# STUDY REGARDING THE INFLUENCE OF THE SALINITY STRESS ON THE ANTIOXIDANT CAPACITY OF *ARTHROSPIRA PLATENSIS*

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**Abstract**. High intensity salt stress mainly decreases the content of phycocyanin in cyanobacteria, including spirulina, and thereby interrupts the energy transfer from phycobiliproteins to PS2 reaction center. Salt stress increases the accumulation of NaCl in the cytoplasm of cyanobacterial cells, affects growth rate, and is often associated with decrease in photosynthetic electron transport activities in photosynthesis. As a result, the high concentration of NaCl results in irreversible metabolic changes in the susceptible strains. The antioxidant capacity of *Arthrospira platensis* (Spirulina) cultivated for 6 day at a temperature of 32-35ºC with different quantities of NaCl added to the medium (0.043M; 0.085 M; 0.342 M; 0.684 M and 0.017 M as control) was measured using Photochem instrument from AnalytikJena. The highest antioxidant value was obtained for NaCl concentration of 0,043 M, 63,55 µg TROLOX/ml product. The lowest antioxidant value was obtained for NaCl concentration of 0,684 M, 9,61 µg TROLOX/ml product. A drastic decrease in antioxidant activity in biomass was observed.

**Keywords**: *Arthrospira platensis,* Spirulina, saline stress, antioxidant capacity

## INTRODUCTION

*Arthrospira platensis* (Spirulina) is considered a miracle of nature. Being part of the cyanobacteria group, one of the oldest on Earth, spirulina is a perfect source of protein. Depending on the strain and growth conditions, the amount of protein in the spirulina can reach values above 60% of the dry mass. The quality of the protein in spirulina is also very valuable (Becker 2007). Thus, all essential amino acids are present in considerable quantities in the protein of spirulina . Spirulina also contains an important amount of phycobiliproteins, especially phycocyanin. These components are characterized by pronounced antioxidant properties. Also antioxidant properties have carotenoids, antioxidant enzymes, γ-linolenic acid, tocooherol and other phenolic components of the biomass (Herrero et al. 2005; Hirata et al. 2000; Kepekçi and Saygideger 2012). The beneficial effects of spirulina as a nutritional and dietary supplement are due in particular on its immunomodulatory and antioxidant effects (Maddaly et al. 2010).

In nature, this cyanobacteria is found in a wide range of habitats largely varying in their salinity. As a result, different spirulina strains possess an unusual and valuable physiological characteristic, such as salt tolerance (Liu et al. 2016; Wang et al. 2013). In the same time, salt stress causes decrease in cyanobacteria growth and productivity by disrupting physiological processes, especially photosynthesis. The accumulation of intracellular sodium ions at salt stress changes the ratio of K : Na, which seems to affect the bioenergetic processes of photosynthesis (Sudhir and Murthy 2004). Chlorophyll is the primary target to salt toxicity limiting net assimilation rate, resulting reduced photosynthesis and reduced growth. In regarding carotenogeneis, at higher NaCl concentration, the grown cells containing higher amount of total carotenoids, which are produced by the cells in stress

condition as cell protecting mechanism decreases (Leema et al. 2010; Kandasamy and Nagarajan 2013). Salinity stress (0.8M NaCl) induced a decrease in oxygen evolution activity, inhibited the electron transport at both donor and acceptor sides of PSII, resulted in damage to phycobilisome and shifted the distribution of excitation energy in favour of PSI. In these conditions phycocyanin content decreased significantly in spirulina biomass. (Lu and Vonshak 2002). Biochemical analysis of salt stressed spirulina revealed that lipid content was slightly increased together with certain saturated and unsaturated fatty acids especially the polyunsaturated ones (γ-inolenic acid, omega 3 fatty acid) (Emad, Sanaa, and Vikramjit 2010).

Essential changes occur in the spirulina proteome under saline stress conditions. The total amount of protein decreases (Volkmann et al. 2008). In a comparative study of protein expression profiles of Spirulina under different salt-stress conditions (0.02 M, 0.5 M and 1.0 M NaCl, respectively), 141 proteins showing significantly differential expression in response to salt-stress were identified. These proteins are involved in photosynthesis, glucose metabolism, cysteine and methionine metabolism, lysine synthesis, fatty acid metabolism, glutathione metabolism. Additionally, the SRPs, heat shock protein and ABC transporter proteins were identified. These proteins are considered responsible for *Arthrospira (Spirulina) plantensis*'s resistance against high salt stress.(Wang et al. 2013)

The strains with high tolerance to salt, such as *Spirulina* strains FACHB-843 (SP843) and FACHB-972 (SP972) produce more carotene, phycocyanin, polysaccharides, proline and betaine in 400–600 mM NaCl than the control. Salt stress also induced them to produce higher activities of superoxide dismutase and peroxidase. Total antioxidant capacities of SP843 and SP972 peaked at 600 and 400 mM NaCl, respectively.(Liu et al. 2016).

## MATERIALS AND METHOD

### Samples

To carry out the experiments, *Arthrospira platensis* CNMN-CB-11 (A. platensis) (strain from National Collection of Nonpathogenic Microorganisms, Institute of Microbiology and Biotechnology of the Academy of Science of Moldova) was used. The cultivation of A. platensis cells was carried out during 6 days at a temperature of 32-35ºC, illumination 37-55 μmoles of photons/m2/s, pH 8-9 and at constant mixing, on mineral nutritive medium SP-1 without NaCl (NaNO3-2.5 g/L; NaHCO3-8.0 g/L; K2SO4-1.0 g/L; Na2HPO4-0.2 g/L; MgSO4·7H2O-0.2 g/L; CaCl2-0.024 g/L; H3BO3-2.86 mg/L; MnCl2·4H2O-1.81 mg/L; CuSO4·5H2O -0.08 mg/L; MoO3 –0.015 mg/L; FeEDTA-1mL/L). In addition, the following quantities of NaCl were added: 0.043M; 0.085 M; 0.342 M; 0.684 M and 0.017 M as control. On stationary growth phase (6th day of cultivation) the cyanobacteria biomass was separated from the culture medium by vacuum filtration, washed with distillated water, and obtained wet biomass was used in analytical experiment.

### Reagents

Ultra-pure water (EVOQVA), Ethanol (Supelco), ACL kit from AnalytikJena, HPLC grade Methanol (Merck).

### Sample preparation

5 grams of wet biomass was extracted using a mixture of methanol and ethanol (50/50) in a 50 ml tube with screw cap. The samples where then centrifuged at 4000 rpm for

10 minute and the supernatant was taken for analysis. Four samples with different NaCl concentration and a witness samples were analyzed.

**Method**

For the determination of the antioxidant capacity expressed in equivalents of Trolox the Photochem instrument from AnalytikJena was used and the specially design ACL kits. ACL kit contains a standard solution of Trolox, stock solution (Photo sensitizer and detection reagent) and buffer solution. The instrument uses the fast photochemical excitation of radical formation combined with sensitive luminometric detection.

A calibration curve of Trolox was done in 4 points 0,5nm, 1 nm, 2 nm and 3 nm. The samples were diluted 1:20-1:50 to be in the range of the calibration curve.

The formula used for calculation is:

$$Concentration\ \left[\frac{\mu g}{ml}\right] = \frac{Quantity \cdot Dilution \cdot M}{Pipetted\ Volume}$$

Where:

*Quantity* - nmol Trolox

*M* - Molar mass trolox = 250,29 ng/nmol

*Pipetted volume*: used volume in the vial in µl

*Dilution* - at 1:10 dilution factor = 10.

## RESULTS

The obtained results are presented in table 1. The highest antioxidant value was obtained for NaCl concentration of 0,043 M, 63,55 µg TROLOX/ml product. The lowest antioxidant value was obtained for NaCl concentration of 0,684 M, 9,61 µg TROLOX/ml product. A significant decreas in the antioxidant capacity was observed when high concentration of NaCl were added to the medium where *Arthrospira platensis was cultivated*.

Table 1

| Crt. No. | NaCl Concentration (M) | Results (µg TROLOX/ml product) |
|---|---|---|
| 1 | 0,043 | 63,55 |
| 2 | 0,085 | 55,83 |
| 3 | 0,342 | 26,20 |
| 4 | 0,684 | 9,61 |
| M | 0,017 | 46,64 |

Figure 1 reveals the correlation between the saline concentration and the antioxidant capacity of the Spirulina. The obtained results revealed that polar antioxidant substances might be present in the polar Spirulina extract to which attributed the antioxidant activity. These substances mainly include phycocyanin pigment, sulphated polysaccharides and phenolic compounds which are largely present in most macro, micro and cyanobacterial species which exhibited pronounced antioxidant activity. These non-polar substances include carotenoids (β-carotene, Astaxanthin, and Zeaxanthin), chlorophylls and fatty acids which were largely enhanced by salinity stress (0.04 and 0.08 M NaCl) and reported to have higher antioxidant activities (Emad et al. 2010).

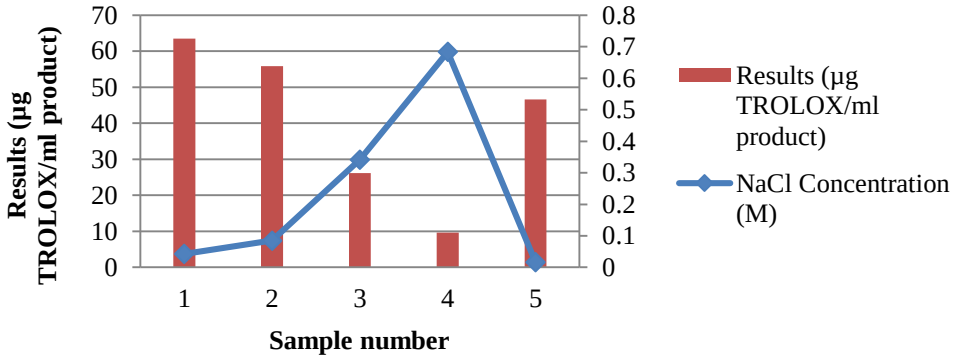


Fig. 1 Saline concentration and the antioxidant capacity of the Spirulina

## CONCLUSIONS

The effect of saline stress on spirulina depends on the strain and the intensity of stress. The basic mechanism of salt acclimation involves the active extrusion of toxic inorganic ions and the accumulation of compatible solutes, including different carbohydtrates. Even when cyanobacteria resist saline stress, an excessive accumulation of free radicals occurs in biomass. Our investigation involved a spirulina strain with low salt tolerance. The nutrient medium for this strain contains 1 g / L NaCl. Low intensity saline (2.5 and 5.0 g / l NaCl) produced an increase in antioxidant activity in biomass. This is a cell protection reaction that activates the synthesis of antioxidant enzymes and low molecular weight antioxidant components. Our results are consistent with the results of other researchers who reported increasing antioxidant activity in different cell types in saline stress conditions (Hernández and Almansa 2002; Liu et al. 2016; Tang et al. 2007).

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