PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF ANTARCTIC ISOLATE *CHORICYSTIS MINOR* DURING OXIDATIVE STRESS AT DIFFERENT TEMPERATURES AND LIGHT INTENSITIES

R. Zidarova and I. Pouneva*

University of Sofia, Faculty of Biology, Department of Botany, 8, Dr. Tzankov Blvd., 1164 Sofia, Bulgaria

**Acad. M. Popov Institute of Plant Physiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria*

Summary. Oxidative stress effects on growth, viability, pigment (chlorophyll *a*, chlorophyll *b*, *β*-carotene) and protein contents in Antarctic microalga *Choricystis minor* were investigated. *C. minor* was cultivated at optimal (23.5°C), or extreme (15°C 27.5°C) temperatures and light intensity of 520 and 1000 μmol m^{-2} s^{-1}. Oxidative stress was developed by adding Sanosil which degrades to H_2O_2 and Ag^+. As a result inhibited growth and viability of algal cells cultivated at optimal and extreme high temperatures was observed and treatment caused a decrease of pigment and protein contents. This negative effect was further increased by the enhancement of light intensity. The combined treatment of algae with low temperature (15°C) and Sanosil resulted in an increase of total pigment and protein contents by 38% and 36%, respectively.

Keywords: Antarctic microalga, chlorophyll *a*, chlorophyll *b*, *β*-carotene, oxidative stress, protein content, viability.

INTRODUCTION

Most of the Antarctic algae could grow in a wide temperature range (Nadeau et al., 1992; Nadeau and Castenholz, 2000) and their temperature-growth response suggests close relationship with algae from moderate temperature regions (Seaburg et

* Corresponding author, e-mail: ipuneva@bio.bas.bg

al., 1981). For this reason Antarctic algae can be used as test objects for studies on different physiological and biochemical processes.

Data concerning the influence of temperature on photosynthetic activity of Antarctic microalgae are contradictory (Hawes, 1990; Anning et al., 2001; Mock and Valentin, 2004). To investigate the temperature effect on polar microalga photosynthesis we studied changes in the concentration of photosynthetic pigments and biomass accumulation in the at Antarctic isolate *Choricystis minor*, cultivated different temperatures, light and oxidative stress conditions. Species of the genus *C. minor* have been recently found in Antarctic habitats as well as among the picoplankton in lakes and small water basins in Europe, Asia and North America in very low percentage which suggests their good adaptive potential to different temperatures. In this aspect *C. minor* is a convenient object for ecological and physiological investigations.

Oxidative stress arises from an imbalance in the generation and metabolism of reactive oxygen species (ROS) with more ROS (such as H_2O_2, $OH^{\cdot}$, and O_2^{-}) being produced than metabolized. Cytosolic H_2O_2 creates toxic environment in the plant cells leading to oxidative stress and cell death. It is established that in the marine alga *Nannochloropsis* the induced oxidative stress increase under higher light intensity.

The aim of the present study was to determine the growth, viability, pigment and protein content of the Antarctic microalgal isolate *Choricystis minor* during oxidative stress at different temperatures and light intensity.

MATERIALS AND METHODS

Choricystis minor (Skuja) Fott was isolated from one of the moss samples collected on Livingston Island, the South Shetland Archipelago.

C. minor was cultivated in 200 cm^3 culture vessels kept in haemostat at suspension temperatures of 15º, 26º and 30ºC under continuous illumination (520 and 1000 ìmol m^{-2} s^{-1}). Cultures were aerated with air (100 dm^3 h^{-1}) enriched with 2% CO_2.

Oxidative stress was applied by adding Sanosil (algicid concentration – 0.125%) which biologically dissociates to H_2O_2 and Ag^+.

The growth, viability, pigment (chlorophyll ***a***, chlorophyll ***b*** and ***β*** - carotene) and protein contents at different temperatures, light intensity and under oxidative stress were examined after 48 h of cultivation. The growth was determined gravimetrically as an absolute dry weight (DW). Viability was tested using the triphenyl tetrazolium chloride (TTC) method (Steponkus and Lanphar, 1967). Pigment extraction was performed with hot methanol; pigment content was assessed spectrophotometrically and estimated according to McKinney (1941). Protein content was tested using the method of Lowry.

RESULTS

The present investigation showed that at both light intensities pigment quantity in *C. minor* cells decreased reciprocally to the temperature enhancement of the cultivation media. The highest pigment content was observed in cultures grown under the lowest temperature of 15°C (Fig.1A, 1B; 2A, 2B and 3A, 3B). Oxidative stress inhibited chlorophyll *a*, chlorophyll *b* and *β*–carotene content at extreme high temperatures. However, at low cultivation temperature H_2O_2 stimulated the pigment synthesis. The pigment synthesis inhibition caused by oxidative stress enhanced si-

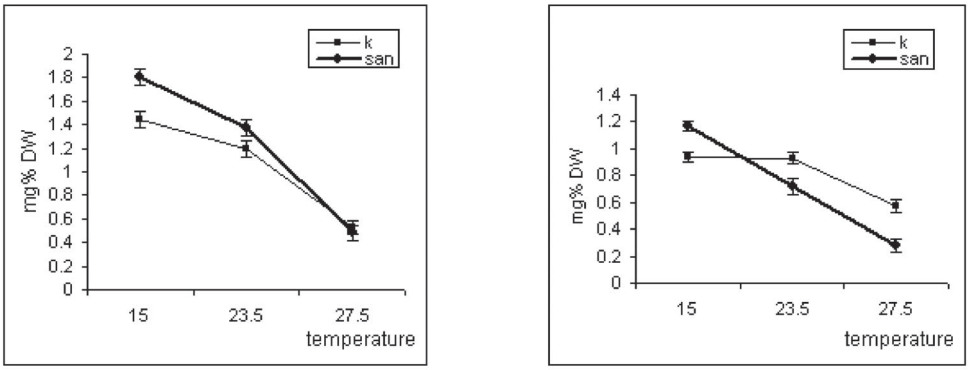


Figure 1. Effect of oxidative stress on chlorophyll content.
A - 520 μmol m^{-2} s^{-1} light intensity; B - 1000 μmol m^{-2} s^{-1} light intensity.

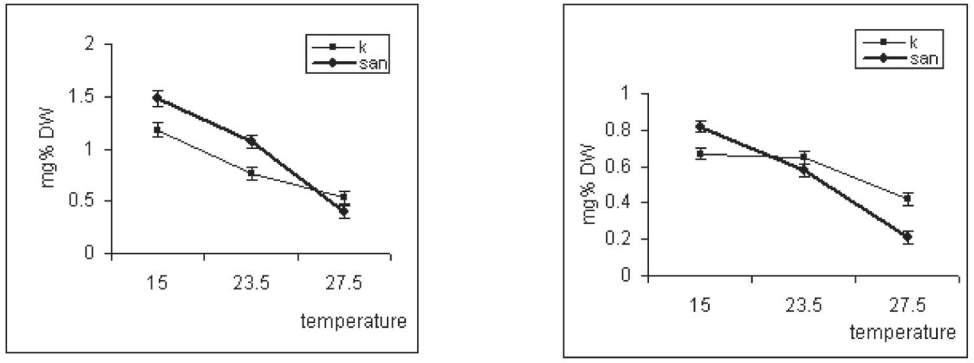
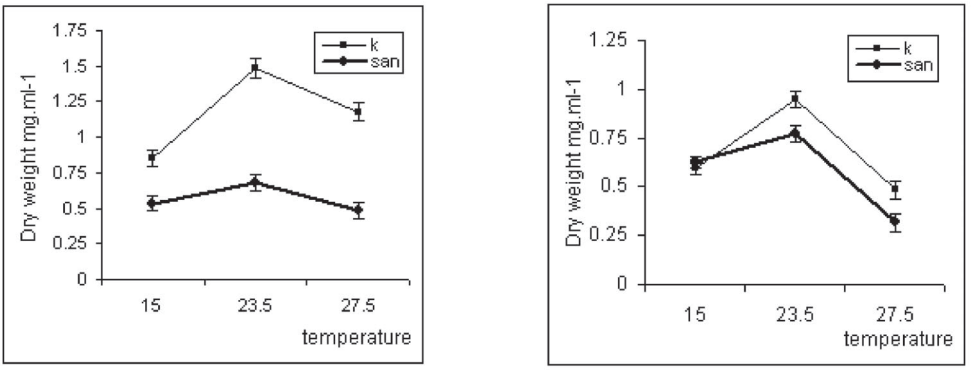


Figure 2. Effect of oxidative stress on chlorophyll *b* content.
A - 520 μmol m^{-2} s^{-1} light intensity; B - 1000 μmol m^{-2} s^{-1} light intensity

multaneously with the light intensity increase (Fig.1A, 1B; 2A, 2B and 3A). *β*–carotene turned to be the most sensitive parameter towards H_2O_2 treatment and it was strongly inhibited under illumination of 1000 μmol m^{-2} s^{-1} (Fig. 3B).

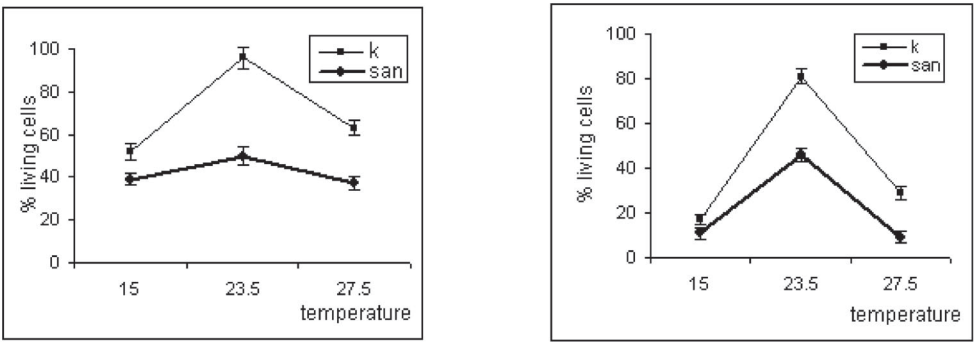
H_2O_2 inhibited protein synthesis at high temperature cultivation. The inhibition rate increased with enhancement of light intensity. However, H_2O_2 treatment at 15°C resulted in an protein content increase in *Choricystis minor* cultures (Fig. 4A, 4B). Under low temperature (15°C) treatment with Sanosil (H_2O_2) resulted in an increase of total pigment and protein contents with 38% and 36%, respectively.

The oxidative stress caused by Sanosil treatment inhibited *Choricystis minor* growth and viability and this effect enhanced with increasing the light intensity (Fig. 5A, 5B**;** 6A, 6B).



A **B**

Figure 5. Shift of biomass production in response to oxidative stress.
A - 520 μmol m^{-2} s^{-1} light intensity; B - 1000 μmol m^{-2} s^{-1} light intensity



A **B**

Figure 6. Influence of oxidative stress on *Choricystis minor* viability.
A - 520 μmol m^{-2} s^{-1} light intensity; B - 1000 μmol m^{-2} s^{-1} light intensity

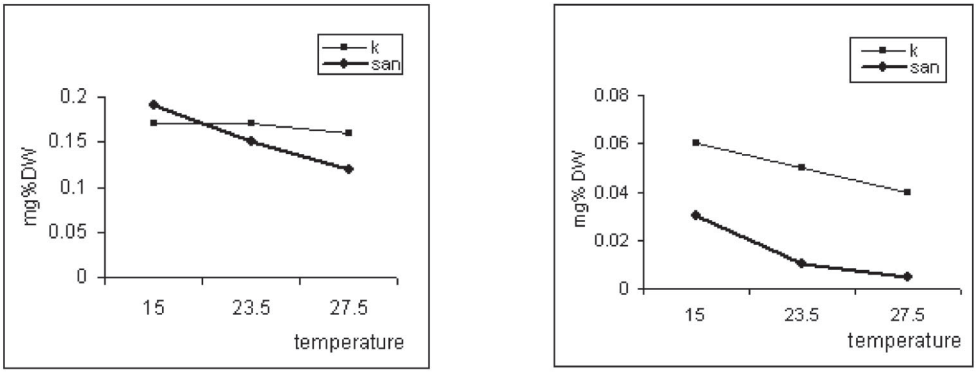
DISCUSSION

Data about the effect of temperature and light on photosynthesis of Antartic microalgae are quite contradictory. Investigation of photosystem II (PSII) of a polar diatom *Fragilariopsis cylindrus* established that the temperature shift to –1,8°C and the enhancement of light intensity caused marginal decrease of photosynthetic quantum yield (Mock and Valentin, 2004). Anning et al. (2001) demonstrated that light limited photosynthesis of the marine diatom *Chaetoceros calcitrans* was associated with even negligible temperature changes. Hawes (1990) found that Antarctic alga *Zygnemasp* was able to maintain its photosynthetic capacity during repeated overnight exposures up to – 4°C. Huiskes et al. (2001) have studied PSII quantum efficiency of six Antarctic species and conveyed the idea that the temperature status of the vegetation obscured any possible influence of UV treatment on the PSII.

Our results strongly suggest that under temperature stres *C. minor* responded with stronger decrease of chlorophyll a, chlorophyll b, and β –carotene concentrations under temperature stress when algae were subjected to intensive light irradiation. This reaction of the Antarctic microalga under study is in concert with the data reported for algae from moderate temperature regions, i.e. the damage effect of extreme temperatures is enhanced under extreme irradiation. Study of photosynthesis changes in response to combined irradiance and temperature stress in cyanobacteria, (Ibelings, 1996) has established that the combination between high temperature and high photon irradiance was more damaging than that of high temperature alone. Conversely, low photon irradiance offered substantial protection against heat injury of the photosynthetic apparatus. Huner et al. (1996) and Ensminger et al. (2006) made the general conclusion that flowering plants, green algae and cyanobacteria respond to subnormal temperatures similarly by electron transport changes caused by a shift in the redox potential of PSII.

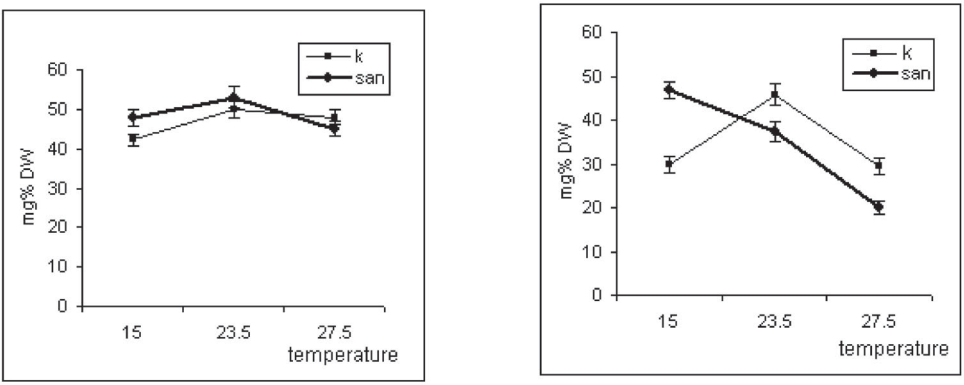
The oxidative stress was associated with decrease of growth, viability, pigment and protein contents of *C. minor* cells cultivated at optimal and extreme high temperatures. Moreover, our results demonstrate that the negative effect increased simultaneously with enhancement of light intensity. Obtained data are in agreement with the suggestion of Mc Minn at al (2005) that the generation of excess active oxygen radicals observed in polar ice microalgae in the presence of free oxygen is mostly responsible for the decline in growth, maximum quantum yield, relative maximum electron transport rate, and photosynthetic efficiency.

Investigating Cd toxicity on *Dunaliella salina* Lee and Shin (2003) have established that prolonged light period resulted in in also significant H_2O_2 and malonyldialdehyde accumulation. Light-induced oxidative stress in *Chlamidomonas reinhardtii*



A **B**

Figure 3. Influence of the oxidative stress on β-carotene content.
A - 520 µmol m^{-2} s^{-1} light intensity; B - 1000 µmol m^{-2} s^{-1} light intensity



A **B**

Figure 4. Changes in protein content after oxidative stress.
A - 520 µmol m^{-2} s^{-1} light intensity; B - 1000 µmol m^{-2} s^{-1} light intensity

has caused translation arrest of the large Rubisco subunit. This process took place after ROS increase (Cohen et al., 2005). High light intensity caused photodamage of photosystem II reaction centers in *Dunaliella salina* cells (Masuda et al., 2002).

***Acknowledgment*:** This study was partially supported by PISA Project (PISA-INI14/01.09.2005).

References

Anning, T., G. Harris, R.J. Geider, 2001. Thermal acclimation in the marine diatom *Chaetoceros calcitrans* [Bacillariophyceae], J. Phycol., 36, 233-241.

Cohen, I., J.A. Knopf, V. Irihimovitch, M. Shapira, 2005. A proposed mechanism for the inhibitory effects of oxidative stress on Rubisco assembly and its subunit expression, Plant. Physiol., 137, 738-746.

Ensminger, I., F. Busch, N.P.A. Huner, 2006. Review Photostasis and cold acclimation: sensing low temperature through photosynthesis, Physiol. Plantarum, 126, 28-44.

Hawes, I., 1990. Effects of freezing and thawing on a species of *Zignema* (Chlorophyta) from Antartica, Phycologia, 29, 326-331.

Huner, N.P.A., D.P Maxwell., G.R. Gray, L.V. Savitch, M. Krol, A. G. Ivanov, S. Falk, 1996. Minireview. Sensing environmental temperature change through imbalances between energy supply and energy consumption: Redox state of photosystem II, Physiol. Plantarum, 98, 358-364.

Ibelings, W.B., 1996. Changes in photosynthesis in response to combined irradianc e and temperature stress in Cyanobacterial surface waterblooms, J. Phycol., 32, 549-557.

Lee ,M.Y., K.W. Shin, 2003. Cadmium-induced changes in antioxidant enzymes from the marine alga *Nannochloropsis oculata*, J. Appl. Phycol., 15, 13-19.

Masuda, T., J.E.W. Polle, A. Melis, 2002. Biosynthesis and distribution of chlorophyll among the photosystems during recovery of the green alga *Dunaliella salina*from irradiance stress, Plant. Physiol., 128, 603-614.

Mc Minn, A., A. Pankowski, T. Delfatti, 2005. Effect of hyperoxia on the growth and photosynthesis of polar sea microalgae, J. Phycol., 41, 732-741.

Mock, T., K. Valentin, 2004. Photosynthesis and cold acclimation; molecular evidence from a Polar Diatom, J. Phycol., 40, 732-741.

Nadeau, T.–L., R.W. Castenholz, 2000. Characterization of psychrophilic Oscilatorians (Cyanobacteria) from Antarctic meltwater ponds, J. Phycol., 36, 914-923.

Nadeau, T.–L., E.C. Mibrandt, R.W. Castenholz, 2001. Evolutionary relationships of cultivated Antarctic Oscillatorians (Cyanobacteria), J. Phycol., 37, 650-654.

Seaburg, K.G., B.C. Parked, R.A. Wharton, G.M. Simmons, 1981. Temperature-growth responses of algal isolates from Antarctic oases, J. Phycol., 17, 353-360.

Steponcus, P.L., F.O. Lanphear, 1967. Refinement of the triphenyl tetrazolium chloride method of determining cold injury, Plant. Physiol., 42, 1423-1426.

Vona, V., V.D.M. Rigano, O. Lobosko, S. Carfagna, S. Esposito, C. Rigano, 2004. Temperature responses of growth photosynthesis, respiration and NADH; nitrate reductase in cryophilic and mesophilic algae, N. Phytol., 163, 325-331.