REVIEW

by Prof. Iskra Vitanova Ivanova, Sofia University "St. Kliment Ohridski" - Faculty of Biology

of the materials submitted by Ivanka Petrova Boyadzhieva for participation in a competition for the academic position of "associate professor" at the Institute of Microbiology "Stefan Angelov" at the Bulgarian Academy of Sciences in the field of higher education 4. Natural Sciences, Mathematics and Informatics; professional field 4.3. Biological Sciences (specialization Microbiology)

In the competition for "associate professor", announced by the Institute of Microbiology "Stefan Angelov" at the Bulgarian Academy of Sciences in the State Gazette, issue 30 of 08.04.2025 for the needs of the Department of "General Microbiology", Laboratory "Extreme Microorganisms", as the only candidate, senior asst. dr. Ivanka Petrova Boyadzhieva participated.

1. General presentation of the received materials and assessment of the fulfillment of the minimum national requirements and additional requirements of the Institute of Microbiology "Stefan Angelov" by the candidate in the competition for the academic position "associate professor".

By order No. I-77 of 28.05.2025 of the Director of the Institute of Microbiology "Stefan Angelov" I am appointed as a member of the scientific jury in the competition for the academic position "associate professor" in the professional field 4.3. Biological Sciences (Microbiology), for the needs of the Department of "General Microbiology", Laboratory "Extremophilic Microorganisms" at the Institute of Microbiology-BAS.

The only candidate for participation in this competition - Senior Asst. Prof. Dr. Ivanka Boyadzhieva from the Institute of Microbiology, has submitted all necessary documents required under Art. 19 of the Act on the Development of the Academic Body in the Republic of Bulgaria (ADRB) and Art. 58(2) of the Regulations on the conditions and procedure for acquiring scientific degrees and occupying academic positions at the Stefan Angelov Institute of Microbiology at the Bulgarian Academy of Sciences.

The materials submitted by candidate Senior Assistant Professor Ivanka Boyadzhieva fully meet the requirements of the competition and cover the entire specific evidentiary part regarding the required criteria of the competition. They present the candidate's overall activity, both through lists of publications and citations, and through the text of the submitted contributions, the CV, etc.

The submitted documentation is well arranged, including digital copies of the publications related to the participation in this competition, as well as their summaries in Bulgarian and English.

Senior Assistant Professor Ivanka Boyadzhieva is a co-author of 31 scientific works, of which 27 scientific articles, 3 book chapters and 1 patent. For the competition she presents - 20 scientific articles, 3 book chapters and 1 patent. 20 articles have been published in journals with IF, the total IF is 42.694 with h index 10 (Scopus). Compliance with the requirements of the ZRASRB:

Reference for the minimum requirements:

 Indicator A: abstract for the ONS "doctor" - 50 points;

 Indicator B: 5 scientific articles (in journals with IF and quartile Q2) - 100 points;

 Indicator D: 340 points

• Indicator D7 - 15 scientific articles (14 in journals with IF; of which 3 with Q1, 6 with Q2, 5 with Q3) and 2 book chapters - 270 points

• Indicator D8 - 3 book chapters - 45 points;

• Indicator D9 - 1 patent - 25 points;

 Indicator D: 163 citations (SCOPUS) - 326 points;

Compliance with additional requirements of the Institute of Microbiology and Biotechnology:

The candidate is a co-author of 23 articles after receiving the NOS "doctor" (required - 20), and in 6 articles she is the first author or corresponding author. The total number of citations is 218 (required - 100).

The report on the fulfillment of the minimum requirements for the academic position "associate professor" shows that the candidate scored 816 points, which covers and exceeds the required 430 points. In addition, Dr. Boyadzhieva also exceeds the additional requirements of the Institute of Microbiology and Biotechnology-BAS. Evidence of 11 participations in Bulgarian and international forums is presented.

All works of Dr. Boyadzhieva are collective. In 8 of them she is the first author, 7 of which are published in refereed and indexed publications. For participation in the competition, a list of 163 citations of her publications is submitted, which cover both the minimum and additional requirements for the position of "associate professor".

2. Brief biographical data about the candidate

Senior Assistant Professor Ivanka Boyadzhieva graduated from the Faculty of Biology of Sofia University "St. Kliment Ohridski" in 1999 with a degree in "Molecular Biology". In the period between 2003-2008 she was a full-time doctoral student, developing a dissertation on the topic "Biosynthesis and properties of superoxide dismutase from thermophilic bacteria isolated from Bulgarian hot springs", which she defended in 2011 and acquired the educational and scientific degree "doctor". At the Institute of Microbiology - BAS she successively held the following positions - microbiologist, assistant, senior assistant.

3. Characteristics of scientific and publication activities

The scientific and applied scientific contributions submitted for participation in the competition can be systematized in four main areas:

 Biodiversity of microbial communities in extreme niches.

Molecular biological methods are a basic and reliable approach for revealing the real microbial diversity. This allows not only taxonomic and phylogenetic description, but also functional analysis of microbial populations in a given ecosystem, especially extreme niches, which are among the least studied in term

of microbial diversity. As a result of a metagenomic analysis, the biodiversity of a bacterial community in the P18 crystallizer basin in the Pomorie salt pans (34% salinity) and the Mirovo rock salt deposit was studied.

The results corresponding to this direction are included in 3 journal articles and 2 book chapters (G7-10, G7-11, G7-17, G7-20, G8-21).

 Biosynthetic potential of extremophiles from Bulgarian extreme niches.

One of the main incentives for the intensive study of extremophiles is their ability to synthesize new metabolites with unusual properties. The evolutionarily developed adaptation to extreme niches of microorganisms makes them extremely interesting with their ability to synthesize compounds with significant potential. Such molecules, thanks to their unique stability and activity at unusual temperatures, pH, high salinity and the presence of organic solvents, find application in biotechnology.

The materials presented by the candidate (3 journal articles and 2 book chapters, G7-11, G7-19, G7-20, G8-21, G8-22), which consider the potential of bacteria and archaea with extremophilic conditions. Data were obtained on the potential of a number of representatives from hot springs and hypersaline waters to synthesize thermostable and halophilic enzymes.

 Microbial enzymes and exopolysaccharides of biotechnological importance.

The candidate is a co-author of studies on the purification and characterization of enzymes and EPZ from extremophilic bacteria with proven application in practice. The growing trend towards environmentally friendly industrial production stimulates the search for new microbial enzymes that degrade a variety of industrially important substrates. The presented articles prove the research strategy – from biodiversity to application (12 journal publications and 1 book chapter - B4-1, B4-2, B4-3, B4-4, B4-5, G7-6, G7-9, G7-12, G7-13, G7-14, G7-15, G7-18, G8-23).

In this section, Dr. Ivanka Boyadzhieva studies a number of enzymes, such as - alkaliphilic phytase from halophilic bacteria, extracellular haloalkalophilic pectinase, nitrilase, cyclodextrin-glucanotransferase. A bacterial community that degrades the plastic ε-polycaprolactone (PCL) has been isolated with a representative of this community - Brevibacillus thermoruber strain 7 with esterase activity.

 Participation in joint research with teams of scientists working in other scientific areas.

Dr. Boyadzhieva works to create immobilized matrices for incorporating whole cells under gentle conditions and high operational stability. She also participates in the development of bacterial nanocellulose membranes using food waste and symbiotic cultures for supercapacitors. Her qualification has been used in the study of the intestinal microbiota of lizard species in Western Bulgaria in relation to their microbiome.All of the above shows that she is a sought-after partner in multidisciplinary research (3 journal articles - G7-7, G7-8, G7-16).

3.2. Evaluation of project activities

Dr. Boyadzhieva has participated in 8 research projects and is a project manager within the framework of International Cooperation of Interacademic Contracts and Agreements. The project participations are thematically closely related to the scientific areas of her research activity and correspond to the specialty of the current competition for the position of "associate professor".

3.3. Evaluation of scientific contributions

I accept the contributions made in the relevant areas of her research activity, as follows:

 Biodiversity of microbial communities in extreme niches:

1. Data on bacterial diversity in the P18 crystallizer basin of the Pomorie Salt Pans are presented for the first time.

2. For the first time, rare and unique genera have been identified in a hypersaline environment, which emphasizes the ecological uniqueness of the Pomorie Salt Pans.

3. 23 operational taxonomic units (OTUs) belonging to 15 genera from 5 main bacterial groups were identified: Firmicutes (47.5%), Proteobacteria (23.1%), Bacteroidetes (22%), Deinococcus–Thermus (2.4%) and Candidate division SR1.

4. For the first time, results on the biodiversity of extremophile bacteria and archaea in the hypersaline waters of the Pomorie salt pans have been included in international databases.

5. For the first time, information on the biodiversity of cultivable halophilic bacteria from the Mirovo rock salt deposit has been presented.

 Biosynthetic potential of extremophiles from Bulgarian extreme niches:

1. Producers of extremozymes from Bulgarian habitats characterized by high temperature and high salinity of the environment have been identified. Producers of xanthine lyase, gellan lyase, arabinase and phytase from the group of halophilic bacteria have been identified for the first time.

2. The isolated pectinase from Anoxybacillus gonensis 357 is the first described for this species and has potential for application in the food and textile industries due to its stability at high temperatures and alkaline pH.

3. The potential of microbial communities of thermophilic and halophilic bacteria to degrade polycaprolactone (PCL), polystyrene (PS) and polypropylene (PP) has been proven.

4. For the first time, PCL degrading activity has been established in a strain of the species Brevibacillus thermoruber, which allows the highest optimal process temperature to date. The results obtained suggest application in the treatment of plastic waste.

5. Producers of exopolysaccharides have been identified and an effective producer from the species Chromohalobacter canadensis has been selected. This is the first report of a halophilic bacterium capable of synthesizing a polymer containing γ-PGA (polyglutamic acid)

 Microbial enzymes and exopolysaccharides of biotechnological importance:

1. A new phytase from Cobetia marina strain 439 was isolated, purified and characterized for the first time. The enzyme has a wide range of temperature and pH stability and a certain substrate specificity. The new enzyme is suitable for application in the feed industry.

2. Virgibacillus salarius strain 434 isolated from the salt pans in Burgas and Pomorie is capable of producing extracellular haloalkalophilic pectinase and the cultivation conditions and the optimal composition of the nutrient medium under which maximum enzyme activity is achieved have been defined. An

electrophoretically homogeneous enzyme preparation was obtained, which was characterized as a haloalkalophilic polygalacturonase. The enzyme is promising for application in the pretreatment of wastewater from the textile, tissue and paper industries.

3. This is the first study on the production, purification and characterization of pectinase from Anoxybacillus gonensis, isolated from a hot mineral spring in the area of the village of Varvara. A purified alkaliphilic enzyme with high thermostability and a temperature optimum at 65°C was obtained. Due to these properties, this enzyme has potential for application in the textile and paper industry, the food industry and processes related to thermal treatment.

4. A new thermophilic strain of the genus Bacillus produces nitrilase showing significant thermostability, which is increased by immobilizing the cells in an agar gel. The strain Bacillus sp. UG-5B has broad substrate specificity and degrades aromatic nitriles (benzonitrile), heterocyclic compounds (4-cyanopyridine), alkenes and dialkyl nitriles, including those with chlorine substituents.

5. The immobilized enzyme shows increased thermostability and resistance to toxic compounds. The new nitrilase is promising for application in detoxification of high-temperature industrial wastewater.

6. For the first time, a cyclodextrin-glucanotransferase (CGTase) has been purified and characterized from the obligately alkaliphilic strain Bacillus pseudalcaliphilus, isolated from soils in Bulgaria. The newly isolated CGTase can be used in the production of industrially preferred β-cyclodextrin and γ-cyclodextrin, necessary for the needs of the pharmaceutical and food industries.

7. For the first time, an extracellular thermostable lipase, produced by a thermophilic bacterial strain Brevibacillus thermoruber strain 7, active on Ɛ-polycaprolactone, has been isolated and purified. The enzyme has high thermostability and substrate specificity, which refer it to the group of esterases in the subgroup of lipases. It is suitable for applications related to recycling and degradation of PCL waste at temperatures close to the melting point of the polymer. This includes treatment in contaminated thermal zones or in situ treatment of plastic waste.

8. The halophilic bacterium Chromohalobacter canadensis 28, isolated from the salt pans in Pomorie, has been identified as a new and efficient producer of exopolymeric substances (EPS). This is the first report of a halophilic bacterium capable of synthesizing a polymer containing γ-PGA (polyglutamic acid).

9. A purified EPS preparation synthesized by Chromohalobacter canadensis 28 was obtained, which was characterized in terms of its composition, which has high hydrophilicity and water-holding capacity, good foaming activity, excellent emulsifying and stabilizing efficiency in oil-in-water dispersion systems.

10. An optimized method for obtaining EPZ from C. canadensis 28 in a laboratory bioreactor in batch and continuous cultivation mode has been developed. The physicochemical and functional properties of EPZ determine its significant potential for use in medicine, pharmacy, cosmetics and the food industry.

 Participation in joint research with teams of scientists working in other scientific areas:

1. Bacillus sp. UG-5B cells are effectively encapsulated in sol-gel hybrid matrices composed of TEOS (tetraethoxysilane) and various organic components (such as polyethylene glycol, glycerol, etc.). The addition of polyethylene glycol (PEG) and glycerol to the matrix improves the viability and enzymatic activity of immobilized cells compared to pure silica matrices.

2. Characteristic bacterial profiles of five species of lizards coexisting in the same low-mountain zone in Western Bulgaria were established: Lacerta viridis, Podarcis muralis, Darevskia praticola, Ablepharus kitaibelii and Anguis fragilis.

3. Through fermentation of a symbiotic culture isolated from a Kombucha drink, nanocellulose was obtained, on the basis of which hybrid membranes impregnated with poly-benzimidazole were created as ecological and effective separators for supercapacitor cells.

4. Assessment of the candidate's personal contribution

The documentation presented by Dr. Boyadzhieva, together with the attached scientific papers, the analysis of the obtained results, the interpretation and derivation of the main scientific and scientific-applied contributions - show her personal contribution to their experimental development.

. The scientific papers are collective, which is usual for a professional field of the competition, but the personal contribution of Dr. Boyadzhieva is clearly distinguishable, given her specialization in the indicated scientific fields and her position in the author team.

5. Critical remarks, recommendations and questions

I have no critical remarks and recommendations in substance.

In some places in the submitted documents, a number of technical inaccuracies have been made in presenting the numerical values of the points from the implementation of some of the indicators, which are enshrined in the requirements for the academic position of "associate professor".

I do not accept the concept of "scientific and theoretical contributions".

I must note, however, that these inaccuracies do not change the positive result of the overall implementation of the indicators, which significantly exceeds the requirements.

I have the following questions for Dr. Boyadzhieva:

• The mechanisms for importing glucose into cells, as well as the proteins involved in this process, have been very poorly studied in haloarchaea. What are the metabolic pathways used in the utilization of carbohydrates?

Do ABC transporters play a key role in glucose import in halophilic archaea?

• What are the features of energy metabolism in haloarchaea?

CONCLUSION

The documents and materials submitted by Senior Assistant Professor Dr. Ivanka Boyadzhieva for participation in the competition for the academic position of "associate professor" meet all the

requirements of the Act on the Development of Academic Staff in the Republic of Bulgaria (ADSRB), the Regulations on the Terms and Procedure for Acquiring Scientific Degrees and Holding Academic Positions at the Bulgarian Academy of Sciences and that of the Institute of Microbiology at the Bulgarian Academy of Sciences.

The candidate in the competition has presented a sufficient number of scientific papers with high scientometric indicators, as well as convincing evidence of her scientific and scientific-applied activity, which I assess entirely positively and highly.

I believe that Dr. Boyadzhieva fulfills and even significantly exceeds the requirements of the ADSRB and the additional ones of the Institute of Microbiology for the academic position of "associate professor".

Her works have a contribution of scientific and scientific-applied significance. They have been evaluated by the international scientific community through the citations received.

All of the above allows me to confirm my positive assessment, given by me in the analysis of her academic activity and to confidently recommend to the esteemed scientific jury, formed by order No. I-77 of 28.05.2025 of the Director of the Institute of Microbiology at the Bulgarian Academy of Sciences and the members of the National Council to evaluate the candidacy of Senior Assistant Professor Dr. Ivanka Boyadzhieva on merit and to vote positively for her to occupy the academic position of "associate professor" in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3. Biological Sciences (Microbiology).

Sofia, 12.08.2025 Signature:

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