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OBTAINING ZINC ENRICHED SPIRULINA BIOMASS AND ESTABLISHING ITS TOXICITY

Вивчено вплив різних концентрацій Цинку у стандартному поживному середовищі Заррука на інтенсивність нарощування біомаси *Spirulina platensis*. Збагачення спіруліни проводили внесенням різних концентрацій сірчанокислого цинку – від 6,0 до 24,6 мг/л стандартного поживного середовища Заррука. Додавання сульфату цинку у дозі 6,0 мг/л до середини культивування проявляє стимулювальний ефект щодо нарощування клітин *Spirulina platensis*, а потім внаслідок акумулювання металу проявляється його токсичний ефект. Додавання до поживного середовища високих доз Цинку призводить до різкого припинення нарощування біомаси спіруліни та загибелі клітин культури з восьмої доби.

Досліджуючи гостру токсичність біомаси *Spirulina platensis*, збагаченої Цинком, у дозі 5000 мг/кг маси тіла шляхом внутрішлункового введення білим мишам та щурам не встановлено загибелі тварин. Клінічна картина реакції як білих мишей, так і білих щурів на біомасу *Spirulina platensis*, збагачену Цинком, була однаковою. В обох випадках навіть повторне введення високої дози добавки у кількості 5000 мг/кг маси тіла не супроводжувалось летальними наслідками. За таких умов біомаса спіруліни у концентрації до 5000 мг/кг маси тіла має значення (DL0).

Ключові слова: біомаса, білі миші, білі щури, культуральне середовище, *Spirulina platensis*, Цинк.

Formulation of the problem. Single-cell algae possess unique metabolic and reproductive systems, sensory and protective mechanisms that allow them to successfully adapt to different environmental factors (temperature, effects of heavy metals, radiation). Microalgae are increasingly used as producers of food and feed protein, amino acids, vitamins and other valuable substances.

A significant advantage of algae is that their trace elements are in organically linked form, that is the most accessible and digestible form, and their quantity is inherent in nature. Colloids, produced by algae, are unparalleled in other organisms, they are difficult to synthesize chemically [3].

Spirulina biomass, rich in macro- and micronutrients, is one way of providing livestock and poultry with Zinc [4, 8]. The cultural conditions largely change the content of trace elements in Spirulina biomass.

Natural Zinc complex composed of algae has significant advantages, primarily because biotic metal went through a biological filter, therefore has most favorable to the body biologically available form. To obtain such a natural complex is important in creating artificial mixtures [13].

Therefore, the development of Spirulina biomass enrichment biotechnology by biotic metal zinc and toxicity study of such additives makes scientific and practical interest.

Analysis of recent research and publications. The physiological effect of Zinc is primarily conditioned by its ability to form biocomplexes and is being accompanied by relative safety of this trace element for biomolecules. This is largely predetermined by the absence of prooxidant properties inherent in metals with variable valence (iron, copper, etc.). However, these properties promote the transporting and metabolism of zinc in the body and implementation of its biological functions in cells [5, 11].

It is known that Zinc is involved in many intracellular molecular processes, characterized by regulatory effects on proliferation, differentiation and functional activity of different cell types. This determines the physiological effects of trace elements, namely: the impact on the growth and development of the body, the immune, nervous, reproductive and other systems functioning [6]. In particular, there is a relationship between zinc metabolism in the body and condition of cardiovascular and respiratory systems [2]. This trace mineral is needed for reproductive function, function of skin and mucous membranes, bone tissue, visual and gustatory analyzers, digestive and pancreatic organs [7]. The pancreas is involved in zinc homeostasis [10], releasing Zn^{2+} into intestinal tract, where the trace element reabsorption occurs in periods of zinc deficiency [9, 12].

The issue of obtaining zinc complexes within *Spirulina platensis* biomass and study of this feed supplement harmlessness remains unexplored.

The purpose and objectives of the study – to obtain Zinc enriched biomass, to study the different doses effect of this metal mineral form on *S. platensis* cells growth, and the study of biochemical parameters in the white mice body in the conditions of harmlessness influence of Spirulina biomass enriched with Zinc.

Material and research methods. Experiments on the development of biotechnology for obtaining Zinc enriched *Spirulina platensis* biomass, were performed by cultivating trichome cyanobacteria strain LHU-603 on modified nutrient medium Zarruka with different concentrations of this biotic metal in mineral form (zinc sulfate) in closed conditions and around the clock lighting.

Enrichment of spirulina was made by introducing various concentrations of zinc sulphate – from 6.0 to 24.6 mg/l of standard nutrient medium Zarruka (Table 1). The biomass activity growth of unicellular alga *Spirulina platensis* depended on zinc sulfate content in the cultural fluid.

Table 1 – **Scheme of experiment on establishing optimal Zinc concentration in the cultural fluid that helps to maximize the accumulation of this metal in Spirulina biomass**

Variant	Environment
Control environment	The standard nutrient media Zarruka (SNMZ) for *Spirulina platensis* containing of zinc sulfate 0.23 mg/l
I experimental medium	SNMZ containing 6.21 mg/l zinc sulfate
II experimental medium	SNMZ containing 9.20 mg/l zinc sulfate
III experimental medium	SNMZ containing 12.42 mg/l zinc sulphate
IV experimental medium	SNMZ containing 25.30 mg/l zinc sulphate

One of the stages of feed additives preclinical study is measuring their acute toxicity. The acute toxicity studies of Zinc enriched *Spirulina platensis* biomass, was carried out on white mice and white rats. The experiments had two stages – preliminary and detailed. Before introducing spirulina solution supplement inside the stomach, the mice were kept on a starvation diet for 3–4 hours, the rats – during the night.

The study of Zinc enriched *Spirulina platensis* biomass harmlessness, was carried out on *Albino* line white mice.

The groups were formed with four mice in each. Laboratory animals were kept in specially designed cages, mice had free water supply from drinkers and balanced food. During the experiment following parameters were taken into account: the external look, behaviour, hair condition, response to external stimuli, food and water consumption.

Animals were injected with test solution through the mouth into stomach by a metal probe with 1 mm diameter tip, on an empty stomach. The I experimental group was once injected with Zinc enriched Spirulina biomass solution, followed by observation for 10 days. The II experimental group mice were administered with zinc sulphate solution. The control group animals were injected with saline.

The acute toxicity of *Spirulina platensis* biomass was studied on 3–4 months old white mice, weighing 20–23 g and on 3–4 months old white rats, weighing 170–185 g.

Before making experiment the animals were quarantined for 14 days. Raising and feeding of animals was carried out according to the generally accepted requirements. Metal enriched spirulina biomass was dissolved in 2 % starch solution and thoroughly mixed.

Results and discussion. Taking into account that zinc has biotic action factors, and is a toxic metal, our studies were conducted on the effects of Zinc different concentrations in the standard nutrient medium Zarruka on intensity increase of *Spirulina platensis* biomass.

Adding zinc sulfate in the nutrient medium at a dose of 6.21 mg/l to 6 days of cultivation, revealed stimulating effect of increasing cell *Spirulina platensis*, after 6 days due to the accumulation of metal manifests its toxic effect. Adding to the nutrient medium of zinc mineral forms in doses of 25.3 mg/l leads to a sharp increase of spirulina culture biomass suspension and cell death on the eighth day.

Thus, the addition of zinc sulfate to the I experimental medium before the middle of cultivation process manifests the stimulating effect of *Spirulina platensis* cell growth, and then due to the metal accumulation its toxic effect is manifested. Adding mineral form of zinc to the nutrient medium of the second and the third experimental medium leads to a sharp suspension of Spirulina biomass growth and cell death starting from the eighth day.

Given that the obtained Spirulina biomass with high content of zinc is a new feed supplement, it is reasonable to study its harmlessness and acute toxicity.

Introduction Zinc enriched *Spirulina platensis* biomass to mice, in the amount of 50 and 5000 mg/kg per body weight in the preliminary experiment was not accompanied by loss of animals during the observation period (14 days). A dose of 50 mg/kg per body weight did not affect animal condition. Behavior, respiratory rate remained normal. Changes in consuming food, water, responding to external factors were not observed. With the 5000 mg/kg dose for white mice, during 3–9 hours there was observed slow

response to external stimuli. Some animals showed the gastrointestinal tract disorder. However, with some time, animals returned to normal.

The full-scale experiment showed that the introduction of Zinc enriched *Spirulina platensis* biomass to white mice, with doses of 50, 500 and 2500 mg/kg per body weight had no adverse effect on the clinical condition of the animals. There were no changes in animal behavior, as in the first hours after the introduction of investigational factor and during 14 days of observation. It was experimentally proved that introduction of 5000 mg/kg dose of *Spirulina platensis* biomass as in the preliminary experiment, caused temporary inhibition of mice, which lasted up to 8 hours. During this period, it was found that the animals were grouped, moved little, did not come to food and water. In addition, the mice had intestine disorders. Ethological parameters recovered in all animals after 18–20 hours. Indigestion in mice was terminated by the 3 day after introducing feed additive (Table 2).

Table 2 – **Toxicity indicators of Zinc enriched *Spirulina platensis* biomass on white mice**

Number of animals in the group, heads	Feed supplement dose, mg/kg	The number of dead animals		
		all	in %	Average time of death
6	50	0	0	0
6	500	0	0	0
6	2500	0	0	0
6 in each (twice)	5000	0	0	0

During the study of acute toxicity of Zinc enriched *Spirulina platensis* biomass on white rats there were alos used preliminary and detailed stages. Introduction of 50 mg Spirulina biomass per kg of body weight did not cause changes in animal behavior. In addition, there was observed active consuming of feed and water. With the dose of 5000 mg/kg the rats mobility was slowed. Animals slowly responded to touch, noise and light. Respiratory rate decreased. Within 5–6 hours their condition improved, in 14 days after feed additive administration to rats, there were no deaths observed.

With full-scale experiment introduction of 500 and 2500 mg/kg doses to white rats their clinical condition was the same as that of the preliminary experiment animals with a dose of 50 mg/kg per body weight. There were no animal deaths within two introductions of Zinc enriched *Spirulina platensis* biomass, with a dose of 5000 mg/kg per body weight. Oppression duration of rats and their attitude to food, water, reaction to external stimuli coincided with the behavior of animals in preliminary experiment with a similar supplement dose (Table 3).

Table 3 – **Toxicity indicators of Zinc enriched *Spirulina platensis* biomass on white rats**

Number of animals in a group, heads	Feed supplement dose, mg/kg	The number of dead animals		
		all	in %	Average time of death
6	50	0	0	0
6	500	0	0	0
6	2500	0	0	0
6 (twice)	5000	0	0	0

Clinical response of both white mice and rats to Zinc enriched *Spirulina platensis* biomass was the same. In both cases, even repeated administration of high-dose supplements in the amount of 5000 mg/kg per body weight was not fatal. Under these conditions, the doses of Spirulina biomass of up to 5000 mg/kg really matter (DL0).

It was experimentally found that Zinc enriched *Spirulina platensis* biomass belongs to the low-toxic substances – Grade 4 GOST 12.1.007-76 [1]. DL50 Spirulina biomass with intragastric administration to white mice and white rats is more than 5000 mg/kg.

The study of cumulative properties of Zinc enriched *Spirulina platensis* biomass feed supplement, showed that during the course of introduction of feed additive the rats rapidly ate food, consumed water, responded to environmental factors. The hair condition, visible mucous membranes, respiratory rate, body temperature of rats did not differ from those in the control group animals. During the research there have been not any fatality.

Totally put the average dose biomass *Spirulina platensis*, enriched Zinc per rat was more than 5000 mg/kg, indicating a lack of cumulative feed additive properties.

Drawing homogeneous biomass *Spirulina platensis*, rich in Zinc, the mucous membrane of the eye of rabbits studied it irritating. Experimentally proved that the biomass of Spirulina after 2 and 24 hours after its application does not cause formation of eye discharge, redness and swelling of the mucous membrane. Thus, according to a point system features: allocation, congestion and edema scored "0" points.

Consequently, it was found that the biomass feed supplement *Spirulina platensis*, enriched with Zinc, not irritating effect on the mucous membranes of the eyes.

Conclusions. 1. The optimal concentration of Zinc in the nutrient medium for *Spirulina platensis*, in which cell proliferation is not terminated and the metal accumulated maximum in algae biomass, is 24.6 mg/l. Under these conditions, Zinc content in the *Spirulina platensis* dry matter is 155.9 g/kg, respectively.

2. *Spirulina platensis* biomass, rich in Zinc, belongs to low-toxic substances and has no cumulative properties. Its intragastric administration DL50 to laboratory animals (white rats and mice) constitutes more than 5000 mg/kg per body weight.

Promising area of research is the study of biochemical parameters in the body of mice by administration of Spirulina biomass enriched with Zinc.

LIST OF LITERATURE

1. ГОСТ 12.1.007–76. Система стандартов безопасности труда. Вредные вещества. Классификация и общие требования безопасности. – Введ. 1977–01–01. – М.: Стандартинформ, 2007. – 7 с.

2. Micronutrients in African-Americans with decompensated and compensated heart failure / M. Arroyo, S.P. LaGuardia, S.K. Bhattacharya [et al.] // Transl. Res. – 2006. – Vol. 148. – P. 301–308.

3. Ben Amotz A. Mode of action of the massively accumulated beta-carotene of Dunaliella bardawil in protecting the algae against damage by excess irradiation / A. Ben Amotz, O. Shaish, M. Avron // Plant Phisiol. – 1989. – Vol. 9, № 3. – P. 1040–1043.

4. Commercial interest of micro- and macroalgae / S. Bonotto, D. Van der Ben, A. Sanfulli [et al.] // Mar. Res. Div. Rapt. – 1987. – Vol. 602. – P. 116–117.

5. Brzóska M.M. Interactions between cadmium and zinc in the organism / M.M. Brzóska, J. Moniuszko // Food Chem. Toxicol. – 2001. – Vol. 39. – P. 967–980.

6. Cousins R.J. Mammalian zinc transport, trafficking, and signals / R.J. Cousins, J.P. Liuzzi, L.A. Lichten // Journal of Biological Chemistry. – 2006. – Vol. 281. – P. 24085–24089.

7. Long-term zinc deficiency decreases taste sensitivity in rats / T. Goto, M. Komai, H. Suzuki, Y. Furukawa // J. Nutr. – 2001. – Vol. 131. – P. 305–310.

8. Gupta R.S. Biotechnology of mass production of Spirulina and Arthrospira in fresh water / R.S. Gupta, M.L. Changwal // Spirulina ETTA Nat. Symp. MCRC. – Madras, India, 1992. – P. 125–128.

9. Krebs N.F. Overview of zinc absorption and excretion in the human gastrointestinal tract / N.F. Krebs // J. Nutr. – 2000. – Vol. 130. – P. 1374–1377.

10. McClain C.J. The pancreas and zinc homeostasis / C.J. McClain // J. Lab. Clin. Med. – 1990. – Vol. 116. – P. 275–276.

11. Vallee B.L. Zinc: biochemistry, physiology, toxicology and clinical pathology / B.L. Vallee // Biofactors. – 1988. – Vol. 1. – P. 31–36.

12. Van Wouwe J.P. The role of the pancreas in the regulation of zinc status / J.P. Van Wouwe, J.J.M. Uijlenbroek // Biol. Trace Elem. Res. – 1994. – Vol. 42. – P. 143–150.

13. Vencataraman L.V. Production of food, feed, biochemical and bioenergy from microalgae / L.V. Vencataraman, E.W. Becker // 8-th Int. Biotechnol. Symp., Paris, 1988; Proc. – Paris, 1989. – Vol. 2. – P. 910–922.

REFERENCES

1. GOST 12.1.007–76. Sistema standartov bezopasnosti truda. Vrednye veshhestva. Klassifikacija i obshhie trebovanija bezopasnosti. – Vved. 1977–01–01. – M.: Standartinform, 2007. – 7 s.

2. Micronutrients in African-Americans with decompensated and compensated heart failure / M. Arroyo, S.P. LaGuardia, S.K. Bhattacharya [et al.] // Transl. Res. – 2006. – Vol. 148. – P. 301–308.

3. Ben Amotz A. Mode of action of the massively accumulated beta-carotene of Dunaliella bardawil in protecting the algae against damage by excess irradiation / A. Ben Amotz, O. Shaish, M. Avron // Plant Phisiol. – 1989. – Vol. 9, № 3. – P. 1040–1043.

4. Commercial interest of micro- and macroalgae / S. Bonotto, D. Van der Ben, A. Sanfulli [et al.] // Mar. Res. Div. Rapt. – 1987. – Vol. 602. – P. 116–117.

5. Brzóska M.M. Interactions between cadmium and zinc in the organism / M.M. Brzóska, J. Moniuszko // Food Chem. Toxicol. – 2001. – Vol. 39. – P. 967–980.

6. Cousins R.J. Mammalian zinc transport, trafficking, and signals / R.J. Cousins, J.P. Liuzzi, L.A. Lichten // Journal of Biological Chemistry. – 2006. – Vol. 281. – P. 24085–24089.

7. Long-term zinc deficiency decreases taste sensitivity in rats / T. Goto, M. Komai, H. Suzuki, Y. Furukawa // J. Nutr. – 2001. – Vol. 131. – P. 305–310.

8. Gupta R.S. Biotechnology of mass production of Spirulina and Arthrospira in fresh water / R.S. Gupta, M.L. Changwal // Spirulina ETTA Nat. Symp. MCRC. – Madras, India, 1992. – P. 125–128.

9. Krebs N.F. Overview of zinc absorption and excretion in the human gastrointestinal tract / N.F. Krebs // J. Nutr. – 2000. – Vol. 130. – P. 1374–1377.

10. McClain C.J. The pancreas and zinc homeostasis / C.J. McClain // J. Lab. Clin. Med. – 1990. – Vol. 116. – P. 275–276.

11. Vallee B.L. Zinc: biochemistry, physiology, toxicology and clinical pathology / B.L. Vallee // Biofactors. – 1988. – Vol. 1. – P. 31–36.

12. Van Wouwe J.P. The role of the pancreas in the regulation of zinc status / J.P. Van Wouwe, J.J.M. Uijlenbroek // Biol. Trace Elem. Res. – 1994. – Vol. 42. – P. 143–150.

13. Vencataraman L.V. Production of food, feed, biochemical and bioenergy from microalgae / L.V. Vencataraman, E.W. Becker // 8-th Int. Biotechnol. Symp., Paris, 1988; Proc. – Paris, 1989. – Vol. 2. – P. 910–922.

Получение биомассы спирулины, обогащенной Цинком, и установление ее токсичности

Г. Мерзлова

Изучено влияние различных концентраций Цинка в стандартной питательной среде Заррука на интенсивность наращивания биомассы *Spirulina platensis*. Обогащение спирулины проводили внесением различных концентраций сернокислого цинка – от 6,0 до 24,6 мг/л стандартной питательной среды Заррука. Добавление сульфата цинка в дозе 6,0 мг/л до середины культивирования проявляет стимулирующий эффект по наращиванию клеток *Spirulina platensis*, а затем в результате аккумулирования металла проявляется его токсический эффект. Добавление в питательную среду высоких доз Цинка приводит к резкому прекращению наращивания биомассы спирулины и гибели клеток культуры начиная с восьми суток.

Исследуя острую токсичность биомассы *Spirulina platensis*, обогащенной Цинком в дозе 5000 мг/кг массы тела путем внутрижелудочного введения белым мышам и крысам не установлено гибели животных. Клиническая картина реакции как белых мышей, так и белых крыс на биомассу *Spirulina platensis*, обогащенную Цинком, была одинаковой. В обоих случаях даже повторное введение высокой дозы добавки в количестве 5000 мг/кг массы тела не сопровождалось летальным исходом. При таких условиях биомасса спирулины в концентрации до 5000 мг/кг массы тела имеет значение (DL_0).

Ключевые слова: биомасса, белые мыши, белые крысы, культуральная среда, *Spirulina platensis*, Цинк.

Obtaining Zinc enriched Spirulina biomass and establishing its toxicity

H. Merzlova

The effect of different zinc concentrations in the standard nutrient medium Zarruka on the intensity of *Spirulina platensis* biomass growth is being stuided. Enrichment of spirulina was made by introducing various concentrations of zinc sulphate – from 6.0 to 24.6 mg/l into standard nutrient medium Zarruka. Adding zinc sulfate in a dose of 6.0 mg/l to the cultivation medium manifests stimulating effect on *Spirulina platensis* cell growth, and then due to accumulation of metal manifests its toxic effect. Adding high doses of Zinc to the nutrient medium leads to a sharp decrease of Spirulina biomass growth and to cell death from the eighth day.

While exploring the acute toxicity of *Spirulina platensis* biomass, enriched with Zinc at a dose of 5000 mg/kg, by intragastric administration to rats and white mice, there were not observed animal deaths. Clinical response both white mice and rats to *Spirulina platensis* biomass, enriched with Zinc, was similar. In both cases, even repeated administration of high-dose supplements in the amount of 5000 mg/kg per body weight was not fatal. Under these conditions, the Spirulina biomass in concentrations up to 5000 mg/kg of body weight really matters (DL0).

Key words: biomass, white mice, white rats, culture medium, *Spirulina platensis,* Zinc.

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ВИЗНАЧЕННЯ ПРИСУТНОСТІ ГЛЮТЕНУ ЗЛАКОВИХ КУЛЬТУР У ХАРЧОВИХ ПРОДУКТАХ МЕТОДОМ ПЛР-РЧ

За використання *TaqMan*-технології методу полімеразної ланцюгової реакції в режимі реального часу (ПЛР-РЧ) розроблено тест-систему для виявлення та ідентифікації у продовольчій сировині і харчових продуктах чотирьох злакових культур (пшениці, жита, вівса і ячменю), глютен яких призводить до виникнення у людини захворювання на целіакію. Тест-система уможливлює аналіз продуктів, які піддавались термічному обробленню, ферментації, гідролізу, її рівень чутливості достатній для виявлення так званого прихованого глютену. Апробацію тест-системи проведено на 53 зразках продовольчої сировини і харчових продуктів шляхом порівняльних випробувань за використання комерційних ІФА- та ПЛР-тест-систем – RIDASCREEN® Gliadin та SureFood® ALLERGEN ID Gluten (R-Biopharm, Німеччина) відповідно. За ефективністю роботи (специфічністю, чутливістю, повторюваністю та відтворюваністю результатів) розроблена система не поступається зарубіжним аналогам і є значно дешевшою.

Ключові слова: глютен, целіакія, ПЛР в режимі реального часу, харчові продукти.