



REVIEW

According to the documents of the competition for the academic position "Associate Professor" in the field of Higher Education area 4. Natural sciences, mathematics and informatics, professional area 4.3. Biological sciences, scientific specialty "Microbiology", announced in SG №, 12/ 12.02.2021.

REVIEWER: prof. Dr. Petya Koycheva Hristova

1. General presentation of the procedure:

The competition for "Associate Professor" in the area of Higher Education 4. Natural sciences, mathematics and informatics, professional field 4.3. Biological sciences, scientific specialty "Microbiology" has been launched for the needs of the Laboratory "Cell Biology", Department of "General Microbiology", at IMicB-BAS in SG no. 12 от 12.02.2021г. The scientific jury was formed in accordance with order 30 / 29.03.2021, based on Art. 4 and Art. 29a of the Act for the Development of the Academic Staff in the Republic of Bulgaria, art. 60 of the Regulations for its implementation and the Regulations for the conditions and the order for acquiring scientific degrees and holding academic positions in IMicB at BAS. The documentation is very well organized and fully reflects the multilateral research activities of the candidate.

2. Biographical data, career growth and professional qualities of the candidate

The only candidate who submitted documents for participation in the competition for the academic position "Associate Professor" in Professional Field 4.3. Biological Sciences, specialty Microbiology is **Assistant prof. PhD. Tsvetelina Paunova-Krasteva**.

The candidate graduated from the Bachelor's Degree in Biology in 2003 at the Faculty of Biology. In 2005 she acquired the specialty of Molecular Biologist after successfully completing the Master's degree in the Ministry of Education and Science in Microbiology and Microbiological Control at the Department of General and Industrial Microbiology at the Bulgarian Academy of Sciences. In the same year Tsvetelina Paunova-Krasteva entered as a biologist in the section "Morphology of microorganisms and electron microscopy" at IMIKB at BAS, where her scientific career continues to this day. The research of the section during this period is aimed at studying the ultrastructure, cytochemistry and morphological changes of microorganisms from different phylogenetic groups: viruses, bacteria and eukaryotes, based on electron microscopy. This contributes to the growth of the candidate as a specialist in the field of microscopic techniques, morphology, microtomy, as well as the application of various methods for processing samples for transmission and scanning electron microscopy.

In 2011 the candidate won a competition for an assistant and after successfully defending a doctoral dissertation (2015) she continued her development as a senior assistant (2016). From 07.12.2020 she is the head of the laboratory "Cell Biology" at IMicB at BAS.

Therefore, Assistant prof. PhD. Tsvetelina Paunova-Krasteva corresponds to Article 56 of the Regulations on the terms and conditions for holding academic positions of IMicB, as she has held sufficient time for each academic position, which ensures the mastery of specific duties and responsibilities, written in their respective job descriptions.

From the presented autobiography and scientific works there is a deep interest and accumulated professional experience in various current issues of cell biology and molecular microbiology.

The professional growth of the candidate is related to expanding and upgrading the knowledge in the field of the competition through purposeful qualifications. As a master (2005) Dr. Paunova participated in the specialization in "Food Microbiology" in Ghent, Belgium and after joining IMicB continues its development in more than ten short-term training and coaching courses in the field of transmission-electronic and confocal laser scanning microscopy, studies molecular methods for identification and typing of bacteria, topics in cell biology and bacterial infections. Experience and innovations in the field of microscopic techniques are presented to a wide audience as a guest lecturer at the Youth School of Electron Microscopy. One of the main contributions of the candidate for the development of the unit is related to the testing of new methodologies for the unit, in the field of fluorescence microscopy, in the selection of suitable fluorochromes for labeling, in the preparation of bacterial and biofilm samples for fluorescence microscopy, incl. two-stage and three-stage protocols for immunofluorescence labeling for observation in two different modes - epifluorescence and / or confocal laser scanning microscopy

The knowledge and experience gained within these specializations are essential for the establishment of the candidate as an excellent microbiologist, striving for new aspects of modern microbiology.

Dr. Paunova actively participates in the dissemination of scientific information as a member of the organizing committees of five scientific forums and is a member of the Union of Scientists in Bulgaria. Proofs of her professional development are the numerous awards received from 2007 to 2019. This indicator distinguishes the grants of the World Federation of Scientists, FEMS for Young Scientist and the Union of Scientists in Bulgaria. These achievements show the professional development of a young scientist who is constantly improving and evolving.

3. Evaluation of the scientific production and the scientometric indicators of the candidate

Assistant prof. Tsvetelina Paunova presents all documents required for the competition in compliance with the requirements of the Law on the Protection of the Rights of Persons with Disabilities and the Regulations of IMicB Tessa arranged neatly, systematically with a clear distinction.

3.1. Characteristics and evaluation of scientific and publishing activities

A total of **32** scientific publications, **46** participations with posters in national and international forums and **16** participations with reports were presented for participation in the competition. On **9** of them she was presented as the first author. It is noteworthy that the

publications are mainly in three main areas of scientific topics concerning various current issues of microbiology and cell biology, which are in strict accordance with the theme of the competition. Publications in specialized, referenced and indexed foreign scientific journals with IF/SJR and quartiles Q1-Q4 are **19**; chapters of books are **3**. Scientific and educational publications referred magazines without IF and SJR are 11. Participation in international scientific forums and poster reports in total are **65**.

Very good impression is made by the presented publications of the candidate publications IF, SJR and quartiles (Q1-Q4), included in the international databases SCOPUS and WEB of Science. The total IF of all publications is 22,091 which shows the importance of the research.

3.2. Evaluations of scientific works

The published scientific results of **16** articles of the candidate are cited **63** times in Scopus and Web of Science. The citation index of the *h factor* is 5 (according to Scopus), and for the whole scientific career the *h-index* is 6.

3.3. Assessment of fulfillment of the minimum national requirements for the respective scientific field and the additional requirements of Sofia University "St. Kliment Ohridski"

The minimum national criteria according to the RASRB are fully met by the presented scientometric indicators:

According to Indicators of group C, the requirement of 100 points is fully met by the submission of **6** publications with Q2, which set **120** points.

According to Indicators of group B, evidence for **264** points is presented for the required 200 minimum points, which are formed by **13** articles, of which 1 -Q1, 4 -Q2, 6 -Q3 and 2 -Q4 and **3** chapters in books.

According to Indicators of group.D evidence was presented for **63** citations, which give **126** points and exceed the minimum criterion of 50 points.

Particularly good is the evidence under Indicators of group E, which is not mandatory for the academic position of "Associate Professor". In this paragraph the candidate forms **338** items with a minimum required 150 points as a participant in **9** national research projects and **4** international projects and as a leader of **3** national and **2** international projects.

Dr. Paunova presents **24** publications (excluding those included in the Doctor's degree) in refereed journals with an impact factor, which covers the additional requirements of at least 20 articles for the position of "Associate Professor" defined in the Regulations on the terms and conditions for acquisition of scientific degrees and for holding academic positions in IMIC.

3.4. Evaluation of teaching and learning activities, scientific guidance students and administrative experience

Dr. Paunova actively participates in the preparation of graduates. Currently, there are two students who have successfully defended their thesis (one bachelor and one master). She actively participates in the "Student Internships" program, where she mentors 13 trainees.

The teaching activity of the candidate is related to conducting practical classes as a part-time lecturer in the Master programme "Cellular Biology and Pathology" to the course "Cellular

Pathogens" for students from Biological faculty and "Microbiology and Virology" for students from Pharmacy in the Faculty of Chemistry and Pharmacy.

3.5. The project activity of the candidate was evaluated

The presented report shows the active participation of the applicant in national and international projects. Evidence for 19 projects is presented, 4 of which are led by Dr. Paunova and one is a consultant. This project activity proves not only the scientific but also the organizational qualities of the candidate.

Conclusion under item 3: *The candidate in the current competition for the academic position of "Associate Professor" Dr. Tsvetelina Paunova-Krasteva meets the minimum criteria of the Law on Public Procurement and the Regulations for its application and exceeds them (898 total number of points).*

4. Evaluate the original scientific contributions of the candidate

The research work of Dr. Tsvetelina Paunova-Krasteva in the Laboratory of Cellular Microbiology is characterized by developments grouped into three main topics:

- ✓ *Bacterial biofilms - development, structural and functional characteristics, inhibition;*
- ✓ *Phenotypes and phenotypic variations in microorganisms - antigenic, superficial*
- ✓ *Cellular interactions between pro- and eukaryotes, structural-functional studies.*

Research on the structure of biofilms, the dynamics of their development, as well as the assessment of the factors influencing the rate of formation is one of the main areas in which Dr. Paunova works. These studies highlight a number of original contributions that have made scientific and applied contributions in this area. The introduction of an optimized and standardized procedure for the study of bacterial biofilms, based on a complex methodology, allows its standardization for both Gram-negative and Gram-positive bacteria. The methodology has been successfully applied in the study of the effect of newly synthesized natural products on the formation of bacterial biofilms. Of particular scientific interest is the determination of strain-specific effects of secretory metabolic products on the formation of biofilms of 17 strains *E. coli*, including uropathogenic, enteroaggregative, enterohemorrhagic, enterotoxigenic, and enteropathogenic. This approach has also been applied to the study of biofilm formation patterns from 42 strains of *P. aeruginosa* isolated from CF patients geographically distant. For the first time, the effect of the culture medium on the dynamics of biofilm formation (attachment, growth, maturation, exfoliation) has been proven. The first comparative study on the phenotypic characteristics (growth, biofilm formation and motility) of *P. aeruginosa* strains is important. from Bulgarian patients with cystic fibrosis isolated before and after inhalation treatment with tobramycin. The candidate deepens her research in applied aspect by searching for polymers for destruction of mature biofilms. Screening of 26 newly synthesized cationic polymer micelles revealed a high potential of polymers based on PDMAEMA poly (2-(dimethylamino) ethyl methacrylate) against active mature biofilms of Gram-positive and Gram-negative strains. An original contribution and scientific-applied achievement in the work of Dr. Paunova are the studies on the activity of newly synthesized polymer micelles loaded with silver nanoparticles. Thus, in practice, a new concept for overcoming the barrier of the biofilm, extracellular matrix has been

realized by creating a synergistic effect of destructive activity and bactericidal action. To determine the metabolic activity of the treated biofilms, Dr. Paunova-Krasteva develops and applies a new methodology with a redox indicator Alamar Blue. The new data obtained have a fundamental contribution to the scientific literature.

Tendencies to increase infections caused by resistant microorganisms are also reported in biofilm bacteria. The application of sub-minimum inhibitory concentrations (sub-MICs) of antibiotics, peptides, plant or synthetic substances to biofilms has a different effect: from inhibition of biofilm formation, to no effect or stimulant effect. These are essential conceptual points that Dr. Paunova-Krasteva discusses in her review article for modulating biofilms from sub-inhibitory concentrations of antimicrobials. The review of the literature outlines the role of antimicrobial peptides for changes in the gene regulation of quorum-sensing signaling, lipopolysaccharide synthesis, mobility and others. Many plant extracts and / or their metabolic products (essential oils, fractions, etc.) inhibit biofilm growth, while others completely inhibit it. Therefore, with regard to the application of plant extracts, the candidate has made an original contribution to the research of sesquiterpenes lactones from Arnica, inhibiting quorum-sensing signaling in *Vibrio harveyi*, which will provide an answer for the autoinduction systems of bioreporter strains.

Another significant part of Dr. Paunova's research is focused on a very topical issue related to the study of changes in the structural and functional characteristics of surface glycom and especially with the characteristics of carbohydrate-binding proteins - lectins. For the first time, a complex methodology for analysis of surface glycoconjugates in pathogenic microorganisms was applied, which revealed significant differences between individual cells in terms of lectin-binding epitopes. Another original contribution of the candidate in this field is related to the study of both cyclic forms of enterobacterial total antigen ECA (tetramer and pentamer), which were first isolated in *E. coli* strain O157: H (-) from aqueous and phenolic fractions. To study the phenotypic variations in *E. coli* O157: H (-), the candidate develops a new molecular genetic approach for typing the genes responsible for the synthesis of surface polysaccharide antigens, mainly with a view to studying the co-expression of ECA and O real-time antigen on a pathogenic strain of this serotype. The scope of this field includes the applied research of Dr. Paunova-Krasteva for the use of biosurfactants that affect the growth, morphology and ultrastructure of another conditionally pathogenic species - *Pseudomonas aeruginosa*. The data obtained that the synergistic combination of rhamnolipid and antimicrobial agent (methylthiosulfonate) is suitable for the production of new, more effective antimicrobial preparations are an important practical contribution of the candidate.

In the third direction of the research activity of the candidate, researches on the cellular interactions between prokaryotes and eukaryotes and the resulting ultrastructural changes are presented. Clarification of the mechanisms of adhesion is of particular importance for understanding the pathogenesis of many diseases and Dr. Paunova's research has made a significant contribution in this area. The results proving the two different models of adhesion in Shiga non-producing strains (*E. coli* O157: H- and *E. coli* O157: H7) are of fundamental and applied contribution. The development and application of a fluorescence microscopic FAS test enriches the range of microscopic techniques introduced by Dr. Paunova in the Laboratory of Cell Biology and reveals important information about both the mechanisms of adhesion of *E. coli* O157 and the ongoing ultrastructural changes in host cells. A series of studies have examined the structure and organization of the components of the intestinal glycocalyx in neonates, prenatal and adult mice. The application of biotinylated or fluorescently labeled lectins proves through TEM

and KLSM the specificity and localization of important carbohydrate components of the apical membrane of enterocytes, as well as their biological role in their interaction with microorganisms.

After successful adhesion of the pathogen begins colonization in the intestinal tract, which are components of the infectious process. Dr. Paunova studies the nutritional factors that ensure the effectiveness and balance of these two processes depending on the type of growth factors. It turns out that in food stimulation with growth factors the adhesion of pathogenic *E. coli* strains increases. Moreover, when stimulated with certain growth factors (EGF and TNF-alpha), bacterial adhesion is significant. These data form an important scientific contribution that, despite the benefits for intestinal differentiation processes, dietary supplements are not recommended for infants. To study the invasiveness and intracellular survival of *P. aeruginosa* PAO1, for the first time Dr. Paunova-Krasteva applied a triparent model system by labeling with green fluorescent protein (GFP). The original results obtained for the intracellular reproduction of the strain and its effect on the actin cytoskeleton identify *P. aeruginosa* PAO1 as an invasive strain that not only survives in host cells but also multiplies intracellularly. The applied fluorescence microscopic analysis showed a clear disintegration of the actin cytoskeleton, and co-cultivation with other strains of this species leads to increased cell permeability and the presence of necrotic areas in eukaryotic cells.

In parallel with the above methodologies and the focused interest of Dr. Paunova-Krasteva to increasingly relevant areas, the candidate conducts research in the field of microbial morphology by studying the ultrastructural and surface characteristics of bacteria and molds in the processes of differentiation and stress factors.

Thus, to study the localization of chitin in the cell wall of *Aspergillus niger* 26, the candidate developed cytochemical methods for labeling with lectin from *Triticum vulgare*, which allow to capture ultrastructural changes in the mycelium during growth, differentiation and aging of hyphae. This method has important practical application in the tracking of strains that are widely used in the production of fermented foods, organic acids and enzymes. Of particular interest are studies related to morphological and physiological changes during temperature stress in *Aspergillus niger* 26 and copper-induced oxidative stress in *Humicola lutea* 103. Data for two patterns of behavior of *Aspergillus niger* 26 at different temperatures have their scientific of practical importance because when cultured at low temperatures the model strain can be a good producer of Cu / Zn superoxide dismutase. The presence of copper ions inhibits the accumulation of biomass from *Humicola lutea* 103 without observing intracellular accumulation. The ultrastructural changes of this strain at lower concentrations of copper are aimed at rupture of the cell wall and the accumulation of lipid droplets, while maintaining the ability to adapt to this stress. At higher levels, severe intoxication is observed, accompanied by cell degeneration, autophagy and probable cell death, which is confirmed by low levels of biomass production. Of fundamental importance are data on the morphological changes in mitochondria, which judge the levels of toxicity and provide a better understanding of the role of copper-induced oxidant in fungal tolerance at high concentrations.

In the scientific publications submitted for review, the candidate's research related to the testing of various substrates with cytoprotective effect on oxidative stress also occupies an important place. Whey proteins have a strong antioxidant effect and help increase glutathione levels. The established cytoprotective effects of whey on the oxidative stress caused by doxorubicin treatment are an important contribution. The development of various platinum-based chemotherapeutics is one of the latest innovative strategies in the fight against cancer. In this

regard, the applicant's studies for the administration of a supramolecular nanocapsule reflect the latest trends in this field. TEM provides evidence of morphological changes in cancer cells expressed in the thickening of the mitochondrial matrix, thickening of the cytoplasm and the appearance of necrotic areas. Prolonged exposure leads to the formation of vacuole-like bodies and a euchromatic nucleus. The good pharmacological characteristics of the nanocapsule are a good basis for the creation of new chemotherapeutics.

CONCLUSION

The analysis of documentation allows me to confirm the positive assessment of the research and project activity, e.g. Assistant Professor Dr. Tsvetelina Paunova-Krasteva. I consider that the candidate fulfills all the requirements of the RASRB and the Rules for its application.

I strongly recommend to the members of the scientific jury, to the Scientific Council of the Institute of Microbiology at BAS to evaluate the candidacy of assistant Dr. Tsvetelina Paunova-Krasteva and to vote positively for the scientific position "Associate Professor" in the professional area 4.3. Biological sciences, specialty Microbiology.

Sofia

REVIEWER:

31.05.2021

Prof. Petya Hristova