REVIEW

of the scientific activity of Assistant Professor, Dr. Tsvetelina Paunova - Krasteva,
participant in a competition for Associate Professor in scientific field 4.3. Biological Sciences (Microbiology), announced in SG No. 12 of 12.02.2021,
for the needs of the Department of General Microbiology, Cellular Microbiology Laboratory at the Stefan Angelov Institute of Microbiology, BAS
Reviewer: Assoc. Prof., Dr. Zlatka Milcheva Aleksieva, Institute of Microbiology, BAS

Dr. Tsvetelina Paunova - Krasteva, Chief Assistant Professor in the Laboratory of Cellular Microbiology at the Department of General Microbiology at the Department of Microbiology, BAS is the only candidate who has submitted documents for the announced competition.

PROFESSIONAL BIOGRAPHY

Dr. Tsvetelina Paunova - Krasteva graduated as MS in "Microbiology and Microbiological Control" at the Department of "General and Industrial Microbiology" of the Faculty of Biology, Sofia University "St. Kliment Ohridski "in 2005. She is appointed as a specialist biologist at the Institute of Microbiology of the Bulgarian Academy of Sciences in the same year, and since the beginning of 2011 she has been an assistant. In 2015 she defended a dissertation on "Phenotypic variations associated with polysaccharide antigens in Escherichia coli O157", and in 2016 she won a competition for the scientific position of "Assistant Professor".

Throughout her career at the Institute, Dr. Tsvetelina Paunova works in the section "Morphology of microorganisms and electron microscopy", which entered the new structure of the Institute called the laboratory "Cell Microbiology" at the Department of General Microbiology. Since the end of 2020, Dr. Tsvetelina Paunova has been the head of the laboratory.

Dr. Tsvetelina Paunova has completed 11 specializations, including 8 specialized training courses in a number of European scientific institutions such as: Federico II University, Naples; Hellenic Pastor Institute; University of Wrocław; San Raffaele Research Institute, Milan; University of Tübingen; Institute of Molecular Pathology, Vienna; FEI NanoPort - FEI Academy, Eindhoven. She has completed 2 Erasmus specializations at the University of Ghent, Belgium and a Joint Unit of the Gregor Mendel Institute for Molecular Biotechnology, the Institute for Molecular Biotechnology (IMBA), the Research Institute for Molecular Pathology (IMP), Vienna, Austria and 1 project specialization PTR-43-16 "Role of nucleotidyl cyclase ExoY in Pseudomonas aeruginosa infections", Institut Pasteur, Paris.

Dr. Tsvetelina Paunova has received 7 awards, including FEMS - Grant for mobility of a young scientist - 2007; One-year grant from the World Federation of Scientists. National Scholarship Competition 2013; High scientific achievements in dissertations for 2015 "by the Union of Scientists in Bulgaria; The most successful project funded by the Program for Support of Young Scientists and Doctoral Students at BAS - 2017; award for 2014 from the Stefan Angelov Foundation and others.

Dr. Tsvetelina Paunova is the leader of 5 research projects, 2 of which are international, 2 - Program for Support of Young Scientists at BAS and 1 funded by the Ministry of Education and Science. He is a participant in 10 national and 3 international projects. She is the secretary and member of the management of 5 scientific forums. She has participated with 16 papers (in 9 - I author) and 46 posters (in 20 – I-st author) in a number of national and international scientific forums. He is a member of the Microbiology Section of the Union of Scientists in Bulgaria.

TEACHING ACTIVITY

Dr. Tsvetelina Paunova has supervised 2 graduates (master, bachelor) from Sofia University "St. Kliment Ohridski ". She participated in the training of 13 graduates, was a mentor in the program "Student Practices". From 2016 to the present he is a lecturer on a civil contract in the specialty "Cell Biology and Pathology" at Sofia University "St. Kliment Ohridski ".

MAIN SCIENTIFIC INDICATORS

Dr. Tsvetelina Paunova - Krasteva presents for the current competition her scientific production, which can be systematized according to the minimum national requirements for scientific and other activities of the candidates for the academic position "Associate Professor". She presented the Abstract of her PhD thesis on the topic: "Phenotypic variations associated with polysaccharide antigens in Escherichia coli O157" which covers the requirements of group "A" indicators.

30 scientific publications, pulished after PhD-thesis defense are presented for the competition. Their total IF is 22.556. To the requirements of group B, indicator 4, the candidate has submitted 7 scientific publications. They have been published in prestigious international scientific journals, referenced and indexed in world databases Scopus and Web of Science (6 - Q2 and 1 - chapter of a book). This results in 125 points, with a required minimum of 100 points in the Regulations for implementation of the Law in the Republic of Bulgaria at the Institute of Microbiology - BAS. According to the indicators from group G, 23 scientific papers are presented (21 publications and 2 bookchapters). The required number of points in the Regulations for implementation of the Law in the Institute of Microbiology - BAS is 200, and the candidate in the competition scores a total of 249 points.By group of indicators D, to indicator D11 "Citation in scientific publications, monographs, collective volumes and patents, referenced and indexed in world-famous databases with scientific information (Web of Science and Scopus)" is presented a list of 9 cited articles from the submitted for evaluation in the competition, with a total of 58 citations. The calculations show that Dr. Tsvetelina Paunova - Krasteva scored 116 points, with a minimum of 100 required.

The results clearly prove the importance of the candidate's scientific results and achievements, as well as the international popularity of the publications.

The candidate's data on the indicators from group E to the highest extent exceed the minimum national requirements. Dr. Tsvetelina Paunova - Krasteva is the leader of the Bulgarian team in 2 international and 3 national research projects, and a participant in 10 national and 4 international research projects. The funds raised as a result of these projects is BGN 40,196. This indicator brings 338 points, with a required minimum of 150 points.

In conclusion, it can be seen that the amount of the minimum required by ZRASRB 400 points is significantly exceeded. The total number of points on indicators from A to E is 878.

Regarding "Additional criteria for the growth of the academic staff in IMikB" it can also be noted that the criteria are over fulfilled. For his entire scientific activity, Dr. Tsvetelina Paunova - Krasteva has published a total of 32 scientific publications with H-index - 6, until the presentation of the documents.

The requirement in the criterion "Number of publications in journals with IF, monographs, chapters of monographs, collections published in full text, patents" is 20 pcs. (Excluding those included for "doctor") 25 are presented, and in 7 of them the candidate is a leading or corresponding author.

The next criterion "Citations" requires 100 for the entire scientific career, and the candidate submits over 111 citations.The requirement for H factor is 5 for the whole scientific internship is fulfilled. The value of the required IF was also exceeded - a total of 20.61.

Regarding the number of research projects managed, as well as the participation in such, it is said in detail above, but the exceeded requirements are indisputable.

SCIENTIFIC CONTRIBUTIONS

One of the main objects of analysis in the research of Dr. Tsvetelina Paunova-Krasteva are the strains of Escherichia coli, especially with serotype O157 LPS, which are often associated with foodborne infections and can damage health with varying severity. Research with these sites is diverse and multidirectional and has a significant contribution to the more complete characterization of these widespread and significant inhabitants of the human body.

Carried out for the first time a study in which by nuclear magnetic resonance (NMR), it was shown that in two isolated and purified from Escherichia coli O157: H cyclic forms of the enterobacterial common antigen - ECACYC-4 and ECACYC-5 no attachment of the following substitute groups was observed, which differs from previously described cyclic antigens from other enterobacteria. Their cyclic structure and degree of polymerization was proved by MALDI MS spectrometry. There is a reasonable hypothesis that ECACYCs may be microbially associated molecular structures that can be recognized by human humoral mechanisms.

Laser scanning confocal microscopy analyzes showed abnormal rearrangement and behavior of cytoskeletal actin and associated ZO-1 and vilin proteins in the HeLa cell line compared to polarized epithelial cells. The absence of F-actin accumulations suggests a different pattern of bacterial pathogenesis in unpolarized epithelial cells. The recorded changes in the cytoskeleton as a result of coincubation of HeLa cells with E. coli strain O157: H7 are important original results.

Comparing the adhesion patterns characteristic of one E. coli strain O157: H- and E. coli strain O157: H7 revealed the presence of different adhesion patterns characteristic of each of the two strains. It was observed that in both cases there was selectivity and not all HeLa cells were associated with bacteria. The results suggest different degrees of change in the cytoskeleton of the host from both strains. E. coli O157: H- has been suggested to be able to modulate the tubulin cytoskeleton and alter the localization of dynein and VDP, two molecules involved in the intracellular transport of vesicles.

The ability of uropathogenic strains of E. coli (UPEC) to form biofilms was also studied and the various effects of biogenic factors (medium containing signals for quorum sensing and epinephrine) on the formation of biofilms were found on them. The effect of Arnica montana extracts (sesquiterpene lactones) on Quorum Sensing (QS) signals was studied. A Vibrio harveyi bioreporter strain system was used to compare the effects of fractions on QS-regulated bioluminescence. The results for two sesquiterpene-rich fractions of A. montana, Am2 and Am3, showed their good potential as QS inhibitors of the expression of certain virulence factors in order to inhibit the development of pathogens.

Much of the research has focused on the question whether pathogens through secreted factors can affect the attached growth of non-pathogenic E. coli strains.Biofilms of a non-producing strain of E. coli O157: H, a laboratory strain of E. coli K-12 producing a biofilm, and a biofilm-forming strain of the pathogen Yersina enterocolitica O : 3 were compared. It was found that only products secreted by pathogens induce an increase in biomass in some strains K-12 with "biofilm deficiency".Proteinase K has been shown to significantly reduce the stimulatory effects of all modulating supernatants, indicating the important role of protein / peptide factors in biofilm status. The use of secreted polysaccharides (rPS) has significant but specific to the target strain of E. coli K-12 depending on the source of rPS.

In a number of other precisely conducted studies, original results have been achieved. Changes in the surface exposure of antigens to pathogenic strains of Escherichia coli as a result of growth temperature and cell differences are shown. The study was performed with

specifically gold-labeled lectins that can distinguish surface polysaccharide antigens. The results obtained by immunofluorescence electron microscopy showed that there was no correlation between concavalin A (ConA) binding and the immunoreactivity of individual cells in the bacterial population. It has been shown that growth temperatures have a significant effect on the binding capacity of concavalin to bacterial glycocalyx in relation to lectin affinity and bacterial adhesion to cultured HeLa cells. The influence of cultivation time, growth medium and temperature on the development of biofilms from a set of model Gram-positive (Staphylococcus aureus ATCC 29213 and Bacillus subtilis 168) and Gram-negative strains of Pseudomonas aeruginosa PAO1 and six strains of Escherichia coli K-12 was tested.

Important and of great health and public importance is the direction of research on the disease mucoviscidose, in which infections, very often caused by Pseudomonas aeruginosa, are the leading cause of exacerbations and early death of patients.

One of the largest studies involves the organization in a reference panel of 42 strains of P. aeruginosa of different origins, in order to harmonize and compare the data from studies on this pathogen. The studies have performed in the larvae of the wax moth G. mellonella, which is often used as a model organism for research. Following a number of growth, physiological and bohemian parameters of different strains, and the high variability of this pathogen was confirmed and characterized. There is a great diversity in the dynamics of biofilm formation and preferences for different conditions of biofilm cultivation between different strains. The study increases knowledge about the virulence and pathogenesis of P. aeruginosa and provides a basis for testing new therapies for the serious diseases caused by this pathogen.

For the first time, a comparative phenotypic study has performed on a panel of 6 paired couples of strains of Ps. aeruginosa isolated from Bulgarian patients with cystic fibrosis before and after antibiotic treatment with tobramycin. Differences have been reported that characterize the ability of bacteria to adapt and prolong the lag phase of bacterial growth after treatment. When working with P. aeruginosa, the effect of a number of agents and substances on the development of the pathogen has studied. In this regard, the influence of biosurfactants (rhamnolipid and trehalose lipid) on the antibacterial activity of methylthiosulfonate has studied. The use of scanning and confocal electron microscopy has shown that the combination of methylthiosulfonate and rhamnolypid biosurfactant significantly increases the therapeutic potential of methylthiosulfonate.

High in vitro cytotoxic effects have been demonstrated as a result of different mechanisms of action in the co-culture of A549 lung epithelial cells with two clinical strains of P. aeruginosa isolated from the same cystic fibrosis patient before and after tobramycin treatment. The interaction of P. aeruginosa strain PAO1 with human lung carcinoma cell line A549 has characterized. Fluorescence microscopic analysis of the PAO1-GFP strain, specifically labeled with green fluorescent protein, after co-culture with A549 cells confirmed intracellular survival and bacterial replication, as well as progressive disruption of the actin cytoskeleton.

The effectiveness of cationic polymer micelles (CPM) against formed in advance biofilms of Escherichia coli 420, Ps. aeruginosa PAO1, Staphylococcus aureus 29213 and Bacillus subtilis 168 has studied. It has been found that the most effective for reducing the biomass of the biofilm (3-4 times) are CPM based on poly (2- (dimethylamino) ethyl methacrylate) copolymers. The results showed the applicability of CPM for disinfection of biofilm contaminated surfaces and wound healing.

In order to remove biofilms associated with serious infections, an original combination of cationic polymer micelles formed from poly (2- (dimethylamino) ethyl methacrylate) -b-poly (ecaprolactone) -b-poly (2- (dimethylamino) ethyl methacrylate) was created.) (PDMAEMA-PCL-PDMAEMA) triblock copolymer. The results show that both the

copolymer and its combination with silver nanoparticles (M_AgNPs) have a bactericidal effect, causing changes in the bacterial surface, significantly reducing the volume of the biofilm and having the potential to treat biofilm infections from P. aeruginosa.

The object of study is also the ultrastructural changes in eukaryotic microorganisms, in which induced oxidative stress is observed.

The induction of a significant increase in oxidative stress levels with increasing temperature to 40–45 ° C for A. niger 26 is characterized by various changes in hyphal morphology, such as intrahyphal hyphae formation, mitochondrial damage, and accelerated hyphal autolysis. The practical conclusion from this is that the decrease in biomass and intracellular protein content, despite the increase in SOD biosynthesis, causes a significant decrease in the yield of Cu / Zn - SOD in the model strain A. niger 26. High concentrations of heavy metals (Cu + 2) cause distinct and widespread ultrastructural changes in the copper-resistant fungal strain Humicola lutea 103. Mitochondria are the first and main target of toxic action. To study the ultrastructural changes and the localization of chitin in the micelles of a strain of Aspergillus niger, cytochemical labeling was performed with a lectin from Triticum vulgaris, suitable for marking chitin.

Another important research area in which Dr. Tsvetelina Paunova - Krasteva actively participates is the improvement of the pharmacological profiles of platinum anticancer drugs. The strong anticancer effect of supramolecular nanocapsules formed by self-assembly of four bis-anthracene ligands with two metal ions, either Pt (II) or Pd (II), has been demonstrated. Studies on the Pt (II) nanocapsule and its uptake into suspension cultures of two types of cancer cells have shown very high nanocapsule cytotoxicity, better selectivity, and lack of cisplatin cross-resistance. Based on morphological changes in HL-60 cells (ultrastructural changes in the cell cytoplasm) induced by the Pt (II) nanocapsule, examined by transmission electron microscopy (TEM), it is assumed that endocytosis is the most likely way to internalize 1Pt.

The protective effect of whey protein concentrate against toxicity and oxidative stress induced by the antitumor chemotherapeutic Doxorubicin (DOX) by stimulating glutathione biosynthesis was studied. Transmission electron microscopy has shown that the biochemical and histological changes that occur are effectively attenuated by pretreatment with this concentrate, which has a cytoprotective effect against the oxidative stress caused by DOX treatment.

Research related to the features and fine structure of the intestinal glycocalyx should be marked.

Fluorescein-labeled lectins binding various non-reducing carbohydrate residues were identified by confocal laser scanning microscopy in the glycocalyx of the small intestine of mice. Biotinylated lectins were used for electron microscopic monitoring of intestinal glycocalyx components at different periods of postnatal development in mice. The distribution and glycosylation of mucins is of interest in analyzing the response of the mucosal barrier to intestinal pathogens causing infection or inflammation. Oligomers of N-acetyl-β-glucosamine were abundant on all surfaces of the microvilli glycocalyx, and residues of α-L-fucosyl were not found in the glycocalyx of the duodenum, jejunum, or ileum of adult mice.

The results of studies on the influence of a set of growth factors on bacterial colonization with E. coli strains in intestinal explants from 5-day-old neonatal mice showed that stimulation of intestinal explants with fibroblast growth factors significantly increased bacterial adhesion. Therefore, they are not recommended as a supplement for artificial formulas for newborns.

The works presented for the competition also include three review chapters from books: Modulation of biofilm growth by sub-inhibitory amounts of antibacterial substances;

The multicellular behavior of Escherichia coli: target for effects; Variability of surface glycoma as an adaptation in host and pathogen interactions.

Dr. Tsvetelina Paunova-Krasteva is a specialist in the field of electron microscopy, microtomy, sample processing for transmission electron microscopy (TEM) and scanning electron microscopy (CEM). One of the main contributions of the candidate is related to the testing of new methodologies for the laboratory of "Cell Microbiology" in the field of fluorescence microscopy. The original methodological contribution of Dr. Paunova-Krasteva was demonstrated by the selection of suitable fluorochromes for labeling, in the preparation of microbial samples for fluorescence microscopy, including immunofluorescence labeling for epifluorescence and / or confocal-laser.

The main original contributions highlighted above can be grouped into three main groups, based on the themes formulated by Dr. Tsvetelina Paunova - Krasteva as: Contributions to the study of bacterial biofilms - development, structural and functional characteristics, inhibition; Contributions to the study of microbial phenotypes and phenotypic variations - antigenic, superficial; and Contributions to the establishment of mechanisms of cellular interactions between pro- and eukaryotes associated with structural-functional studies.

I will highlight some of the most important of them:

- The main scientific contribution of Dr. Tsvetelina Paunova-Krasteva is the modification and standardization of methods for analysis of biofilm formation.

- For the first time, an innovative approach has been developed for loosening and / or destroying mature biofilms, using cationic polymer micelles, but additionally loaded with silver nanoparticles.

- The metabolic activity of the treated biofilms has been determined, with a method with a redox indicator Alamar Blue, newly introduced for the unit by Dr. Paunova-Krasteva.

- As an original scientific contribution of the candidate in this direction, is the characterization of the biofilm potential of the panel of strains of P. aeruginosa isolated from different geographical regions and subsequent development of new strategies in the fight against them.

- 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.

- The original contribution of the candidate in this field 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.

- To monitor the intracellular localization of P. aeruginosa bacterial cells, Dr. TsvetelinaPaunova-Krasteva applied for the first time a triparental, model system for labeling RAO1 with green fluorescent protein (GFP). Transformed PAO1-GFP strains have been successfully isolated.

- The cytoprotective effects of whey on oxidative stress caused by doxorubicin treatment have been established.

CONCLUSION

The scientometric data of Dr. Tsvetelina Paunova - Krasteva exceed the minimum national and additional requirements of Academic Staff Development Low of RB, and the relevant Rules of IMikB for holding the academic position "Associate Professor" in the Professional field 4.3. Biological sciences. The researches and the results are distinguished by originality, topicality, fundamental and applied significance; fully correspond to the scientific field and scientific direction of the present competition.

The achievements of Dr. Tsvetelina Paunova-Krasteva have been published in various authoritative scientific journals, have been reflected in numerous and well-funded research

projects and have found a wide positive response in the scientific community in Bulgaria and around the world, which undoubtedly confirms the importance of tasks and the applicability of the obtained results.

The analysis of the submitted documents and materials gives me grounds to support the candidate and to convincingly recommend to the respected members of the Scientific Jury to evaluate positively and to propose to the Scientific council of IMicB to award Dr. Tsvetelina Paunova-Krasteva, the academic position of "Associate Professor".

Sofia, May 26, 2021 REVIEWER:

(Assoc. Prof. Dr. ZlatkaAleksieva)