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Assessment of the effect of the extract *Arthrospira platensis* on human skin microbiota

Zinurov M. R [1], Manzhelei O. A [2], Sametova I. N [2], Bagaeva T. V [3] and Zinurova E. E [4,*]

[1] *Kazan National Research Technological University, Food Production Technology Department, K.Marx str.68, 420015, Russian Federation Kazan.*

[2] *National Research Center "Kurchatov Institute", Department of Biotechnology and Bioenergy,Ac.Kurchatov square,1,123182, Russian Federation, Moscow.*

[3] *Kazan Federal University,Institute of Fundamental Medicine and Biology, Kremlyovskaya str.,18,420008, Russian Federation, Kazan*

[4] *Federal State Budgetary Institution of Science "FRC Nutrition and Biotechnology", laboratory of biosafety and analysis of nutrimicribiome, Ustinsky passage, 2/14, 109240, Russian Federation, Moscow*

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Abstract

A new direction in the development of pharmaceutical and cosmetic products, provides for the widespread use of pigment containing components of extracts of microalgae and blue-green algae. However, the use of these components requires a deeper study of their effect on humans, including on their microbiota. In our experiments, it was found that the extract of the blue-green algae *Arthrospira platensis* (commercial name of the drug *Spirulina*), with a phycocyanin content of 38%, is able to influence the overall microbiota of human skin, and to varying degrees of effectiveness depending on the concentration of the drug. It is shown that the effect of the studied extract has a dose-dependent effect, both when affecting the general microbiota of human skin and on representatives of its gram-positive microbiota. The gram-negative microbiota of human skin is less sensitive to the action of the extract of the blue-green algae *Arthrospira platensis* (commercial name of the drug *Spirulina*), since only at a concentration of 200 mcg/ml there was a significant low increase in biomass.

Keywords: Microorganisms; Microbiota; Skin; Blue-green algae; Arthrospira platensis; *Spirulina* extract

1. Introduction

In recent years, researchers in many countries have seen an increase in significant interest in microalgae and blue-green algae. These producers have a wide range of secondary metabolites that have a wide variety of effects on the human body, including wound healing, anti-inflammatory, and antitumor effects. Some species of blue-green algae and microalgae, such as *Arthrospira, Spirulina, Chlorella, Haematococcus, Dunaliella* and *Porphyridium* are used as a source of natural dyes or additives in cosmetics [1]. Thus, *Arthrospira platensis* – bioactive cyanobacteria, blue-green algae with beneficial properties for human health, are an important ingredient of natural cosmetic products. Under the commercial name *Spirulina*, it is included in creams for topical skin care as a moisturizer, anti-wrinkle, anti-aging and anti-acne [2,3]. In addition, blue-green algae is used by cosmetic developers as a component of sunscreen, for the treatment of skin pigmentation disorders and for wound healing [4]. By chemical composition, it contains: proteins (55-70%), carbohydrates (15-25%), essential fatty acids (18%), vitamins. *Arthrospira platensis* (commercial name *Spirulina*) is considered the richest natural source of vitamin B12. It consists of various minerals, pigments. Among these components of cyanobacteria, phycocyanin, a pigment that is part of the phycobiliprotein complex with a powerful antioxidant effect, is of particular interest at present [5,6]. It has been established that phycocyanin beneficially modulates apoptotic pathways in skin cells exposed to UV rays, its antimelanomic and wound-healing effect is noted

* Corresponding author: Zinurova E. E

[7]. In general, there is a significant potential of phycocyanin in terms of its cosmetic properties [8]. However, for its wide application, a deeper study of the effect of this pigment, including on the microbiota of human skin, is necessary. Maintenance of commensal bacteria to avoid skin dysbiosis correlating with the frequency of more severe skin diseases, including due to hyper-proliferation of some bacteria that colonize hair follicles and the same [9], is an important stage in both cosmetic and dermatological fields.

This work is devoted to the effect of *Arthrospira platensis* (commercial name *Spirulina*) extract, with a phycocyanin content of up to 38%, on the microbiota of human skin.

2. Material and methods

The work used samples of skin washes on the neck of conditionally healthy volunteers who do not use cosmetics, as well as strains of microorganisms of the genera *Staphylococcus spp*. and *Klebsiella* spp., the Museum of Cultures of Kazan Federal University, isolated earlier from the skin surface.

Flushing was carried out according to the standard procedure using sterile swabs filled with sterile buffer. Samples of microorganisms were taken from 15 volunteers 25-28 years old, and combined to one common sample. The treated area 10 cm^2. The washing microbiota from the cellulose tips of the swaab was placed in a sample with buffer and used for sowing on a nutrient medium.

The cultivation of microorganisms was carried out on meat-peptone broth (MPB), in a TS 1/80 SPU thermostat at a temperature of 37 °C for 72 hours.

A preparation of blue-green algae extract *Arthrospira platensis* (commercial name *Spirulina*) by Natec (China), with a phycocyanin content of 38%, was used as the pigment-containing component under study.

Arthrospira platensis extract was introduced into the initial nutrient medium for the growth of microorganisms of the obtained samples in various concentrations. The final concentration of pigment in nutrient media was 10 mcg/ml, 100 mcg/ml and 200 mcg/ml respectively. Similarly, the effect of *Arthrospira platensis* extract (commercial name *Spirulina*) on individual strains of the studied bacteria was studied. During the entire cultivation period, samples were taken to determine the increase in biomass. The increase in biomass was determined by a standard technique using a Varian Cary 50 spectrophotometer at a wavelength of 540 nm.

All experiments were carried out in three parallel repetitions.

The purity of the microorganisms used was monitored using a Meiji MT5200I microscope.

3. Results and discussion

The skin is the largest organ of the human body and is most susceptible to external factors [7]. This peculiar system, closely related to the internal environment of the body, is an ecological niche for the development of microorganisms, which primarily perform a protective function [10]. Changes in the composition of microorganisms under the influence of external factors can lead to the appearance of various dermatoses. Therefore, any substances that are used to affect the skin must be investigated, including the skin microbiota.

In our experiments, we investigated the effect of *Arthrospira platensis* (commercial name *Spirulina*) extract, with a phycocyanin content of up to 38%, in different concentrations on the total microbiota of human skin (Figure 1).

The results of the studies have shown that a sufficiently long period of the lag phase (up to 24 hours) is necessary for the development of the general microbiota of the human skin on a nutrient medium (MPB). It can be assumed that this is due to the presence in the studied sample of various microorganisms that compete for the initial substrate. After 36 hours of cultivation, active growth of microorganisms began in the studied objects, and after 72 hours the cells entered the stationary phase of population growth.

The introduction of *Arthrospira platensis* (commercial name *Spirulina*) extract into the nutrient medium in various concentrations had a different effect on the growth of human skin microbiota cells. Thus, the introduction of an extract in an amount of 10 mcg/ml led to the active growth of human skin microorganisms from the very first hours of sample cultivation. The increase in biomass was 1.6±0.2 times higher compared to the control. An increase in the concentration

of *Arthrospira platensis* (commercial name *Spirulina*) extract in the nutrient medium to 100 mkg/ml also contributed to the growth of cells relative to the control, but to a lesser extent by 1.2 ± 0.1 times than the increase in cells at an extract concentration of 10 mkg/ml.

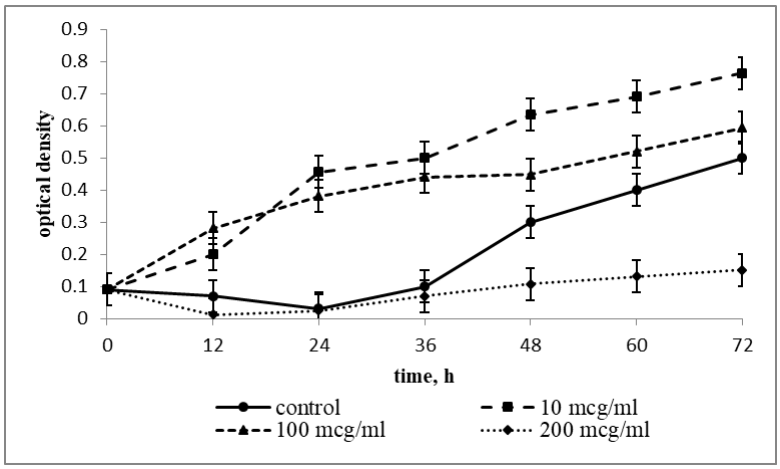

Figure 1 Dynamics of growth of the general microbiota of human skin on MPB (control) with the addition of *Arthrospira platensis* (commercial name *Spirulina*) extract in different concentrations (experiment)

A significant decrease in cell growth was observed when *Arthrospira platensis* (commercial name *Spirulina*) extract was introduced into the nutrient medium at a concentration of 200 mkg/ml. This concentration reduced the growth of microbial cells by 3.3± 0.2 times (for 72 hours) compared to the control variant.

Thus, it can be concluded that *Arthrospira platensis* (commercial name *Spirulina*) extract has a dose-dependent effect on the increase in biomass of the general culture of the microbiota of human skin. In small doses, namely up to a concentration of 100 mcg/ml, *Arthrospira platensis* (commercial name *Spirulina*) extract had a stimulating effect. Higher concentrations of *Arthrospira platensis* (commercial name *Spirulina*) extract, namely 200 mcg/ml, had an inhibitory effect on cells.

The stimulating effect of *Arthrospira platensis* (commercial name *Spirulina*) extract on the growth of microorganisms can be explained by the introduction of additional components in the form of a pigment-protein complex into the culture medium. The inhibitory effect of the extract on cell growth corresponds to the literature data on its ability to antibacterial action [11,12,13].

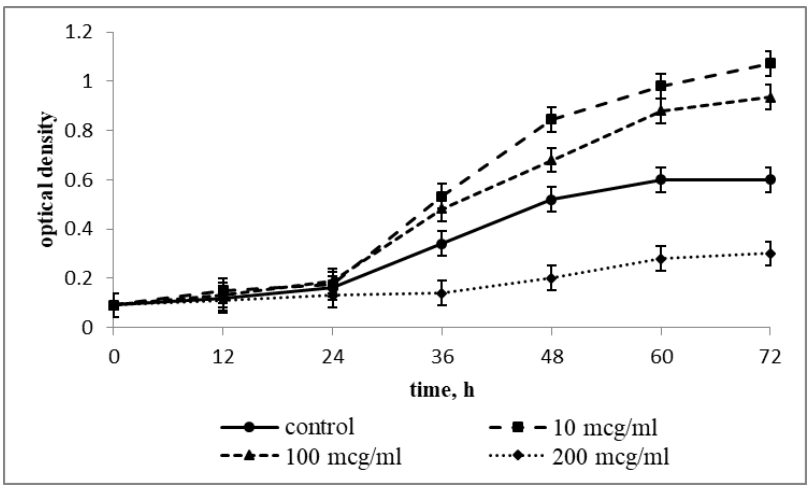

Figure 2 Dynamics of growth of bacterial culture of the genus *Staphylococcus spp.* on MPB (control) and with the addition of *Arthrospira platensis* (commercial name *Spirulina*) extract in different concentrations (experiment)

The skin microbiota is a collection of many microorganisms that form stable communities [14,9,15]. Among the frequently encountered microorganisms of the resistant microbiota of the skin surface are various species of the genus *Staphylococcus*, as well as coliform bacteria [16].

In our experiments, we studied the effect of *Arthrospira platensis* (commercial name *Spirulina*) extracts on microorganisms of the genus *Staphylococcus*, as a representative of gram-positive microbiota and the genus *Klebsiella*, as a representative of gram-negative microbiota, which occurs in the composition of the skin biocenosis to a lesser extent [8].

The addition of *Arthrospira platensis* (commercial name *Spirulina*) extract to the nutrient medium for the growth of microorganisms of the genus *Staphylococcus spp*., at a concentration of 10 mcg/ml, 100 mcg/ml, 200 mcg/ml, showed that the results obtained were consistent with the effect of the extract on the overall microbiota of human skin. The introduction of the extract at a concentration of 10 mcg / ml increased the biomass growth of *Staphylococcus sp*. by 1.8 ± 0.2 times compared with the control, and at a concentration of 100 mcg/ml by 1.5 ± 0.1 times. An increase in the concentration of *Arthrospira platensis* (commercial name *Spirulina*) extract to 200 mcg/ml led to a decrease in cell growth by 2.0± 0.2 times and a smaller accumulation of bio-mass (Figure2).

Another dependence was observed when adding *Arthrospira platensis* (commercial name *Spirulina*) extract to the nutrient medium for the growth of a microorganism of the *Klebsiella spp*. (Figure3).

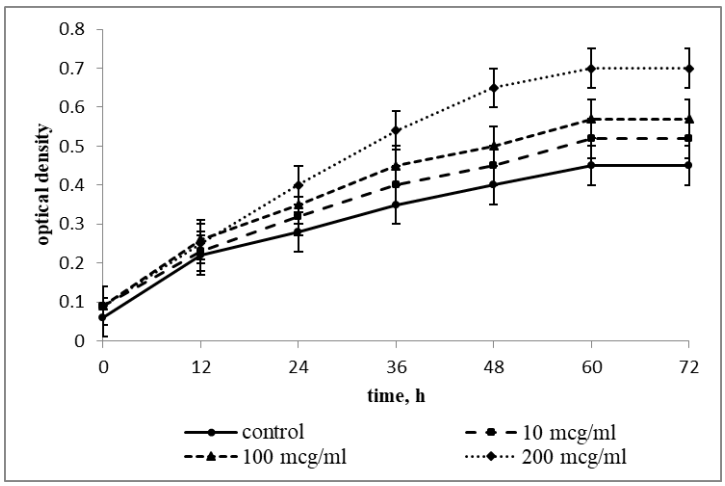

Figure 3 Growth dynamics of the culture of bacteria of the *Klebsiella spp*. on MPB (control) and with the addition of *Arthrospira platensis* (commercial name *Spirulina*) extract in different concentrations (experiment)

Arthrospira platensis (commercial name *Spirulina*) extract had significantly less effect on the growth of bacteria of the genus *Klebsiella*. In addition, the introduction of *Arthrospira platensis* (commercial name *Spirulina*) extract into the culture medium of these bacteria led to an increase in biomass relative to the control, regardless of the concentration of the extract. Thus, the addition of *Arthrospira platensis* (commercial name *Spirulina*) extract at a concentration of 10 mcg/ ml led to an increase in biomass growth for 72 hours of bacterial development by 1.2 ± 0.2 times, when the extract was applied at a concentration of 100 mcg/ml by 1.3 ± 0.2 times, and at a concentration of 200 mcg/ml by 1.5 ± 0.4 times compared with the control.

Thus, in the case of the introduction of *Arthrospira platensis* (commercial name *Spirulina*) extract into the culture medium of *Klebsiella* bacteria, a stable stimulating effect of this substance is observed at all the concentrations studied.

Comparative data on the biomass growth of the studied microorganisms for 72 hours are presented in the diagram (Figure4).

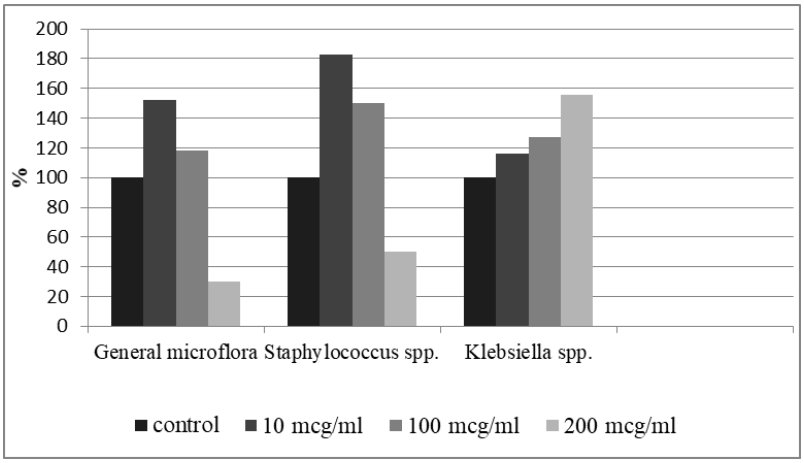

Figure 4 Comparison of microbial biomass growth for 72 hours of cultivation

When applying *Arthrospira platensis* (commercial name *Spirulina*) extract in different concentrations (in %)

The research results have shown that the effect of the *Arthrospira platensis* extract (commercial name *Spirulina*) has the most effect on the general microbiota of human skin, namely its gram-positive microbiota. Nevertheless, it is necessary to take into account the effect of *Arthrospira platensis* extract on gram-negative microbiota, since at high concentrations of 200 mcg/ml, its growth is possible and, consequently, the balance between the separate groups of the skin microorganisms can be broken.

4. Conclusion

The extract of the blue-green algae *Arthrospira platensis* (commercial name *Spirulina*), with a phycocyanin content of 38%, has an effect on the overall microbiota of human skin. This action has a dose-dependent effect, since the concentration of the extract in the range of up to 100 mcg / ml contributes to an increase in the number of microbiota cells in the nutrient medium, and higher concentrations inhibit their growth.

The main effect of the extract of the blue-green algae *Arthrospira platensis* (commercial name *Spirulina*), with a phycocyanin content of 38%, is noted on the gram-positive microbiota of human skin, which is shown when cultivating bacteria of the genus *Staphylococcus* spp. and the genus *Klebsiella*. However, it is necessary to take into account the ability of the extract to increase the amount of biomass of gram-negative microbiota regardless of concentration.

Thus, it can be said that using different concentrations of the extract of the blue-green algae *Arthrospira platensis* (commercial name *Spirulina*), it is possible to regulate both the total content of cells on the surface of the skin and its individual representatives.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

A statement on the absence of a conflict of interest is attached

Statement of ethical approval

'The present research work does not contain any studies performed on animals/humans subjects by any of the authors'.

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