REVIEW

on a Dissertation submitted for acquiring the educational and scientific degree of "Doctor" Scientific specialty 01.06.16. - Plant physiology

Author of the Dissertation: **Tanya Stavreva Toshkova-Yotova**, PhD student at the Institute of Plant Physiology and Genetics - Bulgarian Academy of Sciences, Laboratory „Experimental Algology"

Topic of the Dissertation: **"Biological activity of products from *Coelastrella* sp. BGV "**

Reviewer: Professor Liliana Georgieva Gigova, PhD, Institute of Plant Physiology and Genetics at the Bulgarian Academy of Sciences

The dissertation of Tanya Toshkova-Yotova covers 135 standard pages and is structured in 8 chapters with the correct proportion between them: Introduction (2 pages), Literature review (28 pages, with 8 subchapters), Purpose and tasks (1 page), Material and methods (14 pages, with 7 subchapters), Results and discussion (50 pages, with 8 subchapters), Conclusions (2 pages), Contributions (2 pages), Literature (32 pages). The list of cited literature contains 322 titles, 7 of which are in Cyrillic. It should be noted that 268 references are from the period 2000-2020, and 17 are from 2019-2020. The dissertation is illustrated with 30 figures and contains 12 tables. Immediately after the description of the Contents, a list of Abbreviations Used is provided for convenience. On the last, 135th page are presented Scientific publications on the topic of the dissertation and Participation in national and international forums of the PhD student.

In recent decades, microalgae have established themselves as a promising and inexhaustible source of biomass and a number of useful products that are increasingly used in medicine, pharmacy and various industrial and agricultural areas. Of particular importance are microalgae products with specific antitumor activity, as well as those with a broad spectrum of action against pathogens, due to the need for natural, safer and more effective alternatives to antitumor therapy and widely used antibiotics. Despite the significant advances in science and industry, the isolation and research of new strains among the vast microalgae biodiversity, as well as the discovery and exploitation of the

potential of this beneficial bioresource, continues and will continue. This determines **the actuality and significance of the dissertation** - the results of the complex investigation on the physiology, biochemistry and biological activity of a new microalgal strain contribute to the enrichment of fundamental knowledge and reveal the prospects of this Bulgarian strain for practical application. **The up-to-date of the topic** is outlined in the Introduction of the dissertation and well substantiated in the extensive but purposeful Literature Review. **The Literature Review** presents a chronology of the mass cultivation of algae and the production of valuable products from algal biomass worldwide. The biotechnological bases for microalgae production are described, including the selection of an appropriate species/strain and knowledge of the main factors (light, temperature, nutrient availability, pH of the medium, stirring and aeration) influencing the growth of microalgae. The mass-cultivated species, valuable commercial products produced by them, and areas of their application are summarized in a Table. Particular attention is paid to microalgae metabolites with biological activity, the variety of biological activities that they exhibit, and which are used or can be used for the human health benefit.

Good literature awareness on the chosen topic and the ability to analyze the available information has allowed Tanya Toshkova-Yotova to focus on a poorly studied object (microalgae of the genus *Coelastrella*), **to justify and formulate clearly the purpose of his scientific work and the specific tasks for achieving the set goal**.

The **methods** applied in the implementation of the dissertation are diverse, scientifically sound, and undoubtedly allow the obtaining of correct scientific results. Appropriately selected and in details described methods cover both cultivation and analytical - weight, spectrophotometric, chromatographic, gas chromatographic-mass spectrometric, colorimetric, spectroscopic, microscopic, etc., as well as extraction methods and methods for determining biological activity – antibacterial, antifungal, antitumor and antioxidant (including MTT test, staining with fluorescent dyes, agar- and disk-diffusion methods, method of micro-dilutions in a liquid medium, etc.) and show the good methodological skills in building the research potential of the PhD student.

The systematically arranged **results** and their discussion are presented on 50 pages. The results are well illustrated in a total of 29 figures and 8 tables. The growth rate, biomass productivity and biochemical composition of four strains of the genus

Coelastrella are shown (in 2 graphs and 1 table) and compared, of which strain *Coelastrella* sp. BGV is reasonably chosen as the object of the subsequent investigations. Extracts and metabolites were obtained from the selected strain, a total of 8 in number. Using contemporary methods, the chemical characteristics of oil extract, fatty acids and exopolysaccharides (presented in 2 tables and 7 figures, including 4 spectra, 2 chromatograms and 1 elution profile) have been determined, and these characteristics are logically related to the biological activity of the products. Original and significant results for antitumor, antibacterial and antifungal activities of all obtained products are presented. The growth-inhibiting specific effect on HeLa tumor cells is illustrated in 10 graphs and 1 table, and the morphological changes in the treated tumor cells are illustrated on 4 panels with fluorescence microscopic images. The differences in the antibacterial and antifungal potential of *Coelastrella* sp. BGV products are demonstrated on 2 light microscope photos and 3 tables. The antioxidant potential (total phenol content, total flavonoid content and total antioxidant activity) of the algae biomass alcohol extract is presented in tabular and graphical form. **The reliability of the obtained results** is guaranteed by the correctly maintained sterile cultivation conditions, by the three-fold experiments and by the application of statistical analyzes such as one-way analysis of variance ANOVA, followed by the Bonferroni test. The results are convincing, competently interpreted, and the **discussion** is based on a comparative analysis of the own data and available in the literature data from similar studies with other, mainly green microalgae.

The **conclusions** (11 in number) and **contributions** (8 in number) formulated by the PhD student are in full accordance with the obtained results. In general, the scientific and scientific-applied contributions can be summarized as **obtaining and proving new facts**, which makes the dissertation extremely valuable. The more significant scientific and scientific-applied contributions of **original** character in my opinion are:

- For the first time an exopolysaccharide of *Coelastrella* sp. BGV is isolated and its molecular weight, functional groups and monosaccharide composition are determined;
- For the first time, it is experimentally proven a high and specific *in vitro* antitumor activity of fatty acids, unsaponifiable matter, alcoholic extract and exopolysaccharides

from *Coelastrella* sp. BGV to HeLa (human cervical adenocarcinoma tumor cell line) by induction of programmed cell death;

- Another important scientific-applied contribution is the established high antibacterial activity of fatty acids and ethanolic extract of *Coelastrella* sp. BGV, against more resistant Gram-negative pathogenic bacteria (*Escherichia coli* ATCC, *Escherichia coli* UPEC, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*);
- It is established for the first time that *Coelastrella* sp. BGV is a rich source of natural antioxidants;
- The comprehensive research shows that the Bulgarian strain of green microalgae *Coelastrella* sp. BGV is a rich and promising source of biomass and biomass products, with the potential for future use in the development of new natural medicines for the treatment of tumors and bacterial infections.

Of the contributions with a **confirmatory** nature, I will note the following:

- The biochemical and morphological characteristics of the Bulgarian strain of green microalgae *Coelastrella* sp. BGV have been expanded and enriched;
- The fatty acid profile of *Coelastrella* sp. BGV has been complemented with new experimental data.

I find it appropriate to make the following **recommendations** regarding one formulated contribution and one achieved contribution, which is not included in the dissertation:

- In contribution № 4 formulated in the dissertation, it would be better to add the mechanism by which the products of *Coelastrella* sp. BGV accomplish their antitumor activity (the disclosure of this mechanism in my opinion is an important contributing result);
- The successful extraction of carotenoids from *Coelastrella* sp. BGV with vegetable oil deserves to be noted as a contribution of a confirmatory nature, due to the previously established advantages of this non-toxic solvent over the standard organic solvents used.

Two articles on the topic of the dissertation are presented, one of which is "in press" in Comptes Rendus de l'Academie Bulgare des Sciences (IF_{2018} - 0.321, SJR_{2018} - 0.21, **Q2**), and the other was published in 2020 in Oxidation Communications (IF_{2019} -

0.361, SJR_{2018} - 0.21, **Q3**). The articles of Tanya Toshkova-Yotova are co-authored. She is the first author in both, which testifies to **her significant role in conducting the researches and presenting the results of the dissertation**. The publications in scientific journals, referenced and indexed in world-famous databases with scientific information, as well as the presentation of the results at scientific forums at home and abroad (oral report at a seminar with international electronic participation and poster reports - two at international conferences and one at an international symposium of ecologists) are indicative of the relevance of the thematic focus of researches and the importance of the results obtained.

The **Summary** (47 pages) is arranged according to the requirements and fully and accurately reflects what was done and achieved in the dissertation.

CONCLUSION

I am convinced that with the up-to-date topic, appropriately selected, mastered and applied diverse methods, precision and quality of research, originality and significance of scientific-applied and scientific contributions, the submitted for review dissertation fully meets the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Rules for its implementation, as well as the Regulations for the specific conditions and the procedures for acquiring scientific degrees and for employment of academic positions at the IPPG-BAS. With her dissertation Tanya Toshkova-Yotova presents herself as an excellent researcher, with theoretical knowledge, methodological competence and practical skills in the field of physiology and biochemistry of microalgae, which can conduct complex research in border scientific fields as well. As a member of the Academic Board, **I confidently vote positively** and dare to strongly recommend to the Honorable Academic Board to appreciate the qualities of the dissertation and its author, supporting the award of the educational and scientific degree "**Doctor**" in the scientific specialty "Plant Physiology" to **Tanya Toshkova-Yotova**.

November 20, 2020 Reviewer:

/Prof. Liliana Gigova, PhD/