Desiccation induced changes in osmolytes production and the antioxidative defence in the cyanobacterium *Anabaena* sp. PCC 7120

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Published online: 28 October 2012
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Abstract Cells of *Anabaena* sp. PCC 7120, a low desiccation tolerant cyanobacterium, was subjected to prolonged desiccation and effect of loss of water was examined on production of osmolytes, and antioxidant response as well as on overall viability in terms of photosynthetic activity. During dehydration (22 h), the organism maintained about 98.5 % loss of cellular water, yet cells remained viable as about 30 % of photosynthetic O₂-evolution activity resumed upon hydrating (1 h) such cells. In desiccated state, cyanobacterial cells accumulated osmolytes within 1 h though their contents decreased thereafter. The highest levels of trehalose (179 nmol mg⁻¹ protein), sucrose (805 nmol mg⁻¹ protein) and proline (23.2 nmol mg⁻¹ protein) were attained within 1 h. Chlorophyll *a* and carotenoid contents also increased within 1 h but phycocyanin level showed opposite trend. The oxygen-evolving activity declined in desiccated cyanobacterial biomass while rehydration led to instant recovery, indicating that cells protect the photosynthetic machinery against desiccation. Notwithstanding, activities of antioxidant enzymes (catalase, peroxidase and superoxide dismutase) attained their peaks after 3 h of desiccation, though within 10 min of rehydration, their levels returned back close to basal activities of the cultured cells. We propose that onset of osmolyte production in conjunction with upshift of antioxidant enzymes apparently protects the cyanobacterial cells from desiccation stress.

Keywords Catalase · Oxygen-evolution · Peroxidase · Superoxide dismutase · Sugars

Introduction

Recurrence of drought with the concomitant drop in crop production is of major concern (Serraj and Sinclair 2002), and this necessitates a thorough understanding of desiccation tolerance in plants. Cyanobacteria contributing fixed nitrogen to soils, proliferate in thermal springs, the Antarctica, paddy fields and tree bark etc., and survive extreme conditions including desiccation (Dadheech 2010). Anhydrobiosis in prokaryotes is well reviewed (García 2011). Desiccation refers to water content less than 10 % of cell dry weight in the organism (Alpert 2005), and the hydration shell of molecules is gradually lost (França et al. 2007). The water loss in the tolerant cell traits is balanced by interactions with other molecules (Rebecchi et al. 2007), and the native conformation is achieved following rehydration (Wolkers et al. 2002).

There are reports that drought and salt stress induce trehalose and sucrose accumulation in cyanobacteria (HersHKovitz et al. 1991; Sakamoto et al. 2009). The induction of trehalose synthesis in *Anabaena* sp. PCC 7120 cells against salt stress is already reported by us (Asthana et al. 2005). Accumulation of sucrose and trehalose in cyanobacteria has dual functions: a) formation of hydrogen bonds to protect biomolecules, and the replacement of water (Carpenter and Crowe 1988), and b) the creation of viscous cytoplasmic matrix (Rudolph et al. 1986). As there is a limitation to store the non-reducing disaccharides in plants (Wingler 2002), alternative mechanisms such as rapid synthesis of proteins is additionally operational

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(Potts et al. 2005). It is proven that protein oxidation is the main cause of desiccation damage in bacterial cells (Fredrickson et al. 2008). Proline, along with the other antiaggregation osmolyte, trehalose may be useful in deciphering as to how the endogenously synthesized intracellular osmolytes could act synergistically to prevent protein aggregation in vivo (Fisher 2006).

Oxidative stress as a consequence of dehydration is little understood though, water stress increased reactive oxygen species (ROS), causing lipid peroxidation, protein denaturation and nucleic acid damage with lethal consequences in the cell metabolism (Hansen et al. 2006). The antioxidative defense system consequently gets activated to alleviate the ROS level (Kranner and Birtić 2005). Photosynthetic status under oxidative stress is assessed in terms of photosynthetic efficiency (Millan-Almaraz et al. 2009). In the resurrection plant *Haberlea rhodopensis*, activities of photosystem (PS) I and PSII and the rate of oxygen evolution were negatively affected by water deficit (Mihailova et al. 2011).

In present study, we have monitored physiological status of *Anabaena* sp. PCC 7120 cells together with the accumulation of non-reducing disaccharides (trehalose and sucrose), imino acid (proline), antioxidative enzymes (catalase, peroxidase and SOD), and relative cellular water content with an aim that these could throw some light on desiccation tolerance mechanisms operative in cyanobacteria.

Materials and methods

Organism and culture conditions

Anabaena sp. PCC 7120 was grown in BG-11 medium (Rippka et al. 1979), free from any combined nitrogen sources under continuous tungsten plus fluorescent illumination ($27.17 \mu\text{mol m}^{-2} \text{s}^{-1}$) at $28 \pm 1^\circ\text{C}$.

Imposition of desiccation conditions

The desiccation was imposed as described by Katoh et al. (2004). Cyanobacterial cells ($A_{750}=1.5\text{--}1.8$), were harvested by filtration through cellulose acetate filters ($0.45 \mu\text{m}$, Sartorius). The cells were desiccated for 1, 3, 6, 10 and 22 h in a Petri dish under laboratory conditions using incandescent light ($45 \mu\text{mol m}^{-2} \text{s}^{-1}$) to maintain 40°C to obtain desired relative humidity (35 %). We have monitored the R.H., temperature and irradiance by Licor-6400 (USA).

Water deficit estimation

The percent water deficit was calculated by following the method of Mansour and Hallet (1981). The original weight (OW) was obtained at selected relative humidity (35 % in

the present case), and saturated weight (SW) by immersing the biomass for 4 h in distilled water. The dry weight (DW) was obtained by transferring the biomass to an oven at 80°C for 24 h.

$$\text{Percent water deficit} = \frac{SW - OW}{SW - DW} \times 100$$

Trehalose and sucrose identification by NMR

Cyanobacterial cells exposed to different durations of desiccation (1, 3 and 6 h), were mixed with 70 % ethanol (100 ml), shaken vigorously and incubated at 65°C (4 h) and centrifuged (10,000 g, 15 min). The supernatant was evaporated under vacuum in a rotary evaporator (40°C), and the residue dissolved in D_2O (1 mL) for NMR spectroscopy. Proton decoupled ^{13}C -NMR spectra were recorded at 500 Hz using a Jeol F X 90 Q FT NMR (Japan) with 5 mm probe at 25°C . Typical acquisition parameters were: acquisition time 0.95 s, pulse delay time 0.5 s, spectral width 5,000 Hz, pulse angle 45° , pulse width 5 μs . The chemical shifts in peaks were measured in ppm relative to methanol as the internal standard (49.9 ppm). The identity of trehalose and sucrose was ascertained by comparing the chemical shifts of the standard trehalose and sucrose (0.2 M each) dissolved in D_2O under identical conditions (Page-Sharp et al. 1999).

Quantitative determination of trehalose and sucrose

Trehalose and sucrose were quantified by gas chromatography. Desiccated cyanobacterial cells were washed, suspended in 80 % ethanol and incubated at 65°C for 4 h. Cell suspensions were overnight incubated at 4°C and centrifuged (10,000 rpm, 20 min). Supernatant was vacuum dried and suspended in 5 mL of water:chloroform (1:0.5, v/v). The suspension was centrifuged to collect the aqueous phase which was dried and converted to oximes (Li and Schuhmann 1981). The carbohydrates were dissolved in 35 μL of hydroxylamine hydrochloride (2.5 % w/v) in anhydrous pyridine. This mixture was heated at 75°C for 30 min and converted to silyl derivatives using hexamethyldisilazane (350 μL) and trifluoroacetic acid (TFA) (35 μL) and heating at 45°C for 30 min. The suspension was centrifuged (10,000 rpm) and 1 μL injected in a gas chromatograph GC-Varian CP-3800 equipped with flame ionization detector fitted with the fused silica capillary column (CPsil 8CB). The injector and detector temperature were set at 250°C . The analysis was carried out using temperature programming (200– 250°C) at heating rate $15^\circ\text{C}/\text{min}$ with an initial holding at 200°C (10 min). N_2 was used as carrier gas, and the injections were made in split mode (split ratio, 1:40). Trehalose and sucrose were the standards.

Estimation of proline

Proline was measured according to Bates et al. (1973). Cells were harvested by centrifugation and subjected to desiccation for various intervals (1,3,6,10 and 22 h). Such cells along with control were resuspended in 10 mL (3 %, v/v) of sulfosalicylic acid and disrupted ultrasonically (Labsonic, Malaysia) for 5 min. The resulting cell extracts were centrifuged to remove cell debris and the supernatant containing proline was treated with freshly prepared acidic ninhydrin at 80 °C for 1 h and the reaction terminated in ice bath. The coloured complex was extracted in toluene and absorbance read at 520 nm. Proline standard was prepared by dissolving proline (Himedia, India) in 3 % (v/v) sulfosalicylic acid. Proline level is expressed in nmol per unit cellular protein.

Assays of peroxide and antioxidative enzymes

The level of total peroxide was measured by method of Sagisaka (1976) with some modifications. The cell pellets were homogenized in 3.5 mL of 15 % TCA followed by centrifugation (10,000 rpm, 20 min). The resulting supernatant (2 mL) was mixed with 0.4 mL of ferrous ammonium sulphate (10 mM) and 0.2 mL of potassium thiocyanate (2.5 M). Following an incubation of 10 min at 25 °C, the absorbance was recorded at 480 nm. The value of total peroxide is expressed in nmol H₂O₂ µg⁻¹ protein.

Activities of catalase, peroxidase and SOD were measured by the methods of Aebi (1984), Gahagan et al. (1968) and Beauchamp and Fridovich (1971), respectively. The cells (desiccated or non desiccated) were broken in liquid N₂ and centrifuged at 4 °C. The pellets were discarded and supernatants taken for enzymatic assays. The cells taken for such studies contained 4 mg protein.

SOD activity was assayed in terms of its ability to inhibit photochemical reduction of nitroblue tetrazolium (NBT). The reaction mixture (3 mL) contained 33 µM NBT, 10 mM L-methionine, 0.66 mM EDTA Na₂ and 0.0033 mM riboflavin in sodium phosphate buffer (0.05 mM, pH 7.8). Riboflavin added lastly and the test tubes with the reaction mixture were incubated for 10 min under 300 µmol⁻¹ m⁻² s⁻¹ irradiance (25 °C). Such reaction mixture with no enzyme developed maximum colour due to maximum reduction of NBT. A non-irradiated reaction mixture did not develop colour, and was used as negative control. One unit of SOD is defined as the amount of enzyme that inhibits 50 % NBT photoreduction.

Catalase activity was measured as consumption of H₂O₂ recorded at 240 nm. Three mL reaction mixture contained 100 µL extract protein and 10 mM H₂O₂ in 50 mM sodium phosphate buffer (pH 7.0). For each measurement, the blank corresponded to the absorbance of the mixture at zero time was subtracted from the actual reading of absorbance after

30 s. One unit of catalase decomposes 1.0 µmol of hydrogen peroxide per minute, and was expressed per mg protein.

For determining peroxidase activity, the reaction mixture (3 mL) contained 16 mM H₂O₂, 10 mM pyrogallol. One unit of peroxidase is defined as the amount of enzyme required to catalyze the production of 1 mg of purpurogallin from pyrogallol in 20 s at 20 °C.

Carbohydrate estimation

Total carbohydrate of cyanobacterial cells was determined by the phenol method of Dubois et al. (1956).

Protein estimation

Protein was estimated by the method of Lowry et al. (1951) as modified by Herbert et al. (1971).

Estimation of photopigments

Chl *a* and carotenoids concentrations in the acetone extract were measured at 665 nm and 460 nm, respectively in UV/Vis 6715 Spectrophotometer (JENWAY, U.K.). Phycocyanin extracted in deionised water, was measured at 620 nm. Pigment concentration (µg/mL) is based on absorbance coefficient of 82.04 for Chl *a*, 200 for carotenoids (Myers and Kratz 1955) and 7.5 for phycocyanin (Brody and Brody 1961), respectively.

Oxygen evolution measurements in rehydrated cells

Cyanobacterial cells were desiccated for pre-determined durations and the dried biomass (10 mg) used to measure oxygen evolution as described by Tamaru et al. (2005). Rehydration of biomass was done in an oxygen electrode chamber (Rank Brothers, Cambridge, U. K.) with 25 mM HEPES-NaOH buffer (pH 7.0) supplemented with 10 mM NaHCO₃. Saturating actinic light (1,600 µmol m⁻² s⁻¹) was applied to monitor photosynthetic O₂-evolution at 33 °C.

Statistical analysis

All experiments were performed in triplicate with standard deviation (SD) represented as bar wherever necessary.

Results

Water deficit percent

Water loss in desiccated cells of *Anabaena* sp. PCC 7120 at different intervals was calculated as water deficit (Table 1).

Table 1 Water deficit and H₂O₂ concentration in *Anabaena* sp. PCC 7120 facing desiccation

Duration	1 h	3 h	6 h	10 h	22 h
Water deficit (%)	97.8	97.98	98.2	98.3	98.5
H ₂ O ₂ (μmol mg ⁻¹ protein)	1.23±0.28	0.59±0.2	0.52±0.11	0.48±0.31	0.39±0.18

Following 1 h of desiccation, the cyanobacterial cells lost 97.8 % of water. However, further desiccation improved water loss only marginally as it was sustained at 98.5 % even after 22 h.

Levels of selected osmolytes (trehalose, sucrose and proline)

¹³C-NMR spectrum for trehalose and sucrose standards revealed designated chemical shifts (δ ppm) of sugars (Fig. 1d and e). The desiccated cells exhibited peaks for trehalose (61.5, 70.6, 72.07 and 72.10) and sucrose (61.2, 70.2, 73.08, 77.9, 82.7 and 104.8) very close to the chemical shifts displayed by the standard sugars (Fig. 1b and c). Control cells however, lacked such peaks (Fig. 1a). Gas chromatographic analysis of such samples showed accumulation of trehalose and sucrose at given time intervals

Fig. 2 Trehalose (-Black square-) and sucrose (-Black circle-) production in *Anabaena* sp. PCC 7120 facing desiccation stress for 1–22 h

(Fig. 2). The trehalose amount rapidly increased to 3.3-fold (179 nmol mg⁻¹ protein) during the first hour of desiccation but gradually decreased thereafter, attaining 76 nmol mg⁻¹ protein by 22 h, still above the basal level of cultured cells (54 nmol mg⁻¹ protein). Sucrose also showed the same pattern of change during desiccation as its level relative to the control cells (117 nmol mg⁻¹ protein) enhanced by 7.1-fold (805 nmol mg⁻¹ protein) during 1 h. However, sucrose level in prolonged incubation of 22 h came down to what was found in the cultured cells.

Fig. 1 ¹³C-NMR spectra from methanolic extract of *Anabaena* sp. PCC 7120 cells: (a) control, (b) exposed to desiccation for 1 h and (c) 3 h, (d) trehalose and (e) sucrose standard

Fig. 3 Proline production in *Anabaena* sp. PCC 7120 facing desiccation stress for 1–22 h

The induction of proline synthesis also followed the trend similar to the osmolytes (Fig. 3). Once again, 1 h desiccation was crucial as apparent from 2.3-fold (23.2 nmol mg⁻¹ protein) rise in cellular proline compared to its basal level in controls (9.93 nmol mg⁻¹ protein). The desiccated cells also retained high proline (3.27 units) over the control following 22 h.

Level of H₂O₂ and activity of antioxidant enzymes

Hydrogen peroxide concentration in desiccated cells increased with the onset of desiccation (1.23 μmol mg⁻¹ protein; 1 h) to approximately 5-times the basal level (0.26 μmol mg⁻¹ protein) although the amount decreased thereafter (Table 1). In desiccated cells, antioxidant enzymes' activities enhanced with the rise in H₂O₂ (Table 2). Catalase activity rose by 2.3-fold (18.5 U mg⁻¹ protein) within 3 h, and decreased to 9.8 U mg⁻¹ protein after 22 h, a value still 1.2-fold higher than controls (8 U mg⁻¹ protein). Similar trend was observed for peroxidase and SOD as well. Rehydration for 10 min caused decrease in activities of all the antioxidant enzymes to values similar to the cultured cells.

Fig. 4 Total carbohydrate level (–Black square–) and protein content (–Black circle–) in *Anabaena* sp. PCC 7120 facing desiccation stress for 1–22 h

Total carbohydrates and protein content

Total cellular carbohydrate content rapidly increased during 1 h of desiccation (33.6 μg mg⁻¹ DW) but decreased thereafter at the rate of 1.75 μg mg⁻¹ DW h⁻¹ until 10 h (Fig. 4). A further decline led the carbohydrate content reach 13.85 μg mg⁻¹ DW at 22 h; a value still 2.57 units higher than the basal level (11.28 μg mg⁻¹ DW).

In contrast to the aforementioned osmolytes, protein content was maximum (301 μg mL⁻¹) at 3 h in desiccated biomass of *Anabaena* sp. PCC 7120 relative to the basal level (129 μg mL⁻¹). However, the curve followed similar trend to that of the osmolytes and carbohydrate contents during desiccation state.

Phycocyanin, carotenoids and Chl *a* under desiccation

Changes in photopigments' contents are shown in Fig. 5. Phycocyanin content decreased 3-fold (17 μg mg⁻¹ DW) after 1 h (rate 34 μg mg⁻¹ DW h⁻¹). A further decrease in the phycocyanin (0.78 μg mg⁻¹ DW h⁻¹) lowered the pigment level to 10 μg mg⁻¹ DW after 10 h, and the declining trend continued until 22 h. By contrast, the cellular carotenoid contents increased to 4.15 μg mg⁻¹ DW over the control

Table 2 Activities of antioxidative enzymes in *Anabaena* sp. PCC 7120 facing desiccation

Antioxidant Enzymes	Hydrated cells (U mg ⁻¹ protein)			Desiccated cells (U mg ⁻¹ protein)			Rehydrated cells (10 min) ^a (U mg ⁻¹ protein)
		1 h	3 h	6 h	10 h	22 h	
Catalase	8±0.27	17.6±0.17	18.5±0.29	13.4±0.26	10.9±0.15	9.8±0.13	12.3±0.18
Peroxidase	0.6±0.31	0.84±0.37	0.91±0.57	0.74±0.23	0.7±0.21	0.64±0.18	0.71±0.25
SOD	11.03±0.33	18.3±0.15	19.8±0.29	15.2±0.28	14.6±0.22	12.3±0.11	14.2±0.26

^a Data from the cells desiccated for 3 h (having maximum antioxidant activity)

Fig. 5 Photopigment contents in *Anabaena* sp. PCC 7120 facing desiccation stress for 1–22 h

($2.12 \mu\text{g mg}^{-1}$ DW) after 1 h of incubation under desiccation. However, the increase could not be sustained as the pigment content decreased within next 6 h ($0.28 \mu\text{g mg}^{-1}$ DW h^{-1}) followed by much gradual decrease ($0.052 \mu\text{g mg}^{-1}$ DW h^{-1}) to attain $1.9 \mu\text{g mg}^{-1}$ DW level at 22 h. The response of Chl *a* in desiccated cells followed a trend similar to carotenoids.

O₂-evolution in recovering desiccated cells

The viability of 1, 3 and 22 h desiccated cyanobacterial cells was monitored in terms of O₂-evolution following rehydration of desiccated cells for either 10 min or 1 h (Fig. 6). Undesiccated cells as controls had high O₂-evolution rate ($552 \mu\text{mol (g DW)}^{-1} \text{h}^{-1}$). The upper curve shows O₂-

Fig. 6 Recovery of photosynthetic O₂-evolution upon rehydration for 10 min (Black circle—Black circle) and 1 h (Black triangle—Black triangle) of the desiccated (1 h, 3 h and 22 h) biomass

evolution to be $527.3 \mu\text{mol}$ after 1 h, $434 \mu\text{mol}$ at 3 h, followed by a sharp decline ($165 \mu\text{mol}$) for 22 h desiccated sets subsequently rehydrated for 1 h. The lower curve corresponds to cells rehydrated for 10 min. The overall trend indicates that cells rehydrated for 10 min had lower recovery of O₂-evolution activity.

Discussion

In the present communication, we report on the changes in some physiological parameters especially the photosynthetic activity, osmolyte accumulation, peroxide production and level of antioxidant enzymes owing to imposition of desiccation conditions in otherwise weak desiccation tolerant cyanobacterium, *Anabaena* sp. PCC 7120. Water deficit (97.8 %) condition could be achieved within 1 h under desiccated state. Among the metabolites examined, trehalose content reached its maximum ($0.25 \mu\text{g mg}^{-1}$ Chl *a*) during 1 h but decreased afterward in the desiccated cells. Likewise, the level of sucrose was also maximum ($1.19 \mu\text{g mg}^{-1}$ Chl *a*) during 1 h. In report by Higo et al. (2006), the dehydrated *Nostoc* PCC 7120 cells accumulated trehalose but not sucrose in response to desiccation stress. Moreover, accumulation of trehalose was also reported for drought resistant variety of *Phormidium autumnale* and *Chroococcidiopsis* (Hershkovitz et al. 1991).

Apart from a protective role of osmolytes during dehydration, trehalose also induced dehydration tolerance regulator chaperones DnaK in *Anabaena* sp. PCC 7120 (Higo et al. 2006).

Intracellular concentration of total amino acids is shown to vary between fresh and desiccated cells of *Tolypothrix scytonemoides* (Rajendran et al. 2007). Fluctuations in proline content in the present cyanobacterium during desiccation followed a trend similar to those of sucrose and trehalose. Possibly proline serves as a reductant (Hare and Cress 1997), ATP source (Atkinson 1977), carbon-skeleton (Köcher et al. 2010), and is instrumental in quenching the ROS (Matysik et al. 2002) generated upon dehydration. Along with these functions, conformation of Hsp70 is reported to be regulated by proline (Vogel et al. 2006) thus indicating a multifaceted role of proline in desiccation tolerance.

Desiccation is reported to trigger ROS (singlet oxygen, superoxide, hydroxyl radical, nitric oxide and H₂O₂) production, and in the dry state, survival of desiccation tolerant organisms is jeopardized once antioxidant mechanism is perturbed (Kranner and Birtić 2005). Although we observed a 5-fold rise in H₂O₂ level after 1 h, its level declined afterwards. In conjunction with this, significant rise in activities of antioxidant enzymes (catalase, peroxidase and SOD) up to 3 h, and the lowering of ROS, clearly indicates role of antioxidant enzymes in scavenging ROS. Desiccation in the cyanobacterium

Tolypothrix scytonemoides showed elevated catalase and SOD activities (Rajendran et al. 2007). In present study, antioxidant enzymes' activities in rehydrated cells (for 10 min) were close to that of hydrated cells, suggesting an instant reversal of the enzymes subject to the changes in cellular water content.

Decline in phycocyanin and the rise in carotenoids and Chl *a* contents in *Anabaena* sp. PCC 7120 cells desiccated for 1 h seemed to be interlinked. Reduced O₂ evolution during 1 h of desiccation and an increased Chl *a* content are in line with those reported for *Nostoc commune* (Tamaru et al. 2005). Phycocyanin is the predominant energy collectors of PS II, and once degraded, energy transfer to PSII gets impaired, as can be evident from decreased O₂ evolution in present study. As desiccation leads to oxidative stress, an enhanced level of carotenoids most likely facilitates quenching of ROS.

It was apparent that cells desiccated for 1 h followed by 10 min of rehydration, evolved about 50 % O₂ suggesting the duration was not enough to block the process. If such cells were rehydrated for 1 h, O₂-evolution reached over 95 % indicating that 1 h dehydration failed to completely inactivate the photosynthetic machinery. Moreover rehydration of 22 h desiccated cells for 10 min and 1 h showed only 13 % and 29 % of O₂-evolution capabilities respectively, indicating that vitality of the organism declined though an inherent capacity of revival existed.

To sum up, desiccated *Anabaena* sp. PCC 7120 cells produced osmolytes (trehalose, sucrose and proline) and antioxidant enzymes that peaked between 1 h and 3 h. These attributes seem to protect the cells from dehydrated state. Further, photosynthetic O₂-evolution, while displaying extraordinary sensitivity to desiccation, maintained some degree of revival upon rehydration. Since desiccation leads to sequential metabolic events in *Anabaena* sp. PCC 7120, selection of genes with hyper-antioxidant activities coupled with overproduction of osmolytes may be chosen for developing desiccation tolerant strains as model for higher plants.

Acknowledgements We are grateful to the Head and Programme Coordinator, Center of Advanced Study in Botany, Banaras Hindu University for lab facilities and Head, Department of Chemistry, BHU for NMR. Financial support by University Grant Commission, New Delhi (F. No. 17/46/98(SA-I) to Priyanka and (19-12/2010 (I) EU-IV) to A. Tiwari is gratefully acknowledged.

References

- Aebi H (1984) Catalase in vitro. In: Packer L (ed) Methods Enzymol. Academic Press 105, pp 121–126
- Alpert P (2005) The limits and frontiers of desiccation-tolerant life. Integr Comp Biol 45:685–695
- Asthana RK, Srivastava S, Singh AP, Kayastha AM, Singh SP (2005) Identification of maltotriose synthase and maltotriose synthase trehalohydrolase enzymes catalyzing trehalose biosynthesis in *Anabaena* 7120 exposed in NaCl stress. J Plant Physiol 162:1030–1037
- Atkinson DE (1977) Cellular energy metabolism and its regulation. Academic, New York
- Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water stress studies. Plant Soil 39:205–207
- Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assays and applicable to acrylamide gels. Anal Biochem 44:276–287
- Brody SS, Brody M (1961) A quantitative assay for the number of chromatophores on a chromoprotein: its application to phycoerythrin and phycocyanin. Biochim Biophys Acta 50:348–352
- Carpenter JF, Crowe JH (1988) Modes of stabilization of protein by organic solutes during desiccation. Cryobiology 25:459–470
- Dadhech N (2010) Desiccation tolerance in cyanobacteria. Afri J Microbiol Res 4:1584–1593
- Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:250–256
- Fisher MT (2006) Proline to the rescue. Proc Nat Acad Sci 103:13265–13266
- França MB, Panek AD, Eleutherio EC (2007) Oxidative stress and its effects during dehydration. Comp Biochem Physiol Part A 146:621–631
- Fredrickson JK, Li Shu-mei W, Gaidamakova EK, Matrosova VY, Zhai M, Sulloway HM, Scholten JC, Brown MG, Balkwill DL, Daly MJ (2008) Protein oxidation: key to bacterial desiccation resistance? ISME J 2:393–403
- Gahagan HE, Holm RE, Abeles FB (1968) Effect of ethylene on peroxidase activity. Physiol Plant 21:1270
- García AH (2011) Anhydrobiosis in bacteria: from physiology to applications. J Biosci 36:939–950
- Hansen JM, Go YM, Jones DP (2006) Nuclear and mitochondrial compartmentation of oxidative stress and redox signaling. Annu Rev Pharmacol Toxicol 46:215–234
- Hare PD, Cress WA (1997) Metabolic implications of stress-induced proline accumulation in plants. Plants Growth Regul 21:79–102
- Herbert D, Phipps PJ, Strange RE (1971) Chemical analysis of microbial cells. In: Norris JR, Ribbons D (eds) Methods in microbiology. Academic Press, London, pp 209–344
- Herskovitz N, Oren A, Cohen Y (1991) Accumulation of trehalose and sucrose in cyanobacteria exposed to matrix water stress. Appl Environ Microbiol 57:645–648
- Higo A, Katoh H, Ohmori K, Ikeuchi M, Ohmori M (2006) The role of a gene cluster for trehalose metabolism in dehydration tolerance of the filamentous cyanobacterium *Anabaena* sp. PCC 7120. Microbiology 152:979–987
- Katoh H, Asthana RK, Ohmori M (2004) Gene expression in the cyanobacterium *Anabaena* sp. PCC 7120 under desiccation. Microb Ecol 47:164–174
- Köcher S, Tausendschön M, Thompson M, Saum SH, Müller V (2010) Proline metabolism in the moderately halophilic bacterium *Halophilus*: differential regulation of isogenes in proline utilization. Environ Microbiol Rep. doi:10.1111/j.17582229-2010-00214-x
- Kranner I, Birtić S (2005) A modulating role for antioxidants in desiccation tolerance. Integr Comp Biol 45:734–740
- Li BW, Schuhmann PJ (1981) Gas chromatographic analysis of sugars in Granola cereals. J Food Sci 46:317–323
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurements with the Folin-phenol reagent. J Biol Chem 193:265–275
- Mansour KS, Hallet JN (1981) Effect of desiccation on DNA synthesis and the cell cycle of the moss *Polytrichum formosum*. New Phytol 87:315–324

- Matysik J, Alia Bhalu B, Mohanty P (2002) Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. *Curr Sci* 82:525–532
- Mihailova G, Petkova S, Büchel C, Georgieva K (2011) Desiccation of the resurrection plant *Haberlea rhodopensis* at high temperature. *Photosynth Res* 108:5–13
- Millan-Almaraz JR, Guevara-Gonzalez RG, Romero-Troncoso R, Osorio-Rios RA, Torres-Pacheco I (2009) Advantages and disadvantages of photosynthesis measurement techniques: a review. *Afri J Biotechnol* 8:7340–7349
- Myers J, Kratz WA (1955) Relation between pigment content and photosynthetic characteristics in blue-green algae. *J Gen Physiol* 20:11–22
- Page-Sharp M, Behm CA, Smith GD (1999) Involvement of compatible solutes trehalose and sucrose in the response to the salt stress of a cyanobacterial *Scytonema* species isolated from desert soils. *Biochim Biophys Acta* 1472:519–528
- Potts M, Slaughter SM, Hunneke FU, Garst JF, Helm RF (2005) Desiccation tolerance of prokaryotes: applications of principles to human cells. *Integr Comp Biol* 45:800–809
- Rajendran UM, Kathirvel E, Anand N (2007) Desiccation-induced changes in antioxidant enzymes, fatty acids and amino acids in the cyanobacterium *Tolypothrix scytonemoides*. *World J Microbiol Biotechnol* 23:251–257
- Rebecchi L, Altiero T, Guidetti R (2007) Anhydrobiosis: the extreme limit of desiccation tolerance. *Invertebr Survival J* 4:65–81
- Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J Gen Microbiol* 111:1–61
- Rudolph AS, Crowe JH, Crowe LM (1986) Effects of three stabilizing agents-proline, betaine and trehalose on membrane phospholipids. *Arch Biochem Biophys* 245:134–143
- Sagisaka S (1976) The occurrence of peroxide in a perennial plant *Populus gelrica*. *Plant Physiol* 57:308–309
- Sakamoto T, Yoshida T, Arima H, Hatanaka Y, Takani Y, Tamaru Y (2009) Accumulation of trehalose in response to desiccation and salt stress in the terrestrial cyanobacterium *Nostoc commune*. *Physiol Res* 57:66–73
- Serraj R, Sinclair TR (2002) Osmolyte accumulation: can it really help increase crop yield under drought conditions? *Plant Cell Environ* 25:333–341
- Tamaru Y, Takani Y, Yoshida T, Sakamoto T (2005) Crucial role of extracellular polysaccharides in desiccation and freezing tolerance in the terrestrial cyanobacterium *Nostoc commune*. *Appl Environ Microbiol* 71:7327–7333
- Vogel M, Bukau B, Mayer MP (2006) Allosteric regulation of Hsp70 chaperones by a proline switch. *Mol Cell* 21:359–367
- Wingler A (2002) The function of trehalose biosynthesis in plants. *Photochemistry* 60:437–440
- Wolkers WF, Tablin F, Crowe JH (2002) From anhydrobiosis to freeze drying of eukaryotic cells. *Comp Biochem Phys* 131A:535–543