### **REVIEW**

of the materials submitted for participation in the competition for

### "ASSOCIATE PROFESSOR"

in the field of higher education 4. Natural sciences, mathematics, and informatics;

professional field 4.3. Biological sciences;

scientific specialty "MICROBIOLOGY" (General Microbiology and Phytopathogenic Bacteria), announced in SG No. 55 from 28.06.2024

### by Associate Professor Ventsislava Yankova Petrova

designated as a member of a scientific jury, according to Order № РД 38-468/22.07.2024 of the Rector of Sofia University "St. Kliment Ohridski".

#### 1. Professional and career development of the candidate

Assistant Professor Dr. Yoanna Krasimirova Kizheva is the only candidate in the competition for Associate Professor, announced for the needs of the Department of "General and Industrial Microbiology", Biological Faculty at Sofia University "St. Kliment Ohridski". She graduated with a bachelor's degree in "Biology and Chemistry" in 2007 and a master's degree in "Microbiology and Microbiological Control" in 2010 at the BF of SU. She successfully defended a Ph.D. thesis in 2014 on the topic: "Phytopathogenic bacteria of the genus *Xanthomonas* on *Solanum lycopersicum*". Since 2015, she has successively held the following positions in the Department of General and Industrial Microbiology: part-time assistant in Microbiology (2015-2016), assistant professor from 2017 to now. In the period 2017-2019, she additionally qualified by participating in 4 specialized interdisciplinary training courses on: "New compounds and nanotechnologies for improving the quality of life" (2017); "Innovative research and techniques in biomedicine" (2017); "Mathematical approaches for application in biomedicine" (2017); and "Entrepreneurs in science"

- a program conducted by the Karol Knowledge Foundation (2018 - 2019).

An important factor in the development of Dr. Yoanna Kizheva as an established teacher and researcher is her scientific research on the molecular identification and intraspecific diversity of phytopathogenic bacteria, isolation, and characterization of bacteriophages as an agent for biocontrol of plant diseases, studying the biology of lactic acid bacteria with probiotic potential, investigation of virulence and antibiotic resistance among opportunistic pathogenic bacteria, testing of new antibacterial agents. Her teaching activity includes a high study load (395 – 477 hours) during the last 3 years. He is the recipient of several scientific awards; actively participates in research and educational projects; has a significant administrative commitment as a member of the Mandate Committee at the General Assembly of the Faculty of Biology, SU (2024 - until now); the council of specialties in "Bachelor's Degree Programmes" (specialty Biomanagement and sustainable development) (2018 - 2024); the council of specialities "Bachelor's Degree Programmes" (Molecular Biology (2024 - until now); the committee for preparing a self-assessment report for the accreditation of the doctoral program "Microbiology" (2022); the committee for determining additional funds for doctoral students at the Faculty of Biology SU (2020 - until now), scientific secretary of the Department of "General and Industrial Microbiology" and responsible for departments: "PhD students", "Scientific research activity" and "Postgraduate qualification". For her professional achievements, Dr. Yoanna Kizheva also received public recognition in the form of 4 awards and honors: i) Diploma from the Foundation "Acad. Prof. Dr. Stefan Angelov" for the best work of a young Bulgarian microbiologist in 2018; ii) "Award for the best poster" (3rd place) at the 14th Congress of Microbiologists in Bulgaria; "Award for the best poster" (1st and 2nd place) at the 15<sup>th</sup> Congress of Microbiologists in Bulgaria. Dr. Yoanna Kizheva established herself as a researcher with a national/international reputation and recognition, participating in a scientific network under the program COST Action CA22158 "Exploiting Plant-Microbiomes Networks and Synthetic Communities to Improve Crops Fitness" 2023-2027; preparing 14 reviews of scientific papers for refereed scientific journals, 7 of which are with quartile Q1; participated as guest editor of the peer-reviewed scientific journal Pathogens, MDPI (Q2).

The overall work experience of Dr. Yoanna Kizheva at the Faculty of Biology, Sofia University "St. Kliment Ohridski" at the time of submission of the documents is 7 years and 4 months, and her entire professional activity is thematically related to the announced competition.

#### 2. Teaching experience

Assistant Professor Dr. Yoanna Kizheva has a large and varied workload in teaching students of both educational degrees - Bachelor's and Master's Degree Programmes and in both forms of education - full-time and part-time. From the attached report, the academic class workload for the last three years is as follows: for the 2020/2021 academic year the total academic workload is 674.1 hours, of which 395.7 hours are class work; for the academic year 2021/2022 – 660.8 hours, of which 425 hours are class work and for the 2022/2023 academic year – 678.4 hours, of which 421.4 are class work. From her admission as an assistant professor until now, Dr. Yoanna Kizheva's class work and extramural work have been related to teaching in the fields of General Microbiology, Microbiological control, and Phytopathogenic bacteria.

In the Bachelor's Degree Programme Assist. Prof. Yoanna Kizheva participates in conducting practical exercises in Microbiology (full-time training) for the specialties Molecular Biology, Ecology and Environmental Protection, Molecular Biology, Biology and Chemistry, Biomanagement and Sustainable Development, Biotechnology, and Biology.

In the Master's Degree Programmes, the candidate participates in teaching:

➢ Practical classes on Biological hazards in food, Microbial metabolism and Microbiological control of food and food products in the Master's Degree Programme"Food Quality and Safety".

➢ Practical classes on Biological hazards in food, Metabolism of prokaryotes, Food microbiology, and Environmental Microbiology in the Master's Degree Programme "Microbiology and microbiological control".

➢ Lectures and practical classes on Phytopathogenic bacteria for Master's Degree Programme" Microbiology and microbiological control".

➢ Lectures and practical classes in Microbiology and Virology for Master's Degree Programme "Cosmetics and Household Chemistry".

➤ Lectures and practical classes on Sanitary microbiology of cosmetic and pharmaceutical products for Master's Degree Programme "Microbiology and microbiological control".

In connection with the educational and teaching work, Dr. Yoanna Kizheva participated in

the upgrade and development of several lecture materials (78 hours) and practical classes (72 hours), all in the field of "General Microbiology and Microbial Phytopathology":

1. Phytopathogenic organisms and integrated pest control - part Bacteria, Bachelor's Degree Programme "Agrobiotechnologies", compulsory - 15 hours of lectures and 15 hours of exercises.

2. Sanitary microbiology of cosmetic and pharmaceutical products - Master's Degree Programme "Microbiology and microbiological control", compulsory - 15 hours of lectures and 15 hours of exercises.

3. Microbiology and virology - Master's Degree Programme "Cosmetics and household chemistry", elective - 30 hours, lectures, and 30 hours of exercises.

4. Selected chapters from microbiology - a special part (Module 4. "Biology of phytopathogenic bacteria") of the doctoral program "Microbiology - 18 hours, lectures and 12 hours of seminar exercises.

An important part of the educational activity is the management of graduates. Under the leadership of Assist. Prof. Dr. Yoanna Kizheva successfully graduated 15 students in the Master's program and 7 in the Bachelor's program. She also participates in the guidance and preparation of students for the international competition in biology "Jugend forscht"; she is the leader of the workshop "Young Biologists from Bulgaria to Japan" at the Japanese Cultural Center "Kokoro"; a member of the scientific jury at the Karol Knowledge Foundation for awarding scholarships to doctoral students.

### 3. Submitted materials for the competition - publication activity and citations

The publication activity of the candidate, presented in the documentation, is reflected in 63 titles, divided into scientific publications, participation with reports and posters in national and international conferences and congresses, and articles in conference proceedings.

In the competition for "Associate Professor" Dr. Yoanna Kizheva participated with 18 scientific publications with a total IF/SJR of 30.503. In 10 of the publications, the candidate is the lead author. Most of the scientific works have been published in prestigious journals such as *Pathogens, Journal of Biosciences, Plants (Basel), Heliyon*, etc. 34 participations in scientific forums are presented in the competition documentation: 5 sectional reports and 29 posters. 111 citations have

been registered in the Scopus database for the period from 2015 to the present time, many of which are in renowned journals with IF/SJR such as *Microbiome., Animals, Molecular Plant Pathology, Scientia Horticulturae, Plant Health Progress, Genome Biology and Evolution, ISME Journal, Parasit Vectors, Foods, Toxins, Frontiers in Nutrition, Insects, Frontiers in Microbiology, Ecotoxicology and Environmental Safety, LWT - Food Science and Technology, Advances in Applied Microbiology, Journal of Biological Macromolecules* и др.

All scientific publications presented in the competition for "Associate Professor" are in refereed and indexed journals. In the scientific database Scopus, the candidate's h-index is 7. In the competition for the academic position "Associate Professor", the distribution by quartiles of publications referenced and indexed in the world-recognized scientific information databases Web of Science or Scopus is as follows: Q1 - 4 pcs., Q2 - 4 pcs., Q3 - 7 pcs., Q4 - 3 pcs.

The provided reference shows that Assist. Prof. Dr. Yoanna Krasimirova Kizheva exceeds the minimum national requirements for holding the academic position "Associate Professor" (indicator A meets the minimum national requirements – 50 points, indicator B is 100 with a minimum value of national requirements – 100 points, indicator D is 221 with a minimum value of 200 points, indicator D is 222 with a minimum value of 50 points). Total for all indicators is 593 points (required minimum: 400 points).

My conclusion regarding this part of the analysis of the scientific activity of Dr. Yoanna Kizheva is that the procedure was followed, and the documentation was prepared according to the requirements of the Act for the Development of the Academic Staff in the Republic of Bulgaria (DASRBA) and the regulations for its application for the occupation of the academic position of "associate professor". She participates in the competition with scientific work that fully corresponds to the professional direction of the discipline in terms of volume and quality, according to the requirements of the law and the regulations for its application.

### 4. Participation in scientific projects and programs

The research of the candidate is supported by participation in the development of 9 scientific research projects financed by the "Scientific Research" Fund at SU "St. Kliment Ohridski"; 7 scientific research projects financed by the Bulgarian National Scientific Research Fund – Ministry of Education and Science; 1 project financed under the National Plan for Recovery and Sustainability and 1 training project under the ERASMUS + program. Dr. Yoanna Kizheva is the leader of two of the above-

mentioned projects. The activity of Dr. Yoanna Kizheva in the development and realization of projects contributed both to her development and those of the students participating in them, as well as to the improvement of the educational and scientific research base of the Faculty of Biology.

### 5. Research activity and main scientific contributions

The main research activity of Assist. Prof. Dr. Yoanna Kizheva is associated with research on various phytopathogenic and opportunistically pathogenic bacteria, their characterization, study of their distribution, and the development of various methods to combat them. She also studies the biology of lactic acid bacteria isolated from different natural habitats to select potential probiotic strains. Evaluates the microbiological quality of various probiotic food supplements. The accumulated scientific research experience of the candidate on microbial molecular taxonomy, the mechanisms of bacterial virulence and antibiotic resistance, as well as the testing of new approaches to combat pathogenic microorganisms, emphasize the presence of a well-established specialist whose research profile fully coincides with the direction of the announced competition - General Microbiology and Phytopathogenic Bacteria.

The main scientific contributions in the publication activity have a fundamental and scientific-applied nature, and can be summarized in the following directions:

### 1. Molecular identification and intraspecific diversity of phytopathogenic bacteria (publications: 15, 16; conferences: 7, 20, 21; award: 26)

The scientific works presented in this direction are related to the development and validation of alternative molecular genetic procedures for interspecies and intraspecies identification of representatives of the phytopathogenic genus *Xanthomonas*. The newly introduced methodology is based on restriction analysis (RFLP) of the amplified region (ITS) between the 16S and 23S genes in the ribosomal operon and the use of a specific combination of three restriction enzymes (MboI, AluI and HpaII). Another important aspect in the study of phytopathogenic xanthomonads, the causative agents of bacterial scab on tomatoes and pepper, is the disclosure of their genetic diversity in our country, an important contribution from an epidemiological point of view to determine disease outbreaks. The scientific research of the candidate in this field is related to the application of pulse gel electrophoresis, with the help of which the intraspecific diversity of the Bulgarian population of the

species *X. vesicatoria* and *X. gardneri* was studied for the first time and a unified and validated approach was proposed for typing of these two species.

### 2. Study of distribution and relationships of phytopathogenic bacteria with primary and alternative host plants (publications: 1, 2, 6, 11, 12; conferences: 6, 8, 13, 16, 18, 29, 34)

Dr. Yoanna Kizheva's research is in two main directions: studying the pathogenesis of X. euvesicatoria and the genetic transformation of the species X. euvesicatoria with a specially constructed plasmid encoding the gene for green fluorescent protein (GFPuv). Archived scientific developments allow easy tracking of bacterial behavior in plant tissues. The response of pepper plants artificially infected with a strain of X. euvesicatoria was also investigated by monitoring a set of biochemical and stress markers (photosynthetic parameters, H<sub>2</sub>O<sub>2</sub> production, expression of the enzymes SOD, catalase, glycol peroxidase, and glutathione reductase, levels of phenols, proline, and chlorophyll). The presence of a series of specific defense reactions, which are activated in conditions of infectious stress and lead to inhibition of the spread of infection in plant tissues has been demonstrated. Another aspect of research in this scientific direction is the proof of new hosts of phytopathogenic bacteria. For the first time in Bulgaria, the possibility of the X. euvesicatoria species causing scabs on tomato plants was shown. These studies provide new knowledge about the range of susceptible hosts of the species and are extremely important from an epidemiological point of view. As a continuation of these studies, a thorough characterization of different strains from the species Curtobacterium flaccumfaciens was carried out and the role of tomato and pepper plants as its alternative hosts and a reservoir for its spread to the main susceptible plants such as beans and soybeans was demonstrated. The isolated Curtobacterium flaccumfaciens pathogens were identified using a polyphasic approach and their key phenotypic characteristics were investigated (ability to hydrolyze casein, starch, and esculin, production of urease, catalase, oxidase, and indole from tryptophan, ability to reduce nitrate and nitrite).

3. Isolation and characterization of bacteriophages with potential as a biocontrol agents of plant diseases caused by phytopathogenic bacteria (publications: 5, 7, 8, 10; conferences: 2, 3, 4, 9, 10, 15, 17, 19, 22, 25, 26, 28, 30, 32, 33)

Another important direction in the research work of Assist. Prof. Dr. Yoanna Kizheva is aimed

at the search for new effective environmentally friendly alternatives for the fight against bacterial plant diseases. The candidate studies for the first time in Bulgaria the possibility of applying bacteriophages isolated from natural habitats in the country to combat the phytopathogenic bacteria *X. euvesicatoria*, *X. vesicatoria* and *X. gardneri*. Accumulated experimental data add to the knowledge in this field worldwide, as bacteriophages have been isolated that are characterized by a broad spectrum of action (SfXv124t/3) and can successfully lyse cells of all three bacterial species. Species capable of destroying target cells only of the species *X. euvesicatoria* have also been studied. Bacteriophages have been characterized by several important key features regarding their potential to be used as biocontrol agents. Their effectiveness and ability to kill the target phytopathogenic species *in vivo* was also investigated and their promising potential as biocontrol agents was established. To improve the production qualities of such potential biocidal preparations, a fast and efficient way to quantify the bacteriophages has been developed. The optimization of this procedure makes it possible to quickly monitor the number of phages in the industrial production of such preparations, as well as to track the emergence of resistance in host bacterium.

### 4. Study of the biology of lactic acid bacteria isolated from different natural habitats in connection with the selection of potential probiotic strains and for the evaluation of the microbiological quality of probiotic food supplements (publications: 3, 13, 17, 18; conferences: 5, 24, 27)

Part of the studies of Dr. Yoanna Kizheva are focused on the selection of strains of lactic acid bacteria (LAB) with potential application as probiotics. Research has been carried out related to the optimization of a general diagnostic algorithm, which would allow fast and correct identification of newly isolated strains of LAB. Using the newly developed approach, Dr. Kizheva identifies species of the genus *Weissella* in probiotic food supplements offered on the Bulgarian market, whose presence is undesirable and, accordingly, not described on the packaging. The method was also successfully applied in the study of the lactic acid microflora of the gastrointestinal tract (GIT) of the garden snail *Cornu aspersum* and in spontaneously fermented rye sourdough. The inclusion of modern sequencing techniques in the conducted analyses leads to the disclosure of the species composition of LAB (sauerkraut, pickles (tomatoes and cucumbers), kefir, white brine cheese, and cottage cheese), including and pending proof of the existence of hitherto unreported species. A set of specific

characteristics of newly isolated LAB strains determining their beneficial and health effects were also investigated, and a diagnostic algorithm was proposed to evaluate the probiotic potential of LAB by stakeholders.

# 5. Investigation of virulence potential and antibiotic resistance among opportunistic pathogenic bacteria isolated from different habitats (publications: 4, 9; conferences: 11, 12, 14, 23, 31)

Another important scientific contribution of Dr. Yoanna Kizheva's research is related to the study of the so-called cross-pathogens that are capable of modifying their mechanisms of action and developing the ability to infect representatives of other biological kingdoms. She identified and characterized in detail opportunistic pathogenic bacteria, representatives of six families (*Enterobacteriaceae, Erwiniaceae, Pectobacteriaceae, Moraxellaceae, Pseudomonadaceae,* and *Enterococcaceae*) isolated from tomato and pepper plants. Regardless of the lack of genetically encoded antibiotic resistance and virulence in them, their discovery in these atypical habitats poses fundamental questions related to clarifying the role of the two crops in the life cycle of these bacterial species. The results obtained by Dr. Kizheva complement the knowledge in this field and represent a solid basis for future more in-depth research.

Another part of the research activities carried out by Assist. Prof. Yoanna Kizheva aimed at isolating and characterizing *Enterococcus* species from different habitats (GIT of *C. aspersum* and different foods). A laboratory collection containing representatives of 11 species of the *Enterococcus* genus was created and all isolates were studied in detail for the presence of antibiotic resistance and virulence genes to assess the biological hazard they represent in these foods.

## 6. Testing new substances with potential as antibacterial agents (publications: 14; conferences: 1)

In connection with the growing antibiotic resistance among pathogenic bacteria, another part of Dr. Yoanna Kizheva's research is assigned to the tests of various biologically active substances of natural origin, as substitutes for traditional antibiotic preparations (AB). Investigation in this direction is related to the discovery of the antimicrobial potential of hemocyanin extracted from the stone crab *Eriphia verrucose*. Fractions of its subunits have been shown to have antibacterial activity against important clinical pathogens: *Staphylococcus epidermidis, Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella enterica subsp. enterica ser. enteritidis* and *Pseudomonas aeruginosa.* Based on the conducted research, it was established that the degree of glycosylation of the hemocyanin subunits also determines their more pronounced antibacterial action.

Based on the accumulated scientific data from the above-mentioned studies, the following contributions stand out:

### I. Contributions of scientific and fundamental importance:

1. A new optimized molecular genetic approach has been developed for the rapid and accurate identification of the four bacterial species causing bacterial scab on tomato and pepper: *X. vesicatoria, X. euvesicatoria, X. gardneri* and *X. perforans*.

2. For the first time the genetic diversity in the Bulgarian population of the *X. vesicatoria* and *X. gardneri* species was investigated and the genetic heterogeneity within the *X. vesicatoria* species was proven.

3. New knowledge was gained regarding the pathogenesis and close interaction between the species *X. euvesicatoria* and its susceptible host plants.

4. For the first time, successful genetic transformation with a plasmid was achieved in the species *X. euvesicatoria*.

5. For the first time in Bulgaria, the species *X. euvesicatoria* was identified by phenotypic and molecular genetic methods as the causative agent of bacterial scab disease on tomatoes.

6. For the first time in Bulgaria, an in-depth phenotypic and genotypic characterization of supposedly pathogenic strains of the species *Curtobacterium flaccumfaciens* on tomatoes and pepper was made.

7. For the first time in Bulgaria, bacteriophages with the potential for biocontrol agents against plant diseases were isolated and characterized in detail, and a laboratory collection of them was created.

8. For the first time in Bulgaria, the presence of LAB of the genus *Lactobacillus* in the gastrointestinal tract of the garden snail *C. aspersum* is reported.

9. The species *Levilactobacillus koreensis* and *Levilactobacillus yonginensis* isolated from fermented vegetable foods, as well as the species *Lactobacillus spicheri*, *Lactobacillus paralimentarius*, *Lactobacillus kimchi* and *Lactobacillus sanfranciscensis* isolated from fermented rye dough are reported for the first time.

10. The diversity of human opportunistic pathogens isolated from plants has been studied and a laboratory collection of them has been created.

11. The distribution of genetically determined antibiotic resistance and virulence among enterococci strains isolated from food was studied.

### **II.** Contributions of applied importance:

1. A procedure was developed to obtain a recombinant plasmid (pWG5) carrying genes for constitutive expression of green fluorescent protein and resistance to gentamicin.

2. The species and strain composition, dynamics, and pathotype of the phytopathogenic bacteria causing bacterial scab on tomatoes and peppers in Bulgaria are described in detail.

3. The role of tomato and pepper plants as alternative hosts of the *C. flaccumfaciens* species has been proven.

4. For the first time in Bulgaria, bacteriophage BsXeu269p/3 was administered *in vivo* to limit the spread of the bacterial scab disease caused by the phytopathogenic bacterium *X*. *euvesicatoria*.

5. A molecular genetic approach was developed for rapid and reliable target detection and quantification of bacteriophage BsXeu269p/3 in natural and laboratory samples.

6. An optimized diagnostic molecular-genetic algorithm was developed for rapid species identification of LAB.

7. Basic characteristics concerning the probiotic potential of both newly isolated Bulgarian strains of the LAB and those included in commercial probiotic products were studied.

8. For the first time in Bulgaria, the antibacterial activity of the structural subunits of hemocyanin extracted from *E. verrucose* was established, having the potential to serve as a basis for the development of antibacterial preparations.

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### **III.** Contributions of educational importance:

1. Students from Bachelor's and Master's Degree Programmes were trained in techniques and laboratory methods, which are not studied in the general courses in the both educational degrees.

2. For the first time within the Faculty of Biology, laboratory procedures have been developed for 1/ cultivation of bacteriophages, 2/ quantitative determination by classical and molecular genetic methods, 3/ preparation of phage suspensions for TEM and for isolation of phage DNA with the participation of students studying at the Faculty of Biology of the SU.

#### Personal impressions of the candidate

I know Dr. Yoanna Kizheva from her admission as a student at the Faculty of Biology of SU "St. Kliment Ohridski", and then during her career development in the field of microbiology and phytopathology in the Department of General and Industrial Microbiology. She established herself as a sought-after specialist and teacher with a clearly defined profile in the field of biology and molecular identification of phytopathogenic and lactic acid bacteria, with authority among teachers, students, and scientific circles. Maintains professional contacts and works with specialists from the country and abroad and is a preferred partner in carrying out scientific research tasks. As a colleague in the Department of General and Industrial Microbiology, she is respected, motivated, and responsive to the needs of her colleagues. She is an extremely capable and disciplined teacher and researcher, respected and sought after by students. Has a high sense of responsibility towards various types of duties in the organizational life of the department and faculty.

### CONCLUSION

Based on the analysis of pedagogical work (class work), active scientific research activity, the volume of scientific production, interpretation of scientific data and contributions, their reflection in international scientific literature, participation in scientific research projects, presentation of results at international and national scientific forums, active administrative commitment and personal qualities, I believe with conviction that Assistant Professor Dr. Yoanna Krasimirova Kizheva fully meets the requirements of the DASRBA and the Regulations and the recommended criteria for

occupying academic positions at the Sofia University "St. Kliment Ohridski". All this gives me a reason to give a **POSITIVE** evaluation for the choice of Assistant Professor Dr. Yoanna Krasimirova Kizheva for "*Associate Professor*". I propose to the honorable Scientific Jury to vote on a proposal to the Faculty Council of the Faculty of Biology at SU "St. Kliment Ohridski" to elect Dr. Yoanna Krasimirova Kizheva for "Associate Professor" in 4.3 Biological Sciences (Microbiology -General Microbiology and Phytopathogenic Bacteria) for the needs of the Department of General and Industrial Microbiology of the Faculty of Biology of the Sofia University "St. Kliment Ohridski".

18. 10. 2024 г. Sofia Reviewer:

(Assoc. Prof. Ventsislava Petrova, PhD)