

# Effect of salinity and light intensity on superoxide dismutase and ascorbate peroxidase activity from *Microcoleus chthonoplastes* Strain SC7B9002-1

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## ABSTRACT

Exposure of filamentous nonheterocystous cyanobacterium *Microcoleus chthonoplastes* to varying daylight (0-1591  $\mu\text{E m}^{-2} \text{s}^{-1}$ ) and varying salinity (2.5, 4.0, 8.0, and 12% NaCl) produced different effects on growth rate, superoxide dismutase (SOD) activity, and ascorbate-peroxidase (AsA-POD) activity. A decrease in SOD and AsA-POD activity was observed under all salt concentrations, though the highest growth rates were in 8.0% NaCl. When *M. chthonoplastes* cells were exposed to different light conditions, a different pattern was observed to those observed in saline solutions. As light intensity increased, there was an increase of total protein, accompanied by a partial photoinhibition.

## INTRODUCTION

Under environmental stress, a variety of reactive oxygen species (ROS) may be produced. These include singlet oxygen ( $\text{O}_2$ ), superoxide radical ion ( $\text{O}_2^-$ ), perhydroxyl ion ( $\text{HO}_2^-$ ), hydroxyl ion ( $\text{HO}^-$ ) and hydrogen peroxide

(H<sub>2</sub>O<sub>2</sub>), some of which can be scavenged either by superoxide dismutase (SOD), catalase (CAT), or peroxidase (POD). Bacteria may be subjected to environmental stress through potential fluctuations in temperature, oxygen, pH, osmolarity, toxic chemicals, nutrients, radiation, and pressure. The cyanobacteria are of special interest because of their capacity to survive and colonize extreme environments (Potts, 1994), especially the genus *Microcoleus*, which is a cosmopolitan cyanobacterium and occurs in many marine and hypersaline environments as a dominant constituent of microbial mats (Garcia-Pichel *et al.*, 1996). Because of this, *Microcoleus* has been the subject of much attention. The aim of this work was to investigate the effect of environmental light intensity and salinity on SOD and AsA-POD activity from *Microcoleus chthonoplastes* strain SC7B9002-1, a dominant cyanobacterium of mats found in tidal channels in Puerto San Carlos in BCS, México.

## MATERIALS AND METHODS

The effect of light intensity on SOD and AsA-POD activity from a *M. chthonoplastes* strain, designated as SC7B9002-1, isolated from the microbial mats in the tidal channels of San Carlos, Baja California Sur, Mexico (López-Cortés, 1990) was tested following a day-night cycle ranging from 0 to 1591  $\mu\text{E m}^{-2} \text{s}^{-1}$  in an open sky. *Microcoleus* was cultured in a 1-L flask containing 200 mL of ASN-III at a temperature of  $26 \pm 2^\circ\text{C}$  and a constant agitation provided by a magnetic stirrer. We took an aliquot of 3 mL from the culture to measure the total protein content, chlorophyll *a* concentration, SOD, and AsA-POD activity every 2 h in a diurnal cycle. The effect of salinity was tested in batch culture in 1-L sterile flasks with 200 mL of ASN-III culture medium (Waterbury & Stanier, 1981) in a range of salinities: 2.5, 4.0, 8.0, and 12% NaCl,  $25^\circ\text{C}$ , 100 rpm agitation, and a light intensity of  $30 \mu\text{E m}^{-2} \text{s}^{-1}$  provided by daylight white cool lamps. Three replicate samples of 3 mL were taken every two days of culture during 18 days to measure chlorophyll *a* (Tandeau de Marsac & Houmard, 1988), total protein (Bradford, 1976), and nucleic acids (Sanbrook *et al.*, 1989) as growth variables. The cells were disrupted by sonication with a COLE PARMER 4710 sonicator using three intermittent pulses of 30 seconds at 20 W power in an ice bath at  $\sim 4^\circ\text{C}$ . SOD activity was estimated according to McCord & Fridovich (1969). The donor specificity of *M. chthonoplastes* POD activity was determined using ascorbate (0.5 mM at 290 nm) according to Nakano & Asada (1981) in a DU-640 Beckman spectrophotometer. Samples in triplicate were used to evaluate total extractable protein, chlorophyll *a*, nucleic acids, SOD, and POD activity. One-way ANOVA, Student's *t*-test and multifactorial analysis ANOVA were made using STATGRAPHICS version 5.1 (1991).

## RESULTS

Despite lacking catalase, *M. chthonoplastes* possesses AsA-POD as a system to eliminate the hydrogen peroxide produced by photosynthesis (Nakano & Asada, 1981). Light intensity of  $700 \mu\text{E m}^{-2} \text{s}^{-1}$  induces the increase of the SOD activity (Fig. 1B). As light intensity increases, SOD and

