#### REVIEW

by PhD Penka Angelova Moncheva, professor, Sofia University "St. Kliment Ohridski" – Faculty of Biology on the materials submitted for participation in a competition for the academic position of "Professor" in

Plovdiv University "Paisii Hilendarski" in: Area of higher education 4. Natural Sciences, Mathematics and Informatics,

Professional field 4.3. Biological sciences (Microbiology)

In the competition for "professor", announced in the SG, no 40/14.05.2021 and on the website of Plovdiv University "Paisii Hilendarski" for the needs of the Department of Biochemistry and Microbiology at the Faculty of Biology, Assoc. Prof. Dr. Sonya Kostadinova Trifonova from the same University is the only applicant that has submitted document for this competition.

#### 1.General presentation of the submitted materials

By order № P33-4296/10.08.2021. of the Rector of Plovdiv University "Paisii Hilendarski" (PU) I have been appointed a member of the scientific jury in a competition for the academic position "Professor" in PU in the area of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3. Biological Sciences (Microbiology), announced for the needs of the Department of Biochemistry and Microbiology at the Faculty of Biology.

The presented materials by Assoc. Prof. Dr. Sonya Kostadinova Trifonova, the only candidate for this competition, on paper and electronic carrier is perfectly prepared and are in compliance with the requirements of the Act for the Development of Academic Staff in Republic of Bulgaria as well as with the requirement indicated in art. 77(1) of the Regulations for Development of the Academic Staff of Plovdiv University and includes:set of administrative documents, list of scientific papers, list of citations of scientific papers, documents for scientific and research activity, reference for compliance with minimum national requirements and additional faculty requirements, extended habilitation reference, incl. and self-assessment of the contributions, documentation for the educational and pedagogical activity, documents according to the additional requirements of the BF of PU, declaration of originality and authenticity of the attached documents, scientific works subject to review, evidence of the observed citations of the publications.

Assoc. Prof. Sonya Trifonova has presented a list of a total of 66 scientific papers, of which monographs - 1, textbooks and manuals for laboratory work - 5, scientific publications – 61, and a list of research projects - 11. Thirty seven (37) scientific works are subject to review and evaluation, which are outside the dissertation and participation in the competition for the academic position of "Associate Professor", as well as project development - 11. The distribution of scientific papers related to this competition is as follows:

- $\blacktriangleright$  Monograph 1
- Scientific publications in journals with impact factor / impact rank 18
- Publications in refereed journals without impact factor / impact rank 12
- > Publications in conference proceedings -3
- Textbooks and manual for laboratory work 3

#### 2.Biographical data about the candidate

Assoc. Prof. Sonya Trifonova graduated from Plovdiv University "Paisii Hilendarski", Faculty of Biology in 1988 and was awarded a Master degree in Biology, qualification Biologist, teacher of Biology and with a second specialization in Chemistry. In the period 1990-1992 she was a doctoral student at the same faculty and after successful defense the PhD thesis on "Purification and properties of phospholipase C and lipases from some Gram-negative bacteria" in 1992 was awarded the scientific degree "Candidate of Biological Sciences" (Doctor, Diploma  $N_{2}$  22805 / 06.01.1993).

In the period 1986-1988 Assoc. Prof. Trifonova worked as a teacher in the Secondary School of Food Technology in Asenovgrad. From 1990 to 1992 she was an assistant in the Department of Biochemistry and Microbiology at the University of Plovdiv, then in the period 1992-2003 – Chief Assistant professor. Since 2003 she has been an Associate professor in the same department (Certificate № 22247 / 30.01.2004).

In the process of acquiring higher education and subsequent professional development, Assoc. Prof. Sonya Trifonova is established both as a university lecturer with more than 32 years of experience and as a scientist-researcher in the field of Microbiology.

In addition to teaching and research, Assoc. Prof. Trifonova has significant organizational and administrative activity. In the period 2007-2011 she was the head of the Department of Biochemistry and Microbiology. From 2011 to 2015 she was Deputy Dean of the Faculty of Law, and since 2015 shas been the Dean of the Faculty of Biology at the University of Plovdiv. In the period 2015-2019 she is a member of UKOA in PU, participates in the preparation of documentation and procedures for program accreditation, in institutional accreditation, in the preparation of a self-assessment report for post-accreditation monitoring and control of PU. She is the coordinator of the master's program "Biotechnological Microbiology" / "Industrial Microbiology", participates in the organization and preparation of curricula in three professional fields in the bachelor's degree and "master's" and ESD "doctor" in the BF of PU. The above is proof of her significant academic activity.

# 3. General characteristics of the candidate's activity

# 3.1. Evaluation of the educational and pedagogical activity

After taking the academic position of "Associate Professor", the candidate in the competition - Assoc. Prof. Trifonova, develops an impressive teaching activity. Since 2004 she has conducted lecture courses at the Bachelor's degree programs in Microbiology and Microbiology and Virology, Physiology and Biochemistry of Microorganisms, Microbial Metabolism, Ecology of Microorganisms, Bioremediation. In the first years after the habilitation as an associate professor she also gave practical classes in Physiology and Biochemistry of Microorganisms, Microbiology and Ecology of Microorganisms. Since the academic year 2008/2009, Assoc. Prof. Trifonova has been counducting lecture courses in Microbiology and Ecology of Microorganisms in the branches of the University of Plovdiv in the cities of Smolyan and Kardzhali. For the current school year she is lecturer in Microbiology, Microbiology and Virology, Ecology of Microorganisms and Microbial Metabolism. I would like tot emphasize the extremely high auditorium workload of the candidate. The average annual auditorium workload for the 17-year period (2004-2021) is approximately 587 hours, and for the academic 2020/2021 year it is 632 hours.

Assoc. Prof. Trifonova conducts lectures and exercises in the Master's degree programs. Since 2004 she has conducted lecture courses in Microbial Metabolism - first only for a master' program "Microbial biotechnologies" and later (since 2012) for program "Microbial biotechnologies" food"; since 2005 - in Biotransformations (for master's programs "Microbial biotechnologies"/Biotechnological microbiology/ Industrial Microbiology and Biopharmacological biochemistry); in Microbiological diagnostics (for master programs Medical Microbiology and Biodiagnostics); in Microbiology (for eight master's programs). For some of these disciplines she also gives practical classes. Its average annual auditorium workload in the Master's degree is approximately 276 hours, and for the current school year - 200 hours. Its total auditorium workload in Bachelor and Master degree programs for current year is extremely high - 832 hours.

Apart from being a lecturer in the above-mentioned courses, Assoc. Prof. Sonya Trifonova is the author of programs of a great number of academic disciplines. In the Bachelor's degree she is the author of the programs of 13 courses - Microbiology, Microbiology and Virology, Microbial Metabolism, Microbial Diagnostics, Bacteriology and Virology, Ecology of microorganisms, Bioremediation, Practicum-microbiology and Undergraduate practicum (compulsory); Physiology of microorganisms, Practice-microbiological diagnostics, Soil microbiology and Microbiology of marine and freshwater reservoirs (elective). In the Master's degree she is the author of the programs of 4 compulsory disciplines - Microbial Metabolism, Biotransformations, Microbiological Diagnostics and Microbiology, included in the curricula of various master's programs.

After the habilitation as an "associate professor" 8 graduates and 3 PhD students successfully defended their BSc, MSc and PhD, respectively. The topics of the diploma theses and the dissertations are thematically connected with the main areas in the scientific activity of Assoc. Prof. Trifonova.

The analysis of the educational and pedagogical activity of the candidate Assoc. Prof. Trifonova allows me to conclude that she is an established university lecturer specializing in teaching disciplines covering a wide range of areas of microbiology - bacteriology, virology, metabolism, biotransformations, ecology of microorganisms, soil and water microbiology, microbial diagnostics, which are interrelated. All lecture courses corresponds very well with the main area of her scientific activity. If we trace the teaching activity of Assoc. Prof. Trifonova after her habilitation as an associate professor, it will be seen that it is characterized on the one hand by sustainability, but on the other hand, with development and upgrading, which I evaluate very positively. This wide range of disciplines she teaches shows her erudition, energy and dedication to the profession of university lecturer.

# 3.2. Evaluation of the scientific and scientific-applied activity

As I have indicated in item 1 of the review, for participation in the competition for the academic position of "professor" Assoc. Prof. Trifonova has presented 37 scientific papers that could be thematically classified in accordance with the main scientific area of her research.

Scientific papers related to <u>microbial enzyme studies</u> - Publications: III.1., III.2.2., III.2.10., III.2.16., III.3.2., III.3.3, III.3.5., III.3.9., III.4.2.

The presented monograph (III.1) deals with issues related to phospholipase enzymes produced by bacteria of the genus *Bacillus*. It presents the personal results of Assoc. Prof. Trifonova on the selection of strains-producers of phospholipases C, belonging to 3 species of this genus - *B. cereus*, *B. thuringiensis* and *B. sphaericus*. The

analysis and interpretation of the obtained results is made in a comparative aspect with the scientific achievements in this field. The hypothesis on which this work is based is that the above-mentioned species are excellent producers of several types of phospholipases C, therefore it can be expected that active producers of these enzymes can be selected among them by optimizing the conditions for their production and the procedures for their purification. Although research on this topic has been conducted for several decades, it is still relevant today, given the importance of these enzymes. The monograph contains original and unpublished results. In addition to assoc. prof. Trifonova's own research, the monograph presents in a well-structured, synthesized, summarized and sufficiently in-depth form the world achievements in this scientific field. Five hundred and fifty five (555) literature sources are cited, mostly after 2000, which demonstrates the excellent knowledge of the current state of research in this area. I believe that this work is very useful for researchers working in this field, as well as a source of additional information for graduate and doctoral students and is of high scientific value.

Papers related to studies on <u>virulence factors and microbial pathogenesis</u> - Papers III.1., III.2.1., III.2.3., III.2.4., III.2.5., III.2.6., III.3.3., III.3.4., III.3.7., III.3.10., III.3.11., III.3.12.

Papers related to research in the field of <u>ecology of microorganisms</u> - publications III.2.6., III.2.7., III.2.8., III.2.9., III.2.11., III.2.12., III.2.13., III. 2.14., III.2.15., III.2.17., III.2.18., III.3.1., III.3.6., III.3.8., III.4.3.

Works related to the *educational activity* - works III.5.1., III.5.2. and III.5.3.

Of the 37 scientific papers presented, 6 were published in Bulgarian and the remaining 31 - in English. 18 of the papers (55%) have been published in international scientific journals with impact factor / rank - total IF = 8.008 and total SJR = 1.179. Among the journals I would mention *Engineering in Life Sciences, Journal of Basic Microbiology, Central European Journal of Biology, Biotechnology and Biotechnological Equipment.* Two publications are in journals Q2, 8 - Q3 and 8 - Q4. Twelve of the papers have been published in Bulgarian scientific journals, three - in proceedings of national scientific forums, and the remaining 4 papers are the presented monograph, textbooks and laboratory manual published by the Plovdiv University Press "Paisii Hilendarski". All works of the candidate, except for 2 (monograph and 1 textbook) are collective. Of these, 3 have one co-author, 7 have two co-authors, and the remaining 25 have three or more co-authors.

For participation in this competition, Assoc. Prof. Trifonova presented a list of participations in 11 projects, She has been a coordinator of 4 projects: 1 - a national project funded by the NSF; 1 - funded by the NSF of the University of Plovdiv and two, in connection with the organization and holding of two Balkan Scientific Conferences in Biology, with partial funding from the University of Plovdiv. In addition, it has participated in 7 other scientific projects, as follows: 1 - funded by the BNSF; 3 - financed by the NSF of PU; 1 - financed by the Financial Mechanism of the European Economic Area; 1 - funded by the Operational Program "Science, Education for Smart Growth, co-financed by structural and investment funds and 1 - funded by the National Science Program" Innovative low-toxic biologically active tools for precision medicine ". The realized research projects are thematically related to the scientific field of the research activity of Assoc. Prof. Trifonova.

# 3.3. Contributions (scientific, scientific-applied, applied) and citations

The scientific works of Assoc. Prof. Trifonova contain contributions to the main areas in her research activity. I would like to highlight the most important of them:

# > Contributions in the field of microbial enzymes

- As a result of extensive screening, producers of type C phospholipases have been selected from strains of *B. cereus*, *B. thuringiensis* and *B. sphaericus*. Representatives of the species *B. cereus* have the widest range of biosynthetic potential, synthesizing all three types of phospholipases type C, followed by strains of the species *B. thuringiensis* and *B. sphaericus*. These results confirm the existing data that the first two species are among the best producers of phospholipases C. For the first time the production of phosphatidylinositol-specific phospholipase C of the species *B. shaericus* has been reported, which expands the existing scientific information (monograph) III.1.). I define the above as a scientific and applied research contribution, because in addition to providing new and complementary scientific information, the results could be used in further research, in a view of the practical use of the biosynthetic potential of these strains for various purposes.
- 2. As a result of optimization of the conditions of cultivation of strains-producers of phospholipases type C, a significant increase in the activity of the enzymes synthesized by them was achieved 54 -58%. This is an applied research contribution and is an important stage in the development of schemes for practical application of these enzymes (in bioremediation processes, in industrial biosynthetic processes, in biodegradation processes of waste from various industries) (papers III.1. and III.2.16.)
- 3. For the isolation and purification of type C phospholipases frm *B. cereus* and *B. thuringiensis* effective schemes have been developed which could be successfully applied to other producers of such enzymes (paper III.1). The main characteristics of the purified enzymes have been determined. This contribution has a scientific and applied research value and is essential in the next stages of research related to the production and application of these enzymes.
- 4. From a scientific and applied point of view an important information for the synthesis of various enzymes from Gram-positive (alkaline phosphatase, alkaline and neutral proteases, amylolytic activity, papers III.2.2, III.2.10, III.3.9) and Gram-negative bacteria alkaline phosphatase, lipase and phospholipase C activity, works III.2 and III.3.5.) was obtained.

#### > Contributions in the field of microbial pathogenesis

- 5. Given the importance of type C phospholipases as factors of virulence and pathogenicity in some pathogenic and opportunistic bacteria, the results obtained, summarized and commented in the presented monograph are a scientific contribution and confirm the role of these enzymes in the mechanisms of microbial pathogenesis (paper III. 1).
- 6. A rich collection of identified bacteria and yeasts isolated from patients with urogenital tract infections has been created. Their virulent determinants have been determined and drug resistance to medicinal preparations used in practice has been established. Information on the influence of various agents on the formation of biofilms in some of the isolated strains has obtained, the antimicrobial activity of alternative therapeutic agents against some of the isolates has been studied. This is a scientific and applied research contribution, as an important database has been obtained that could be used in comparative studies, as well as in the selection of

appropriate therapy and in the development of new approaches in the treatment of these infections (papers III.3.10, III .3.7, III 2.1, III.2.3, III.2.4, III.3.4, III.3.11, III.3.12, III.2.5).

# > Contributions in the field of microbial ecology

- 7. The microbiological status of two large and economically significant water reservoirs Dospat and Kardzhali dams (publications III.2.7, III.3.6 and III.3.8) has been determined in view of the assessment of its sanitary and hygienic quality, as well as that of the waters used for aquaculture. Given the importance of these dams for fish farming, as well as the increased urbanization of their coastal areas, the results fill the lack of information in Bulgaria on the impact of aqua farms on the microbiological status of the hydroecosystem of water bodies in which they are located. These studies represent a scientific and applied research contribution, as they provide scientific information on the current state of the waters in the two dams, which will be used as a basis for comparison in further microbiological monitoring.
- 8. Scientific information is provided on the relationship between abiotic environmental factors, microbiological indicators of water quality and phytoplankton, with a view to the development of intensive cell aquaculture (paper III.2.14). Based on the applied modern statistical methods of processing the results it is proved that in dams with developed intensive cell aquaculture, long-term operation can lead to local changes in the physico-chemical quality of water, which in turn can lead to quantitative and qualitative changes in phytoplankton and bacterial communities. These results are important both for the institutions controlling the water quality in the dams and for the aquaculture producers.
- 9. Contribution to the study of microbial diversity in freshwater environments are the results for the taxonomic status of the microbial community of two large dams in Bulgaria Batak and Tsankov Kamak by a modern molecular genetic method metagenomic analysis (paper III. 2.8). This contribution is of scientific value and expands existing data on microbial diversity in freshwater environments.
- 10. A contribution to the study of microbial diversity is the characterization of microbial communities in wetlands in southern Bulgaria (paper III.2.11.) and in the Maritsa River basin (paper III.2.18.). For the first time, data on bacterial diversity in two different wetlands in the Maritsa River basin are provided by applying a modern molecular genetic method massive parallel sequencing (paper 2.15). The contribution has scientific value.
- 11. A contribution of practical importance is the construction of a new composite biosorbent for removal of heavy metals from aqueous solutions based on waste biomass from *Bacillus cereus* immobilized with activated carbon in alginate beads (paper III. 2.13), as well as the result of the study of the possibilities for use of waste biomass from *B. thuringiensis* as a biosorbent for heavy metals (work III.2.17).

# > Contributions to academic education

- 12. Contributions to academic education are the presented textbooks and manual, which provide access to students of Bachelor's and Master's degrees to modern knowledge in specific disciplines, which is one of the conditions for a good university education.
  - The textbook "Microbial Metabolism" (scientific work III.5.1.) is intended for students of bachelor's and master's degree programs. The content of this textbook is consistent with the curriculum of this discipline. The textbook is very well illustrated. It is written in good scientific language, understandable, without unnecessary volume, which makes it easy to read and

understand. I believe that the writing and publishing of this textbook is an important contribution to the successful and responsible training of the students in this field.

- The Manual in Microbiology (paper III.5.2.), Co-authored by Assoc. Prof. Trifonova is constructed thematically in accordance with the curriculum of the course of Microbiology for different specialties in the bachelor's and master's degree of University of Plovdiv. The exercises are grouped thematically, which allows to make the necessary set of topics corresponding to the needs of the specialty. The manual provides knowledge and skills for both the basic classical techniques of microbiological laboratory work and basic modern molecular genetic methods, which are widely used in microbiological research. This guide provides an opportunity for modern training of students in the discipline of Microbiology.
- The textbook "Biological Membranes" presents and summarizes in three sections the current knowledge about the structure and functions of biological membranes biochemical composition, structure of biological membranes and the features of membrane components; the main functions of the membranes; specific membranes in the organismic world. The textbook is intended for students studying the discipline "Biological Membranes". Given the role of biological membranes, in addition to being a textbook, this scientific work can be used by a wide range of students from different specialties, teachers, PhD students and others as a source of additional knowledge, in addition to those included in more general biological disciplines.

The publications of Assoc. Prof. Trifonova receive recognition from the international scientific community through the received citations. A list of citations in scientific journals, monographs, collective volumes, referenced and indexed in world-famous databases of scientific information (Web of Science and Scopus) is presented. The list contains 101 citation, all of which are by foreign authors. 19 of the papers covering research from the three main scientific fields of the candidate were cited. No negative citations were observed. The papers III.2.12. and III.2.13 presenting results from the development of a heavy metal biosorbent have the highest number of citations so far (20 and 14 citations, respectively), as well as the papers III.2.8., III.2.4. and III.2.1. (12, 9 and 8 citations, respectively), related to studies of bacteria isolated from the urinary tract and the study of microbial diversity in two Bulgarian dams. According to Scopus, the h-index is 7. Most of the citations of the scientific works of Assoc. Prof. Trifonova are in scientific journals with a high impact rank (Current Pharmaceutical Biotechnology, Food control, Environmental Microbiology, Aquatic Science, Carbohydrate polymers, Bioresource technology, Current Pollution reports, Journal of water process Engineering, Journal of Cleaner production, Ecotoxicology and Environmental Safety, Journal of Agricultural and Food Chemistry, desalination and water Treatment Chemosphere, Scientific Reports, Industrial Crops and products, Frontiers in Microbiology, Science of the Total Environment, Annals of the American Thoracic Society BMC Microbiology, all in Quartile: Q1).

Based on the analysis of the research activity of the candidate in the competition I would conclude that Assoc. Prof. Trifonova has presented scientific papers of scientific and scientific-applied significance, reflecting the results of her research in three main scientific areas, as well as those with contributions to academic education. Her scientific works is recognized by the international scientific community. On the basis of the submitted scientific papers and their evaluation, I strongly believe that the candidate meets the minimum national requirements for scientific and teaching activities for the academic position of "professor", defined in accordance with Act for the Development of Academic Staff in Republic of Bulgaria, as follows:

Group of indicators	Contents	Requirements for the academic position "professor"	Fulfillment by the candidate
А	Indicator 1	50	50
В	Indicator 2	100	0
С	Indicator 3 or 4	100	100
D	Sum of indicators from 5 to 10	200	256
Е	Sum of points in indicator 11	150	202
F	Sum of indicators from 12 to the end		248
Total		600	856

The candidate fully meets the additional requirements of the Faculty of Biology for the academic position of "professor", adopted by Decision of the FC, protocol №246 / 12.07.2019. Her activities, according to the cited document are discussed above in the review.

# 4. Assessment of the personal contribution of the candidate

The scientific works presented for review in the predominant part (excluding the monographic work and one of the textbooks) are collective works. The authors of these publications are researchers with different specific competence, which allows the successful complex research, such as the presented scientific works. In most of the publications doctoral students participate, whose research supervisor is Assoc. Prof. Trifonova. I believe that the candidate's personal contribution to the presented scientific publications is obvious, not only as a direct participant, but also as a generator of ideas and leader of the research team.

# 5. Critical remarks and recommendations

I have no critical remarks and recommendations to the materials and documentation submitted by the candidate for participation in the competition, as well as to his overall academic activity.

## 6. Personal impressions

My personal impressions of Assoc. Prof. Trifonova are based on our joint participation in scientific juries for the defense of doctoral dissertations at the Faculty of Biology at Sofia University and at the University of Plovdiv. My impressions are that she is an organized, accurate and ethical university lecturer and colleague, responsible for all aspects of her academic activity, which was fully confirmed after reviewing the materials presented for participation in the competition for the academic position "professor".

# CONCLUSION

The documents and materials submitted by Assoc. Prof. Dr. Sonya Kostadinova Trifonova for participation in the competition for the academic position of "professor" meet all the requirements of the Act on the Development of Academic Staff in the Republic of Bulgaria, Regulation for its implementation, Regulations for development of the academic staff of University of Plovdiv "Paisii Hilendarski". The candidate in the competition has submitted a sufficient number of scientific papers published after the materials used in the defense of PhD thesis and after holding the academic position of "Associate Professor". The candidate's scientific works contain original scientific, applied research and applied contributions and are recognized by the international scientific community through citations, mostly in international scientific journals with a high impact rank. The teaching activity of Assoc. Prof. Trifonova is impressively diverse in qualitative and quantitative terms. The textbooks and laboratory manual, of which she is the author / co-author, are a contribution to the academic education at the University of Plovdiv, which I highly appreciate. I believe that the scientific and teaching qualification of Assoc. Prof. Trifonova is undoubted.

The results achieved by Assoc. Prof. Trifonova in her overall academic activity - teaching, research, administrative and organizational activity, comply with the minimum national requirements and the additional requirements of the Faculty of Biology, adopted in connection with the Regulations of the University of Plovdiv for implementation of Act on the Development of Academic Staff in the Republic of Bulgaria.

The review and evaluation of the materials and scientific papers presented in the competition, the analysis of their significance and of the scientific, applied research and applied contributions allow me to confirm my positive assessment, presented above and recommend to the Scientific Jury to prepare a report-proposal to the Faculty Council of the Faculty of Biology for the election of Assoc. Prof. Dr. Sonya Kostadinova Trifonova to the academic position "Professor" at the University of Plovdiv "Paisii Hilendarski" in: Area of higher education 4. Natural Sciences, Mathematics and Informatics, Professional field 4.3. Biological sciences (Microbiology).

29.10. 2021 Sofia Reviewer: .....

(prof. PhD Penka Moncheva)