REVIEW

by Dr. Penka Angelova Moncheva, prof., SU "St. Kliment Ohridski" - Faculty of Biology

on the materials submitted for participation in a competition for the academic position of "Associate Professor" at Plovdiv University "Paisii Hilendarski"

in: Area of higher education 4. Natural sciences, mathematics and informatics

Professional field 4.3. Biological Sciences (Microbiology – Microbial pathogenesis)

In the competition for "Associate professor", announced in the SG, no. 92/18.11.2022 and on the website of Plovdiv University "Paisii Hilendarski" for the needs of the Department of "Biochemistry and Microbiology" at the Faculty of Biology, Assistant Professor Dr. Mariana Ivanova Marhova-Koseva, from the same university is the only applicant that submitted documents for this competition.

1. General presentation of the received materials

By order No. PD 21-332/ 15.02. 2023. of the Rector of the University of Plovdiv "Paisii Hilendarski" (PU), I have been appointed as a member of the scientific jury in a competition for the academic position of "Associate Professor" in the PU in the area of higher education 4. Natural sciences, mathematics and informatics, professional field 4.3. Biological Sciences (Microbiology - Microbial Pathogenesis), announced for the needs of the Department of "Biochemistry and Microbiology" at the Faculty of Biology.

The presented materials by Assistant Professor Dr. Mariana Marhova-Koseva, the only candidate for this competition on paper and electronic carrier are very well prepared and 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: a set of administrative documents (application, curriculum vitae, diplomas required for the case, certificate of work experience, list of scientific papers, list of citations of scientific works, documents for scientific and research activity, references for compliance with the minimum national requirements and the additional faculty requirements, annotation of the materials according to Art. 65 of the PRASPU (in Bulgarian and English), self-evaluation of the contributions (in Bulgarian and English), references for the educational and research activities, declaration of originality and authenticity of the attached documents), as well as the scientific works subject to review, evidence of the observed citations of the works, curricula of bachelor's and master's disciplines prepared by Dr. Marhova.

Dr. Mariana Marhova, Assistant Professor, has presented a list of a total of 42 scientific works, of which monograph - 1, scientific publications - 39, a textbook and a manual for laboratory work – a total of 2, and a list of scientific research projects - 8. Thirty six (36) scientific works, which are outside the dissertation for obtaining the "PhD" degree, are the subject to review and evaluation, as well as the project developments – 8. The distribution of scientific works related to the present competition is as follows:

- **Monograph – 1**
- **Scientific publications in journals with impact factor/impact rank – 15**
- **Publications in refereed journals without impact factor/impact rank – 15**

- **Publications in conference proceedings – 5**
- **Textbook and manual for laboratory work - 2**

2.Кратки биографични данни на кандидата

Assistant Professor Dr. Mariana Marhova graduated from Plovdiv University "Paisii Hilendarski", Faculty of Biology in 1985 and was awarded a Master degree in Biology, qualification Biologist, teacher of Biology and with a second specialization in Chemistry. In the period 1986-1990 she was a doctoral student at The Institute of Physiology and Biochemistry of Microorganisms at the Academy of Sciences of the USSR, and after successful defense the PhD thesis in 1991 was awarded the scientific degree „doctor" (Diploma № 009839/18.02.1991 г.). From 1992 to the present, she has been successively Honorary Assistant, Assistant, Senior Assistant, Assistant Professor, respectively in Genetics and Cell Biology, Microbial Genetics, Immunology and Microbial Pathogenesis.

In the process of obtaining a doctoral degree and subsequent professional development, Assistant Professor Dr. Marhova builds and establishes herself as a researcher-scientist and university teacher, with almost 32 years of experience, of which almost 30 years as a lecturer (of at least 5 years, according to the additional requirements of the PU for the academic position "Associate Professor").

In addition to teaching and research, Dr. Marhova has significant organizational and administrative activity - participation in the Program Accreditation Commission in professional field 4.3. Biological Sciences, Faculty Committee on Quality, Faculty Committee on Attestation, participation in activities to prepare and update study documentation, etc. The above shows that she has an active attitude to the overall academic activity of the PU and fulfills the additional requirements of the PU for the academic position "Associate Professor".

3. General characteristics of the candidate's activity

3.1.Evaluation of educational and pedagogical activity

As stated above, Dr. Marhova's teaching activity started as early as in 1992. From the provided report on her educational activity during the last 5 academic years, it can be seen that she has a total classroom employment at the educational and qualification degree (EQD) "Bachelor" to 2866 hours (equated to exercises), of which 1634 hours are lectures, and in the EQD "Master" - 1670 hours, of which 1170 are lectures. Its average annual auditorium workload is 907 hours (573 hours in the EQD "Bachelor" and 334 hours in the EQD "Master"), which significantly exceeds the required minimum. For the last 5 academic years, Dr. Marhova has conducted lectures on the following disciplines: two courses in Immunology (for specialties Medical Biology, Biology and Ecology and environmental protection; and for Molecular biology, Ecology of biotechnological productions, and Pharmaceutical biotechnologies and Bioinformatics, respectively); Immunogenetics (for specialty Molecular Biology); Microbial Genetics (for specialties Molecular Biology and Pharmaceutical biotechnologies); Microbial pathogenesis (for Medical biology). In addition, she also led practical classes in the disciplines of Microbial Genetics and Plasmids (for Molecular Biology and Pharmaceutical biotechnologies), Microbial Pathogenesis (for Medical Biology) in the EQD „Bachelor". In the EQD "Master" she gives lectures on 9 academic disciplines, 5 of which are compulsory, in different master's programs: Genetics of industrial microorganisms (MSc Programme Biotechnological Microbiology), Immunology (MSc Programme Medical Biology - non-specialists), Infection and Sterility (MSc

Programme Reproductive Biology), Genetics of Microorganisms and Immunogenetics (MSc Programme Genetics), as well as the optional disciplines Clinical Microbiology (MSc Programme Biotechnological microbiology), Clinical and Sanitary Microbiology and Microbial Pathogenesis (MSc Programme Biopharmaceutical Biochemistry), Biology of Infectious Diseases (MSc Programme Medical Biology). She also conducts practical classes in 8 of the compulsory and optional disciplines. It could be concluded that the teaching activity of Dr. Marhova is extensive and has a wide profile, as it covers a wide variety of academic disciplines.

In addition to being a lecturer and conducting practical classes in the disciplines mentioned above, Dr. Marhova has developed the curricula of an impressive number of disciplines. In the EQD "Bachelor" she is the author of the curricula of 6 disciplines (3 compulsory - Immunology, Microbial Genetics, Microbial Pathogenesis and 3 optional - Immunogenetics, Plasmids and Microbial Genetics) for various specialties (Microbiology and Virology, Pharmaceutical Biotechnologies, Medical Microbiology, Molecular Biology). In the EQD "Master" she is also the author of the curricula of 6 disciplines (5 compulsory and 1 elective) - Genetics of industrial microorganisms, Genetic engineering of industrial microorganisms, Medical microbiology, Genetics of microorganisms, Immunogenetics and immunogenomics and Clinical microbiology, respectively for various Master's programs (Microbial Biotechnology, Microbiology and Microbiological Control and Genetics).

Under her scientific supervision, 23 students have successfully defended their diploma theses, 15 of them in the EQD "Bachelor" and 8 in the EQD "Master" (minimum number 5, according to the additional requirements of the PU for the respective position). She was the scientific supervisor of a doctoral student who has successfully defended a dissertation for the degree "Doctor".

The review and analysis of Dr. Marhova's educational and pedagogical activities give me reason to conclude that she is a well-established university lecturer, specializing in teaching disciplines covering a wide range of interrelated areas of microbiology - bacteriology, genetics, immunology, microbial pathogenesis, medical microbiology, immunogenetics, clinical microbiology, etc.

3.2. Evaluation of the scientific and scientific-applied activity

As I have already indicated in item 1 of the review, for participation in the competition for the academic position "Associated proffesor" Dr. Dr. Marhova has presented 36 scientific research works that combine research results in two scientific directions (Microbial Pathogenesis and Ecology of Microorganisms) in which the candidate works.

Microbial pathogenesis. The scientific works reflecting the results of research in this field are 18 - monographic work (1.1.) and publications 2.1. – 2.5., 3.1., 3.2., 3.5., 3.7., 3.8., 3.11., 3.13.-3.15., 4.1., 4.2. and 4.5.

The monograph "Drug resistance and virulence in uropathogens from *Enterobacterales.* Research in the period 1997 - 2021" provides a summary of modern scientific information on: the taxonomic status of the former family *Enterobacteriaceae*; the development of molecular biological methods and their role in the identification of bacteria; modern ideas about the urobiome; the mechanisms of drug resistance, incl. and the multiple one in uropathogens of the order *Enterobacterales*, its importance and the reasons why it has grown into a global problem, threatening the lives of an extremely large number of people in the world, due to the impossibility of treating a number of infectious diseases, in particular those related to UTI. In addition, this work contains results of own studies of isolates from samples of outpatients with infections of the urogenital tract, conducted in the period 1997-2021, which are the first such studies both in terms of volume and duration for the region of the city of Plovdiv and Southern Bulgaria. The data from these studies have been analyzed and compared with the publicly announced

information from the NCIPD and BulSTAP. An etiological map of urogenital tract infections in outpatients was made, as a result of which the species *Escherichia coli* was determined as the dominant etiological agent in the urogenital tract, followed by *Klebsiella* spp., *Proteus* spp., *Enterobacter cloacae*, *Staphylococcus* spp., *Enterococcus* spp., as well as, to a lesser extent, *Candida* spp., which dominated the genital samples. The results of the study of the antibiotic sensitivity of the urinary isolates give the author reason to show the need to improve the system for its monitoring both at the regional and national level by improving the National BulSTAR program. The studies presented in the monographs confirm that uropathogenic *E. coli* (UPEC) express chromosomally and plasmid-encoded virulence determinants. The studies carried out on the virulence characteristics of uropathogens from *Enterobacterales* confirm the importance of their adhesive potential for the success in the colonization of the urinary tract and the formation of biofilms as a defense strategy. On the basis of the obtained results, related to the antibiotic sensitivity to different groups of antibiotics of the studied isolates, the main conclusions were drawn.

I highly appreciate the enormous and consistent research work carried out over a long period of time which has resulted in very rich and representative information having both practical and scientific value and which has been analyzed and discussed in the light of modern scientific achievements in the specific field. I highly appreciate its scientific and practical value. 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.

Apart from the monographic work, Dr. Marhova's research in the field of microbial pathogenesis and their results are presented in 17 scientific papers. These works reflect the results of studies of the sensitivity of uropathogenic isolates to antibiotics, incl. and multiple such, as well as the production of broad-spectrum beta-lactamases (ESBL) by different species of bacteria isolated from the urogenital tract of patients (2.2., 2.3., 3.1., 3.5., 3.13., 3.14.). In some of these publications, as well as in others, the results of a study of the serum sensitivity of uropathogenic bacteria are presented (2.1., 3.2., 3.11., 3.13.). Results of research on virulence factors and proof of genetic determinants for them are presented in publications 2.3., 3.11., 3.14., 3.15. (biofilm-forming capacity, type I pili formation, for PAP pili, capsular antigens, for toxins and serum resistance). Research related to the search for alternative approaches to antibiotics for the treatment of UTI is reflected in publications 2.4., 2.5., 3.8. and 4.1. Three review articles address the quorum sensing mechanism by which the expression of virulence characteristics in pathogens is regulated, incl. and uropathogens (3.7., 4.2.) and a review of lectin-based studies of bacteria and helminths (4.5.).

Ecology of microorganisms. Research in this scientific field covers 18 scientific publications (2.6. – 2.15., 3.3., 3.4., 3.6., 3.9., 3.10., 3.12., 4.3. and 4.4.).

A survey of the microbiological status and sanitary condition of several large Bulgarian dams was carried out in response to the interest in recent years in their use for aquaculture production, on the one hand, and the increased urbanization and development of coastal areas, which increases the risk of pollution by domestic waste water, on the other. The of these studies are presented in 6 scientific publications (2.6., 2.7., 2.8., 2.12., 3.10. and 4.4.). A complete metagenomic analysis of the planktonic bacterial community was made for two large and economically important dams for Bulgaria - Batak Dam and Tsankov Kamak Dam, and the results are reflected in publication 2.8.

An assessment of the ecosystems of protected wetlands in Southern Bulgaria was made, and its results were included in 2 scientific publications (2.10 and 2.14.).

Dr. Marhova has investigated enzyme production and optimization of the cultivation of strains with potential for application in bioremediation and biotechnological productions. The results of these studies have been published in 8 scientific works (2.9., 2.13., 3.3., 3.4., 3.6., 3.9., 3.12. and 4.3.).

Research on the condition and qualities of soils in urban environments and the creation of sustainable models for bioremediation and their management are published in 2 scientific papers (2.11. and 2.15.).

Papers related to the **educational activity** - papers 5.1. and 5.2. (minimum 2 according to the additional requirements of the PU).

The textbook "Biological Membranes" (work 5.2.) co-authored by Dr. Marhova is intended for students studying the discipline "Biological Membranes".

The Manual in Microbiology (work 5.1.), co-authored by Dr. Marhova, is intended for students from various specialties in the EQD "Bachelor" and various master's programs in EQD "Master".

Of the 36 scientific papers presented, 9 were published in Bulgarian, and the remaining 27 in English. Fifteen of the works (about 42%) were published in international scientific journals with an impact factor/rank - total IF=7.3 and total SJR=0.934. Among the journals I would mention *Land*, *Journal of Basic Microbiology*, *Bulgarian Journal of Agriculture Science*, *Biotechnology and Biotechnological Equipment*, *Comptes Rendus de l'Academiie bulgare des Sciences*. Two publications are in Q2 journals, 5 in Q3 and 8 in Q4. Fifteen of the works have been published in Bulgarian scientific journals, 5 - in proceedings of scientific forums, and the remaining 3 works are the monographic work, 1 textbook and 1 manual, published by the "Paisii Hilendarski" University Publishing House. All works of the candidate, with the exception of the monograph and 1 publication, are collective. Of these, 4 have one co-author, 10 - with two co-authors, and the remaining 20 have three or more co-authors, which reflects the multidisciplinary nature of the research conducted. Dr. Marhova is the first author of 8 of the scientific publications.

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

The scientific works of Dr. Marhova contain scientific contributions to the main areas in her research activity. The contributions are well differentiated and I fully agree with their formulation in the presented reference. Below I will summarize those that I consider more important.

Contributions to the field of Microbial pathogenesis

1. For the first time in the country, a representative, large-scale and original study of the etiological structure of the causative agents of urogenital infections in outpatients in the city of Plovdiv was conducted, covering a 24-year period, and an etiological map of urinary tract infections was compiled in southern Bulgaria. A correlation was established with BulStar and NCIPD data (monograph - 1.1.). The contribution has a scientific and scientific-applied nature.
2. On the basis of a systematic evaluation of the drug sensitivity of a large number of isolates from samples of outpatients, an increasing resistance to fluoroquinolone preparations, a wide spread of ESBL-producing strains, as well as those with multiple resistance were found, which is of extreme importance for the treatment of these infections. The contribution is of scientific and applied importance.
3. It has been shown that the resistance of isolates from people with UTI to quinolone antibiotics is plasmid determined. The importance of bacterial adhesive potential in urinary tract pathogenesis has been confirmed, and biofilm-forming ability and serum resistance have been identified as significant factors in bacterial adaptation to the urinary tract. This is a contribution of scientific importance.

4. An advanced experimental protocol was developed for the evaluation of serum resistance of *Enterobacterales* isolates, which could be proposed for use in clinical practice in the diagnosis of UTI. The contribution is of scientific and applied importance.
5. Studies of alternative approaches to antibiotics show that they change or modulate virulence characteristics of uropathogenic *E. coli* or exhibit activity also on other uropathogens, which could serve as a basis for their use in the treatment of UTI. The contribution is of scientific and applied importance.

Contributions to the field of Microbial Ecology

1. For the first time in Bulgaria, the applicability of microbiological indicators for water quality in ecological assessment of complex dams has been proven. A classification system adapted for the country has been developed for assessing water quality according to the number of sanitary indicators, total number of heterotrophic microorganisms, *E. coli*, fecal coliforms and streptococci. The contribution is of scientific and applied importance.
2. For the first time in Bulgaria, data from a metagenomic analysis of the structure of microbial communities in complex and economically significant dams are presented, which is progress in elucidating the microbial ecology of water bodies. This contribution is of scientific value.
3. For the first time, the ecological potential of economically significant dams has been determined, according to the information available in the Basin Directorates (DB) and the direct monitoring carried out in the water area of these dams. This contribution is of scientific and applied importance.
4. For the first time, results of studies of spatial differences and physiological diversity of soil microbial communities in natural wetlands and constructed rice fields in the Maritsa River Basin, protected under the Birds Directive 2009/147/EC as natural habitats, are presented. The contribution is of scientific value.
5. A scientific-applied contribution is the selection of a promising strain of *Bacillus thuringiensis* - producer of extracellular proteases and the development of laboratory technology for obtaining a partially purified enzyme preparation.

Contributions to academic education

1. Contributions to academic education are the presented textbook 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 prerequisites for a good university education.

- The textbook "Biological Membranes" presents and summarizes in three sections the modern knowledge about the structure and functions of biological membranes - biochemical composition, structure of biological membranes and features of membrane components; basic functions of membranes; specific membranes in the organismal world. It is intended for students studying the discipline of the same name. In addition to being a textbook, this work can be used by students from various specialties, doctoral students, teachers, etc. as a source of additional knowledge about biological membranes beyond that included in more general biological disciplines.
- The Manual in Microbiology is constructed thematically in accordance with the curricula of the course of Microbiology for different specialties in the bachelor's and master's degree of PU. The exercises are grouped thematically in such a way as to provide the necessary knowledge for the relevant specialty. The

manual provides knowledge and skills for the classic techniques in microbiological laboratory practice, but also for basic modern molecular genetic methods widely used in microbiological research.

The publications of Dr. Marhova receive recognition from the scientific community through their citation. A list of citations in scientific publications, monographs, collective volumes, referenced and indexed in world-famous databases of scientific information (Web of Science and Scopus) is presented, which contains 66 citations, almost all of which are from foreign authors. I would like to note that 2 self-citations are included in the citations of paper 2.5. So, the actual number of citations is 64. Eleven of Dr. Marhova's scientific works, which cover both scientific fields in her research activity, received citations. No negative citations were observed. With the highest number of citations to date are 5 scientific publications, presenting results from the field of microbial pathogenesis (2.4., 2.3., 2.2. - a total of 28 citations) and microbial ecology (2.8 and 2.6. - a total of 17 citations). According to Scopus, the h-index is 6. Most of the citations of the scientific works of Dr. Marhova are in scientific journals with a high impact rank (*Antimicrobial Resistance and Infection Control*, *Advanced Functional Materials*, *Osong Public Health and Research Perspectives*, *Food Control*, *Molecules Frontiers in Microbiology*, *Journal of Ethnopharmacology*, *Plants*, *Chemosphere* (Q1); *Evidence-based Complementary and Alternative Medicine*, *Microbial Pathogenesis*, *Journal of Microbiology and Biotechnology*, *Water Environment Research*, *Antibiotics* (Q2) and others).

On the basis of the presented scientific works and their assessment, I firmly believe that the candidate fulfills the minimum national requirements for scientific and teaching activities for the academic position of "Associate Professor", 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 „Associate Professor“	Fulfillment by the candidate
A	Indicator 1	50	50
B	Indicator 3 or 4	100	100
C	Sum of indicators from 5 to 10	200	211
D	Sum of points in indicator 11	50	128
E	Sum of indicators from 12 to the end	-	124
Total		400	613

The candidate also fully meets the additional requirements of the Faculty of Biology for the academic position "Associate Professor", adopted by Decision of the FC, protocol No. 246/12.07.2019. Her activities, required by the cited decision, are noted in the relevant places in the review.

4. Assessment of the candidate's personal contribution

The scientific works presented for review are mostly collective works (excluding the monographic work and one of the publications). The co-authors of the collective publications possess different specific competence, which is a prerequisite for the successful implementation of complex scientific research, such as the majority of the presented scientific works. I believe that the personal contribution of the candidate in the works presented is clearly distinguishable and visible, given the scientific specialization in the mentioned two scientific fields of Dr. Marhova.

5. Critical remarks and recommendations

I have no critical remarks and recommendations on the materials and documentation submitted by Dr. Marhova for participation in the competition, as well as on her overall academic activity.

6. Personal impressions

I do not have direct personal impressions of Dr. Marhova, but as a reviewer of a doctoral thesis developed under her scientific supervision, I was left with the feeling of a hardworking, serious, responsible and very demanding researcher and supervisor. These impressions of mine have been confirmed after a thorough review of her teaching, research, publication and organizational activities in connection with the current competition for the academic position "Associate Professor".

CONCLUSION

The documents and materials presented by Assistant professor, Dr. Mariana Marhova, to participate in the competition for the academic position "Associate Professor", meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Rules for its Implementation and the Regulations for the Development of the Academic Staff of PU "Paisii Hilendarski".

The candidate in the competition has submitted a sufficient number of scientific works published after obtaining the degree "Doctor". The candidate's scientific works contain scientific and scientific-applied contributions and have received recognition from the scientific community through citations in prestigious international scientific journals. Dr. Marhova's educational and teaching activity is voluminous and diverse. The textbook and the laboratory manual of which she is the co-author, are a contribution to academic education at Plovdiv University, which I highly appreciate. I believe that the scientific and teaching qualification of Dr. Marhova is undoubted.

The achievements of Dr. Marhova in her overall academic activity - teaching, research, administrative and organizational, correspond to the minimum national requirements, as well as to the additional requirements of the Faculty of Biology, adopted in connection with the Regulations of the PU for the application of The Law on the Development of the Academic Staff in the Republic of Bulgaria and even significantly exceed them.

The review and evaluation of the materials and scientific works presented in the competition, the analysis of their significance and of the scientific, scientific-applied contributions contained in them give me arguments to convincingly give my positive assessment and to recommend to the esteemed Scientific Jury to prepare a report-proposal to the Faculty Council of the Faculty of Biology to awered the academic position "Associate Professor" to Assisant Professor Dr. Mariana Ivanova Marhova-Koseva at Plovdiv University "Paisii Hilendarski" in: Area of higher education 4. Natural sciences, mathematics and informatics, Professional field 4.3. Biological Sciences (Microbiology – Microbial Pathogenesis).

01.04. 2023 Reviewer: .....

Sofia (Prof. PhD Penka Moncheva)