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The influence of some Ca(II) and Ba(II) with Co(II) compounds on the productivity of the cyanobacteria *Spirulina platensis*

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Abstract. The article includes experimental results related to the productivity of the *Spirulina platensis* (Nordst.) Geitl - CALU - 835 strain, which was cultivated for 8 days on modified nutrient media supplemented with two coordination compound containing Ca and Ba. A greater amount of biomass was recorded when growing spirulina on the Zarrouk culture medium supplemented with these coordination compounds in a concentration of 5 mg/L, accumulating a fresh biomass of about 13,3 g/L and 12,8 g/L, respectively, or about 14,5% and 9,9% higher than that accumulated by the witness.
Keywords: *Spirulina platensis*, trichomes, productivity, stimulation, coordination compound, biomass.

Influenţa unor compuşi ai Ca(II) şi Ba(II) cu Co(II) asupra productivităţii cianobacteriei *Spirulina platensis*

Rezumat. Articolul include rezultate experimentale ce ţin de productivitatea tulpinii *Spirulina platensis* (Nordst.) Geitl – CALU – 835, care a fost cultivată timp de 8 zile pe medii nutritive modificate şi suplinite cu doi compuşi coordinativi cu conţinut de Ca şi Ba. O cantitate mai mare de biomasă s-a înregistrat la cultivarea spirulinei pe mediul de cultură Zarrouk suplimentat cu aceşti compuşi coordinativi în concentraţie de 5 mg/L, acumulând o biomasă proaspătă de circa 13,3 g/L şi 12,8 g/L respectiv, sau cu circa 14,5% şi 9,9% mai mare decât cea acumulată de către martor.
Cuvinte cheie: Spirulina platensis, trihomi, productivitate, stimulare, compus coordinativ, biomasă.

1. Introduction

Recent international and national biotechnology research has increased interest in obtaining pure cultures of some species of cyanobacteria and microalgae with an increased content of biologically active substances, that can be widely applied in agriculture as biofertilizers, plant growth stimulators, as well as in zootechnics, medicine, food industry, etc. Among the cyanobacteria under investigation there are species from the genera

Spirulina, *Calothrix*, *Nostoc, Anabaena, Cylindrospermum, Phormidium, Synechocystis*, etc., as well as autotrophic protists from the genera *Chlorococcum, Scenedesmus, Chlorella, Dunaliella, Porphyridium*, etc.

Cyanobacteria and microalgae participate in the formation of organismal associations from the composition of aquatic and edaphic biocenoses, that influence the organoleptic qualities of surface basin waters. Enriching them with oxygen, organisms are involved in the process of self-purification of contaminated streams including organic chemicals, heavy metals, radionuclides, etc. Nitrogen-fixing cyanobacteria play an active role in boosting soil fertility. By fixing atmospheric nitrogen, they contribute to the buildup of atmospheric nitrogen in the soil in the form of easily available compounds in plant mineral nutrition [12; 14].

Spirulina platensis (Nordst.) Geitl. is a blue-green trichomed filamentous cyanobacterium. In its natural development, it has spiral trichomes, with more or less regular spirals, with a diameter of 26-36 μ, and a spacing between the spirals of 43-57 μ. When it is cultivated, it loses the ability to form a spiral and transforms into filaments with a linear morphology [1]. Trichomes are able to move along their axis. They don't have any heterocysts. Filaments are solitary but can form clusters and bundles as large as 2-5 mm in diameter. The trichomes are 350-1000 μ long and 6-8 μ wide, with mild strangling between adjoining cells in the region of the transverse walls. The trichomes' heads are rounded. Numerous granules may be seen in the cells along the dividing walls using a photon microscope. These are the gas vacuoles, that appear as darker and brighter spots under the microscope. It grows by fragmenting the thallus and generating hormogons. The life cycle is divided into three major stages: trichome fragmentation with the generation of hormogonia; hormone development and maturation; and trichome elongation [4; 8]. It differs from other *Spirulina* species since it endures high levels of sodium bicarbonate in the growth media, up to 22-25 g/L. It can survive temperature variations of 20-25°C during the day without considerably decreasing production.

Research on the optimal chemical parameters of nutrient medium for the cyanobacteria culture under laboratory circumstances can serve as a benchmark for the development of new technologies for their intense cultivation. By improving their nutritional circumstances, cyanobacteria boost their daily biomass growth rate in a very short period of time (8-10 days). Thus, *Spirulina platensis* strains are also used as microbiological objects to evaluate the activity of various chemical agents that can either stimulate or hinder the development and accumulation of their biomass. Thus, *Spirulina platensis* strains are also used as microbiological objects for evaluating the activity of various chemical agents that can either stimulate or hinder the development and accumulation of their biomass.

Coordination compounds are complex molecules that contain both inorganic cations of some bioelements and an organic component which is made up of various functional groups containing electron-donating atoms and can increase the properties of influence on physiological systems through a synergistic effect [5]. Previous studies have shown that certain coordinating chemicals have a favorable effect on biosynthetic processes in specific microbes [2; 5-7].

The goal of this study was to investigate how coordination compounds with Ca and Ba content affected the productivity of the strain *Spirulina platensis* (Nordst.) Geitl - CALU - 835.

2. Materials and Methods

The investigations were carried out in the "Ecological Biotechnologies" scientific laboratory of the "Ion Creangă" State Pedagogical University in Chisinau. The cyanobacteria strain *Spirulina platensis* (Nordst.) Geitl - CALU - 835, which was taken from the "Vasile Şalaru" scientific research laboratory in Algology at the State University of Moldova, served as the object of research. For cultivation, the Zarrouk liquid nutrient medium was used with the following chemical composition (g/L): $NaHCO_3$ – 16,8; $NaNO_3$ – 2,5; $K_2HPO_4 \cdot 3H_2O$ – 0,5; K_2SO_4 – 0,1; NaCl – 1,0; $MgSO_4 \cdot 7H_2O$ – 0,2; $CaCl_2 \cdot 6H_2O$ – 0,04; $FeSO_4$ – 0,01; EDTA – 0,08; solution of trace elements – 1 mL/L (H_3BO_3 – 2,86; $MnCl_2 \cdot 4H_2O$ – 1,13; $ZnSO_4 \cdot 7H_2O$ – 0,222; $NaMoO_4 \cdot 5H_2O$ – 0,39; $Co(NO_3)2 \cdot 6H_2O$ – 0,049; $CuSO_4 \cdot 5H_2O$ – 0,079), pH – 9,5 [9]. Part of the experimental variants, from the assembled experience, were supplemented with the coordination compound containing Ca – $[CaL_3][Co(NCS)_4]$ (1), and another part of the experimental variants – with the coordinating compound containing Ba – $[BaL_3\text{-}\mu\text{-}(NCS)_2\text{-}Co(NCS)_2]$ (2), where L – pyridine-2,6-dimethyldicarboxylate. The coordination compounds were synthesized according to the method described in [3].

Cultivation was carried out in Erlenmeyer flasks with the 100 mL volume, in which 75 mL of Zarrouk nutrient medium with *Spirulina platensis* suspension (starting biomass 0,09 g/75 mL) was poured. Over 2 days, the spirulina stem, from these flasks, was treated with coordinating compounds 1 and 2 and cultivated for another 8 days at an artificial lighting of about 4000 lx and at a temperature of about 27°C (Fig. 1). Chemical compounds 1 and 2 were administered in concentrations of 50 mg/L, 10 mg/L, 5 mg/L, 1 mg/L and 0,5 mg/L. The samples grown in the absence of these chemical compounds, respecting the other cultivation parameters, were considered control samples.

Figure 1. Erlenmeyer flasks with the inoculated culture of *Spirulina platensis* on Zarrouk nutrient medium supplemented with coordinating compounds of Ca and Ba.

The data collected were statistically analyzed using the "STATISTICA 7" computer software, and the standard error of the average was calculated. Spirulina productivity was calculated using the current approach [10, 11].

3. Results and Discussions

It is important to provide macro- and microelements to *Spirulina platensis* when cultivating it in the laboratory. For the synthesis of the main cellular components, spirulina requires macroelements such as N, P, K, Mg, S, Ca, etc., as well as microelements such as Fe, Mn, B, Sr, Cu, Zn, Ba, Ti, Mo, etc., which are present in varying concentrations in the Zarrouk liquid nutritional medium [15].

Calcium, for example, is administered only in low concentrations and represents an essential chemical element necessary for the normal growth and development of all autotrophic organisms, including cyanobacteria. Calcium ions contribute to the stabilization of cell membrane structures, and serve as a messenger in many physiological processes of growth and development of microalgae and cyanobacteria, allowing the cells to absorb anions and cations from the nutrient environment [13]. This element favors shifting the pH of the environment towards alkaline values, inhibits the release of potassium from senile cells, etc., creating suitable conditions for the normal growth and development of the *Spirulina platensis* culture [9].

In the case of testing the coordination compound with Ca content, it was established that its effect on the *Spirulina platensis* culture depends on the concentration added to the Zarrouk nutrient medium, as well as on its period of action. Thus, on the 8th day

of cultivation, a greater amount of biomass of the researched crop was recorded in the experimental version with a coordination compound with a Ca content administered in a concentration of 5 mg/L, which produced 13,37 g/L, or 14,5% more than the control variant, as well as the variants with coordination compound 1 with concentrations of 10 mg/L and 1 mg/L, accumulating respectively 13,01 g/L and 13,09 g/L, or with 11,4% and 12,1% more than the control variant (Tab. 1; Fig. 2).

Table 1. The fresh biomass accumulated by the *Spirulina platensis* strain when treated with the compound $[CaL_3][Co(NCS)_4]$.

Nr.	Variant		Fresh start biomass g/1000 mL	Fresh biomass after 8 days of cultivation, g/L		Δ
				x±mx	σ	
1.	control		0,9	11,67±0,4	0,8	-
2.	$[CaL_3][Co(NCS)_4]$	50 mg/L	0,9	11,91±1,5	2,1	2,0
3.		10 mg/L	0,9	13,01±0,8	1,6	11,4
4.		5 mg/L	0,9	13,36±0,7	1,4	14,5
5.		1 mg/L	0,9	13,09±1,0	2,0	12,1
6.		0,5 mg/L	0,9	11,82±0,5	0,6	1,2

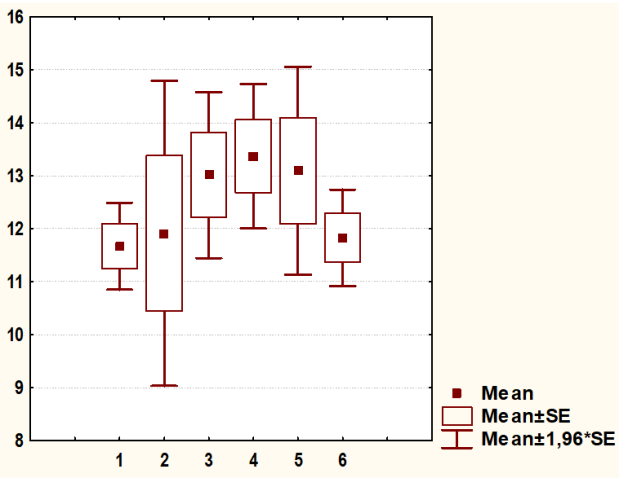

Figure 2. The fresh biomass accumulated by the *Spirulina platensis* strain when treated with the compound $[CaL_3][Co(NCS)_4]$ (g/L); 1. – control; 2. – 50 mg/L; 3. – 10 mg/L; 4. – 5 mg/L; 5. – 1 mg/L; 6. – 0,5 mg/L.

The concentrations of 50 mg/L and 0,5 mg/L of administered complex 1, proved to be ineffective because the productivity of the spirulina strain was almost equal to that of the control - 11,91 g/L and 11,82 respectively g/L. Concentrations of coordinating compound 1 higher than 50 mg/L have a negative effect on the strain of *Spirulina platensis*, the productivity being lower than in the control variant.

The findings of the eighth day of the *Spirulina platensis* strain cultivation in Zarrouk liquid nutritional medium supplemented with the chemical compound [BaL_3-μ-$(NCS)_2$-$Co(NCS)_2$] showed that its effect is highly dependent on the concentration provided. Thus, compound 2 provided at the concentration of 5 mg/L revealed to be the most appropriate for the investigated culture, ensuring a spirulina production of about 12,83 g/L, or 9,9% more than the collected biomass by the control variant. Coordination compound 2 enhanced the production of the spirulina strain, collecting 14 times more biomass in 8 days than was initially supplied (Tab. 2; Fig. 3).

Table 2. The fresh biomass accumulated by *Spirulina platensis* during 8 days when treated with the coordinating compound containing Ba.

Nr.	Variant		Fresh start biomass g/1000 mL	Fresh biomass after 8 days of cultivation, g/L		Δ
				x±mx	σ	
1.	control		0,9	11,67±0,4	0,8	-
2.	[BaL_3-μ-$(NCS)_2$-$Co(NCS)_2$]	50 mg/L	0,9	11,31±1,0	1,4	-3,0
3.		10 mg/L	0,9	11,74±0,3	0,7	0,5
4.		5 mg/L	0,9	12,83±0,9	1,7	9,9
5.		1 mg/L	0,9	12,74±1,3	2,5	9,1
6.		0,5 mg/L	0,9	12,15±0,3	0,4	4,1

An obvious stimulatory effect was also observed in the experimental version with a concentration of 1 mg/L, where the cyanobacterium strain generated 12,74 g/L of biomass, 9,1% more than the control. Concentrations of coordination compound 2 of 10 mg/L and 0,5 mg/L resulted in a 0,5% and 4,1% increase in strain production, respectively, which was slightly higher than that produced by the control variant. At the same time, higher concentrations of coordinating chemical 2 of 50 mg/L, as well as higher amounts provided to the Zarrouk culture medium, hinder *Spirulina platensis* strain growth and development.

During the first 2-3 days of cultivation, the *Spirulina platensis* strain goes through the phase of latency and growth acceleration. In the microscopic preparations, numerous

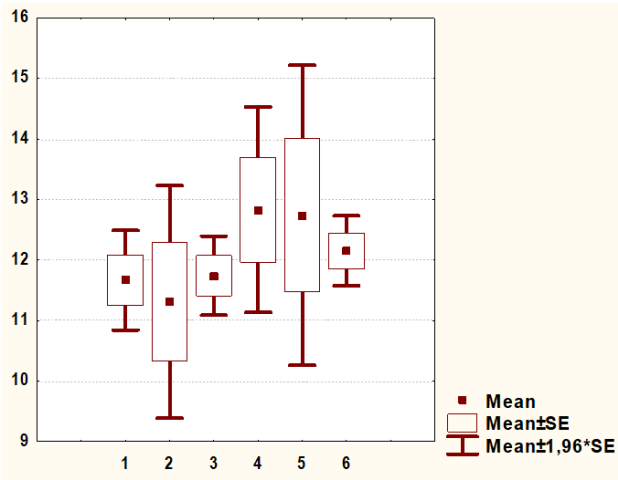

Figure 3. The fresh biomass accumulated by the *Spirulina platensis* strain when treated with the compound [BaL_3-μ-$(NCS)_2$-$Co(NCS)_2$] (g/L); 1. – control; 2. – 50 mg/L; 3. – 10 mg/L; 4. – 5 mg/L; 5. – 1 mg/L; 6. – 0,5 mg/L.

fragments of trichomes (hormogons) with variable lengths from 45 μ to 120 μ and with a thickness of 4,1-5,4 μ were highlighted. At the same time, trichomes exceeding the length of 500 μ were recorded. On the 3rd-4th day of cultivation, the phase of exponential multiplication of the culture is established, which is manifested by rapid growth (binary division of cells) and maturation of the trichomes. Starting from the 10th day, the logarithmic growth phase of the culture decreases obviously, and the produced biomass decreases. Under the microscope, most of the trichomes exceeded the length of 500 μ, being highlighted cells devoid of content, from which the thallus fragmented forming hormogons.

4. Conclusions

(1) The coordination compounds with a Ca and Ba content of 5 mg/L significantly increased the productivity of spirulina, registering after 8 days of action a fresh biomass of about 13,3 g/L and 12,8 g/L, being higher about 14 times and 12 times compared to the initial biomass administered in the Erlenmeyer flasks and about 14,5% and 9,9% higher than that accumulated by the control variant.

(2) The productivity of the *Spirulina platensis* culture decreases as the concentration of coordination compounds increases, and concentrations more than 50 mg/L have an inhibitory effect.

(3) The concentrations of the tested compounds of 0,5 mg/L resulted in an insignificant increase in the productivity of the strain of just 0,5% and 4,1%, respectively, being slightly higher than that produced by the control.

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