To:
The Chair of the Scientific Jury,
appointed by Order № I-142/01.10.2025
of the Director of the Institute of Microbiology, BAS

OPINION

by
Assoc. Prof. Lyubomira Dimitrova Yocheva, Ph.D.
Sofia University "St. Kliment Ohridski", Faculty of Medicine,
Department "Biology, Medical Genetics and Microbiology"

Regarding: Competition for the academic position of "Associate Professor" in Higher Education Field 4. Natural Sciences, Mathematics and Informatics, Professional Field 4.3 Biological Sciences, Scientific Specialty Microbiology, announced in State Gazette no. 66 of 12.08.2025, for the needs of the Department of Infectious microbiology, Laboratory "Bacterial virulence, resistance and new antimicrobial agents" at Institute of Microbiology, BAS (IMicB-BAS).

Chief Assistant Dr. Lyudmila Dimitrova is the sole candidate in the competition.
I declare that there is no conflict of interest between myself and the candidate, Chief Assistant Dr. Lyudmila Dimitrova, in accordance with §1, items 2a, 3, 4, and 5 of the Additional Provisions of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB). The documents provided to me for the preparation of this opinion meet the requirements of IMicB-BAS and were submitted on time.

1. Candidate's Biographical Data

a. Education

Chief Assistant Dr. Lyudmila Dimitrova received her higher education at Sofia University "St. Kliment Ohridski", obtaining a Bachelor's degree in specialty "Ecology and environmental protection " in 2011 and a Master's degree in the same specialty in 2013.
In October 2013, she was enrolled as a full-time PhD student at the Department of Infectious Microbiology, IMicB-BAS and in 2019 she obtained the degree of Doctor with a dissertation titled "Biological activity of extracts and compounds isolated from *Geum urbanum* L.".

b. Professional Development

From 2016 to 2020, she worked as an "Assistant" in the above-mentioned department. In 2020, she was promoted to "Chief Assistant" in the same department. To date, she has over 9 years of professional experience.

c. Qualification

Throughout her scientific career, Chief Assistant Dimitrova has acquired significant research experience and expertise in the field of microbiology; scanning, transmission and fluorescence microscopy; bacterial genetics and microbial ecology.

2. Submitted Documents and Fulfillment of Minimum Requirements for the Academic Position

The submitted documents are complete and well-organized. The accompanying tables and summaries show that the candidate not only meets the national minimum requirements but also fulfills the additional criteria of IMicB-BAS for the academic position of Associate Professor.

Dr. Dimitrova exceeds the required 100 points for indicator C and 220 points for indicator D under the LDASRB requirements, as the points under the indicators are 140 and 254, respectively. The high ranking of the scientific journals where the presented articles are published is notable. They are peer-reviewed and indexed in globally recognized databases and have a high impact factor. These facts demonstrate that Dr. Lyudmila Dimitrova is well-recognized in both the Bulgarian and international scientific communities. This is further confirmed by the high citation count - 491 citations in the Scopus database.

In this competition, Dr. Dimitrova has presented a total of 20 scientific papers, refereed in Web of Science and Scopus with a total impact factor of 62.435.

Seven publications were presented as habilitation work, and 13 publications with an impact factor/SJR were included under indicator "D". All publications are distributed as follows: 11 papers - in Q1 journals, 3 - in Q2, 4 - in Q3 and 2 - in Q4.

Dr. Dimitrova's scientific works have been published in prestigious international journals such as *Molecules, Heliyon, Plants, Antibiotics, Toxics, Biomedicines*.

Additional criteria for "Associate Professor" at IMicB-BAS

Dr. Lyudmila Dimitrova meets the additional requirements specified in the Regulations of IMic-BAS. She has held the position of "Chief Assistant" for five years. The 20 publications presented in the competition do not overlap with those used to obtain the PhD degree. In six of the scientific works, Dr. Dimitrova is the first author. She exceeds the criteria in terms of number of citations (491 - Scopus; 660 - Google Scholar at requirement 100), impact factor (62.435 at requirement 20), h-index: 10 - Scopus (at requirement 5), as well as participation in scientific projects (26 of which 24 national and 2 international projects at requirement 3).

Her scientific work as a young microbiologist has been honored with a number of awards, the most significant of which are the Awards of the "Acad. Prof. Dr. Stefan Angelov" Foundation for the best work of a young microbiologist for 2017, 2019, 2020 and 2023. In 2022, she was among the nominees for the "Pythagoras" Award for a young scientist in the field of Life Sciences and Medicine.

3. Teaching Activities

Dr. Lyudmila Dimitrova leads practical exercises at the Faculty of Medicine of Sofia

University "St. Kliment Ohridski" in the discipline of *Microbiology* for students in specialty "Medicine".

4. **Project Activities**

Dr. Dimitrova has participated as both a leader and a team member in **26 scientific projects**, including:

1) Two projects under European and international programs and funds
2) Eleven projects funded by the Bulgarian National Science Fund at the Ministry of Education and Science of the Republic of Bulgaria; in one of which she serves as the project leader
3) Two projects under National Programs financed by the Ministry of Education and Science of the Republic of Bulgaria
4) Two institutional projects financed by the Bulgarian Academy of Sciences; in both of them she serves as the project leader
5) Four projects with the Faculty of Medicine, Sofia University "St. Kliment Ohridski"
6) One project financed by inter-academic contracts and agreements
7) Three projects under the Standards Development Program
8) One project with the Antibiotic company, Razgrad

5. **Scientific and Applied Contributions**

The research interest of Chief Asst. Dimitrova is focused mainly on the study of the antimicrobial activity of various extracts and compounds of natural origin and testing their influence in animal models. The scientific topic in this direction is particularly relevant in view of the increasing prevalence of multidrug-resistant strains not only in obligate pathogens, but also in opportunistic bacteria such as *Pseudomonas aeruginosa*. Therefore, the scientific interests of Dr. Dimitrova are directed to new preparations of natural origin, such as plant extracts, which are phytocomplexes, to which it is considered that the bacteria are practically impossible to develop resistance. In her research, antineoplastic, antiviral and *in vivo* toxicological evaluation of extracts from the plant *Geum urbanum* L. was performed. The scientific contributions in this area are of a fundamental nature. They are associated with the demonstration of the phenotypic inhibition of the Quorum sensing system of *P. aeruginosa* after treatment with ethyl acetate extracts of *G. urbanum* L. Their antineoplastic activity on bladder cancer cell lines, as well as moderate antiviral activity of butanol and methanol extracts from aerial parts and roots against *Herpes simplex virus* 1 (HSV-1) and of ethyl acetate extracts - against *Human adenovirus* (HAdV-5) and *Coxsackie* B (CVB1) viruses were established. At the same time, the safety of the extracts was proven due to the absence of acute and subacute toxicity at the highest applied test concentration. A strong redox-modulating capacity of two ethyl acetate extracts was established. Extract from roots inhibits α-glucosidase, which has a key role in glucose absorption in the gastrointestinal tract. Also, a better cytotoxic profile of butanol extracts against Burkitt lymphoma was established, compared to ethyl acetate extracts from aerial parts and roots, which induce cell apoptosis and suppress the “oncogenic” superoxide.

Traditionally, volatile organic solvents with significant toxicity are used for the extraction of biologically active substances from natural sources. Dr. Lyudmila Dimitrova is involved in the development of modern approach in “green” chemistry for the exclusion of ethanol in the extraction of phenolic compounds from poplar propolis, as well as in the extraction of total phenols and total flavonoids from *Plantago major* and *Sideritis scardica*. Natural deep eutectic solvents (NADESs) are primary metabolites and represent a new alternative to toxic solvents. The results obtained by Dr. Dimitrova confirm the potential of NADESs for the extraction of biologically active substances from medicinal plants and show that they improve the effect of bioactive extracts against the test microorganisms *S. pyogenes*, *E. coli*, *S. aureus* and *C. albicans*. For the first time, her research demonstrates the potential of NADESs to be used as solvents for the extraction of biologically active compounds of poplar-type propolis.

These studies are particularly promising for the development of new healthy phytopreparations and biocontrol agents for the prevention of nosocomial infections caused by biofilm-forming and multidrug-resistant bacteria.

The second field in Dr. Dimitrova's scientific investigations is related to the establishment of microbiological diversity in different ecological niches - water, soil and animals, as well as their potential for application in biotechnology, such as the isolation of cellulose-degrading microorganisms and optimization of environmental conditions.

Anaerobic degradation is carried out by mesophilic and thermophilic microbial communities. Dr. Dimitrova is involved in the study of species diversity in such microbial consortia. Through metagenomic analysis, the main taxa involved in lignocellulose degradation have been identified. She proves that hydrogen generation is mainly due to *Proteiniphilum saccharofermentans*. On the other hand, methane production is mainly associated with several species of methanogenic archaea (*Methanobacterium formicicum, Methanosarcina spelaei, Methanothrix soehngenii* and *Methanobacterium beijingense*). In a system of two continuously stirred bioreactors used for the degradation of corn extract, it has been shown that bacteria of the *Firmicutes* type predominate, and the dominant methanogens are the genera *Methanothrix* (8.03%) and *Methanosarcina* (3.39%). Dr. Dimitrova also participated in the successful isolation and enrichment of two aerobic (at 37 °C and 50 °C) and one anaerobic microbial consortium from an anaerobic bioreactor for biogas production by continuous subculturing. Through sequence analysis it was shown that the dominant phyla are *Bacillota* and *Bacteroidetes* and genera - *Paraclostridium, Defluvitalea, Anaerobacillus, Acetivibrio, Lysinibacillus, Paenibacillus, Romboutsia, Terrisporobacter, Clostridium, Sporanaerobacter, Lentimicrobium*, etc. in different proportions depending on the cultivation conditions and the stage of the process. Some of these representatives are cellulolytic and hemi cellulolytic microorganisms. The lyophilization performed proves to be a suitable method for long-term storage of the most active consortium. In another publication by Dr. Dimitrova, a bacterial consortium was selected and genetically identified for the purpose of cellulose biodegradation and waste recycling during space flights, with the most numerous species being *Bacteroides oleiciplenus*, *Clostridium butyricum* and *Ruminiclostridium papyrosolvens*.

Dr. Dimitrova's studies on the spread of pathogenic strains of *E. coli* and *Yersinia enterocolitica* among pigs, their antimicrobial resistance and potential for biofilm formation confirm the carriage of blaSHV/blaTEM and ampC genes in *E. coli* and ampicillin resistance in *Y. enterocolitica*. These results confirm the need to review the globally applied policy on antibiotics in human medicine and animal husbandry.

The original scientific contributions presented reflect Dr. Lyudmila Dimitrova's the long-term systematic and research in the field of antimicrobial activity of various extracts and compounds of natural origin, related to solving current issues for the control and prevention of nosocomial infections. The presented metrics and significant contributions in the field of microbiology demonstrate that the candidate Dr. Lyudmila Dimitrova has acquired extensive knowledge and experience, providing a solid foundation for her future academic development.

6. Critical Notes and Recommendations

I have no critical remarks or recommendations regarding the submitted documentation.

I am convinced that Dr. Lyudmila Dimitrova is a responsible and competent scientist, sought after as a collaborator for team-based research and as a reviewer in prestigious journals (she has prepared 42 anonymous reviews so far). I believe that she has the necessary competence, professional experience and scientific enthusiasm to meet the challenges of a higher academic position. I believe that she is fully capable of managing and leading national and international scientific projects, as well as mentoring young scientists, graduate students and doctoral students, encouraging their career development in the field of microbiology.

7. Conclusion

Dr. Lyudmila Dimitrova meets and even exceeds in all indicators the minimum requirements of the Academic Staff Development Act (ASDA) and the internal criteria of IMicB-BAS for occupying the academic position of "Associate Professor". The scientific achievements attest to her extensive professional experience and competence in the field of microbiology. Considering her successful scientific research, teaching and expert activity in scientific areas related to solving important issues for epidemiology, medicine and ecology, **I give my strong positive evaluation and recommend to the esteemed scientific jury to award Dr. Lyudmila Lyudmilova Dimitrova the academic position of "Associate Professor" in the scientific specialty of *Microbiology***, for the needs of the Department of Infectious Microbiology, Laboratory "Bacterial Virulence, Resistance and New Antimicrobial Agents" at the Stephan Angeloff Institute of Microbiology – BAS.

Date: November 21, 2025 Signature:
Sofia Assoc. Prof. Lyubomira Yocheva, PhD