R E V I E W

For the competition of the academic position "**Associate professor**" in professional direction **4.3. Biological sciences**, specialty **Plant Physiology** (01.06.16), announced in SG No. 44/19.05.2023 for the needs of the laboratory "Experimental and Applied Algology" of the Institute of Plant Physiology and Genetics - BAS, with a candidate **chief assistant Yuliana Ivanova, Ph.D.**

Reviewer: Desislava Aleksandrova Todorova, Ph.D., Associate professor in the "Regulators of plant growth and development" laboratory, IPPG-BAS

Dr. Yuliana Ivanova is the only candidate for the announced competition. The presented materials in terms of form and content meet the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations on the Terms and Conditions for the Acquisition of Scientific Degrees and the Occupancy of Academic Positions at IPPG-BAS.

1. General data on the candidate's career and thematic development

The candidate for the academic position "docent" Yuliana Georgieva Ivanova was born in 1967 in the city of Sofia. In 1992, she obtained a MSc degree in the specialty "Biotechnology" and speciality "Microbiological processes" at the Faculty of Biology of Sofia University "St. Kliment Ohridski", where she successfully developed and defended a diploma thesis on the topic: "Sucrose degradation with PAAG-immobilized glucoamylase". She began her scientific career in 1994 in the "Experimental Algology" section of the IPP "Acad.M. Popov" as a biologist specialist. After a successful defense in 2006 of a Ph.D. thesis on the topic: "Physiological-biochemical characteristics of *Rhodella reticulate* and its relationship with a bacterial pathogen" under the supervision of Ph.D. Tonka Toncheva-Panova, Yuliana Ivanova acquired the educational and scientific degree "Doctor" in the specialty "Plant Physiology". During the period 2007-2008, she was appointed to the position of researcher II in IPP "Acad. M. Popov", and after the structural transformations of the scientific units at the BAS, she successively held the position of assistant (2011-2012) and chief assistant (2012-present) at IPPG-BAS.

The scientific interests of Dr. Ivanova are mainly focused in the research field of the biological activity of metabolites (and more specifically polysaccharides) isolated from microalgae; optimization of the conditions for cultivating algae; immobilization of microalgae in different sorbent matrices, in order to improve their biological activity; relationships between bacteria and algae; purification of water and soil from heavy metals and organic pollutions.

Most of the articles (49 articles and 1 useful model) from her entire scientific career are related to the topic of the microalgae unit. Of these, 6 publications were used in the defense of the dissertation, and 19 articles and the useful model were presented for participation in the current competition.

2. Evaluation of the submitted reference for the minimum and specific requirements of the competition.

For participation in the current competition, 19 scientific papers and one utility model certificate (Reg. No. 4295 U1, issued by the Patent Office of the Republic of Bulgaria) have been submitted, which do not repeat these, used for doctor. In 8 of these articles, Dr. Ivanova is listed as the first or corresponding author. According to the reference submitted by the candidate for the fulfillment of the minimum national requirements and the specific conditions of the Regulations of the IPPG-BAS for occupying the academic position "Associate professor" it is evident that Dr. Ivanova collects the required minimum points in all indicator groups. In indicator group **B**, she collects **112 points** (out of the minimum required 100 points) from 7 publications equivalent to a habilitation thesis. Of these, 2 numbers fall into quartile Q2 (B4_1 and B4_5), 4 number in quartile Q3 (B4_2, B4_3, B4_4 and B4_6), and one number in quartile Q4 (B4_7). The total **impact factor** of these publications is **8.298**. I would like to point out here that another publication was submitted for review instead of the described in the list under number B4_3. Both publications were published in 2021 in the journal "Oxidation communications", and also the topic of both articles is similar, therefore I agree to review the one presented by Dr. Ivanova and include it with her contributions. In a group indicators **C,** Chief Assistant Ivanova collects **223 points** out of the required 220 points. In this group, she includes 12 publications and the utility model certificate. The distribution of articles by quartiles is as follows: Q1 – 2 publications; Q2 – 2 publications; Q3 – 4 publications and Q3 - 4 publications. The total **impact factor** of these publications is **8.182**. Dr. Ivanova is the first and/or corresponding author in 8 of these publications (she collects a total of **86 points** with a required minimum of 70 points, in a presence in a scientific project, according to Appendix 1 of the Regulations of the IFRG-BAS on the specific conditions for occupying academic positions), which shows that she has achieved the necessary professional experience for leadership, and in-depth research work in her chosen scientific direction. The citations in scientific publications, referenced and indexed in the world databases with scientific information, which the candidate submits for the competition, are of **13 publications with a total of 56 references,** of which, however, I eliminate **5** (numbers 1, 16, 18, 20 and 47) due to non-existent or duplicate citations. Thus, I calculate the points for group **D** indicators at **102**, with a required minimum of 100 points. It should be noted that in the reference for the citations for the last 15 years in Scopus as of 04.08.2023, Dr. Ivanova appears with 86 citations in a total of 18 publications, as publication B4_5 collects 18 citations not listed in the reference of her documents. This shows that her investigations have received a sufficiently wide response in the world scientific community. According to Appendix 1 of the Regulations of IPPG-BAS on the specific conditions for occupying academic positions, candidates for the academic position of "Associate professor" should collect a minimum of 70 points from participation and/or leadership of a scientific or educational project. According to group **E** indicators, Dr. Ivanova indicates 120 points in the reference for the competition, of which 20 points for participation, 80 points for leading/coordinating a team from IFRG-BAS as a partner in the implementation of scientific projects and 20 points from attracted funds. I take this as a sufficient argument that, according to this indicator, Yuliana Ivanova meet and significantly exceed the requirements, although there is some discrepancy between the data in the reference and what is described in the attached CV. Copies of the contracts are not provided to verify the stated information, but I trust the candidate. According to the reference she filed in the documents, the results of her scientific work were presented in 3 oral and 13 poster presentations at a total of 12 international and national scientific forums.

The analysis of the scientometric data shows that the points with which Dr. Ivanova presented herself in the competition cover, and in category E, exceed the requirements for occupying the academic position of "Associate professor" at IPPG-BAS. **With a required minimum of 540 points for an "Associate professor" position, Dr. Yuliana Ivanova presented herself in the competition with 607 points**.

3. Analysis of the main research topics in the candidate's research work

The main contributions of Dr. Ivanova's scientific activity are summarized in 7 pages, providing systematized information about the experimental work performed, and she clearly defines her active participation in almost all the publications presented. Chief. Assistant Ivanova has no publications in which she is the sole author. All are collective with the participation of colleagues, mostly from other scientific units. This is explicable, as her research is part of multidisciplinary research projects and investigations, and in fact allows easily differentiate her personal contribution to these publications. According to the presented reference, the publications with which Dr. Ivanova appears in the competition can be conditionally divided into 2 thematic areas:

1. Biological activity of metabolites isolated from microalgae.

2. Optimizing the physiological-biochemical parameters of algae cultivation, as this thematic area is divided into 3 sub-topics:

A. Optimizing the physiological conditions for microalgae cultivation;

B. Immobilization of microalgae for their practical application.

C. Cultivation of microalgae in waste water from biogas production.

In principle, I agree with the reference made by the candidate about her scientific contributions, and I accept it. In addition, I believe that some of the articles fit within both main thematic scientific areas.

The main contributions to the **first thematic area** are related to studies on the antitumor effect of extracellular heteropolysaccharides, extracted mainly from red microalgae. Dr. Ivanova contributed with 5 publications to this topic: four of them with the object of research polysaccharides from microalgae **B4_2, B4_5, B4_6, B4_7**, and one publication with the object of research trehalose tetraester (disaccharide) from the bacterium *Rhodococcus wratislaviensis* (**G7_3**). I also refer publication **B4_3** to this direction.

The candidate's most significant personal contribution to this direction concerns the extraction, fractionation and purification of polysaccharides from different strains of red microalgae, which were tested *in vitro* on the proliferation and viability of cancer cells from different human tumor lines - carcinoma-related HeLa cells of the cervix; HT-29 cells, from colon carcinoma; MCF-7 (non-invasive) and MDA-MB-231 (invasive) cells, respectively associated with the formation of hormone-dependent and hormone-independent adenocarcinoma of the mammary gland (**Publ. B4_2; B4_3; B4_5**), and A549 human lung adenocarcinoma tumor cells (**Publ. B4_6**). Heteropolysaccharides have also been tested *in vivo* on Graffi's myeloid tumor in hamsters **(Publ. B4_7**). The results in these publications unequivocally show that treatment with heteropolysaccharides derived from microalgae suppresses the proliferation and viability of tumor cells and also induces changes in cell morphology. At the same time, no side effects or negative influence of these biologically active metabolites on healthy cells from the reference (control) cell lines were registered. The new facts in these publications prove that the antitumor activity of polysaccharides depends on the chemical composition of the molecule, the dose of application and the duration of treatment. Electroporation treatment enhances the antitumor activity of these biological compounds.

I believe that the presented publications in the first scientific topic represent in-depth studies that shape the specific scientific profile of Dr. Ivanova as a perspective researcher in the field of extraction, purification, characterization and testing of extracellular heteropolysaccharides as potential biotherapeutics with antitumor activity.

The second research topic refers to publications related to research on optimizing the physiological-biochemical parameters of microalgae cultivation. It is related to improving the conditions for growing algal biomass in order to increase production, reduce cost and, as well as apply targeted biosynthesis to obtain desired metabolites. This direction includes publications **B4_1, B4_4, G7_1, G7_2, G7_4 - G7_12** and **a useful model**. Of greatest importance to this research area, I can attribute the candidate's personal contribution to the selection of suitable strains of microalgae, varying the temperature, illumination, composition of the cultivation medium and the concentration of carbon dioxide, which is essential for improving the cultivation technology of algae and accumulation of specific biologically active substances. Dr. Ivanova also made a significant personal contribution to the development of hybrid matrix carriers with the inclusion of microalgae cells or extracellular heteropolysaccharides extracted from microalgae, which have promising applications in a number of fields.

In her studies, Dr. Ivanova shows that the optimization of the cultivation conditions allows the accumulation of algal biomass from the red microalgae *Rhodella reticulata* (Publ. **G7_6 and G7_7**) and *Porphyridium cruentum* (Publ. **G7_4**) and the blue-green microalgae *Chroococcus R-10* (Publ. **G7_2**), which is accompanied by a significant increase in the production of target metabolites (polysaccharides and phycobiliproteins). The candidate proves that unfavorable conditions for growing microalgae, including the presence of contaminating agents in the nutrient medium, such as bacteria or heavy metals, suppress the growth of the algal culture, and pathogens also lead to the death of the microalgae (Publ. **G7_9, G7_12, G7_5**). In fact, the ability of microalgae cells or their extracellular heteropolysaccharides, immobilized in gel or sol-gel hybrid nanomatrices, to accumulate heavy metals such as copper, zinc, cadmium, lead and nickel, also allows the purification of waters and soils from such pollutants (Publ. **G7_8 , G7_11, G7_5**), which makes the usage of algal biomass or heteropolysaccharides derived from it promising components for bioremediation.

The candidate also participates in other research of a scientific and applied nature, forming the scientific contribution of the proposed useful model. During the anaerobic decomposition of organic matter from waste, including biomass of plant origin (corn stalks, wheat straw) through the biogas and biomethane installations, a by-product named biosludge is obtained, rich in organic nitrogen- and phosphorus-containing compounds, macro and microelements. The cultivation conditions of selected strains of green, yellow-green, blue-green and red microalgae were optimized by including in the nutrient medium biosludge pre-treated with microwaves, ultrasound, high temperature, chemical digestion with a base and purified and decolorized with activated carbon, which helps to augment the biomass and to increase the concentration of some metabolites such as proteins, lipids, carbohydrates, pigments, etc. in *Porphyridium cruentum*, *Porphyridium aerugineum*, *Synechocystis salina*, *Trachydiscus minutus*, *Klebsormidium flaccidum*, and *Scenedesmus acutus*. The results of the complex studies carried out are reported in three of the publications (**B4_1, B4_4 and G7_1**) and in one utility model for invention.

4. Analysis of the scientific topic and significance for science and society.

I believe that the scientific interests of Dr. Yuliana Ivanova are focused on a very current scientific field and important for society. The use of microalgae as a useful and healthy food in the human diet dates back many years. The diversity and uniqueness of microalgal metabolites has been established over the past few decades. Many of them have useful qualities and high biological activity, therefore they quickly become the object of medical and economic interest. In response to the ever-increasing fields of application of algal biomass and its useful components, three main directions are developing in modern algology: isolation and characterization of new species and strains from nature; improvement of cultivation technology; search for approaches to affect the metabolic activity of algae. The topic that Dr. Ivanova develops is related to the extraction and study of the biological activity of products from microalgae. She investigates the antitumor

properties of polysaccharides from red microalgae (from *Rhodella* and *Porphyridium* species) on various cancer cell lines, which is important in discovering potential drugs to treat cancer and improve human and public health. According to the author's reference, the topic also includes studies related to the antioxidant activity of different strains of green and red microalgae, as well as their polysaccharide fractions. The second scientific direction, which she is developing, is related to the optimization of the conditions for cultivating microalgae, as well as the use of non-traditional sources of nutrients (waste products from some industries). The utilization of cheaper or unusable raw materials for the accumulation and extraction of biologically active substances and their subsequent inclusion in a continuous process determines the lower cost of the products, therefore her work in this direction is of economic importance for the introduction and the development of circular economy. Last but not least, her investigations on the inclusion of algal biomass or its products in hybrid nanomatrices, enabling an environmentally friendly method of cleaning water contaminated with heavy metals.

5. Organizational and training activities

Yuliana Ivanova is the coordinator of a scientific team from IFRG-BAS as a partner organization in 3 projects of other institutes of BAS, funded by BNSF at MES for the last 5 years, and head of one contract. Her role in the implementation of funded projects under the Program for "Supporting Young Scientists, Doctoral Students and Postdoctoral Students" of the BAS, financed by the MES, is not clear described. In the reference of fulfillment of the minimum requirements, she indicated that she was a participant, and in her CV that she was the leader of such projects. The funds attracted and her function as a scientific team coordinator of a partner organization in the implementation of scientific projects is evidence that she has a significant experience in acquiring funding for her scientific developments. There is no data presented for supervising students, bachelors and masters to develop student internships and diploma theses.

6. Critical remarks and recommendations

I recommend to Dr. Yuliana Ivanova greater rigor and precision when preparing official documents. There are abundance of technical errors, inconsistencies and inaccuracies. Instead of the publication number B4_3 described in the list of publications for the contest, another one is attached. Copies of the contracts certifying the participation and/or management of scientific projects financed by the specified sources, proving the attracted funds, are not attached.

I also recommend a higher activity of Yuliana Ivanova in publishing the scientific results, such as taking a greater commitment in the key function "corresponding author", which is inherent to the relevant academic position, and also in her capacity as a team leader/coordinator when developing scientific investigations.

I also recommend that she should transfer her experience in the isolation, characterization and testing of the biological activity of microalgal polysaccharides to young colleagues - students and PhD students, as their scientific supervisor or consultant in the development of M.Sc. or Ph.D. theses.

The critical remarks and recommendations made do not belittle the candidate's contributions to the development of the scientific topic, but aim improvement of her personal qualities, which will be useful in her future work.

7. Conclusion

After the analysis of the scientific activity of Chief Assistant Dr. Yuliana Ivanova, I express my positive opinion that the candidate fully meets the profile of the announced competition. She participates with scientific publications sufficient in terms of volume and quality, and the points she collects from scientific

activity satisfy the requirements of the legal requirements and the Regulations for the specific conditions and procedure for occupying the academic position "Associate professor" at IPPG-BAS. The candidate has a clear scientific profile in a scientific field relevant to plant physiology and important to society. In addition to high scientific value, her research has an very promising applied orientation. All her investigations can be an excellent basis for practical application and further developed in the future for the creation of antitumor drugs, increased production of microalgae biomass and targeted synthesis of valuable secondary biologically active metabolites, full utilization of waste products from other industries, bioremediation of water and soils, etc. All this gives me motivation to recommend to the respected members of the Scientific Jury and the Scientific Council of IPPG-BAS to award the academic position of "Associate Professor" to Chief Assistant Dr. Yuliana Georgieva Ivanova in professional direction 4.3. Biological Sciences, specialty Plant Physiology.

05.09.2023 Reviewer:

Sofia /Assoc. Prof. Dr. Desislava Todorova/