

REVIEW

by Prof. Velizar Kostadinov Gochev, PhD,

Head of the Department of Biochemistry and Microbiology, Plovdiv University “Paisii Hilendarski” of the materials submitted for the competition for appointment to a Professor’s academic position at the Biological Faculty of the Plovdiv University “Paisii Hilendarski” in higher education area 4. Natural sciences, mathematics and informatics, professional field 4.3 Biological sciences (Microbiology)

The only candidate in the competition for the appointment to the academic position of "Professor", announced in the State Gazette No. 40 of May 14, 2021 and on the web site of Plovdiv University “Paisii Hilendarski” for the needs of Department Biochemistry and microbiology at the Biological Faculty is Assoc. Prof. Dr. Sonia Kostadinova Trifonova, from the same department.

1. General presentation of the materials submitted

By Order № P33-4296 of 10/08/2021 of the Rector of the Plovdiv University “Paisii Hilendarski” (PU) I was appointed as a member of the scientific jury of a competition for the appointment to the academic position of "Professor" in higher education area 4. Natural sciences, mathematics and informatics, professional field 4.3 Biological sciences (Microbiology) advertised for the needs of the Department Biochemistry and microbiology at the Biological Faculty.

The complete set of materials submitted by Assoc. Prof. Dr. Kostadinova, both in paper and in electronic form is in accordance with the Regulation on the Implementation of the Academic Staff Development Act in the Republic of Bulgaria (RIASDARB) and the Regulations of the PU on the Implementation of the Academic Staff Development Act in the Republic of Bulgaria (RPUIASDARB), and includes the following documents:

- Application form to the rector of PU for admission to the competition;
- Curriculum vitae European form;
- Master's Degree diploma;
- Diplomas for awarding of the educational and scientific degree “Doctor”, at that time a “Candidate of biological sciences”, and the appointment to the academic position “Associate Professor”, issued by Higher Attestation Commission;
- List of scientific publications, list of citations, handbooks, etc.;
- Information on the fulfillment of the minimum national requirements in accordance with the RIASDARB and the relevant supporting evidence;
- Information on the fulfillment of the specific requirements of the Biological Faculty laid down in the RPUIASDARB and the relevant supporting evidence;

- Declaration of originality and non-plagiarism;
- Extended author's habilitation reference for scientific contributions;
- Certificate of work experience;
- Documents for educational activities
- Document for scientific and research activity, including official note of NPD at PU

The applicant has enclosed a list of a total 37 materials, including 1 habilitation monography, 33 scientific publications, 2 textbooks and 1 laboratory manual. They were not included in the dissertation thesis for the awarding of the educational and scientific degree “Doctor and the materials for the competition for the appointment to an Associate Professor’s academic position and all of the submitted materials are under review for the participation in the present competition.

The content of the applied documents and the manner of their organization allow me to categorically define the procedure as lawful.

2. Short biography of the candidate

Sonia Kostadinova Trifonova graduated from RLS “Ivan Vazov” in Plovdiv and the Faculty of Chemistry and Biology at the Plovdiv University, acquiring higher education with a Master’s degree in Biology with a professional qualification Biologist, teacher on Biology with a second specialty in Chemistry. In 1993, after successfully defending a dissertation, the Higher Attestation Commission awarded her the educational and scientific degree “doctor”, at that time a “Candidate of biological sciences”, in the scientific specialty 01.06.12 Microbiology.

Professional career of Assoc. Prof. Dr, Kostadinova started as a teacher and her academic growth followed the whole hierarchy starting from position of biologist, following Senior Assit. Prof., Chief Assist. Prof. and in 2003 she successfully acquiring the academic position of Assoc. Prof. in the scientific specialty 01.06.12 Microbiology. Administrative career of Assoc. Prof. Dr. Kostadinova is impressive. She has successfully held the positions of Head of the Department of Biochemistry and microbiology (2007-2011), Vice Dean of the Faculty of Law at the PU (2011-2015) and two terms Dean of the Faculty of Biology at PU (2015 and present).

The creative biography of the candidate is entirely in the filed of microbiology and fully covers the scope of the announced competition. The entire creative path of Assoc. Prof. Dr. Kostadinova is tightly related to the Faculty of Biology and the main academic unit Department of

Biochemistry and microbiology, which stated the need for announcement of competition for an academic position of “professor”.

3. General characteristics of the candidate's activities

I am deeply convinced that in a review of a professor selection procedure in an institution such as University where teaching activities are dominant, it is more appropriate to start with an assessment of the candidate's teaching activities, but due to the legal requirements for implementing the minimum national criteria for reaching the academic position of “professor” and betting as leading to the criteria reflecting the research activity, I will comply with the requirement and will focus first on it.

Assessment of the candidate's research activity

Group of indicators A: Indicator 1 Dissertation thesis for acquiring educational and scientific degree “doctor” – requirement of RIASDARB – 50 points, implementation by the candidate – 50 points.

Group B indicators (Sum of indicators 3 or 4): Indicator 3 Habilitation thesis – monograph or Indicator 4 Scientific publications that are published in journals referred and indexed in the world scientific databases Scopus and Web of Sciences – requirements of RIASDARB – 100 points, implementation by the candidate – 100 points.

For participation in the competition Assoc. Prof. Dr. Kostadinova submitted habilitation thesis – monograph entitled “*Phospholipases C, produced by Bacillus species*”. The monograph 170 pages, includes a Preface, Introduction, Main part, presented in five chapters – Characteristics of genus *Bacillus* (Chapter 3), Characteristics of phospholipases (Chapter 4), Phospholipases C produced by the genus *Bacillus* (Chapter 5), Functions and physiological effects of phospholipases C (Chapter 6) and Application of phospholipases C (Chapter 7), Summary and references. I strongly appreciate the author's decision, even at the beginning of the preface, to clearly define the scope of the study, without unnecessary pretentious and to declare the originality of own results, which are presented in the monograph.

The Introduction is a natural continuum of the Preface and deeply motivates the necessity of the habilitation study by importance of phospholipases, their wide possible applications and advantages of bacteria belonging to genus *Bacillus* as prospective enzyme producing organisms. The main part of the monograph starts with detailed characteristics of these enzymes (Chapter 3). Historical development of *Bacillus* taxonomy is also discussed. Morphological, physiological, biochemical and genetic characteristics of two major groups *Bacillus sensu stricto* and *Bacillus sensu lato* are discussed. The main focus of this chapter is on the ecology, morphology, physiology, pathogenity and genetic determinants of *B.cereus*, *B.thuringiensis* and *B.sphaericus* (*Lysinibacillus sphaericus*). Chapter 4 of

the monograph is dedicated to the classification of phospholipases, substrate specificity and mechanism of action. Author's decision to predominate the amount of information about phospholipase producing bacteria in comparison with the amount of information about biochemical mechanisms of phospholipase action is appropriate, because the main focus of the monograph is producing of bacterial phospholipases C. The conclusions in Chapter 4 highlight the advantages of species belonging to genus *Bacillus* as the most prospective phospholipase C producing organisms. Chapter 5 is the biggest and it is strongly original. Phospholipase, lipolytic proteolytic activity of 166 strains belonging to the species *B.cereus*, *B.thuringiensis* и *B.sphaericus* is studied. Prospective PC-PLC producing strains are selected. The dynamics of enzyme production is monitored and the regulator role of the quorum-sensing peptides on the gene expression are discussed. The nutritive media composition and cultivation conditions for maximum PC-PLC production are optimized, also the effect of different metal ions on the enzyme production are studied. As a result of optimization procedure the enzyme production of PC-PLC by *B.cereus* and *B.thuringiensis* is increased by 54-58 %. Original laboratory schemes for enzyme purification are composed. The schemes include the following steps: ultrafiltration, gel-filtration on Sephadex G-75 and FPLC HiPrep DEAE FF. High levels of enzyme purification are reached and major enzyme characteristics such as molecular weight, substrate specificity, temperature optimum, pH optimum and ranges of temperature and pH stability are determined. The effect of metal ions and detergents on the enzyme reaction, K_m and V_{max} are also studied. Obtained results for PI-PLC and Smase are presented in the same analytical frame. The interpretation of the obtained results is definitely presented against the background of a significant amount of knowledge on the subject of the specific research of many other authors. This form has allowed the author to summarize, compare and bring to the fore the specific characteristics of PC-PLC, PI-PLC and Smase, produced by the selected original strains of *B.cereus*, *B.thuringiensis* and *B.sphaericus*. Functions and the physiological effects of bacterial PLC are deeply discussed in Chapter 6. The knowledge about possible applications of bacterial PLC, presented in Chapter 7, opens up new directions for future studies.

The habilitation monograph submitted in the present competition presents sufficient volume of original scientific results, interpreted against the background of a large-scale reference information on the studied problem. The style is scientifically sophisticated without creating difficulties in perceiving the information. The volume of the monograph fully corresponds to the title and the predefined scope.

Group of indicators G (sum of indicators from 5 to 10) - requirement of RIASDARB – 200 points, implementation by the candidate – 256 points.

Indicator 7 Scientific publications that are published in journals referred and indexed in the world scientific databases Scopus and Web of Sciences.

To fulfill the requirements of the indicator Assoc. Prof. Dr. Kostadinova submits 18 original scientific publications, divided into quartiles as follows: 2 in scientific journals of quartile Q2 (2 x 20 points., publications III.2.8 и III.2.12); 8 in scientific journals of quartile Q3 (8 x 15 points., publications III.2.2, III. 2.3, III.2.4, III.2.5, III.2.7, III.2.9, III.2.13 and III.2.15); 8 in scientific journals of quartile Q4 (8 x 12 points., publications III.2.1, III.2.6, III. 2.10, III.2.11, III.2.14, III.2.16, III.2.17 и III.2.18). For participation in the announced competition Assoc. Prof. Dr. Kostadinova submits 12 additional scientific publication that are published in referred scientific journal without impact-factor and impact range and 4 publication in proceedings books of scientific conferences, which also are referred in NACID. These publications are taken into consideration for assesement of reasearch activities of the candidate.

Analysizes of the reasearch activities of Assoc. Prof. Dr. Kostadinova allows me to underline scientific contributions in three major directions: (1) Biosynthesis, isolationa and purification of bacterial enzymes; (2) Isolation, identification, virulence factors and pathogenity of microorganisms associated wuth urinary tract infections and (3) Ecology of microorganisms – microbial biodiversity and possibilities for purification of waste waters contaminated with heavy metal by microbial bisorbents.

The production of bacterial enzymes stands out as an essential direction in the research activity of Assoc. Prof. Dr. Kostadinova (publications III.2.2, III. 2.10, III.2.16, III.3.2, III.3.5, III.3.9 and III.4.2), which is natural continuation of the results and contributions mentioned in the habilita-tion-monograph “*Phospholipases C, produced by Bacillus species*”. The following more important achievements can be highlighted in the publications of the candidate:

- New active bacterial strains for production of alkaline phosphatase (*B.cereus* and *E.coli*), protease (*B.thuringiensis*), lipolytic enzymes (*B.thuringiensis*, *B.cereus* и *Ps.fluorescens*) and amylase (*B.cereus*, *B.thuringiensis* и *B.sphaericus*) are selected;
- Nutritive media composition and process parameters for cultivation of newly isolated strains for reaching a maximum enzyme yield are optimized;
- New original and effective laboratory schemes for enzyme isolation and purification are proposed and some of the major enzymes characteristics such as molecular weight, temperature optimum, pH optimum, range of termal and pH stability are determined.

Major contributions in direction microbial pathogenesys (publications III.2.1, III.2.3, III.2.4, III.2.5, III.3.4, III.3.7, III.3.10, III.3.11 and III.3.12) are as follows:

- Identification of bacterial strains, isolated from patients with urogenital infections. The virulence determinants and drug susceptibility of the isolated strains are determined. The obtained results are an essential base for development of effective antimicrobial therapy;
- Biofilm forming capacity of enormous number of clinical isolates is studied and the effect of antibiotics, natural and synthetic substances and probiotic strains are estimated.

Major contributions in direction microbial ecology (publications III.2.6, III.2.7, III.2.8, III.2.11, III.2.12, III.2.13, III.2.14, III.2.15, III.2.17, III.2.18, III.3.6 and III.3.8) are as follows:

- Assessment of microbial status and influence of antropogenic activities on the level of distribution and dynamics of hygienic-indicator and pathogenic microorganisms in dams Kardzhali, Dospat, Cankov kamak, Batak, Arda river and wet zones near Maritza river (Zlato pole and Tzalapitsa) is carried out;
- New e composite biosorbents of waste microbial biomasses co-immobilized with activated carbon and bentonite in alginate gel are constructed. The process parameters for purification of waste water contaminated with Pb(II), Cd(II) and Hg(II) by new biosorbents are optimized.

Group of indicators D (Sum of points in indicator 11) - requirement of RIASDARB – 100 points, implementation by the candidate – 202 points.

An important attestate for scientific achievements of researchers is the number of positive citations of their publications. Assoc. Prof. Dr. Kostadinova applies list of 101 positive citations in database of Scopus and Web of science.

I define the contributions reached in the scientific publications of Assoc. Prof. Dr. Kostadinova as scientifically applied and applied with an original character and those that complement, expand and build on existing knowledge.

Assessment of the candidate's educational and pedagogical activity

Group of indicators E (Sum of indicators from 12 to 20) - requirement of RIASDARB – 150 points, implementation by the candidate – 248 points.

Unfortunately, RIASDARB in any way does not evaluate the teaching activities in the aspect of student education, creation of curricula and new study specialties, nevertheless that by definition the academic position "professor" is precisely for a university lecturer. In my desire to compensate for this legal omission, I want to evaluate the first teaching activity of the candidate before taking into account the fulfillment of the legal norm.

The teaching activity of Assoc. Prof. Dr. Kostadinova is impressive in every respect. During her seventeen years of teaching activity, after acquiring the academic position of "Associate

Professor", the candidate has taught an average of over 900 academic hours per year, of which an average of 824 academic hours of lectures, with an annual rate of between 270 and 360 academic hours. Assoc. Prof. Dr. Kostadinova is the author of 13 curricula in the Bachelor's degree (Microbiology, Microbiology and Virology, Bacteriology and Virology, Physiology of Microorganisms, Ecology of Microorganisms, Microbial Metabolism, Bioremediation, Microbiology Diagnostics, Microbiology Diagnostics, Microbiology of sea and freshwater reservoirs, etc.) and 4 curricula in the Master's degree (Microbiology, Microbial biotransformations, Microbiological diagnostics, Microbial metabolism).

Assoc. Prof. Dr. Kostadinova is the supervisor and co-supervisor of three successfully defended PhD students, each of whom in the main areas of research activity of Assoc. Prof. Dr. Kostadinova - microbial enzymes (Yodran Stefanov), microbial pathogenesis (Marinela Tsankova) and ecology of microorganisms (Ivan Iliev).

Assoc. Prof. Dr. Kostadinova has been a participant in 2 national and 1 international research project and leader of 1 national research project. All research projects have been successfully completed. Participations are certified with a relevant official note. Assoc. Prof. Dr. Kostadinova is an independent author of a university textbook on Microbial Metabolism and co-author of a university textbook on Biological Membranes, in the part of microbial membranes, co-author and editor of the Manual for laboratory exercises in Microbiology, published by the Department of Microbiology. PU. I extremely appreciate the textbook on Microbial Metabolism, which is unique in its nature in Bulgaria. The textbook in a synthesized but comprehensive form, with a style combining scientific correctness and clarity, presents all the main and original metabolic pathways characteristic of the microbial world and its importance for the formation of life on Earth in its current form. The presented information is richly illustrated, presented in many figures and tables, which contribute to its easier assimilation. In terms of content, the textbooks and the laboratory manual are at a level that unequivocally shows the high level of theoretical training and the vast teaching experience of Assoc. Prof. Dr. Kostadinova.

The teaching and pedagogical activity of Assoc. Prof. Dr. Kostadinova is at an extremely high level, meeting all national and international standards for a university professor.

To hold the academic position of "Professor", the Faculty of Biology of the University of Plovdiv has introduced additional requirements related to the authorship of a textbook, management of doctoral students, work experience over 10 years, management of a national research project, organizational and academic experience. From all that has already been said, it is more than clear that Assoc. Prof. Dr. Kostadinova definitely covers all the additional requirements of the BF.

Within the framework of this review, I couldn't hardly appreciate the great efforts and contribution of Assoc. Prof. Dr. Kostadinova to maintain and raise the image of BF, but they really deserve admiration.

4. Assessment of the candidate's personal contribution

I believe that at the present stage of development of science, especially in the field of natural sciences, evaluation of the personal merit of an individual author for the realization of a publication or formulated contribution is impossible and unnecessary, because working in a team. In the spirit of the standard review formats I will share that I appreciate the personal contribution of Assoc. Prof. Dr. Kostadinova for the realization of the presented publications as fully relevant to her experience and competence, which are the result of a career of over thirty years in microbiology.

5. Critical notes and recommendations

I have no critical notes on the content of the materials presented in the competition.

6. Personal impressions

My daily professional contacts with Assoc. Prof. Dr. Kostadinova, in the Department of Biochemistry and Microbiology, for more than 15 years, allow me to form an objective opinion about her personal and professional qualities. She is an indisputable authority in the department and the faculty, among the students and the college. Assoc. Prof. Dr. Kostadinova is an extremely efficient administrator, meticulous researcher and dedicated lecturer.

CONCLUSION

The documents and materials presented by Assoc. Prof. Dr. Sonia Kostadinova Trifonova meet all the national and institutional requirements. The candidate has submitted a considerable number of scientific papers published after the defense of the PhD thesis and the appointment to an Associate Professor's academic position. The candidate's works have original scientific and applied contributions that have received international recognition. The scientific and teaching qualification of Assoc. Prof. Dr. Kostadinova, is undoubted.

The results achieved by Assoc. Prof. Dr. Kostadinova, in the teaching and research activities fully comply with the specific requirements of the PU and faculty of Biology for the appointment to a "Professor's" academic position.

After getting acquainted with the materials and scientific output presented in the competition, performing analysis of their importance and the scientific, science applied and applied contributions contained therein, I find it justifiable to give my positive assessment and to recommend to the Sci-

entific Jury to prepare a report with a proposal to the Faculty Council of the Biological Faculty for the appointment of Assoc. Prof. Dr. Sonia Kostadinova Trifonova, to the academic position of "Professor" at the PU in higher education area 4. Natural sciences, mathematics and informatics, professional field 4.3 Biological sciences (Microbiology)

24.10.2021

Reviewer:

(Prof. Dr. V. Gochev)