesbet, G. and Hennion, F.C. (2015) Abiotic stressors and stress responses: What commonalities appear between species across biological organization levels? *Environ Pollut.*, 202: 66-77.
- Thajuddin, N. and Subramanian, G. (2005). Cyanobacterial biodiversity and potential applications in biotechnology. *Curr Sci.*, 89(1): 47-57.
- Trinity, L.H.; Judith, M.K.; Dirk, B. and Jennifer, L.M. (2018). Cyanobacterial photosynthesis under sulfidic conditions: insights from the isolate *Leptolyngbya* sp. strain *hensonii*. *The ISME Journal*, 12: 568–584
- Whitton BA, Potts M (2000) Introduction to cyanobacteria. In: Whitton BA, Potts M, editors. The ecology of cyanobacteria: Their diversity in time and space. Netherlands: Kluwer Academic, Dordrecht
- Wen-yan, S.; Chang-mei, I. and Chang-fang, Z. (2011). Antioxidant systems of *Spartina alterniflora* and *Phragmite australis* responded differently to environmental sulfur stress. *J Natural Sci.*, 5:1-3.
- Zhang, H.; Ye, Y.K.; Wang, S.H.; LUO, J.P.; Tangg, J. and Fu-Ma, D. (2009). Hydrogen sulfide counteracts chlorophyll loss in sweet potato seedling leaves and alleviates oxidative damage against osmotic stress. *Plant Growth Regul.*, 58: 243-250.