

**OPINION**  
**on holding an academic position "ASSOCIATE PROFESSOR"**

presented to a scientific jury formed by order № 30 / 29.03.21 of the Director of the Institute of Microbiology "Stephan Angeloff" at BAS

**Subject:** Announced competition: for associate professor in professional field 4.3. Biological Sciences (Microbiology), announced for the needs of the Laboratory of Cellular Microbiology, Department of General Microbiology, Institute of Microbiology, BAS.

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An application for participation in the announced competition has been submitted by a single candidate - Ch. Assistant Professor Dr. Tsvetelina Paunova-Krasteva from the Department of General Microbiology, Institute of Microbiology, BAS.

All documents for the competition are prepared precisely and accurately and are presented according to the requirements.

**1. GENERAL INFORMATION ABOUT THE CANDIDATE'S PROFESSIONAL CAREER**

The candidate graduated with a bachelor's degree in biology from the Faculty of Biology at Sofia University in 1999 and a master's degree in microbiology and microbiological control from the Department of General and Industrial Microbiology at the Faculty of Biology at the same University in 2005.

In 2005 she started working as a biologist at the Stefan Angelov Institute of Microbiology, BAS and in 2011 she was elected as an assistant. She defended his doctoral thesis in 2015 and since 2016 she has been a senior assistant. Since 2020 and currently she is the head of the laboratory "Cell Microbiology" at the Institute of Microbiology "Stephan Angeloff", BAS.

In the process of her working Tsvetelina Paunova has realized 11 specializations in leading training institutions. In the course of her education and professional development, Chief Assistant Dr. Tsvetelina Paunova has acquired professional experience and skills in two main areas: research and teaching.

The professional realization of the candidate so far is entirely related to the topic of the competition and reflects current and promising areas of general microbiology.

Ch. as. Dr. Tsvetelina Paunova has been a member of the research team of the Institute of Microbiology since 2005 and has developed as a scientist entirely in it where she actively participates in his scientific life.

She is a member of the Union of Scientists in Bulgaria, was secretary and member of the organizing committees of five scientific forums in the field of microbiology, such as the 10th Balkan Congress of Microbiology, the 14th Congress of Microbiologists in Bulgaria and others. She has received awards for high achievements for the most successful project funded by the "Program for Support of Young Scientists and Doctoral Students at BAS - 2017", "High Scientific Achievements in Dissertations for 2015", Union of Scientists in Bulgaria, best work of young

Bulgarian microbiologist in 2014 - Stephan Angeloff Foundation, grants under FEMS, Erasmus +, One-year grant from the World Federation of Scientists and others .

## **2.ANALYSIS OF THE MATERIALS SUBMITTED FOR PARTICIPATION IN THE COMPETITION**

The analysis of the materials submitted for participation in the competition is in accordance with the requirements of the Law on the Development of the Academic Staff of the Republic of Bulgaria as well as in accordance with additional requirements of the Institute of Microbiology, BAS.

The analysis of the references submitted by the candidate and the evidence to them shows that she has a total number of scientific papers 24 as follows:

articles in journals with IF - 16;

articles in journals without IF - 3;

chapters in monographs and books - 5

The total IF of the publications is 22.091.

A reference for the citations of the candidate's publications -111

**Conclusion under item 2.1:** The candidate fully meets the criteria of Law on the Development of the Academic Staff of the Republic Bulgaria and the Regulations for its application for the academic position of Associate Professor and significantly exceeds its requirements.

According to the additional requirements of Institute of microbiology for academic position of Associate Professor (20 papers, 100 citations, IF-20, H index -5, the data of the candidate are as follow: papers -24, citations – 111, IF- 22.09, H-index – 6. The candidate has participated in scientific forums as follows: 16 participations in international and national forums with oral presentations and 46 poster participations.

A detailed reference for participation in research projects is presented: head of national projects - 3; manager of international projects - 2; participation in national projects - 10; participation in international projects -4.

**Conclusion under item 2.2:** Chief Assistant Dr. Tsvetelina Paunova meets all the additional requirements of the rules of Institute of Microbiology, BAS for the academic position of Associate Professor.

## **3. BRIEF CHARACTERISTICS OF THE RESEARCH WORK OF THE CANDIDATE**

The research activity of the candidate can be summarized in several main topics:

**1. Morphology of microorganisms and electron microscopy** (papers 1, 3, 4, 5, 6, 7, 10, 11, 12, 13, 14, 15, 16, 17, 19, 20, 23, appendix №14).

With such research started the professional career of the candidate as a biologist in the department with a similar title at the Institute. She trains and conducts research on ultrastructure, cytochemistry and morphological changes of viruses, bacteria and eukaryotes. She developed a specialist in the field of microscopic techniques, morphology and application of various methods for processing samples for transmission electron microscopy (TEM) and as a specialist in fluorescent techniques with significant methodological contributions related to selection of suitable fluorochromes for marking, preparation of bacterial and biofilm samples for fluorescence microscopy, epifluorescence and confocal laser scanning microscopy.

**2. Bacterial biofilms - development, structural and functional characteristics, inhibition** (papers 1, 2, 3, 4, 5, 6, 7, appendix №14)

One of the first works of the candidate on the topic of the competition is related to studies of biofilm formation in 17 strains of *E. coli*, including uropathogenic, enteroaggregative, enterohemorrhagic, enterotoxigenic and enteropathogenic. The obtained data reveal variation in the mechanisms of biofilm formation, which is a strain-specific process in different pathotypes. Important data were obtained on the biofilm-modulating effect of sterile supernatants from stationary phase strains of *E. coli* O157: H, *E. coli* K-12 and *Y. enterocolitica*. Original, scientific data were obtained on the influence of the tested supernatants on the growth and biofilm formation in *E. coli*; the lectin affinity of BF was determined by applying a new enzyme-linked lectin assay (ELLA) procedure. The candidate develops an experimental design for screening studies on variable parameters, such as nutrient medium, cultivation duration, variations in growth temperature in a model group of Gram-positive and Gram-negative strains. Such a standardized experimental scheme on strain-specific, biofilm-modulating characteristics of a model group is extremely valuable before testing new substances, such as putative anti-biofilm effectors. A new approach has been developed related to the application of polymer micelles (PM) for disinfection of contaminated with biofilms, surfaces and wound treatment. The obtained results are of scientific and applied significance against biofilm-contaminated surfaces in medicine (medical implants, superficial lesions) and the food industry.

For the first time, an innovative approach has been developed for the destruction of mature biofilms by cationic polymer micelles, but additionally loaded with silver nanoparticles. The changes in the surface characteristics of the biofilms have been studied by SEM and CLSM.

As a partner in a project of COST BM1003 program "Microbial surface determinants of virulence as targets for new therapies for cystic fibrosis" Dr. Paunova-Krasteva participated in determining the phenotypic characteristics of an international panel of 42 strains from geographically remote areas and serial isolates from one and the same patient at different stages of *P. aeruginosa* development. An original scientific contribution of the candidate is the characterization of the biofilm potential of a panel of strains, on different nutrient media, at different time intervals. For the first time, original, scientific data on growth, motility, lag phase elongation have been obtained which is relevant to the survival of the strains as well as to the mechanisms of biofilm formation, which is essential for lung colonization.

**3. Phenotypes and phenotypic variations in microorganisms - antigenic, surface** (publications 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 23, appendix №14)

The candidate's research on phenotypes and phenotypic variations is of considerable interest. For the first time, a complex methodology for analysis of surface glycoconjugates by lectins in pathogenic microorganisms is included. Large differences were found between individual cells in terms of lectin-binding epitopes available on the surface. In studies on *E. coli* O157, which is a serotype of important epidemiological significance.

By microbiological, immunocytochemical, immunological, microscopic and other methods, phenotypic variations in surface glycom have been demonstrated in connection with changes in growth conditions, as well as between cells within the same population in *E. coli* strain O157: H (-).

Changes in surface characteristics have been demonstrated in pathogenic strains cultured at different growth temperatures.

Another original contribution of the candidate is related to the study of the two cyclic forms of ECA (tetramer and pentamer), which were first isolated in *E. coli strain O157: H (-)* from

aqueous and phenolic fractions. Using ELISA, flow cytometric examination of phagocytosis with antigen-laden latex spheres and CLSM, it was found for the first time that the two purified cyclic antigens from *E. coli* O157: H (-) bind to two of the major humoral receptors of innate immunity - MBL and C3b. Their reactivity is significantly higher than that of O157-specific LPS.

An essential element in the candidate's research is the one related to the resistance of biofilm-forming microorganisms. A review of the data on antimicrobial peptides (colistin, peptide LL-37, synthetic peptide 1018 with biofilm-inhibitory potential as well as plant extracts, some of which inhibit biofilm growth and others completely inhibit it. The candidate's contribution to the research of sesquiterpene lactones from Arnica, inhibiting quorum-sensing signaling in the bioreporter strain *Vibrio harveyi* is original.

#### **4. Cellular interactions between pro- and eukaryotes, structural-functional studies (publications 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 appendix № 14)**

This part of candidate investigations include studies related to the type of adhesion of bacteria, structural and functional studies of fungi in normal conditions and after various stress factors, as well as ultrastructural studies on other eukaryotic objects.

TEM and CLSM provided data on the specificity of important carbohydrate components of the apical membrane of enterocytes, as well as their biological role in their interaction with microorganisms. Using lectins, localization of N-acetyl- $\beta$ -glucosamine oligomers was found in the microvilli in newborn mice.

Studies on the invasiveness and intracellular survival of *P. aeruginosa* PAO1 in alveolar carcinoma - A549 cells in dynamics were performed. To monitor the intracellular localization of bacterial cells, Dr. Paunova-Krasteva applied for the first time a triparental model system for labeling PAO1 with green fluorescent protein (GFP). Transformed PAO1-GFP strains have been successfully isolated (on selective media). Data on the intracellular reproduction of the strain were obtained and the effect of PAO1 on the actin cytoskeleton was evaluated. The original results identified *P. aeruginosa* PAO1 as an invasive strain.

Other studies with pathogenic strains of *P. aeruginosa* (clinical isolates from Bulgarian patients with cystic fibrosis) have been performed on model, eukaryotic, cell lines. The cytotoxic effect of a pair of strains isolated from the same patient before and after inhalation treatment with tobramycin was compared.

The localization of chitin in the cell wall of *Aspergillus niger* 26 and its subsequent changes during growth, differentiation were studied. The obtained results give greater clarity about the distribution of chitin in the processes of hyphae differentiation.

Ultrastructural changes in cell organization have also been studied in copper-induced, oxidative stress in *Humicola lutea* 103.

Of great interest are studies related to the use of supramolecular nano capsule (Pt (II) 1Pt in the fight against cancer.

The analysis of the scientific production of the candidate clearly shows that she is a well-established specialist with expertise in the study of different groups of microorganisms, with significant methodological and scientific contributions in the field in which she works. A significant part of these contributions is of an original nature. It is also obvious that the candidate has the potential for successful collaborations with colleagues from the country and with leading research units abroad in the direction of the competition.

#### **4. PERSONAL IMPRESSIONS ON THE CANDIDATE**

I know the chief assistant Dr. Tsvetelina Paunova as a student at the Faculty of Biology where I taught her in a number of microbiological disciplines. She prepared and defended a master diploma thesis in the laboratory I lead. My impressions both from the training courses and during the preparation of the diploma work were for a purposeful young person, disciplined and consistent. By the way, she clearly proves this during her professional development at the Institute of Microbiology.

These impressions were strengthened and during her work with students as a part-time lecturer at the Faculty of Biology.

Analyzing the materials of this competition, I find that my assessments of the capabilities of the candidate for science were quite correct. I am glad that Dr. Tsvetelina Paunova is already an established scientist, I am convinced of her abilities and inexhaustible potential for science and sure of her future success as a scientist.

#### **CONCLUSION**

In terms of volume, content and quality the presented scientific production and teaching activity of the only candidate in the announced competition for the academic position of associate professor, Chief Assistant Dr. Tsvetelina Paunova - Krasteva fully meets the requirements for this position and significantly exceeds them.

The complex evaluation of the submitted materials, as well as the overall activity of the candidate gives me reason to convincingly propose to the scientific jury and the esteemed scientific council of the Institute of Microbiology at BAS to choose Chief Assistant Dr. Tsvetelina Paunova - Krasteva as Associate Professor in a professional field 4.3. Biological Sciences (Microbiology), announced for the needs of the Department of General Microbiology, Laboratory of Cellular Microbiology at Institute of Microbiology, BAS.

Prof. Dr. Veneta Ivanova Groudeva

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Sofia

