

Review

for the competition for the academic position "associated professor", announced in SG No. 44/19.05.2023, in professional field 4.3. Biological sciences, specialty "Plant Physiology", for the needs of the "Experimental and Applied Algology" laboratory of IPPG-BAS

with candidate chief assistant Dr. Yuliana Georgieva Ivanova (only candidate)
reviewer Maya Petrova Stoyneva, Ph.D., Professor in the Department of Botany,
Faculty of Biology, Sofia University "St. Kliment Ohridski"

This review was prepared in my capacity as a member of the Scientific Jury, appointed by Order of the Director of IPPG-BAS, Prof. Dr. V. Vasileva, and is structured in accordance with the Requirements of SC-IPPG for formulating reviews and opinions on a competition for occupying the academic "associated professor" position.

General data on the candidate's career and thematic development

General Assistant Dr. Juliana Ivanova has submitted a CV in European format attached to her documents. Therefore, in view of the limited volume of the review, according to item 1 of the IPPG-BAS Requirements, I will give summarized data to outline the general features of the candidate. Born on 24.03.1967, she is a graduate of the Faculty of Biology of the Sofia University "St. Kliment Ohridski" (1987-1992), where she graduated as a Master of Biotechnology with a specialization in microbiological processes. General Assistant Ivanova had a consistent development from specialist-biologist (1994-2006) in the n.s. II degree (2007-2008), assistant (2011-2012) and chief assistant (from 2012 until now) at IPPG-BAS, where in November 2006, under the supervision of Assoc. Dr. T. Toncheva-Panova, she successfully defended her dissertation for obtaining a doctor's degree on the topic "Physiological-biochemical characteristics of *Rhodella reticulata* and relationships with the bacterium pathogen". Apparently, this detailed "meeting" with the red coccal unicellular microalga *Rhodella reticulata* Deason, G. L. Butler et C. Rhyne 1983 (separated from the genus *Porphyridium* and known currently as *Dixoniella grisea* (Geitler) J. L. Scott, S. T. Broadwater, B. D. Saunders, J. P. Thomas et P. W. Gabrielson 1992 - https://www.algaebase.org/search/species/detail/?species_id=42807), popular due to its rich content of unsaturated fatty acids and especially EPA, has contributed to the

definitive formation of the candidate's interests in the study of the physiology and biochemistry of algae with a view to their application in medicine and bioremediation. This largely predetermines the logic of the overall scientific and professional development of Dr. Ivanova, in the course of which the scientific research activity naturally intertwines cultivation, physiological and medical-biological studies of selected algae from both prokaryotic and eukaryotic evolutionary lines in view of their biochemical composition, biological activity and productivity under different conditions, and also research on algae with a special focus on obtaining nanomaterials and other products with high added value, water purification (incl. absorption of heavy and toxic metals, assimilation of nitrogen and phosphorus compounds) by algae cultures, cultivation of microalgae in wastewater and in fermentation products with potential for biogas production, and last but not least, algae-bacteria relationships. In addition to the notable medical importance of specific algal BACs (for example, the antitumor activity of their unique polysaccharides), the other practical-applied aspects in the research of the assistant professor. Ivanova are currently particularly relevant in connection with the development of green processes important for the circular economy, Blue and Green deals. The thematic development thus determined fully corresponds to the needs and perspectives open to the Experimental and Applied Algology section of IPPG-BAS, for which the current competition has been announced.

According to the attached Service Note from IPPG-BAS as of the date of the competition, the seniority of the chief assistant Dr. Ivanova is 26 years, 1 month and 14 days - a period not only sufficient, but also necessary for the accumulation of serious experience in the field of cultivation, isolation and research of microalgae strains. Therefore, I specifically note this fact as positive for the overall assessment of the activity of the only candidate in the competition.

Assessment of the submitted reference for compliance with the requirements of Art. 29 of ZRASRB and the specific requirements reflected in Appendix 1 of PSURPNSIZAD in IPPG-BAS

According to the Certificate of compliance with the minimum requirements of the RSARB presented in the tender materials, Chief Assistant. Ivanova has a total of 617 points, distributed by groups of indicators as follows: A - 50 points, B - 112 points, D - 223 points, D - 112 points, E - 120 points. These specified 617 points exceed the required 540 points, and the exceedance is in

indicator groups B, D, D and E. The compliance assessment for this review was made for each of the indicator groups as follows:

1. 50 items for group A are fully accepted;
2. Group B – 0 points, as indicated in the documents;
3. Group C and Group D: Out of a total of 50 publications prepared by Dr. Ivanova during her entire research activity, 19 selected publications in journals, indexed and referenced in global scientific databases, are presented for participation in the current competition. Part of these publications (7 in number) are assigned to criterion B, and part (12 in number) to criterion D. This division, given the possibility of redistribution and internal compensation, provided for in the ZRASRB, does not seem necessary to me, but I accept it as the candidate's choice according to the possibilities given in the ZRASRB tables. In any case, by itself this reallocation does not change the total number of points that the submitted publications receive according to their quartiles. In the competition materials, Dr. Ivanova herself states that she participated with 2 publications in magazines with Q1, 4 with Q2, 8 with Q3 and 5 with Q4. The specified quartiles exactly correspond to the data on the website <https://www.scimagojr.com>, which is standardly required by the RSARB. The points distribution for these posts is as follows: 2 with Q1 ($2 \times 25 = 50$), 4 with Q2 ($4 \times 20 = 80$), 8 with Q3 ($8 \times 15 = 120$) and 5 with Q4 ($5 \times 12 = 60$), i.e., a total of 310 items. The patent with registration number 4295U1 is evaluated with 25 points, and so the total number of points for indicators B and D is exactly 335, as Dr. Ivanova also indicated. She shared in detail her publications in two groups: one in which he is the first or corresponding author - 86 points, and the second group of the remaining publications - 112 points. The article in Polymers (Q1, submitted under number Г7_3) refers only to the gram-positive bacterium *Rhodococcus wratislaviensis* and does not correspond to the theme of the competition. Therefore, the total number of points for indicators B+D is 310;

4. Group of indicators D: According to the presented Scopus and WOS reference, the citations until 10.07.23 are a total of 56 and are in prestigious, indexed scientific journals, with 13 publications cited. Of all the citations, 35 are to five articles from 1997, 2000, 2002, 2006 and 2008, which are outside the list of publications for participation in the current competition. The candidate correctly reflected the noticed citations, removing the self-citations and the number of 112 points fully corresponds to the presented reference;

5. Group of indicators F: This group of indicators presents detailed data on the projects according to their type and the funding received, the participation of the candidate (member/leader) and the amount received. The data presented exactly correspond to the points in the reference for the minimum national requirements under the RRSP

The overall assessment of compliance is: Dr. Ivanova, with a total of 592 points, exceeds both the minimum national requirements of ZRASRB and the specific requirements established in IPPG-BAS.

Analysis of the main directions in the candidate's research work and the most important results in each of them, outlining his personal contribution

The present analysis was made on the basis of the Author's Reference for the contributions presented in the documents when compared with the publications selected for participation in the competition. Here I would like to point out that the 18 publications analyzed below have gone through a serious review and editing process in prestigious, indexed and quartile-refereed scientific journals, which in itself testifies to their high quality.

The author's report on the contributions of Dr. Yu. Ivanova has been prepared in detail and correctly reflects the original results of a contributing nature obtained by her. It fully corresponds to the presented scientific publications and convincingly proves the essential role of her research in two main areas: 1) study of the composition and activity of biologically active substances isolated from microalgae (mainly polysaccharides) and 2) optimization of the cultivation conditions of selected strains of microalgae. With this, Dr. Ivanova contributes not only to the enrichment of knowledge about the physiology and biochemistry of microalgae, but also to the development of various aspects of biotechnology and its application for medical purposes with a view to treating or improving the condition of socially significant diseases, among which is cancer. I fully accept the presented reference, and for the needs of this review I will pay attention to some particularly important points and I would like to note that the scientific and scientific-applied contributions refer first of all to the category of obtaining and proving new facts, and secondly - to the category of methodological contributions, which determines the value of published works.

At the beginning of the reference, Dr. Ivanova has outlined the relevance of the topics and objects on which her research work, conducted in the Experimental and Applied Algology section at IPPG-BAS, is focused. This work is related to the biotechnology, biochemistry and physiology of algae, focusing primarily on their microscopic representatives - microalgae and, together with the presented publications and participation in scientific topics and forums, gives grounds to claim that the candidate has a clearly defined profile of his research work.

It is noteworthy that the objects selected for research, despite having the same morphological type of thallus structure (coccal), are different in the type of cytological structure (cyanoprokaryotes and typically eukaryotic algae), in the nature of the ecological group (edaphophyton, hydrophyton, thermophyton), but are also representatives of different evolutionary directions - blue-green, red and green, collected in different taxonomic divisions (Cyanoprokaryota, Rhodophyta, Chlorophyta) and from different regions (Europe, Antarctica). In addition, among the sites selected by the candidate there are newly isolated strains of microalgae from the Bulgarian nature, including from endangered habitats from the Red Book of Bulgaria, such as thermal springs. All this makes the general scope of research carried out at all levels more valuable, enabling comparisons of data for different levels of evolution and a good basis not only for future physiological, genetic and biochemical independent studies, but also for general taxonomic analyzes and conclusions. The problems developed by the candidate are extremely relevant in a scientific and scientific-applied sense, and due to the fact that in recent years microalgae have been increasingly used in medicine, pharmacy, cosmetics and various branches of industry and agriculture, especially in the conditions of modern Green and Blue Deals, and also in the circular economy. This necessitates their mass cultivation, which, in turn, requires not only the so-called phycoprospecting (i.e., the discovery of new local strains of promising producers among the huge biodiversity of microalgae from different ecological and taxonomic groups), but also the study of their "behavior" and biological and biotechnological potential under different cultivation conditions with a view to increasing productivity and yields at maximum economic benefit.

Currently, as a result of numerous and diverse studies, a huge amount of information has been accumulated on the metabolites of microalgae and on their various activities, sometimes with a combined spectrum of action, as well as on the changes in the physiology and biochemistry of

cells and thalli under the influence of various factors, and hence – the amount of synthesized biologically active metabolites.

. A particularly important point is that the changes are not only species-specific, but also strain-specific. At the same time, still the vast majority of microalgae remain almost completely unexplored in terms of the detailed composition of their metabolites, especially BAV. In this sense, the study of rare, poorly studied or completely unstudied algal components (e.g. external heteropolysaccharides of red non-marine algae) with the elucidation of the specific composition of individual polysaccharides (e.g. exopolysaccharides of species of the genus *Porphyridium*) is essential (article group C4).

This type of research complements the research in the first thematic direction in the research work of the Assistant Professor. Yu. Ivanova: Biological activity of metabolites isolated from microalgae. Among the red algal metabolites studied are the exopolysaccharides secreted by the species *Porphyridium sordidum* Geitler 1932 in relation to breast cancer tumor cells (Article B4–5), by the species *Porphyridium purpureum* (Bory) K. M. Drew & R. Ross 1965 (Syn. *Porphyridium cruentum* (S. F. Gray) Nägeli 1849 - https://www.algaebase.org/search/species/detail/?species_id=564) against cervical carcinoma, colorectal carcinoma and two breast adenocarcinomas (Article B4–2), and from the species *Dixoniella grisea* (Geitler) J. L. Scott, S. T. Broadwater, B. D. Saunders, J. P. Thomas et P. W. Gabrielson 1992 against Graffi's myeloid tumor in hamsters and against human lung adenocarcinoma A549 (articles B4–6, B4–7). The methods selected and used to solve the problems are scientifically based, standard and without a doubt allow obtaining correct scientific results. Of particular importance is the establishment of not only a pharmacological effect affecting only tumor cells, but also the proof of the absence of side effects when using the mentioned heteropolysaccharides.

The second (not in order of importance) type of research conducted and published by Dr. Ivanova in the indicated thematic direction is related to clarifying the non-enzymatic antioxidant activity of green coccal microalgae from two different ecological groups: the thermophyton (strain *Chlorella vulgaris* R-06/2, originally isolated from Rupite) and hydrophyton (strains *Scenedesmus acutus* M Tomaselli 8 and *Scenedesmus obliquus* BGP). As *Scenedesmus acutus* Meyen 1829 and *Scenedesmus obliquus* (Turpin) Kützing 1833 are synonyms of *Tetradesmus obliquus* (Turpin) M. J. Wynne 2016 (https://www.algaebase.org/search/species/detail/?species_id=158061), both

investigated strains refer to the same species. The result obtained in the comparative study of the three strains (article B-3) for the highest antioxidant potential with more BAC of *Chlorella vulgaris* R-06/2 is, in my opinion, absolutely logical in view of the extremophilic nature of the strain.

All the results obtained in the first thematic area clearly show the perspective of future research in the field of microalgae metabolites with an expansion of their scope, which deserves a high evaluation. The candidate's personal contribution is clearly visible and has a strongly expressed algological character: selection of strains from different microalgal species and optimization of their cultivation with a view to future diverse applications in biotechnology and medicine, as well as isolation and purification of exopolysaccharides from the mucous membranes of selected strains.

The second thematic direction in the research work of Dr. Yu. Ivanova is related to the optimization of the physiological-biological parameters of microalgae cultivation. The research in it is correctly divided by the researcher herself into three thematic subgroups, which I will examine in sequence using the accepted taxonomic names. Before that, I would like to strongly emphasize that in this thematic direction, the contributions are not only of an original nature with the acquisition of new data and facts, but also of a methodical nature, which is of particular importance for the needs of the Experimental and Applied Algology section.

- 1) Optimizing the cultivation conditions - this refers to experiments carried out with algae from two evolutionary lines: the blue-green thermal strain *Chroococcus* R-10, introduced for the first time under intensive laboratory cultivation conditions (article G7_2) and the red *Dixoniella grisea* и *Porphyridium purpureum* (articles G7-6, G7-7). In addition to the establishment of optimal cultivation conditions and the impact of specific biogens and chemical compounds on the development of the strains and the production of polysaccharides, described in the publications mentioned above, Dr. Ivanova has worked on the interrelationships of algae in cultures with available bacteria by developing a method to normalize the balance in the environment in order to prevent the pathogenic development of bacteria (Articles G7-9, G7-12);
- 2) Immobilization of algae for practical purposes - the experiments performed here include working with the red microalgae *Dixoniella grisea* and *Porphyridium purpureum* (articles G7-6, G7-8, G7-11), with a synthesized sol-hybrid nanomatrix (article G7-10)

as methods have been developed for long-term storage by immobilization in an innovative macroporous cryogel (article G7–6), preparation of polysaccharide from immobilized cells (G7–10). This subgroup also includes research related to the absorption of ions of heavy and toxic metals from the water environment (articles G7–8, G7–10), as a special nanomatrix with embedded *Dixoniella* cells was synthesized for the purpose of biosorption of copper ions (article G7-8). Regarding the bio-absorption of heavy metal ions, experiments were also carried out on the uptake of cadmium by the green microalga *Scenedesmus incrassatulus* Bohlin 1897, which is currently a synonym of *Tetradesmus incrassatulus* (Bohlin) M. J. Wynne 2016 (https://www.algaebase.org/search/species/detail/?species_id=158059) and the possibility of its use for bioremediation has been demonstrated (article D7–5).

- 3) Cultivation in waste water for biogas production – this subgroup includes studies of the green microalga *Tetradesmus obliquus* and the red *Porphyridium* (articles B4–1, B4–5 and D7–1), which clearly show the perspective of the simultaneous cultivation of algae with microorganisms for wastewater treatment and for obtaining biogas. Here again, there is a methodical contribution by creating a closed loop when adding microalgal biomass to the substrate and its subsequent return to the bioreactor (Article B4–1).

All the original and reliable data obtained are of a contributory nature or are methodological contributions, and all of them are essential to modern practices applied in agriculture, industry and environmental protection, and therefore should be highly appreciated. The personal contribution of Dr. Ivanova in all these studies is clearly expressed and is algological in nature: related to the selection of strains, to the optimization of cultivation conditions, to the extraction of bio-products.

Motivated answer to what extent the candidate has a clearly defined current scientific topic, indicating its significance for science and society

The answer to this question is positive and is categorically contained in the analysis presented above - the topic chosen by Dr. Yu. Ivanova for her research activity related to the cultivation of algae, the study of their metabolites and their role in human health and dealing with socially significant diseases such as cancer, as well as the possibilities of using algae for water

purification, for obtaining biofuel and extracting additional products are some of the hottest topics not only of modern algology, but also of society as a whole

The results obtained by Dr. Ivanova are of a contributing nature and are undoubtedly a basis for future application of the studied species and strains in industrial cultivation and are promising for biotechnological practice in the conditions of the modern circular economy, Blue and Green deals.

The evidence for the importance of the results obtained by Dr. Ivanova and for her international recognition is not only in the noted citations and in the articles published in prestigious journals (19), but also in the participation in international and national scientific forums (a total of 12 forums with 6 reports and 10 posters), in the management of 4 and participation in 2 prestigious scientific projects on topics related to the competition.

Organizational and training activity

In the presented documents, there is no data on conducted training activities, and the data on the management of successfully implemented research projects testify to the organizational skills of the candidate in the competition.

Critical notes and recommendations

I have only one wishful recommendation for the candidate for her future work, and it is for greater activity in working with students and doctoral students, if the objective circumstances allow it.

I have no personal impressions of Dr. Ivanova and her work at IFRG-BAS, but after familiarizing myself with the works and the presented documents, I can claim that she is a responsible modern researcher-scientist with great potential.

Reasoned positive or negative conclusion for choice

In conclusion, I believe that the only candidate in the current competition fully meets the requirements of the National Assembly of IFRG-BAS, the Regulations for occupying academic

positions at BAS and the ZRAS in the Republic of Bulgaria for obtaining the academic position "associate professor" in area 4. Natural sciences, mathematics and informatics, professional direction 4.3 Biological sciences, specialty Plant physiology for the needs of the "Experimental and applied algology" section of the IFRG-BAS, therefore I strongly recommend to the honorable members of the Scientific Jury to vote for awarding this position to ch . assistant Yuliana Georgieva Ivanova.

Sofia, 31.08.2023

Signature:

(Prof. DrSc. M. P. Stoyneva)