OPINION

by Assoc. Prof. Tsvetelina Sashkova Paunova-Krasteva,
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Concerning: Materials submitted for participation in a competition for the academic position of "Associate Professor", Area 4. Natural Sciences, Mathematics, and Informatics,
Professional field 4.3. Biological Sciences (Microbiology – General Microbiology and Phytopathogenic Bacteria).

Brief Information About the Competition

According to the announcement in the *State Gazette* No. 55 of 28.06.2024, for the academic position of "Associate Professor", there is one candidate: Chief Assistant Dr. Joana Krasimirova Kizheva. I am participating in this competition as a member of the Scientific Jury, formed by Order No. RD-38-468/22.07.2024, issued by the Rector of Sofia University "St. Kliment Ohridski", and based on the decision of the Faculty Council of the Faculty of Biology, protocol No. 12 of 16.07.2024. The documents presented by Dr. Kizheva comply with the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for its application, as well as the Regulations for the terms and conditions for acquiring scientific degrees and occupying academic positions at Sofia University "St. Kliment Ohridski".

Professional CV of the Candidate

Dr. Kizheva obtained her master's degree at Sofia University "St. Kliment Ohridski", Faculty of Biology, in the "Microbiology and Microbiological Control" program in 2010. She enrolled as a full-time doctoral student in the Department of "General and Industrial Microbiology", Faculty of Biology at Sofia University, where she obtained the educational and scientific degree of "Doctor" in 2014 with the dissertation: "*Phytopathogenic Bacteria of the Genus Xanthomonas on Solanum lycopersicum*". Since 2016, Dr. Kizheva has been working as a Chief Assistant in the Department of General and Industrial Microbiology, where she carries out both teaching (3 lectures and 12 practical courses) and administrative activities. She has supervised 7 bachelor's and 15 master's graduates. She has participated in interdisciplinary training modules, and has received several prestigious scientific awards, including one for "Best Work by a Young Bulgarian Microbiologist" in 2018. Dr. Kizheva is the author and co-author of 23 scientific publications in refereed and indexed journals, with a total impact factor of 30.503. She has participated in 18 national and internationally funded projects. Her research results have been presented at 40 national and international scientific forums, with 5 oral presentations and 35 posters. In 2024, Dr. Kizheva was invited as a guest editor for the journal *Pathogens* (MDPI) and has prepared 14 reviews for Bulgarian and international journals.

Implementation of Scientometric Indicators for the Academic Position of "Associate Professor"

Dr. Kizheva participated in the competition with 18 publications (12 of which she is the first author), with a total impact factor of 30.503 and an H-index of 7, which indicates that she meets and exceeds the minimum national requirements according to the ZRASRB. Specifically grouped:

- **Group A (minimum requirements – 50 points)**: Dissertation for the award of the "Doctor" degree: *Phytopathogenic Bacteria of the Genus Xanthomonas on Solanum lycopersicum* (2014). **Collected points: 50**

- **Group B, indicator 4 (minimum requirements – 100 points)**: this group includes 4 publications in quartile Q1. **Collected points: 100**

- **Group D, indicator 7 (minimum requirements – 200 points)**: This group includes refereed and indexed publications under indicator 7, as follows: 4 in quartile Q2, 7 in quartile Q3, and 3 in quartile Q4. **Collected points: 221**

- **Group D, indicator 11 (minimum requirements – 50 points)**: This indicator has been significantly exceeded, with 96 citations reported. **Collected points: 192**

- **Group E**: While no points are required for this group to hold the position of "Associate Professor", Dr. Kizheva has participated in numerous national and international scientific projects and programs.

As evidenced by Dr. Kizheva's presented scientometric indicators, she fulfill the required criteria set forth in the ZRASRB and its applicable regulations. Furthermore, her candidacy not only complies with these requirements but exceeds the minimum criteria in several areas.

Characteristics of the Main Scientific Directions and Most Important Contributions

The main scientific activities of Dr. Kizheva can be grouped into the following research areas:

- **Molecular identification and intraspecific diversity of phytopathogenic bacteria, and the study of their distribution and relationships with primary and alternative host plants**.

  Dr. Kizheva developed, optimized, and validated an approach based on restriction fragment length polymorphism (RFLP) analysis, which utilizes a specific combination of restriction enzymes in a defined sequence to determine the species identification of phytopathogenic bacteria from the *Xanthomonas* genus. The results led to the establishment of a unique restriction profile for rapid species differentiation. A significant aspect of the study of *Xanthomonas* species is the data related to their genetic diversity. Additionally, studies on genetic transformation and pathogenesis of *X. euvesicatoria* were carried out to investigate the bacteria's behavior in plant tissues. Information on the species and race composition of phytopathogenic bacteria causing scab in tomato and pepper, including their dynamics and pathotypes, has been

reported. A detailed phenotypic and genotypic characterization of *Curtobacterium flaccumfaciens* representatives was performed, and the role of their alternative hosts was confirmed.

- **Isolation and characterization of bacteriophages with potential for biocontrol of plant diseases caused by phytopathogenic bacteria**.

  The genome of bacteriophage BsXeu269p/3 was isolated and sequenced, providing new insights into its ability to inhibit the spread of the phytopathogen *X. euvesicatoria*, thus limiting bacterial scab disease. An efficient molecular genetic approach was optimized for the detection and quantification of bacteriophages, both in natural and laboratory samples. A laboratory collection of isolated and characterized bacteriophages was created, with the aim of applying them in the biocontrol of plant diseases.

- **Study of the biology of lactic acid bacteria isolated from various natural habitats, in connection with the selection of potential probiotic strains and the evaluation of the microbiological quality of probiotic nutritional supplements**.

  Using a molecular genetic algorithm, newly isolated strains of lactic acid bacteria from commercially available probiotic products, kvass, and traditional fermented foods (such as kefir, white brined cheese, and pickles) were identified at the genus and species level. The microflora of the gastrointestinal tract of the garden snail *Cornu aspersum* was also successfully studied. A range of sequencing techniques was applied, leading to the identification of new species from their natural habitats and the characterization of their probiotic potential.

- **Investigation of virulence potential and antibiotic resistance in opportunistic pathogenic bacteria isolated from various habitats**.

  A detailed characterization of genes encoding key virulence factors and antibiotic resistance was conducted in pathogenic bacteria isolated from tomatoes and peppers. Using conventional and multiplex PCR analysis, genes for antibiotic resistance and virulence were identified in *Enterococcus* species isolated from traditional Bulgarian foods and human breast milk. A rich laboratory collection of these isolates was established.

- **Testing new substances with potential as antibacterial agents**.

  The antimicrobial potential of hemocyanin, obtained from the hemolymph of the stone crab *Eriphia verrucosa*, was tested. Chromatographic methods were employed to obtain glycosylated structural units that demonstrated antibacterial activity against various pathogenic bacteria, in contrast to native hemocyanin.

In my opinion, the original contributions of a scientific, fundamental, and applied character can be summarized as follows:

• A molecular genetic approach based on restriction analysis (RFLP) was developed for the identification of bacterial species causing bacterial scab on tomato and pepper, which also demonstrated the genetic heterogeneity within the *X. vesicatoria* species.

• For the first time in Bulgaria, the species *X. euvesicatoria* was isolated and identified, and the stability of a constructed recombinant plasmid in the cells of this pathogen was evaluated.

• Strains belonging to the species *Curtobacterium flaccumfaciens* have been characterized phenotypically and genotypically, and the role of their natural hosts has been demonstrated.

• A molecular-genetic approach was developed for the characterization and quantification of bacteriophages, and their potential to prevent bacterial scab disease on plants was studied. A laboratory collection of these bacteriophages was also created.

• The presence of lactic acid bacteria of the genus *Lactobacillus* isolated from the gastrointestinal tract of the garden snail *C. aspersum* was reported for the first time.

• Data on the presence of lactic acid microflora in traditional fermented Bulgarian foods and rye dough with proven probiotic potential were reported for the first time.

• The diversity, genetically determined antibiotic resistance, and virulence of *Enterococci* strains isolated from traditional Bulgarian foods and breast milk were studied.

• For the first time in Bulgaria, the antibacterial activity of structural glycosylated subunits of hemocyanin obtained from *Eriphia verrucosa* was established.

Conclusion

The documents and materials presented by Dr. Kizheva meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the regulations for its implementation. Based on the presented extended reference of scientific publications and the contributions therein, I believe that Dr. Kizheva emerges as an established scientist in the fields of: identification, sequencing, and expression of genes; construction and transformation of recombinant plasmids; determination of phenotype and pathotype of various phytopathogenic bacteria; detailed study of the probiotic potential of the lactic acid microflora of various foods; and study of antibiotic resistance and virulence of opportunistic pathogens.

Her scientific publications, along with their high impact in renowned journals, are proof of her international contribution to science. In addition, Dr. Kizheva has made significant contributions to educational and administrative activities at "St. Kliment Ohridski." I am confident that Dr. Kizheva possesses all the necessary qualities of an authoritative scientist with a clear scientometric profile and interests in Microbiology, with an emphasis on phytopathogenic bacteria.

Based on the above, I confidently give my positive assessment and recommend that the honorable Scientific Jury approve the candidacy of Chief Assistant Professor Joana Krasimirova Kizheva, Ph.D., for the academic position of "Associate Professor" in professional direction 4.3. Biological Sciences (Microbiology – General Microbiology and Phytopathogenic Bacteria).

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Reviewer:

/Assoc. Prof. Tsvetelina Paunova-Krasteva/