OPINION

from **Prof. Dr. Rumiana Dimitrova Tzoneva** Department

„Lipid-protein interactions", IBPhBME-BAS

on the Dissertation work for acquiring educational and scientific level PhD in professional field 4.3. Biological sciences, Scientific specialty 01.06.16. "Plant Physiology"

of Tanya Stavreva Toshkova-Yotova, PhD student of independent training at the Laboratory of Experimental Algology, Institute of Plant Physiology and Genetics - BAS

Dissertation topic: Biological activity of products from COELASRELLA sp. BGV

1. **Topicality of the topic**

The scientific interest in microalgae is determined by their multifaceted role as an inexhaustible source of a number of biologically active substances - pigments, polysaccharides, sterols, fatty acids, vitamins, which find their role in biomedicine.

The growing interest of the pharmaceutical industry and medicine in microalgae is due to the demand for new natural products with antitumor and antibacterial action, combining on the one hand high specificity and a wide range of action, and on the other - a low degree of side effects.

Despite the growing achievements in the field of experimental and applied algology, the potential of this useful bioresource (microalgae) remains largely untapped.

The topic of the dissertation, which is aimed at revealing the possibilities of the Bulgarian strain of green microalgae to produce substances with antibacterial, antifungal and cytotoxic action is relevant and fully corresponds to the scientific specialty.

2. **Knowing the state of the problem**

The present Dissertation consists of 135 pages and is organized and presented according to the accepted standards. 318 scientific papers are cited, a significant part of which are from the last 10 years. The literature review is extensive and in-depth, including an overview of the biological basis for the production of microalgae and their practice-relevant products, as well as their antioxidant, antiangiogenic, cytotoxic, anti-tumor and anti-microbial activity.

The literature review is thorough, very well structured and comprehensive, which is a testament to the good handling of modern information reflecting research in this area. For greater illustration, the overview presents 3 tables and 1 figure.

3. Purpose, tasks and methodology of the research

The aim of the present Dissertation is clearly formulated - to study the biological activity of products from a selected strain of green microalgae *Coelastrella sp.* In connection with this goal, 5 tasks are formulated, ensuring the fulfillment of the set goal: comparison of the growth characteristics and biochemical composition of strains of the genus *Coelastrella* and selection of an experimental object; preparation of products from the selected strain of *Coelastrella sp.;* characterization of oil extract, fatty acids and exopolysaccharide from the selected strain of *Coelastrella sp*.; study and evaluation of the biological activity of the products obtained from the selected strain of *Coelastrella sp.*

The Materials and Methods section describes correctly and in detail the numerous chemical, biochemical and molecular methods used, as follows: phenol-sulfur method for determination of carbohydrate content, determination of total lipids, gas chromatography and mass spectrometry for determination of fatty acids, method for determination of chlorophyll a, b and carotenoids content, method of Blumenkrantz and Asboe-Hansen for determination of uronic acids, characterization of extracellular polysaccharide, MTT test, acridine-orange/ethidium bromide staining, DAPI staining, agar and disc diffusion method, etc.

4. Evaluation of the obtained results and their interpretation

The section "Results and discussion" (47 pages) includes nine subsections, in which the results obtained regarding the determination of the biological activity of products from a selected strain of green microalgae *Coelastrella sp.* The conclusions are based on a huge amount of experimental work, which is illustrated with 29 figures and 12 tables. Of the studied 4 strains of green microalgae of the genus *Coelastrella* - *Coelastrella sp.* it was found that the Bulgarian strain *Coelastrella sp.* BGV. has the highest growth potential in laboratory conditions. In a lipid extract of *Coelastrella sp*. 11 fatty acids have been identified in BGV, including oleic, linoleic and palmitic. It was found that the ratio of unsaturated to saturated fatty acids is 3: 1, and of monounsaturated to polyunsaturated fatty acids is 1: 1, the exopolysaccharide from *Coelastrella sp.* BGV is a low molecular weight, composed mainly of neutral sugars, among which two

monosaccharides have been identified - galactose and fucose. The highest statistically significant antitumor effect was reported for fatty acids, followed by alcohol extract, unsaponifiable matter and exopolysaccharides. It was found that the antitumor effect caused by extracts and metabolites of *Coelastrella sp.* BGV is manifested by varying degrees of morphological changes in the cell and nucleus of the treated cells, leading to induction of apoptosis. High activity of extracts and metabolites of *Coelastrella sp*. BGV to a wide range of Gram-negative and Gram-positive bacteria, as well as a high level of total antioxidant activity.

5. Evaluation of the contributions of the dissertation

The Contributions are adequate and comprehensive. The 8 contributions summarize the most important results of the Dissertation, emphasizing the possibility of using extracts and metabolites of *Coelastrella sp.* BGV in medical practice based on the results obtained for high antitumor, antibacterial and antifungal activities.

6. Personal contribution of the doctoral student and publications

In connection with the Dissertation, two articles have been published, in which Tanya Toshkova-Yotova is the lead author. They have been published in peer-reviewed scientific journals, one of which has an impact factor (in press,*Comp. Rend. Acad. Bulg. Sci.,* IF-0.321), and the other with SJR - 0.121 (*Oxidation Communication*). The results of the Dissertation are presented at four scientific forums.

Conclusion:

The dissertation introduced by Tanya Toshkova-Yotova presents a current study related to the use of green technologies in biomedicine. The PhD student shows mastery of a wide range of methodologies and an excellent sense of analyzing and summarizing the results. The dissertation has both scientific and applied scientific contributions. The Dissertation and the accompanying documents fully meet all the requirements of LAW on the development of the academic staff in the Republic Bulgaria and the regulations of BAS and IPPG for its application

Taking into account all the above, I strongly vote positively and recommend to the esteemed Scientific Jury to support the award of the educational and scientific degree PhD

in the professional field 4.3. Biological sciences, Scientific specialty 01.06.16. "Plant Physiology" by PhD student Tanya Stavreva Toshkova-Yotova.

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Sofia (Prof. Dr. Rumiana Dimitrova Tzoneva)