Tropical Ecology **44**(2): 257-259, 2003 ISSN 0564-3295

Sensitivity of the cyanobacterium *Tolypothrix scytonemoides* isolated from temple rocks to low water potentials

R. UMA MAHESHWARI* & N. ANAND**

Centre for Advanced Studies in Botany, University of Madras, Chennai – 600 025, India

Key words: Cyanobacterium, growth, nitrogen fixation, osmotic control, photosynthesis, *Tolypothrix scytonemoides*, water potential, water stress.

Desiccation tolerance is more prevalent in prokaryotes than in eukaryotes (Bewley 1979). Many cyanobacteria of both marine and terrestrial origin express a capacity to tolerate water stress and desiccation (Potts 1985). Low water activity can be expressed as osmotic water stress [high salt concentrations] (Reed *et al.* 1986) and matric water stress [dry environments] (Potts & Friedmann 1981). The water potential of a system may be controlled either through the direct addition of solutes like NaCl, to the medium [osmotic control] or by equilibration above a solution of defined water potential [matric or isopiestic] (Potts *et al.* 1984). Potts and coworkers have investigated the effects of acute water stress on nitrogen fixation (Potts *et al.* 1984), photosynthesis (Potts & Friedmann 1981), intracellular ATP levels (Potts *et al.* 1984) and protein synthesis (Potts 1985). This paper deals with the impact of acute water stress effected by the osmotic control method on growth, rates of photosynthetic oxygen evolution and nitrogenase activity in the cyanobacterium, *Tolypothrix scytonemoides* thriving under extreme drought conditions and high temperature on the exposed rock surfaces during summer.

Axenic culture of *T. scytonemoides* (Gardner) Geitler obtained from Dr. S.P. Adhikary, Utkal University was grown autotrophically in BG-11 medium (Rippka *et al.* 1979) in Erlenmeyer flasks at 34 ^{o}C under 30 μE m^2s^{-1}. The water potential of the medium was controlled adding NaCl to the medium in different molal concentrations, which correspond approximately to different water potentials (Potts & Friedmann 1981). Exponentially growing cells were concentrated by low-speed centrifugation (2856 x g) and the pellet was resuspended in fresh medium containing a range of molal concentrations of NaCl. After an incubation of 24 h and 72 h, cells were examined for the effect of different water potential on growth, photosynthesis and nitrogen fixation.

Growth of the cyanobacterium was measured in terms of chlorophyll *a* (Mackinney 1941). The method of Allen & Holmes (1986) was used to determine the rate of photosynthesis and nitrogenase activity was estimated by the acetylene reduction assay of Stewart *et al.* (1967). All the experiments were conducted in triplicate and repeated at least twice to confirm the reproducibility of the results.

T. scytonemoides although isolated from an arid environment (Tripathy *et al.* 1999) is not resistant to low water potential. A decline in growth of the organism in terms of chlorophyll *a* was observed with decreasing water potential. The decrease was linear after incubation of 24 h but after 72 h there was a sharp drop below –2000 kPa. (Fig. 1a). The colour of the cells changed at different water potentials and these changes were persistent. Cells maintained at –90 kPa (control) retained the greenish-brown colour whereas cells at – 1000 kPa were dull green and brown. Cells incubated between –1000 and –4000 kPa assumed a

* *Present Address*: Department of Industrial Biotechnology, Shri Andal Alagar College of Engineering, Mamandur – 603 111.
** *Corresponding Author*

brownish-green colour and below –4000 kPa they appeared bright blue-green. These colour changes at different water potentials may be associated with different pigment concentrations. A substantial loss of chlorophyll *a* accompanied the bleaching of cells. Change in colour from green to red or brown was observed in *Dunaliella* after a transfer to higher salinity due to a stimulated synthesis of carotenoids and the loss of chlorophyll (Mironyuk & Einor 1968). The relative sensitivities of photosynthesis and nitrogen fixation processes of *T. scytonemoides* resemble some of the findings reported earlier (Potts & Friedmann 1981; Potts *et al.* 1984). Potts & Friedmann (1981) subjected strains of *Chroococcidiopsis, Chroococcus* (both from extreme arid rocks) and a marine *Chroococcus* to water potential stress by the osmotic control method. Both the strains of *Chroococcus* showed optimum photosynthesis between –3000 and –4000 kPa but

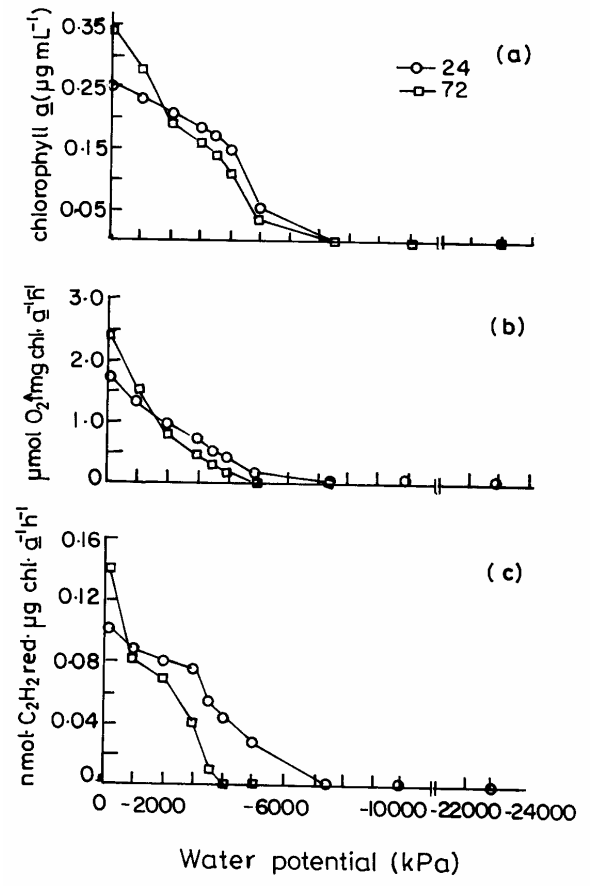

Fig. 1. Effect of water stress on (a) growth (b) rate of photosynthesis and (c) nitrogenase activity in cells of *T. scytonemoides.*

in strains of *Chroococcidiopsis*, the photosynthetic rates decreased with decreasing water potentials. *Chroococcus* strain from desert did not grow at low solute concentration in freshwater media and in its response to water stress, is similar to marine *Chroococcus*. Optimum photosynthetic efficiency of both the strains of *Chroococcus* occurred near the water potential at which the *Chroococcidiopsis* strains were inhibited completely. For the strains of *Chroococcus* from desert, it is possible that the adaptation to desert conditions is due to its ability to photosynthesize at rather low water potenitals, a situation different from that of *Chroococcidiopsis. Chroococcidiopsis* strains were not resistant to low water potentials. Although these two genera do not seem especially adapted for growth and photosynthesis at low water potentials, they show a considerable ability to tolerate severe drought conditions. In *T. scytonemoides,* photosynthetic rates decreased with decreasing water potentials (Fig. 1b) as in the case of *Chroococcidiopsis* strains. There was a sharp decrease in the rate of photosynthesis between – 2000 and – 3500 kPa as a result of loss of chlorophyll *a*. Incubation for a longer period (72 h) further reduced the rate of photosynthesis. The impairment of photosynthesis may be due to the reduction in the photosynthetic pigment content. *T. scytonemoides* thus resembles the strains of *Chroococcidiopsis* which are not resistant to low water potentials. Another sensitive organism is a *Microcoleus* sp., a dominant organism in desert soil crusts (Brock 1975). Nitrogen fixation is restricted to a narrow range of water potential and is sensitive to changes in water potential. After 24 h incubation, nitrogenase activity was found to be lower in cells maintained at -1000 kPa and below in comparison with control (Fig. 1c). In *T. scytonemoides*, nitrogenase activity was impaired and became negligible at very low water potentials and declined with increase in time given for incubation as in *Nostoc commune* (Potts *et al.* 1984). *T. scytonemoides* failed to withstand very low water potentials in the laboratory although it occurs predominantly in extremely dry conditions in nature.

Acknowledgements

We thank the Director for providing the facilities. We also thank Prof. V.N. Raja Rao and Prof. R. Rengasamy for useful suggestions.

References

Allen, J.F. & N.G. Holmes. 1986. The liquid phase oxygen electrode. pp. 108-116. *In*: M.F. Hipkins & N.R. Baker (eds.) *Photosynthesis Transduction a Practical Approach,* IRL Press.

Bewley, J.D. 1979. Physiological aspects of desiccation tolerance. *Annual Review of Plant Physiology* **30**: 195-238.

Brock, T.D. 1975. Effect of water potential on a *Microcoleus* (Cyanophyceae) from a desert crust. *Journal of Phycology* **11**: 316-320.

Mackinney, G. 1941. Absorption of light by chlorophyll solution. *Journal of Biological Chemistry* **140**: 315-322.

Mironyuk, V.L. & L.O. Einor. 1968. Oxygen exchange and the content of pigments in various forms of *Dunaliella salina* Teod. under conditions of increased NaCl content. *Gidrobiologichesky Zhurnal* **4**: 51-58.

Potts, M. 1985. Protein synthesis and proteolysis in immobilized cells of the cyanobacterium *Nostoc commune* UTEX 584 exposed to matric water stress. *Journal of Bacteriology* **164**: 1025-1031.

Potts, M. & E.I. Friedmann. 1981. Effects of water stress on cryptoendolithic cyanobacteria from hot desert rocks. *Archives of Microbiology* **130**: 267-271.

Potts, M., M.A. Bowman & N.S. Morrison. 1984. Control of matric water potential (ψ_m) in immobilised cultures of cyanobacteria. *FEMS Microbiological Letters* **24**: 193-196.

Reed, R.H., L.J. Borowitzka, M.A. Mackay, J.A. Chudek, I. Foster, S.R.C. Warr, D.J. Moore & W.D.P. Stewart. 1986. Organic solute accumulation in osmotically stressed cyanobacteria. *FEMS Microbiological Reviews* **39**: 51-56.

Rippka, R., J. Deruelles, J.B. Waterbury, M. Herdman & R.Y. Stanier. 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Journal of General Microbiology* **111**: 1-61.

Stewart, W.D.P., G.P. Fitzgerald & H. Burris. 1967. *In situ* studies on nitrogen fixation using the acetylene reduction technique. *Proceedings of National Academy of Science, USA* **58**: 2071-2078.

Tripathy, P., A. Roy, N. Anand & S.P. Adhikary. 1999. Blue-green algal flora on the rock surface of temples and monuments of India. *Feddes Repertorium* **110**: 133-144.