

## Review

by Prof. Iskra Vitanova Ivanova,

Member of the NJ according to an order of the Rector of the University of Plovdiv "Paisii Hilendarski" № P33-42968 / 08.2021

concerning:

participation in a competition for the academic position of "professor" in the field of higher education 4. Natural sciences, mathematics and informatics, professional field 4.3 Biological sciences (Microbiology).

Only one candidate participates in the competition for "Professor":

Assoc. Prof. Dr. Sonya Kostadinova Trifonova

### **Brief biographical reference and assessment of the publishing activity**

Associate Professor Dr. Sonya Kostadinova Trifonova was born on October 4, 1962. She graduated with honors from the University of Plovdiv (PA) "Paisii Hilendarski" (Bachelor and Master) in 1988. Since 2003 she has been elected associate professor in the department. in Biochemistry and Microbiology. The candidate's work experience is 35 years, including from 2007 to 2011 he was the head of the Department of Biochemistry and Microbiology, from 2011 to 2015 she was the Deputy Dean of the Faculty of Law of the University of Plovdiv, and from 2015 she is currently the Dean of the Faculty of Biology at the Paisii Hilendarski University of Plovdiv.

### **General description of the submitted materials in the competition.**

The materials presented by the sole candidate Assoc. Prof. Dr. Sonya Kostadinova Trifonova fully cover the requirements of the competition and present the specific evidence regarding the required criteria for the competition, as well as present the overall production of the candidate, both through lists of publications and citations and through the text. of the submitted contributions, CV, etc. The presented documentation is extremely well arranged and very detailed, including digital copies of the publications related to the participation in this competition, as well as their summaries in Bulgarian and English.

For participation in the competition for the academic position "Professor" 36 scientific publications were presented, which were not used for the acquisition of ONS "Doctor" and the

academic positions "Chief Assistant" and "Associate Professor". The scientific papers are distributed as follows:

- 18 publications in publications with impact factor or impact rank, which are referenced and indexed in the databases with scientific information - Web of Science and Scopus;
- 12 publications in refereed editions without impact factor or impact rank;
- 3 publications in proceedings of scientific conferences with scientific review;
- 2 textbooks (1 independent and 1 - co-authored);
- 1 guide (co-authored).

The scientific works of Assoc. Prof. Dr. Sonya Kostadinova can be distributed in accordance with the criteria for the minimum national requirements of the Law on the Republic of Bulgaria and the Regulations to it, as well as with the additional requirements of the Bulgarian Academy of Sciences, as follows:

1. Criterion "A" - an abstract of a dissertation for awarding the educational and scientific degree "Doctor" - (50 points);

2. Criterion "B" - a monograph is presented, which is not used for obtaining the educational and scientific degree "Doctor", and for holding the academic position "Associate Professor" (100 points)

3. Criterion "D" includes 18 publications in publications that are referenced and indexed in world-famous databases of scientific information (Web of Science and Scopus), which are in categories Q2 - Q4 (256 points).

4. Criteria "E" includes 101 citations in scientific journals, monographs, collective volumes and patents, referenced and indexed in world-famous databases of scientific information (Web of Science and Scopus) (202 points). According to the additional requirements of BF, Assoc. Prof. Sonya Kostadinova is the head of three successfully defended doctoral students (total points 125), project manager, participation in two national projects and participation in international (total points 60). The funds raised from projects amount to BGN 70,000 (14 points) and for the publication of a textbook or textbook, which are used in the school network (49 points). Assoc. Prof. Kostadinova significantly exceeds the minimum requirements.

## **Overview of the scientific and scientific-applied contributions of the candidate**

The contributions of the submitted materials for participation in the competition for the academic position of "professor" in the scientific specialty Microbiology can be grouped in several areas:

- **Contributions to the analysis of microbial enzymes:**

The monograph "Phospholipases C produced by *Bacillus* species" presents its own results and summaries on the production and purification of phospholipases C from *Bacillus cereus*, *B. thuringiensis* and *B. sphaericus* species, comparing them with scientific achievements in this field. The monograph is presented on 144 standard pages of text and 354 cited literature sources. I can confidently say that the monograph is an original scientific development, with the potential for scientific achievements that have a rapid practical realization. The proposed monograph is written in scientific language, brings new contributions and ideas, logically and consistently presented, is understandable for professionals and those working in this field, as well as for a wider range of biologists.

The important contributions are as follows:

**(Publication III. 1).**

*B. cereus* strains synthesize all three types of phospholipases type C - phosphatidylcholine-specific (PC-PLC), phosphatidylinositol-specific (PI-PLC) and sphingomyelinase C (SMase C). A highly efficient scheme for purification of phospholipase C strain B has been developed. *B. cereus* No. 51. The purified phospholipase C has a molecular weight of 23-26 kDa, determined by gel filtration and SDS-PAGE electrophoresis, respectively. The enzyme hydrolyzes phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine.

*B. cereus* strains synthesized in addition to phosphatidylcholine-specific and phosphatidylinositol-specific phospholipase C, but there is no correlation in the production of the two enzymes. Strain *B. cereus* No. 93 was selected as a producer of the enzyme, which is secreted into the medium at the end of the exponential growth phase and purified by a four-step scheme including ultrafiltration, isopropanol precipitation, ion exchange chromatography and gel filtration.

Sphingomyelinase C production was detected only in *B. cereus* species. There is a correlation between the phospholipase and sphingomyelinase activity of the cultures. Strain *B. cereus* No. 79 was selected as a producer of SMase C. Sphingomyelinase C was purified in 31%

yield by a combination of ultrafiltration and chromatography of DEAE-cellulose and Sephadex G-75, respectively.

*Bacillus thuringiensis* strains exhibit phosphatidylcholine-hydrolyzing and phosphatidylinositol-specific phospholipase C activity. Eighty-six percent of the analyzed cultures of this species produce phosphatidylcholine-specific phospholipase C, which in strain *B. thuringiensis* var. *Thuringiensis* 17. The optimization of the composition of the medium by varying the source of nitrogen, the addition of metal ions and sugars increases the initial activity by 58%. PC-PLC from strain *B. thuringiensis* 17 was purified by a combination of ultrafiltration, gel filtration and FPLC (HiPrep DEAE) chromatography. A homogeneous enzyme preparation with a specific activity of 190 U / mg was obtained.

The gene encoding phospholipase C in strain *B. thuringiensis* var. *thuringiensis* 17 was isolated and sequenced. The highest degree of homology was demonstrated with the *B. cereus* gene encoding PC-PLC.

The production of phosphatidylinositol-specific phospholipase C in *B. sphaericus* strains has been demonstrated for the first time. The approximate molecular weight determined by gel filtration is  $34 \pm 1.5$  kDa. The optimal pH value for the action of the enzyme, which shows high specificity with respect to phosphatidylinositol, is 7.2-7.5.

Studies and the results obtained confirm that the species *B. cereus* and *B. thuringiensis* are among the best bacterial producers of phospholipase C. From strains of *Bacillus cereus* can be isolated all three types of phospholipases C - PC-PLC, PI-PLC and SMase C. and provide good prospects for their use as enzyme producers.

Detailed information on the optimization of the culture conditions for the synthesis of phospholipase C in strain *B. thuringiensis* 17 is presented, commenting on the possibilities for application of the enzyme in the processes of bioremediation (**Publication III. 2.16.**).

The activity of the enzyme alkaline phosphatase in *Bacillus cereus* has been studied and the production of extracellular (AP I) and membrane-bound (AP II) alkaline phosphatase has been established (**Publication III. 2.2.**).

The activity of the enzyme alkaline phosphatase was also analyzed in strains isolated from soil and identified as *E. coli*, based on biochemical, morphological and cultural characteristics. All strains produce membrane-bound alkaline phosphatase and  $\beta$ -galactosidase (**Publication III. 3.2.**).

In the analysis of the proteolytic activity of 166 strains of the genus *Bacillus*, activity was proved in 90% of them. The activity varies widely, but in five strains high values were found, in the range of 8-9 U / ml. Partial purification of the enzyme was performed by ultrafiltration and Sephadex G-75 chromatography (**Publication II. 2.10.**).

Strains of the genus *Bacillus* were also analyzed for extracellular amylolytic activity, which was found in 31% of the cultures - 61% of the studied *B. cereus*, 31% *B. thuringiensis*, 3% *B. sphaericus* (**Publication III. 3.9.**).

Positive lipase and phospholipase C activity of *P. fluorescens*, *P. putida* and *Pseudomonas* sp. was detected. (**Publication III. 3.5.**).

- **Contributions in the field of microbial pathogenesis, which are related to the identification of microorganisms associated with infections of the urogenital tract (enterobacteria, Enterococcus, Candida), determination of virulence and pathogenicity factors in microorganisms and elucidation of the mechanisms of microbial pathogenesis.**

318 strains of enterobacteria associated with urogenital tract infections were studied and it was confirmed that the most common etiological agent was *Escherichia coli* (64.8%), followed by *Klebsiella* spp. (17%) and *Proteus mirabilis* (10.37%) (**Publication III. 3.10.**).

Phenotypically and multiplexed PCR virulent determinants of twenty strains of *Escherichia coli* isolated from the urine of patients with various urinary tract infections, asymptomatic bacteriuria, and pregnant women showed that the strains expressed different factors of virulence, such as the statistically most found structure is the type pili proved in 75% of isolates (**Publication III. 3.7.**).

As part of the topic for proving the virulent determinants of strains associated with urinary tract infections, a biofilm-forming capacity of 50 strains of *E. coli* isolated from patients with various symptoms of urinary tract infections - cystitis, pyelonephritis, prostatitis was analyzed (**Publication III. 2.3**

The effect of extracts of medically important plants (*Rhodiola rosea*, *Arnica montana*, *Petasites albus*, *Petasites hybridus*) on the biofilm-forming capacity of three clinical isolates of *E. coli* has been shown (**Publication III. 2.4.**).

The results show that urinary tract infections caused by enterococci are more common in children up to 10 years of age, while genital tract infections (GTI) are most common in women of reproductive age. **(Publication III. 3.11.)**

A study on the taxonomy, resistance and some virulence factors of 97 *Candida* strains isolated from outpatient samples showed that antifungal resistance was low and mainly related to *C. glabrata*, and the overall susceptibility of the strains to the tested antifungal drugs was over 95 % **(Publication III. 3.12.)**

The newly synthesized benzimidazole derivatives analyzed for antimicrobial activity against seven strains of microorganisms showed antimicrobial activity (bactericidal, bacteriostatic and antimycotic) against *Staphylococcus aureus*, *Enterobacter aerogenes* and *Candida albicans* **(Publication III. 3.4)**.

- **Contributions to Contributions in the field of ecology of microorganisms:**

Contributions in the field of microbial ecology can be identified in several different ways. One of them is the determination of the microbiological condition of two large dams - Dospat **(Publication III. 2.7.)** And Kardzhali Dam **(Publication III. 3.8. And Publication III. 3.6.)**. The total coliforms (TC) and coli-titer were determined at two stations in the water area of the dam and one station in the Arda River in August 2011. The research in this area was continued by monitoring the seasonal dynamics of the main indicator groups (*Esherichia coli*, fecal streptococci (FS and *Clostridium perfringens*) in the waters of Kardzhali Dam in the period April - March 2011/12, as the established species composition of the group of coliforms - gives reason to assume that microbiological contamination is mainly due to increased environmental pressure caused by human activity in the area.

In the Kardzhali dam, studies have been conducted to establish the relationship between abiotic environmental factors, microbiological indicators of water quality and phytoplankton, with a view to the development of intensive cage aquaculture. Multifactor analyzes were performed based on data for eighteen indicators from 5 monitoring points obtained in the period 2016-2018. The conducted multifactor analysis (RDA) clearly shows that in dams with developed intensive cage aquaculture the long-term operation of farms is associated with local changes (limited near farms) in the physico-chemical quality of water, which lead to quantitative and qualitative changes in phytoplankton. and bacterial communities **(Publication III. 2.14.)**.

The analysis of the waters of Dospat Dam aims to establish the microbiological condition of the water body and possible secondary sources of pollution in the water area have been identified. The high values of the number of indicators are related to the discharge of fecal and domestic wastewater. Their secondary increase in the cage farm indicates significant anthropogenic pressure in the area (**Publication III. 2.7.**).

Microorganisms inhabiting the freshwater environment are an integral part of the aquatic ecosystem. The first comprehensive metagenomic analysis of the planktonic bacterial community of two large and economically important for Bulgaria dams - Batak Dam and Tsankov Kamak Dam - is presented. The analysis showed that 78.45% of the microbiome between the two dams overlapped. The data obtained may contribute to a better understanding of microbial diversity in freshwater environments and serve as a basis for future comparisons (**Publication III. 2.8.**).

Another area in the field of microbial ecology is the characteristics of microbial communities in wetlands in southern Bulgaria and in the basin of the Maritsa River. Studies of interspecific communities and their ability to develop metabolic networks and biofilms may be useful in the processes of bioremediation of contaminated habitats (**Publication III. 2.11. and Publication III. 2.18.**).

The condition of the microbial communities and their ability to form a biofilm in two wetlands protected by Natura 2000 - Zlato Pole and Tsalapitsa rice fields in Southern Bulgaria were analyzed. The number of heterotrophic bacteria (TVC 22 and TVC 37), actinomycetes, fungi, and sanitary-indicative microorganisms in dry soil samples and sediments were determined. The cluster analysis showed great similarity between the soils, around the rice fields and the Gold Field sediments (ZP2) due to the low organic load. Biofilm formation analysis showed a good correlation between the structure of microbial communities and biofilm formation capacity (**Publication III. 2.11.**).

The bacterial microbiome in the natural wetland of Zlato Pole and protected, periodically flooded rice fields in the Maritsa River basin were studied by mass parallel sequencing (NGS). Bacterial consortia in rice fields are dominated by proteobacteria, followed by actinobacteria and acidobacteria (**Publication III. 2.15.**).

The various techniques and carriers for immobilization are analyzed, the main characteristics and possibilities for the use of immobilized microbial biosorbents for removal and concentration of metals from aqueous solutions are determined (**Publication III. 2.12.**).

The results of the attempts to construct a new composite biosorbent for the removal of heavy metals from aqueous solutions are presented. For this purpose, waste biomass from *Bacillus cereus* was immobilized in sodium alginate and co-immobilized with activated carbon or bentonite in alginate gel. The composite biosorbent was tested to remove Pb (II), Cd (II) and Hg (II) from aqueous solutions. The most promising sorbent, consisting of waste biomass of *B. cereus*, immobilized with activated carbon in alginate beads. Immobilization increases both the removal capacity and the mechanical stability of the biosorbent (**Publication III. 2.13.**).

The possibility of using waste biomass from *Bacillus thuringiensis* as a biosorbent for Pb (II), Cd (II) and Hg (II) from model aqueous solutions was also investigated. The studied biosorbent is promising because it successfully removes heavy metals from solutions containing more than one type of ion, which means that it is effective under model conditions closer to real wastewater (**Publication III. 2.17.**).

Contributions of ecological but also practical significance could include the results concerning the effect of new edible coatings based on low molecular weight chitosan (**Publication III. 2.9.**).

Summarizing the contributions made with confidence, I emphasize that some of them are original in nature and others confirming and expanding knowledge in the field of microbial enzymes, ecology of microorganisms and in the field of microbial pathogenesis, which are associated with the identification of microorganisms associated with urinary tract infections. - genital tract.

### **Learning activity**

Assoc. Prof. Sonya Kostadinova Trifonova gives the lecture courses in Microbiology for all for all specialties in the Faculty of Philosophy of the University of Plovdiv and in the master's programs for non-biologists. In "ACS" Master Assoc. Prof. Kostadinova teaches the following courses: microbial metabolism; biotransformation; microbiological diagnostics.

Assoc. Prof. Sonya Kostadinova is the author of the textbook "Microbial Metabolism", University Publishing House "Paisii Hilendarski", 2021, 287 pages, ISBN 978-619-202-641-7. The textbook on Microbial Metabolism is intended for students with a bachelor's and master's degree in the specialties at the Faculty of Biology of Plovdiv University "Paisii Hilendarski".



The presented textbook "Metabolism of prokaryotes" is a unified synthesis of many years of experience and erudition. The textbook includes 20 chapters on the metabolism of microorganisms and its regulation, namely chemoorganotrophic metabolism, metabolic transformations, biochemical energy, carbohydrate fermentation. The catabolic and anabolic processes in microorganisms are presented, with an emphasis on prokaryotes, which are characterized by a large metabolic diversity, enabling them to assimilate a variety of substrates and develop in all possible habitats on Earth. The unique metabolism of specific groups of microorganisms, such as methanogenic archaeobacteria, sulfate-reducing bacteria, halobacteria, phototrophic prokaryotes, which occupy specific ecological niches and are of great importance in physiological processes in the biosphere, is considered. This textbook corresponds to its purpose - to promote the knowledge and education of the young generation of scientists and for this reason I believe that Assoc. Prof. Sonya Kostadinova has fulfilled her teaching duty.

The candidate is a co-author and in management with a team Kostadinova S., V. Gochev, M. Markhova, T. Girova, D. Georgiev, I. Iliev. - Manual of Microbiology, 2017, Paisii Hilendarski University Publishing House, 265 pages, ISBN 978-619-202-240-2. The exercises in the manual are grouped thematically, which allows the teachers to compile the necessary set for the respective course. The manual includes 10 main sections - "Microscopic techniques", "Basic laboratory culture methods", "Morphology of microorganisms", "Biochemical activity of microorganisms", "Effect of environmental factors on microorganisms". Exercises in the field of "Sanitary Microbiology", "Role of microorganisms in the cycle of substances", "Microbial genetics" and "Medical microbiology" are also included in the manual.

Assoc. Prof. Kostadinova is also a co-author of the textbook "Biological Membranes": I. Denev, St. Spasieva, D. Stefanova, E. Daskalova, M. Gevezova, M. Markhova, S. Kostadinova. - Biological Membranes, 2016, Electronic Edition, Paisii Hilendarski University Press, 181 pages, ISBN 978-619-202-111-5. The textbook presents modern knowledge about the structure and functions of biological membranes, based on scientific discoveries in recent decades.

### **Critical remarks and recommendations**

I have no critical remarks to the materials presented by Assoc. Prof. Dr. Sonya Kostadinova Trifonova!

## **Personal impressions**

I have known Assoc. Prof. Kostadinova personally for many years. She is an impressive person with many talents and teaching skills. A public figure and figure in science and education, Assoc. Prof. Kostadinova contributes to the high level of knowledge transfer not only to students, but also serves the cause of the prosperity of the Faculty of Biology at Plovdiv University. I am personally fascinated by Assoc. Prof. Kostadinova, a modest woman, but at the same time a scientist and disseminator of knowledge in the field of microbiology.

## **Conclusion**

The documents and materials presented by Assoc. Prof. Dr. Sonya Kostadinova Trifonova meet all the requirements of ZRASRB, the Regulations for implementation of ZRASRB and the additional requirements of the Faculty of Biology at Plovdiv University "Paisii Hilendarski". The achieved scientific and scientific-applied contributions are at a high professional level, which is confirmed by the list of publications with her participation in journals in the international databases Scopus and WoS and citation of the results.

The educational activity is represented by: defended doctoral students, long-term teaching activity, authorship of textbooks corresponding to the modern science achievements, as well as participation in projects.

The candidate is an established scientist in the field of microbiology, has the ability to focus on current issues in line with modern science, to get to the heart of them and to present innovative ideas

Based on everything noted so far, I strongly recommend to the members of the esteemed scientific jury, formed by a decision of the Faculty of Biology, protocol № P33-42968 / 08.2021 to propose to the Faculty to award Assoc. Prof. Dr. Sonya Kostadinova Trifonova to the academic position "Professor" in professional field 4. Natural sciences, mathematics and informatics, professional field 4.3. Biological sciences, scientific specialty Microbiology.

Sofia, October 29, 2021

Reviewer: .....

/ Prof. Iskra Ivanova /

