R E V I E W

by **Prof. Maria Bogomilova Angelova**, DSc, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences
Scientific area 4. Natural Sciences, Mathematics and Informatics, 4.3. Biological sciences, Scientific specialty Microbiology
Subject: Competition for the academic position "**ASSOCIATE PROFESSOR**" in professional direction 4.3. Biological sciences (Microbiology - General microbiology and phytopathogenic bacteria), for the needs of the Department of General and Industrial Microbiology, presented to a scientific jury, formed by order No. RD-38-468/22.7.2024 of the Rector of Sofia University "St. Kliment Ohridski"

In the competition for the position of "ASSOCIATE PROFESSOR", announced in the State Gazette no. 55 dated 28.06.2024, received the documents of Dr. Ioana Krasimirova Kizheva, Chief Assistant Professor of Microbiology at the Department of General and Industrial Microbiology of the Faculty of Biology of Sofia University "St. Kliment Ohridski".

1. GENERAL PRESENTATION OF THE PROCEDURE AND THE APPLICANT

For participation in the competition, Dr. Ioanna Kizheva has submitted the necessary documents and materials proving the fulfillment of the requirements for the academic position "Associate Professor" on electronic media. All of them are in accordance with the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for its implementation, as well as the Regulations of the Sofia University "St. Kliment Ohridski". I would like to emphasize that the documents have been prepared correctly, precisely, and with great attention to detail.

Chief Assistant J. Kizheva received her higher education at the Faculty of Biology, Sofia University. Kliment Ohridski". In 2007 she graduated with a Bachelor of Science in Biology and Chemistry, and in 2010 she received a Master's degree from the MP Microbiology and Microbiological Control with the qualification Molecular Biologist - Master of Science in Microbiology and Microbiological Control. In 2014 she received the scientific and educational degree "Doctor" and immediately began working as a visiting Assistant Professor in the Department of General and Industrial Microbiology. Since 2017, the candidate has been a Chief Assistant Professor in the same department. The successful completion of 4 training courses that built on her university training has contributed to her growth as a scientist. The candidate is proficient in English and has excellent computer skills.

Dr. Kizheva also has serious administrative experience, she actively participates in the administrative activities of the Faculty of Biology and the Department. She is a member of several committees of the Faculty of Biology at SU - the Mandate Committee of the General Assembly, the Council of Bachelor's Degree Programs (specialization in Biomanagement and Sustainable Development and Molecular Biology); the Committee for the Preparation of the self-evaluation report for the accreditation of the PhD Program in Microbiology; the Committee for the

determination of additional funds for PhD students. In the department, Kizheva is the scientific secretary and is responsible for the PhD students, research, and Postgraduate qualification sections. She is also a member of the Scientific Committee of the Karoll Knowledge Foundation for the awarding of scholarships to doctoral students. Dr. Kizheva received an honorary diploma for her work.

The candidate's scientific career is fully related to the theme of the competition and reflects current and promising areas of general microbiology, molecular biology, molecular taxonomy, biocontrol of plant pathogens, and antibiotic resistance.

2. COMPLIANCE WITH THE MINIMUM REQUIREMENTS OF THE LDASRB

Assistant Professor Ioana Kizheva is a co-author of 23 scientific articles published in indexed and refereed journals with a total IF of 31.485. The results of the candidate have received a wide response in the international scientific community, they have been cited 96 times and form h-index (Scopus) 7/(Google Scholar) 9. For the competition for Associate Professor Kizheva submitted 18 scientific articles, which do not include those related to the acquisition of the Ph.D. I accept for peer review all 18 articles attached to the candidate's list for the competition. I would like to note that they have been published in specialized reputable journals, such as Pathogens, and J. Biosci. Plants Heliyon J. Plant Pathol. Z. Naturforsch., Biotechnol. Biotechnol. Eq. etc.

The report on the fulfillment of the minimum requirements for the academic position of "Associate Professor" shows that the candidate meets and exceeds the required points in the individual indicators, scoring 663 instead of the required 400:

Meeting the requirements of LDASRB

- **Indicator A** - successfully defended dissertation for the PhD degree, **50 pts**.
- **Indicator C4** - 4 scientific articles with SJR in Web of Science and Scopus refereed journals (Q1 - 4;) = **100 pts.** with 100 points required.
- **Indicator D** – **total - 221 pts.** in total with 200 points required
  - ✓ 14 scientific papers with IF/SJR and quartile in Web of Science and Scopus refereed journals (Q2-4; Q3-7; Q4-3)
- **Indicator E** - 96 citations (Scopus) = **192 pts.** out of 100 points required.
- **Indicator F total** – **total 100 points** (not required for Associate Professor)
  - ✓ Participation in the national scientific or educational projects – 7 X 10 = **70 pts.**
  - ✓ Participation in international scientific or educational projects - 1 X 20 = **20 pts.**
  - ✓ Participate in National Recovery and Resiliency Plan projects - 1 X 10 = **10 pts.**

Meeting the requirements of the additional requirements of St. Kliment Ohridski

1. Administration of successfully defended graduates - 22
2. Teaching experience as an Assistant Professor - 7 years
3. Preparation of curricula for bachelor's, master's, and doctoral degree programs - 4
4. Preparation of reviews of scientific papers for peer-reviewed scientific journals - 14
5. Participation as guest editor of a peer-reviewed scientific journal Pathogens, MDPI
6. Coached students for international biology competition - 2

7. Leader of the workshop "Young Biologists from Bulgaria to Japan" at the Japanese Cultural Center "Kokoro".
8. Member of the scientific jury of the Carol Knowledge Foundation for awarding scholarships to Ph.D. students
9. Participation in training programs - 2
10. Awards received – 4
11. Administrative experience in the last 5 years
    - at SU - a member of 3 committees
    - to the Department - Scientific Secretary and responsible for the Doctoral Students, Research, and Postgraduate qualification sections.

As can be seen from the reference, Dr. Kizheva meets and exceeds the requirements of the LDASRB, as instead of the required 400 points, she presents evidence of 663 points. In addition, the candidate fulfills the additional criteria of the Sofia University "St. Kliment Ohridski".

3. EVALUATION OF THE CANDIDATE'S EDUCATIONAL AND PEDAGOGICAL ACTIVITY

Teaching and learning activity is one of the main activities of the candidate and it is completely in the field of the competition. It can be analyzed in several aspects - as a teacher, as a trainer of skills in laboratory practice, as a compiler of curricula, and as a supervisor of graduates. Dr. Kizheva has been teaching students since 2015 as a part-time assistant, and then from 2017 to date as the Chief Assistant Professor in Microbiology at the Department of "General and Industrial Microbiology" of Sofia University. For the last 3 academic years, the candidate has an average total workload of 670 hours, 432 of which are classroom work. A major part of Dr. Kizheva's teaching activity relates to the teaching of students of Programs "Bachelor" and "Master" in laboratory skills and experimental work.

- EQD "Bachelor" - Microbiology /sp. Molecular Biology and Biomanagement/; Microbiology and Virology/sp. Biology and Chemistry, Biology, Pharmacy/; Microbiology and Microbiological Methods of Purification/sp. Ecology and Environmental Protection/;
- EQD "Master" - Biological Hazards in Food, Microbial Metabolism, Microbiological Control of Food and Food Products/sp. Food Quality and Safety/; Metabolism of Prokaryotes, Food Microbiology, Sanitary Microbiology, Environmental Microbiology, Phytopathogenic Bacteria /sp. Microbiology and Microbiological Control/; Microbiology and Virology/; Cosmetics and Household Chemicals/.

Kizheva gives lectures at the master's level (Sanitary Microbiology of Cosmetic and Pharmaceutical Products, Phytopathogenic Fungi, and Microbiology and Virology) to students from the Faculty of Biology and the Faculty of Chemistry and Pharmacy of Sofia University.

The candidate's activity in developing new materials for lectures and practical exercises should also be noted. Dr. Kizheva has prepared curricula for the MSc programs in Agrobiotechnology (Phytopathogenic organisms and integrated pest management - part bacteria),

Sanitary Microbiology (Microbiology and Microbiological Control), Cosmetics and Household Chemistry (Microbiology and Virology), as well as for the PhD program in Microbiology (Selected chapters in microbiology - special part).

At the same time, Dr. Kizheva is very intensively involved in the training of young staff. She is the supervisor of 22 successfully defended graduates of Bachelor (7) and Master (15) programs. The topics of all these are related to the theme of the competition. The candidate has also prepared students to enter an international biology competition.

For her two years of work as the leader of the Young Biologists Workshop - Bulgaria to Japan at the Japanese Cultural Center Kokoro, Dr. Kizheva received a letter of appreciation for her significant contribution to the implementation of the program and for promoting the ideas among younger children and students.

Based on the above, I highly appreciate the teaching and learning activities of the candidate, I believe that they are significant in volume, cover important areas in the field of the announced competition, and fulfill the mission of a teacher at the University.

4. EVALUATION OF THE CANDIDATE'S RESEARCH ACTIVITY

The scientific works of Dr. Ioana Kizheva fully cover the topic of this competition, namely General Microbiology and Phytopathogenic Bacteria. They reflect the activities of the candidate in many topical areas of microbiological science - molecular identification, intraspecific diversity of phytopathogenic bacteria, biocontrol of plant diseases, new probiotic strains, and new antibacterial agents. The candidate's publications can be grouped and analyzed into five sections.

I. Molecular identification and intraspecific diversity of phytopathogenic bacteria and study of their distribution and relationships with primary and alternate host plants (1, 2, 6, 11, 12, 15, 16).

Bacteria of the genus *Xanthomonas* are one of the most common plant pathogens worldwide. They cause significant financial losses for important crops, including vegetables with high nutritional value for consumers. Studies on the prevention of diseases related to representatives of the genus *Xanthomonas* (the bacterial scab) and their importance for the economy are of increasing interest in scientific and business circles. The noted problems concern Bulgaria to a large extent as well. Taking into account the scale of vegetable production in our country and the annual damage caused by compromised produce, the relevance and significance of the conducted research can be clearly outlined.

This section presents an alternative molecular genetic approach to identify and correctly differentiate the species *X. euversicatoria* and *X. perforans*. The problem of their high genetic identity was solved by using restriction analysis (RFLP) of an amplified region (ITS) between the 16S and 23S genes of the ribosomal operon. The appropriate combination of three restriction enzymes in a well-defined sequence greatly increases the level of discrimination. The advantages of the method and its significance for correct identification were demonstrated by its application to a large number of isolates (262) of the genus *Xanthomonas*, isolated from plants with signs of

bacteriosis over a very long period (1985 - 2013). Belonging to the species *X. vesicatoria* (115 strains), *X. euvesicatoria* (132 strains), and *X. gardneri* (15 strains) was established.

Another very important aspect of the section is a study of the intraspecies genetic diversity of the causal agents of bacterial scab, which has been the subject of research and reclassification over the past 20 years. Published results have been inconclusive. DNA-DNA hybridization identified 4 genotypes, and genome-wide comparative nucleotide sequence analysis suggested grouping of strains with >99.6% identity. The candidate research used the highly discriminatory molecular genetic technique of pulsed gel electrophoresis, which for the first time in Bulgaria investigated the intraspecific diversity within the Bulgarian population of the species *X. vesicatoria* and *X. gardneri*. The results of the studied 100 bacterial isolates (for the period 1985-2012) from different hosts define the method as reliable and unified for typing these two species. Based on this method, two haplotypes were found for *X. vesicatoria* and one haplotype for *X. gardneri* strains.

Research into new approaches to plant protection is another modern and relevant aspect. To achieve early diagnosis and limit the epidemiological spread of *X. euvesicatoria*, experiments were carried out to study the pathogenesis of strains of this species. A new qPCR procedure was developed to detect the presence of *X. euvesicatoria* and to re-isolate viable bacterial cells. The possibility of obtaining stably transformed cultures of a wild strain of *X. euvesicatoria* (269p) constitutively expressing the gene for green fluorescence (GFPuv) was investigated, allowing easy monitoring of the behavior of the bacterium in plant tissues. Two commercial synthetic plasmids (pBbB13k-GFP and pWVR5) and a recombinant plasmid constructed for this study (pWG5) were used. The transformation was performed by electroporation and thermal shock. PCR and RFLP were used for species and plasmid identification.

The results of biochemical studies and the role of oxidative stress in the invasion of the pathogen and the defense of pepper plants artificially infected with *X. euvesicatoria* should also be noted. It was found that the intermediate phenotype in the studied pathosystem is an example of a long and successful co-evolution for both species.

Another aspect of research in this direction is the identification of new hosts for phytopathogenic bacteria. Five actinobacterial strains putatively belonging to the species *Curtobacterium flaccumfaciens* isolated from tomato and pepper were identified and characterized. Identification was performed by MALDI-TOF MS, 16S rDNA sequencing, and PCR. The role of both plants as natural reservoirs of this phytopathogen was established. In this direction are the results, which prove for the first time *X. euvesicatoria* is a cause of BS on tomatoes in Bulgaria. Based on 27 strains isolated from different regions of the country and identified by species-specific PCR amplification, two pathotypes were established - tomato (T) with three races and pepper tomato (PT) with five races. These studies provide new epidemiologically important information on the range of susceptible hosts for *C. flaccumfaciens* and *X. euvesicatoria*.

The following original theoretical contributions were made in this section:

1. New information has been obtained that contributes to the knowledge of the pathogenesis and the close interaction between the species *X. euvesicatoria* and its susceptible host plants.

2. A molecular genetic approach based on restriction analysis (RFLP) of an amplified region between the 16S and 23S genes of the ribosomal operon (ITS) was 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. perforan*.

3. For the first time in Bulgaria, information was obtained on the genetic diversity in the Bulgarian population of the species *X. vesicatoria* and *X. gardneri*, based on the restriction profile of the genomes of the two species with two rare-cutting endonucleases (SpeI and XbaI) and an optimized method of performing pulsed gel electrophoresis (PFGE).

4. A procedure for obtaining a recombinant plasmid (pWG5) carrying genes for constitutive green fluorescence and resistance to gentamicin was developed and the probability of its successful transfer into *X. euvesicatoria* by electroporation was studied.

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 on tomatoes, thus completing the circle of sensitive hosts of this pathogen.

6. A thorough review of the species and race composition, their dynamics, and pathotype of the phytopathogenic bacteria causing bacterial scabs on tomatoes and peppers in Bulgaria was made based on research carried out in the period 1999-2016.

7. For the first time in Bulgaria, a detailed phenotypic and genotypic characterization of suspected pathogenic strains of the species *Curtobacterium flaccumfaciens* was carried out.

8. The role of tomato and pepper plants as alternative hosts of *C. flaccumfaciens* species has been demonstrated.

II. Isolation and characterization of bacteriophages with potential for biocontrol of plant diseases caused by phytopathogenic bacteria (5, 7, 8, 10).

The negative economic impact of phytopathogenic bacteria on agriculture and the environment is undisputed. However, the widespread use of antibacterial agents is the cause of the phenomenon of multidrug resistance (MDR) also concerning the pathogens of plant diseases. Biological control is a promising method to suppress plant pathogens, enhance plant immunity, and modify the environment. Bacteriophages have been successfully used to control several plant diseases and offer possible alternatives to antibiotics. The use of phages would reduce environmental contamination with antimicrobial compounds and pathogen resistance caused by chemical bactericides. Phages are highly specific and infect only the appropriate host bacteria, so they do not affect other beneficial microorganisms. The genus *Xanthomonas* is particularly important because it is associated with several important diseases that cause enormous losses in agriculture. Although phage control in *Xanthomonas* is of great interest, the problem is greatly underestimated in our country. The team, including Dr. Kizheva, is investigating for the first time the potential of bacteriophages isolated from natural habitats in the country that are capable of destroying *X. euvesicatoria*, *X. vesicatoria* and *X. gardneri*, pathogens of crops in Bulgaria. It was found that one of these phages (SfXv124t/3), belonging to the family *Podoviridae*, has not been reported as an effective agent against the known phytopathogenic bacteria. It has been characterized as a potential candidate for pest biocontrol. A significant achievement was the

characterization of 11 phage isolates from the rhizosphere of tomato plants with bacterial scab symptoms, capable of destroying only target cells of the *X. euvesicatoria* species. The circular representation of a linear but circularly permuted phage genome among those known for the indicated plant species is reported in this study for the first time.

Of interest are the results of a study of the pathosystem pepper plant (*Capsicum annuum* L.) - phytopathogenic bacterium *X. euvesicatoria* (wild strain 269p) - bacteriophage BsXeu269p/3. Based on the two new model systems established using modern methods such as qPCR and SEM, the possibility of *in vivo* bacteriophage-mediated biocontrol of the disease was demonstrated. This is the first report on *in vivo* evaluation of the potential of locally isolated phages for biocontrol of *X. euvesicatoria*, the causal agent of bacterial scab in Bulgaria.

The accumulated knowledge of many years of research on phage therapy and phage control for the suppression of plant pathogens is presented in a very detailed review based on current data. The scientific information in this work is extremely useful for researchers and students.

I consider the more important contributions in this section to be the following:

Theoretical Contributions

1. For the first time, bacteriophages with the potential for application in phage control of xanthomonads were isolated from Bulgarian crops and characterized in detail. A laboratory collection of them was established.

2. New information was obtained on the morphology of the virion and the organization of the genome of some of the isolated phages, which contributes to the clarification of phage biology in general.

3. Evidence for circular permutation of a linear genome is presented for the first time among known *X. euvesicatoria* phages.

4. The potential of a bacteriophage (BsXeu269p/3) to prevent the spread of bacterial scab caused by the phytopathogenic bacterium *X. euvesicatoria* was demonstrated *in vivo* for the first time in Bulgaria.

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

Methodological Contributions

1. Development of laboratory procedures for the cultivation of bacteriophages, quantification by classical and molecular genetic methods, preparation of phage suspensions for TEM, and isolation of phage DNA.

III. Study of the biology of lactic acid bacteria isolated from different natural habitats and selection of potential probiotic strains with evaluation of the microbiological quality of probiotic food supplements (Publ. 13, 17, 18).

The topicality and perspective of this direction are undeniable. Probiotics are an important keyword in the health portfolio of people of the 21st century. Their enormous health potential has attracted the attention of scientists, producers, and consumers. This, in turn, has inspired researchers to improve methods for assessing their quality. The studies presented in this section focus on the optimization of a general diagnostic algorithm for the rapid and correct identification

of newly isolated LAB strains by modern molecular biological methods. The algorithm includes a series of PCR reactions aimed at first grouping LAB strains at the genus level by amplification of 16S-23S ITS rDNA, followed by confirmation of genus affiliation with genus-specific primers, multiplex PCR to group the strains, and PCR with species-specific primers. It was used to study the LAB content of 26 probiotic products, 16 commercially available and 10 from a local manufacturer. Based on the obtained results, it was established that they do not contain all the labeled LAB, and in some of them, unacceptable microorganisms are proven. The presence of *Weissella* species in probiotic food supplements offered on the Bulgarian market is undesirable and is not marked on the packaging. The species of this genus are close to representatives of the genera *Streptococcus* and *Lactococcus*, which is why they are difficult to differentiate from them by applying a shorter identification scheme.

The proposed algorithm was successfully used to identify newly isolated LAB strains from rye sourdough and the edible snail *Cornu aspersum*. This aspect also includes the results proving the species composition of lactic acid microflora in traditional fermented Bulgarian foods. It should be noted that the application of the method allowed the detection of LAB species not previously reported in the studied habitats. The newly isolated LAB strains were characterized by key features regarding their probiotic potential. Tolerance was evaluated in simulated conditions of GIT, production of antibacterial substances, autoaggregation and coaggregation with pathogenic microorganisms, biofilm formation, hemolytic activity, and phenotypic and genetically determined antibiotic resistance.

The contributions can be formulated as follows:

Theoretical Contributions

1. The lactic acid microflora of the genus *Lactobacillus* in the gastrointestinal tract of the garden snail *C. aspersum* was identified and characterized for the first time.

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

3. The main characteristics concerning the probiotic potential of the newly isolated Bulgarian strains of LAB were studied.

Scientific and applied contributions

1. Effective optimization of a diagnostic molecular genetic algorithm for rapid species identification of LAB was achieved.

2. A detailed characterization of LAB in commercial probiotic preparations was performed, underestimated values of probiotic bacteria and the presence of undesirable species of the genus Weissella were detected.

IV. Investigation of the virulence potential and antibiotic resistance of opportunistic pathogenic bacteria isolated from different habitats (Publ. 4, 9).

The problem of cross-pathogenicity is of interest to scientists because of its importance to human and animal health and safety. These pathogens have adapted to plants without losing their

virulence to humans. Their study helps to clarify their pathogenic spectrum and to take appropriate measures to control them. Examples of recognized cross-pathogens are several bacterial species commonly found on the surface and in the rhizosphere of plants, such as *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Dickeya* spp., *Enterococcus faecalis*, *Serratia marcescens*, and others. This section presents the results of experiments on the presence of cross-pathogens in infected tomato and pepper plants. 21 samples from seven different parts of the plants and three from the rhizosphere of tomatoes were studied. The isolated strains were characterized phenotypically and identified by MALDI-TOF. A high bacterial diversity was observed, with nine species belonging to six families: *Enterobacteriaceae, Erwiniaceae, Pectobacteriaceae, Moraxellaceae, Pseudomonadaceae,* and *Enterococcaceae*. An approach based on PCR amplification of the rpoB gene was used for rapid detection of cross-pathogens in plant samples. All isolates showed different resistance to the tested antibiotics. No genes for virulence factors were found in the population.

Of interest are the studies related to the cross-pathogenicity of Enterococcus bacteria isolated from different foods and *C. aspersum* GIT. They were identified by PCR and MALDI-TOF and analyzed for the presence of antibiotic resistance and virulence genes. A correlation between genotypic and phenotypic resistance to macrolides was found for two strains of *E. faecalis*.

The contributions made can be summarized as follows:

Theoretical Contributions

1. New information was obtained on the diversity of human opportunistic pathogens isolated from plants.

2. New data were obtained on the distribution of genetically determined antibiotic resistance and virulence among enterococcal strains isolated from food.

3. Research results contribute to the problem of cross-pathogenicity.

Applied Contributions

1. A laboratory collection of human opportunistic pathogens isolated from plants has been established.

V. Testing of New Substances with Potential as Antibacterial Agents (Publ. 14)

The candidate's scientific research includes work on the major problem of antibiotic resistance and the search for new antibacterial agents. The object of study is the native hemocyanin (EvH) isolated from *Eriphia verrucosa* and its five structural units (SU). The antimicrobial activity of glycosylated SU was determined against clinically relevant pathogens: *Staphylococcus epidermidis, S. aureus, Bacillus subtilis, Escherichia coli, Salmonella enterica* subsp. enterica ser. Enteritidis and *Pseudomonas aeruginosa*. The dependence of the effect on the degree of glycosylation was also demonstrated. SU1 (with the highest carbohydrate content) has the potential to replace some commonly used antibiotics.

I consider the more important contributions in this section to be the following:

Theoretical Contributions

1. The antibacterial activity of the structural subunits of hemocyanin from E. verrucose against clinically relevant pathogens has been demonstrated.

2. The relationship between the degree of glycosylation of the structural subunits of hemocyanin and the level of antibacterial activity has been demonstrated.

Applied Contributions

1. The structural unit SU1 of hemocyanin from E. verrucose can be used as a basis for the development of an antibacterial preparation.

I positively evaluate the research activity of Dr. Ioana Kizheva in terms of content, methodological approaches, and achievements. I would like to emphasize that the research is complex and involves specialists with different qualifications, as required by today's science. However, the candidate's contribution is very well outlined. This, in turn, proves that it is in line with the theme of the contest. We should add that out of all 18 articles, Dr. Kizheva is in first place in 10 and in second place in 2, which is proof of her significant contribution to the presented developments.

The contributions of Chief Assistant Professor Ioana Kizheva I rate as innovative and significant, both original scientific and applied. Achievements of obvious theoretical and methodological character, including obtaining new information and confirming known data in the main directions of microbiology, are on the face. Undoubtedly, the achieved results are a solid scientific basis for future research. In my opinion, Dr. Kizheva has her place in the activities of the teams she works with, her qualifications and experience greatly contribute to the realization of ideas and achievement of goals. This also defines her share in the contributions as significant.

The results presented for the competition are available to the international scientific community, as evidenced by the number of citations (136, h factor 7/9). This is also a recognition of the level and importance of Dr. Kizheva's activity. Some of the articles have been cited multiple times in such prestigious journals as *Pathogens, Environmental Research, Antibiotics, Microorganisms, Frontiers in Microbiology, Plos One, Toxins*, etc. It is noteworthy that 98% (133) of the citations are from authors outside the country.

5. EDITORIAL AND REVIEW ACTIVITY

It is necessary to emphasize the activity of Chief Assistant Professor Kizheva as a guest editor of a special issue of the journal Pathogens, MDPI, which shows her willingness to engage in additional activities. The serious review work of the candidate for scientific works in refereed scientific journals is also in this direction. For the period 2019-2024, she prepared 14 reviews for articles in the field of her competence (*Plants, Pathogens, Resources, BBQ, Folia Medica, Annual of Sofia University, Curr. Microbiol., Acta Microbiol. Bulg*.).

6. PARTICIPATION IN SCIENTIFIC RESEARCH PROJECTS

The candidate presents information on participation in 18 scientific research projects for the entire scientific internship, 13 of them after receiving the CIS "Doctor". They are financed as follows

- Fund "Scientific Research" at SU "St. Kliment Ohridski" - 9, of which she is the head;

- Fund "Scientific Research" at the Ministry of Education and Culture - 7, of which she is the head;
- National Recovery and Sustainability Plan - 1;
- International Projects - 1.

7. CRITICAL REMARKS AND RECOMMENDATIONS

I have no critical remarks about the presented materials. Considering Dr. Kizheva's qualifications in current scientific fields and her skills as a teacher and researcher, I recommend more active participation as a project manager.

8. PERSONAL IMPRESSIONS

I have known Assistant Professor Dr. Joanna Kizheva since her Ph.D. studies as a very active and enthusiastic young scientist. During these years she grew into a competent and active microbiologist with her field of scientific interest. I would like to emphasize that Dr. Kizheva continued the work of her teachers in the field of phytopathogenic bacteria, developed and brought the research to a new level, and included the subject in the teaching of the department. I familiarized myself with the materials presented for the competition in great detail, and now for me, Dr. Kizheva is a qualified teacher and scientist in the field of microbiology. The subject of her publications is contemporary and very relevant, at the center of important issues of the 21st century. Her participation in collective development characterizes her as a researcher with good teamwork skills.

9. CONCLUSION

The documents and materials submitted by Dr. Joana Kizheva, Assistant Professor, meet the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for the Implementation of the LDASRB, and the relevant Regulations of the University of Sofia. The candidate has a sufficient number of scientific works for the competition, published according to the materials used in the defense of the ONS "Doctor". The results achieved in the educational and scientific research activities fully correspond to the minimum national and additional requirements of the SU, adopted in connection with the application of the LDASRB.

I would like to emphasize that the teaching activity of Dr. Ioana Kizheva is in the field of the current competition and corresponds to the current requirements of higher education. She is the author of curricula and works actively with graduates and students. She is a sought-after partner in the development of scientific projects and an active member of the teams she works with. The presented scientific works define her as a professionally competent specialist. They have been published in prestigious publications and have become known in our and the international scientific community. Formulated scientific and applied contributions are a basis for further developments.

After getting acquainted with the materials and scientific works presented in the competition, after analyzing their significance and the scientific and scientifically applied contributions contained in them, I give my positive assessment and strongly recommend the Scientific Jury to prepare a report proposal to the Faculty Council of the Faculty of Biology for the appointment of

the Chief Assistant Professor **Dr. Joana Krasimirova Kizheva** to the academic position of "**ASSOCIATE PROFESSOR**" at the Sofia University "St. Kliment Ohridski" by the field of higher education 4. Natural Sciences, Mathematics, and Computer Science, specialization 4.3. Biological Sciences, Specialization Microbiology (General Microbiology and Phytopathogenic Bacteria).

October 15, 2024
Sofia

Reviewer: ……
/Prof. Maria Angelova, DSc/