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Oxidative stress in Microcystis aeruginosa as a consequence of global climate change

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ABSTRACT: Cyanobacteria are phototrophic organisms with great ecological and economical importance. Species of the genus *Microcystis* are known for their potential ability to synthesize toxins, notably microcystins. There is a growing interest in the evaluation of oxidative stress in relation to the impact of global climate change on natural ecosystems in different trophic levels. Several studies have focused on the analysis of organismal responses to mitigate the damage by controlling the generation of reactive oxygen species. Variations in environmental factors caused by climate change generate a situation of oxidative damage in *Microcystis aeruginosa* as a direct or indirect consequence. In this study we evaluate the effects of ultraviolet radiation and temperature on physiological and biochemical responses of a native *M. aeruginosa* (strain CAAT 2005-3). The results from the exposure to ultraviolet radiation doses and temperature changes suggest a high ability of *M. aeruginosa* to detect a potential stress situation as a consequence of reactive species production and to rapidly initiate antioxidant defenses. Increased catalase activity is an antioxidant protection mechanism in *M. aeruginosa* for short and long term exposure to different changes in environmental conditions. However, we found a ultraviolet-B radiation threshold dose above which oxidative stress exceeds the antioxidant protection and damage occurs. In addition our results are in agreement with recent findings suggesting that microcystins may act as protein-modulating metabolites and protection against reactive oxygen species.
It is concluded that cyanobacteria have adaptative mechanisms that could lead to the replacement of species highly susceptible to oxidative stress by others with a higher system of antioxidant protection.

Cyanobacteria are phototrophic organisms that have great ecological and economical importance. They have lived on Earth for about 2500-3500 Ma, during which they had to support extreme conditions, mainly due to high levels of ultraviolet radiation (UVR, Schopf, 2000). In relation to global climate change, it has been proposed that these organisms are the best adapted to increases in temperature and UVR levels (Ding *et al.,* 2013), which are the main outcomes of this anthropogenic phenomenon. Increased ultraviolet-B radiation (UVRB, 280-315 nm), as a consequence of decreased ozone layer, is potentially harmful to all forms of life but is more detrimental to photosynthetic organisms, including cyanobacteria (Latifi *et al.,* 2009, Shina *et al.,* 2001). Species of the genus *Microcystis* are known for their potential ability to synthesize toxins, notably microcystins (MC) (Dziallas and Grossart, 2011). *Microcystis* sp. blooms are frequently found in eutrophicated waters affecting fishes, zooplanctonic communities, aquatic plants and vertebrates as well as to hepatocyte necrosis and liver hemorrhage in mammals after acute exposure (Dawson, 1998). Over 70 structural analogues of these heptapeptides have been identified up to date (Pearson *et al.,* 2010), but only a few occur frequently at high concentrations. In addition, different temperatures can also be correlated with production of different chemical MC forms (Rapala *et al.,* 1997). There is growing interest in the evaluation of oxidative stress in relation to the impact of global climate change on natural ecosystems in different trophic levels, through the study of the organism's responses to mitigate the damage, controlling the generation of reactive

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oxygen species (ROS). The balance between the mechanisms of damage and defense has ecological relevance since it keeps the ecosystem productivity and contributes to the regulation of potential climate change. Despite the large number of studies that report the damage by UVR on cyanobacteria, diatoms and chlorophytes, it is only possible in very few to distinguish between direct and indirect oxidative damage induced by radiation.

Bloom forming cyanobacteria are exposed to UVRB on a mass scale, particularly during the surface bloom. All buoyant species of cyanobacteria are at least periodically exposed to higher irradiation during their vertical migration to the surface (Oliver and Ganf, 2000). In order to evaluate the effects of UVR on physiological responses of a native *Microcystis aeruginosa* (strain CAAT 2005-3 (Rosso *et al.,* 2014a), three radiation treatments (by using different filters) were implemented in short time exposure (hours): (i) P (PAR, 400-700nm), (ii) UVA (PAR+UVA, 315-700 nm) and, (iii) UVB (PAR+UVA+UVB, 280-700 nm). Reduction in photosynthetic pigments like chlorophyll-*a* (Chl-*a*) of the test microorganisms were accompanied with an increment in their total reactive species (measured by intra and extracellular 2´-7´-dichlorodihydrofluorescein diacetate oxidation) and enzymatic antioxidants like superoxide dismutase (SOD, measured by enzymatic kit) and catalaze activity (CAT, measured spectrophotometrically) in higher UVR doses. It has been shown that hydrogen peroxide induces apoptotic-like cell death in *M. aeruginosa* in a dose-dependent manner (Ding *et al.,* 2012). However, we found a threshold for 305 nm and 380 nm doses of 1.13 and 9.78 kJ m^{-2} respectively, below which no UVR effects were observed. In addition for 305 nm doses between 1.13 and 1.22 kJ m^{-2}, despite the decrease of Chl-*a,* a significant decrease in 2´-7´-dichlorodihydrofluorescein diacetate oxidation was evident as a consequence of a decrease in SOD activity (Thomas *et al.,* 1998) as well as an increase in assimilation number (μgC (μg Chl-*a*)$^{-1}$ h^{-1}) in UVB treatment compared with P and UVA treatments. For 305 nm higher doses than 1.22 kJ m^{-2}, all stress parameters and photosynthesis inhibition (%), measured by ^{14}C inoculation, increased significantly, being higher the reduction on carbon assimilation due to UVA radiation (315-400 nm).

Increasing fossil fuel emissions and deforestation over the past 250 years have increased atmospheric CO_2 concentrations and caused a rise in global average temperatures (Feng *et al.,* 2009). In the current century, global temperatures are expected to increase an additional 2–5ºC (Houghton *et al.,* 2001). Harmful cyanobacteria such as *Microcystis* have been found to have an optimal temperature for growth and photosynthesis at, or above, 25ºC (Paerl and Huisman, 2008). The growth response of toxigenic and non-toxigenic strains of cyanobacteria to increasing water temperature in an ecosystem setting has been poorly explored. A high temperature stimulates the metabolic rate of the plankton (Zinser *et al.,* 2007) and such activation implies an increase in oxygen consumption that could lead to intracellular oxidative stress conditions and different MC isoforms production. The purpose of this study was to elucidate the effects of changing temperatures on CAT in *M. aeruginosa* in culture conditions. For long term experiments (7 days exposure) we used cells from cultures during the exponential growth phase. Three treatments were applied: 23ºC ("low temperature"), 26ºC ("control") and 29ºC ("high temperature"). The average irradiance in each incubator was 30 μE m^{-2} s^{-1} (daily monitored with an IL spectroradiometer) under 14/10 h light/dark photocycle. ROS and CAT were evaluated like was explained for UVR experiments. Exponential growth rate (μ) was significantly different at each temperature, reaching 0.32; 0.33 and 0.43 d^{-1} at 23º, 26º and 29ºC respectively. In addition, there was a delay in the start of exponential growth at 23ºC. Chl-*a* decreased significantly (30%) as a consequence of high temperature exposure. A significant increase in 2´-7´-dichlorodihydrofluoresce in diacetate oxidation intracellular was observed in coincidence with the activation of CAT activity during the first two days of exposure to 23ºC and 29ºC in comparison to the control, decreasing thereafter to nearly initial values. Increased level of CAT activity indicated its involvement in ROS scavenging. HPLC/MS (liquid chromatography coupled with tandem mass spectrometry) analysis revealed five MCs variants produced by the strain. The highest MC cell concentration, measured as [Leu^1] MC-LR equivalents, was significantly lower on days 2 and 4 in cells exposed at 29ºC than in controls. The same trend was observed for all other MC except for the least abundant MC-LR which showed a continuous increase being the cellular concentration significantly higher at the end of the exposure compared with previous days measurement.

The results from the exposure to high UVR doses and temperature changes suggest a high ability of *M. aeruginosa* to detect a potential stress situation as a consequence of reactive species production and to rapidly initiate antioxidant defenses (Hernando *et al.,* 2015), and lead to the conclusion that these cyanobacteria avoid oxidative stress damage as consequence of UVBR daily doses lower than 99 kJ m^{-2} (4-5 hours exposure) and temperature changes in the range of +/- 3ºC. CAT activity would be one of the antioxidant-protection mechanisms in this cyanobateria for short and long term exposure to different changes in ambient conditions (Rosso *et al.,* 2014b). In addition, our results are in agreement with recent findings from Dziallas and Grossart (2011) who proposed that MCs could act as protein-modulating metabolites and protection against ROS. Variations in environmental factors caused by climate change generate a situation of oxidative damage in *M. aeruginosa* as a direct or indirect consequence. Cyanobacteria have adaptative mechanisms that could lead to the replacement of highly susceptible species to oxidative stress by others with a higher system of antioxidant

defenses that would be able to resist oxidative damage driven by the new environmental conditions.

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