



SCIENTIFIC OPINION

From Assoc. Prof. Dr. Anna Atanasova Tomova

Sofia University „St. Kliment Ohridski“, Faculty of Biology

of the materials submitted for participation in a competition for occupying the academic position of "Associate Professor", announced in State Newspaper No. 55/28.06.2024 for the needs of the Department of General and Industrial Microbiology at the Faculty of Biology, Sofia University "St. Kliment Ohridski".

in the field of higher education 4. Natural Sciences, Mathematics and Computer Science;
Professional field **4.3. Biological Sciences; Scientific specialty "Microbiology" (General Microbiology and Phytopathogenic Bacteria)**

By order № 38-468/ 22.07.2024 of the Rector of Sofia University "St. Kliment Ohridski" (SU) I am included in the Scientific Jury to select an "Associate Professor" for the needs of the Department of General and Industrial Microbiology. The only candidate under the announced competition was Dr. Yoana Krasirimova Kizheva, currently holding the position of Assistant Professor at the Department of General and Industrial Microbiology at the Faculty of Biology (BF), Sofia University "St. Kliment Ohridski" (SU). As a member of the scientific jury, I declare that I have no common articles with the applicant.

Brief information about the career development of Dr. Yoana Kizheva

Dr. Kizheva graduated with a Bachelor's degree in Biology and Chemistry, after which she obtained a master's degree with the qualification of Molecular Biologist - Master of Microbiology and Microbiological Control at Sofia University "St. Kliment Ohridski". She successfully defended her PhD thesis on "Phytopathogenic bacteria of the genus *Xanthomonas* on *Solanum lycopersicum*" in 2014 to obtain the educational and scientific degree "Doctor", BF, SU. Until February 2017 Dr. Kizheva worked as a part-time assistant in microbiology at the Department of General and Industrial Microbiology, after which she won a competition for chief assistant professor at the same department. Her teaching work is combined with several administrative activities at the Faculty of Biology as a Member of the Mandate Committee at the General Assembly (2018 – 2024); Member of the Council of Specialties in the Bachelor's degree (Specialty Biomanagement and Sustainable Development) (07. 2024); Member of the Council of Specialties in the Bachelor's degree (specialty Molecular Biology) (2022), Member of the Commission for the preparation of a self-assessment report for accreditation of the doctoral program "Microbiology" (2020 - present); Member of the committee for determining additional funds for PhD students. In the Department of „General and Industrial Microbiology“, Dr. Kizheva performs the activities of Scientific Secretary and is responsible for "Doctoral Students", "Scientific Research Activities" and "Postgraduate Qualifications". In her scientific career so far, she has participated in the implementation of 18 projects, for two of which she was a leader. The active research work of Dr Kizheva is confirmed by her participation in 40 conferences, of which 19 are international, she was awarded a Diploma by the Foundation „Acad. Prof. Dr. Stefan Angelov" for the best scientific work of a young Bulgarian microbiologist in 2018 and won three awards from participation in congresses in Bulgaria. As an expert, Dr. Kizheva is an editor of the scientific journal Pathogens, prepares reviews of

scientific papers and is involved in the implementation of national and European scientific networks. Since 2020, she has been a member of the scientific jury for awarding a doctoral scholarship from the Carol Knowledge Foundation.

Description of the presented scientific papers and scientometric indicators

The set of materials provided includes all the necessary documents according to the Rules of Sofia University for admission to participate in the competition.

Table 1. Minimum national requirements for "Associate Professor" in Field 4: Natural Sciences, Mathematics and Informatics according to the Development of Academic Staff in Republic of Bulgaria Law and Regulations on the implementation of the Law.

Indicators group	Content	Requirements for Associate Professor	Indicators of Assist. Prof. Dr. Yoana Kizheva
A	Indicator 1	50	50
Б	Indicator 2	-	-
В	Indicators 3 и 4	100	100
Г	Sum of indicators from 5 to 9	200	221
Д	Sum of indicators from 10 to 12	50	222
Е	Sum of indicators from 13 to the end	-	-

Dr. Kizheva participated in the competition for "Associate Professor" with 18 scientific papers with a total impact factor of 30.503, and they do not include the publications for the PhD Degree. As a habilitation work, 4 publications are included, all in journals with rank Q1. Of the 14 publications with impact factor/SJR presented under indicator "D", four are in journals with Q2, seven with Q3 and three with Q4. Dr. Kizheva's scientific works have been published in prestigious international journals such as Pathogens, Plants, Journal of Bioscience and Heliyon.

The 18 publications submitted for participation in the competition were cited 111 times without autocitations of all authors in Scopus, corresponding to 222 points in indicator "D", which significantly exceeds the 50 points required for participation in the competition for associate professor. More than 80% of citations are in journals with Q1 and Q2. The reviewed scientific production shows that the candidate fully meets the minimum national requirements for acquiring the academic position of "Associate Professor" and even exceeds the required number of points in indicators G and D.

Additional criteria for "Associate Professor" at Sofia University "St. St. Kliment Ohridski".

The candidate meets the additional requirements reflected in the Regulations of Sofia University. She has held the position of "Chief Assistant Professor" for 8 years and the publications presented as a habilitation work fully correspond to the field of the announced competition. The report on Dr. Kizheva's workload for the previous three years shows annual classroom occupancy of more than twice as much as required for the position. The total academic workload of the candidate on average for the year, for the specified period, amounts to 671 hours, with a standard of 360 hours. This includes lectures and practical classes for Bachelor's and Master's degrees, full-time and part-time education, and scientific supervision of Dr. Kizheva of 22 graduates, of which 15 in the Master's Degree and 7 in the Bachelor's Degree. The great thematic diversity of the disciplines in the field of microbiology and microbiological control is impressive.

Main directions in research work and most important contributions

In the scientific activity of Dr. Kizheva, five main directions are formed: 1, Molecular identification and intraspecies diversity of phytopathogenic bacteria and study of their distribution and relationships with basic and alternative host plants; 2, Isolation and characterization of bacteriophages with the potential for biocontrol of plant diseases caused by phytopathogenic bacteria; 3, Study of the biology of lactic acid bacteria isolated from different natural habitats concerning the selection of potential probiotic strains and with the assessment of the microbiological quality of probiotic food supplements, 4, Study of virulent potential and antibiotic resistance among opportunistic pathogenic bacteria isolated from different habitats; 5, Testing of new substances with potential as antibacterial agents.

For these areas of investigations, Dr. Kizheva formulated a number of scientific contributions of a fundamental or applied nature, most of which are original. The inclusion of students - bachelor and master in part of the research carried out allows the assimilation of new laboratory methods for them, which gives the candidate grounds to make theoretical and methodological contributions to the educational process.

Direction 1:

Research in this area is divided into two subdirections. The subdirection "Molecular Identification and Intraspecific Diversity of Phytopathogenic Bacteria" includes experiments aimed at optimizing existing routine molecular biological approaches for species identification and revealing intraspecies diversity among representatives of phytopathogenic bacteria of the genus *Xanthomonas*. As an alternative to the application of PCR amplification with species-specific primers, which does not achieve a correct distinction between the species *X. euvesicatoria* and *X. perforans*, Dr. Kizheva developed an approach with higher discrimination and allowing accurate and rapid species differentiation. The study of genetic diversity among phytopathogenic xanthomonads, which is important from an epidemiological point of view, is another focus of Yoana Kizheva's research. For the first time in Bulgaria, she studied the genetic diversity in the Bulgarian population of the species *X. vesicatoria* and *X. gardneri* and proved genetic heterogeneity within the species *X. vesicatoria*. Under the second sub-direction "Study of the distribution and relationships of phytopathogenic bacteria with basic and alternative host plants", the research is aimed at studying the pathogenesis of *X. euvesicatoria*, studying the possibilities for genetic transformation of this phytopathogen, as well as proving new hosts of phytopathogenic bacteria.

Based on the results of the research, original contributions of fundamental and applied nature have been formulated, namely:

- expanding knowledge about the pathogenesis and close interaction between *X. euvesicatoria* and its sensitive host plants
- development of a methodological approach for obtaining a recombinant plasmid carrying genes for green fluorescence and gentamicin resistance and its transfer to *X. euvesicatoria*.
- for the first time in Bulgaria, the causative agent of bacterial tomatoes scab *X. euvesicatoria* has been identified by phenotypic and molecular genetic methods, which complements the range of hosts of this pathogen.
- an in-depth review of the species and racial composition, dynamics and pathotype of phytopathogenic bacteria causing bacterial tomatoes and peppers scab in Bulgaria has been made.
- for the first time in Bulgaria, a detailed phenotypic and genotypic characterization of pathogenic *Curtobacterium flaccumfaciens* strains has been made and the role of tomato and pepper plants as alternative hosts of this species has been proven

Directions 2:

Dr. Kizheva's research in this area is aimed at studying the potential of bacteriophages isolated from natural habitats in Bulgaria to combat phytopathogenic *Xanthomonas* bacteria. The results obtained complement the knowledge gained so far in the field worldwide. The original contribution of these investigations is that for the first time in Bulgaria, bacteriophages with the potential of biocontrol agents have been isolated and characterized and a laboratory collection of them has been created. Dr. Kizheva developed a molecular-genetic approach for rapid and accurate quantification of bacteriophages, in connection with their potential industrial application for the development of phage preparations for plant protection.

Direction 3:

The rapid and accurate species identification of lactic acid bacteria is a key stage in their research for use in probiotic food supplements and traditional fermented Bulgarian foods. In this regard, Dr. Kizheva focuses her attention on optimizing an existing molecular-genetic algorithm for accurate species identification. She also applied this approach when studying the lactic acid microflora in the gastrointestinal tract of the garden snail *C. aspersum* and for the first time reported the presence of species of the genus *Lactobacillus* in it. For the first time, she detected the presence of the species *Levilactobacillus koreensis* and *Levilactobacillus yonginensis* in fermented vegetable foods, as well as *Lactobacillus spicheri*, *Lactobacillus paralimentarius*, *Lactobacillus kimchi* and *Lactobacillus sanfranciscensis* as microflora of fermented rye doughs.

Research in this direction continues with the characterization of key characteristics concerning the probiotic potential of lactic acid bacteria. A scientific fundamentally-applied contribution of the candidate's work is the study of the main characteristics concerning the probiotic potential of both newly isolated Bulgarian lactic-acid strains of and those included in commercial probiotic products.

Direction 4:

In addition to phytopathogens, Dr. Kizheva studies the diversity and pathogenic potential of opportunistic human pathogens isolated from atypical habitats, such as plants. The work contributes to the enrichment of the existing knowledge in the scientific literature about the diversity of the so-called cross-pathogens in plants and their ability to infect representatives of other biological kingdoms. Another part of the research in this area is related to the study of genes for antibiotic resistance and virulence in enterococci, isolated from food products and the gastrointestinal tract of snails. A significant original contribution of the work is the explored distribution of genetically determined antibiotic resistance and virulence among the studied enterococci strains and the created laboratory collection of them.

Direction 5:

The investigations in this area are conducted in response to the growing global demand for new biologically active substances with antimicrobial activity. Dr. Kizheva investigated the antibacterial effect of hemocyanin derived from the stone cancer *Eriphia verrucose* against significant clinical pathogens. For the first time, the antibacterial effect of the individual structural subunits of the studied hemocyanin has been proven, which determines their application in the development of antibacterial preparations. .

Conclusion

Based on the analysis of the presented scientific production's content and quality and the active teaching activity, the only candidate Assistant Prof. Kizheva exceeds the quantitative criteria for occupying the academic position of Associate Professor, according to the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations to him. She is an established scientist

with international recognition, publishing mainly in prestigious international journals. Assist. Prof. Kizheva has made significant contributions to phytopathogenic bacteria, bacteriophages as biocontrol agents, and antibiotic resistance and virulence spreading among bacteria. Her scientific work is accompanied by active editing and publishing activities. The professional competence of the candidate is evidenced by her participation in numerous conferences, training programs, scientific networks and commissions. Based on the above, I confidently support her candidacy and recommend to the members of the scientific jury to propose to the Faculty Council of the Faculty of Biology, Sofia University St. "Kliment Ohridski" to elect Assist. Prof. Yoana Kizheva for the academic position "Associated Professor" in the scientific specialty "Microbiology" for the needs of the Department of General and Industrial Microbiology, BF, SU.

10.10.2024 г.

Prepared the scientific opinion:

Assoc. Prof. Anna Tomova