

## EVALUATION REPORT

on the thesis for obtaining a DOCTORAL degree  
in professional field 4.3. "Biological Sciences", scientific specialty "Plant Physiology"

### DOCTORAL CANDIDATE:

TANYA STAVREVA TOSHKOVA-YOTOVA, Laboratory "Experimental Algology", Research Department "Experimental Algology", Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences (IPPG-BAS)

### TITLE OF THE THESIS:

BIOLOGICAL ACTIVITY OF PRODUCTS FROM *COELASTRELLA* SP. BGV

### EXAMINER:

Prof. Dr. VALYA NIKOLOVA VASSILEVA, Laboratory "Regulation of Gene Expression", Research Department "Molecular Biology and Genetics", IPPG-BAS

### RELEVANCE OF THE TOPIC

The thesis investigates the biological activity of extracts and metabolites from a local newly isolated microalgae strain, *Coelastrella* sp. BGV, and disclosing its potential antibacterial, antifungal and cytotoxic effects. The biotechnological capacity of green microalgae remains poorly studied due to difficulties in their cultivation in laboratory conditions. The topic of the thesis is relevant, and of high scientific and practical importance, which is linked to the growing interest in microalgae as a source of natural substances for various industries, medicine, agriculture, and also their use as model research objects. In particular, the disclosure of the potential of new Bulgarian microalgae strains is especially pertinent in light of the growing demand for natural products that are safe to humans and endowed with antitumor, antibacterial and antifungal activities.

### STRUCTURE AND CONTENT OF THE THESIS

The thesis is structured according to the generally accepted scientific standards, and consists of the following main sections: introduction, literature overview, main goal and objectives, materials and methods, results and discussion, conclusions and contributions. The thesis ends with an impressive bibliography of 315 sources, which includes both the titles from the last decade and older works with relevant to the topic theoretical and methodological information.

- **SECTION "LITERATURE OVERVIEW"** is presented on 27 pages and provides a logical overview on important aspects of microalgae production and practical use, and also various biological effects of microalgae products, such as antioxidant, antiangiogenic, cytotoxic and anticancer activities, including their potential in preventing and combating obesity. Three tables and one figure illustrate the main points concerning the annual production of microalgae, the systematic position of the genus *Coelastrella*, the use of microalgae, and the chemical structure of microalgae compounds. The overall impression from the literature overview is of in-depth knowledge of the topic including a detailed analysis of the existing challenges and perspectives that lead to the formulation of the main thesis goal and specific objectives.

- The **MAIN OBJECTIVE** is broadly formulated, emphasising on the investigation of the biological activity of products from *Coelastrella* sp. Five **experimental objectives** are outlined, the implementation of which lead to the achievement of the thesis goal.

- The “**MATERIALS AND METHODS**” SECTION is described on 13 pages and presents in great detail the used techniques, allowing to be reproduced by other colleagues. To achieve the goal and objectives of the thesis, a complex interdisciplinary approach is used including physiological, biochemical, chemical, microbiological and cell biology techniques and approaches. The methodology is adequate to the set experimental tasks, as some of them (isolation of exopolysaccharides, evaluation of cytomorphological changes by staining with fluorescent dyes, etc.) are used for the first time in the laboratory "Experimental Algology". The reliability of the obtained results is statistically proven by one-way analysis of variance (ANOVA), followed by Bonferroni's test using GraphPAD PRISM software, which allow appropriate data interpretation. It is obvious that in the course of her work, the doctoral student Tanya Toshkova-Yotova has mastered and successfully applied a large number of classical and modern techniques, which will undoubtedly be useful for her future research carrier.

- In the “**RESULTS AND DISCUSSION**” SECTION, the experimental data are presented together with their discussion within 50 pages. The section is very well illustrated with 8 tables and 29 figures, including graphs, histograms, photographs and microscope images of excellent quality, which facilitate the understanding of the obtained quantitative data and the perception of the presented information. The whole section is described very precisely and concisely. The results are discussed in the context of research developments of other teams working on the topic. This shows capability of the doctoral student not only to produce and process experimental results, but also to critically analyse and properly interpret them.

- The dissertation ends with the formulation of 11 **CONCLUSIONS** and 8 **CONTRIBUTIONS**, which adequately reflect the results obtained and their significance.

#### ***SUMMARY OF THE THESIS AND DISSEMINATION OF RESULTS***

The thesis summary has a total volume of 47 printed pages, and according to the requirements, presents fully and accurately the content of the thesis. In addition to the information about the performed experiments, and the obtained data and main contributions, the dissemination of the results through scientific publications and participations in scientific forums are also included. The only remark I have is about the small size of the printed font, which makes the text difficult to read. The results are published in 2 articles in journals that are indexed in reputable databases. One of the articles is published in *Comptes rendus de l'Academie bulgare des Sciences* (Q2, IF 0.321) and the second in *Oxidation Communications* (Q3, SJR 0.21). The doctoral candidate is the first author in both publications showing her leading contribution to the research activities. Some of the results are presented at 4 scientific forums through three posters and one oral presentation.

#### ***CRITICAL NOTES AND QUESTIONS TO THE DOCTORAL CANDIDATE***

I have no remarks on the substance of the work, but I have a few questions:

1. Biochemical composition of the biomass of the four studied *Coelastrella* strains in the two growth phases (Fig. 4) shows that in the stationary phase only *Coelastrella* sp. BGV has an increased lipid content and a decreased accumulation of all other components, compared to the exponential phase. Does this increase have any biological significance/advantage? There is mentioning of changes based on the physiological characteristics and different adaptive capabilities of the strains towards the reduced food resources during longer cultivation. Could you please explain this point in more details.

2. Why propidium iodide is not used in the double staining with fluorescent dyes, as it is done in the original method of Abdel Wahab et al., but ethidium bromide, which is far more toxic? I recommend, where possible, to quantify the data from microscopic observations with fluorescent dyes. A similar

quantitative comparison of the percentages of viable, apoptotic, and necrotic cells is performed in the original publication. Such a quantification would considerably contribute to the data publication.

3. Will the work with the investigated *Coelastrella* strains continue and in what direction?

#### **OVERALL ASSESSMENT AND CONCLUSIONS**

The presented thesis is original in design, well planned, and developed using a variety of experimental techniques. The obtained results are of sufficient quantity and quality, and fully correspond to the set goals and objectives, which has allowed to outline original scientific and practical contributions. The competent discussion of the results, the clear and scientifically correct language, as well as the language literacy of the doctoral candidate make an excellent impression. All this shows that in the course of the thesis development, the doctoral candidate Tanya Toshkova-Yotova has formed herself as a precise, capable and promising young scientist.

The presented materials meet the requirements of the Law for development of the academic staff of the Republic of Bulgaria and the Regulations for its implementation, as well as the specific requirements of the respective Regulations in IPPG-BAS. Therefore, I allow myself to give my positive assessment and recommend to the members of the esteemed Scientific Jury to award the educational and scientific degree "DOCTOR" to TANYA STAVREVA TOSHKOVA-YOTOVA in professional field 4.3 "Biological Sciences", scientific specialty "Plant Physiology".

22.11.2020

Sofia

Prof. Dr. Valya Vasileva