SCIENTIFIC OPINION

from **Prof. Maria Bogomilova Angelova, DSc**, the Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences
Regarding: Competition for the Academic Position of "ASSOCIATE PROFESSOR", Professional Field 4.3 Biological Sciences, Scientific specialty Microbiology for the needs of the Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences (SAIM-BAS), presented to a scientific jury, formed by order No. I-77/28.05.2025 of the Director of SAIM-BAS

In the competition for "ASSOCIATE PROFESSOR", announced in the State Gazette, issue 110/31.12.2024, documents have been submitted by **Dr. Ivanka Petrova Boyadzhieva**, Chief Assistant Professor at the Laboratory of Extremophilic Microorganisms, Department of General Microbiology at SAIM-BAS.

1. GENERAL OVERVIEW OF THE PROCEDURE AND THE CANDIDATE

To participate in the competition, Dr. Ivanka Boyadzhieva has submitted the necessary documents and materials proving the fulfillment of the requirements for occupying the academic position of "Associate Professor" on an electronic medium. 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 SAIM-BAS. The competition documentation comprehensively reflects the candidate's research activity.

Dr. Ivanka Boyadzhieva has a Master's degree in Molecular Biology, specialization in Virology from Sofia University "St. Kliment Ohridski", Faculty of Biology (1999). In 2008, she received the educational and scientific degree "Doctor" at the SAIM-BAS. Her scientific career began in 2000 as a microbiologist at the Institute of Microbiology in the section/now the Laboratory of Extremophilic Microorganisms, and continued successively as an assistant (2011) and Chief Assistant from 2015 to the present. The candidate's work experience is more than 25 years.

Dr. Boyadzhieva also has administrative experience. For 2 years (from 2023 to the present), she has been responsible for the Laboratory of Extremophilic Microorganisms at the Institute.

The candidate's scientific research activity is entirely related to the topic of the competition and reflects current and promising areas of Microbiology.

2. CHARACTERISTICS OF THE CANDIDATE'S ACTIVITY

The Chief As. Prof. Ivanka Boyadzhieva is a co-author of 31 scientific papers, of which 27 are scientific articles, 3 book chapters, and 1 patent. For the competition, she presents 20 scientific articles, 3 chapters of a book, and 1 patent. Twenty 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 LDASRB

Reference for the minimum requirements:

- **Indicator A** - - successfully defended dissertation for the PhD degree - 50 points;
- **Indicator C** - 5 scientific articles (5 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 E** - 163 citations (SCOPUS) - 326 points;

Compliance with additional requirements of the SAIM-BAS

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

The certificate of 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 SAIM-BAS.

3. EVALUATION OF THE CANDIDATE'S RESEARCH ACTIVITY

The scientific works of Dr. Ivanka Boyadzhieva fully cover the topic of this competition. They reflect the candidate's activity in a very relevant aspect of modern Microbiology - extremophilic microorganisms in the aspect of their biodiversity, biotechnological potential, and application. Four scientific directions are outlined, in which important scientific and applied contributions have been formulated.

3.1. Biodiversity of microbial communities in extreme niches

The results corresponding to this direction are included in 3 journal articles and 2 book chapters (D7-10, D7-11, D7-17, D7-20, D8-21). I would like to note that studies on the diversity of microorganisms capable of surviving in extreme habitats are some of the most relevant today. Dr. Boyadzhieva is a follower of scientists from the SAIM-BAS, who initiated this direction in Bulgaria. She is a co-author of the first results characterizing the presence of extremophile bacteria and archaea in a number of unexplored niches in our country, characterized by their extreme conditions such as high temperature and high salt content. The microbial biodiversity in the largest salt lake in the Pomorie Salt Pans and the Mirovo rock salt deposit was studied. Unlike previous reports, these articles demonstrate an unusually large number of taxa in hypersaline environments. Molecular biological methods have identified cultivable and uncultivable bacterial and archaeal genera and species, some of which are reported in the scientific literature for the first time.

<u>The following contributions are outlined here:</u>

1. New knowledge has been obtained about extremophilic microorganisms from the group of thermophilic and halophilic bacteria and archaea.

2. For the first time, results on the biodiversity of extremophilic bacteria and archaea in the hypersaline waters of the Pomorie Salt Pans have been included in international databases.

3. New information has been obtained about the high content and unexpectedly large diversity of bacteria and archaea in a hypersaline environment, refuting the then-current scientific postulates about the microflora in such habitats.

4. 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.

5. Information on the biodiversity of cultivable halophilic bacteria from the Mirovo rock salt deposit has been presented for the first time.

3.2. Biosynthetic potential of extremophiles from Bulgarian extreme niches

The scientific interest in extremophiles is also due to their role as an important reservoir of genetic diversity, unique metabolic pathways, and biological molecules that allow them to cope with multiple environmental stress factors. The enzymes synthesized by such microorganisms can exhibit their catalytic function under the extreme environmental conditions in which they live. Such research is a promising direction in the development of white and green biotechnologies, offering innovations in medicine, industry, ecology, energy, agriculture, etc. In this context, the materials presented by the candidate (3 journal articles and 2 book chapters, D7-11, D7-19, D7-20, D8-21, D8-22), which consider the potential of bacteria and archaea isolated from Bulgarian habitats with extremophilic conditions. Data were obtained on the ability of a number of representatives from hot springs and hypersaline waters to synthesize thermostable and halophilic enzymes.

The contributions made can be formulated as follows:

1. Producers of extremozymes from Bulgarian habitats characterized by high temperature and high salinity of the environment have been identified.

2. For the first time, biosynthesis of pectinase from the species *Anoxybacillus gonensis* with high stability at alkaline pH and high temperature has been proven with application in the food and textile industry.

3. Effective producers of enzymes degrading 18 different substrates have been identified - polygalacturonase, catalase, phytase, and lipase.

4. For the first time, producers of xanthine lyase, gellan lyase, arabinase, and phytase from the group of halophilic bacteria have been identified.

5. The potential of microbial communities of thermophilic and halophilic bacteria to degrade polycaprolactone (PCL), polystyrene (PS), and polypropylene (PP) has been proven. PCL degradation activity has been demonstrated for the first time in a strain of the species *Brevibacillus thermoruber*, which allows the highest optimal process temperature reported so far. The results obtained suggest an application in the treatment of plastic waste.

6. Producers of exopolysaccharides have been identified, and an efficient 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).

3.3. Microbial enzymes and exopolysaccharides of biotechnological importance

This direction is logically related to the above. The presented articles prove the research strategy - from biodiversity to application (12 journal publications and 1 book chapter - C4-1, C4-2, C4-3, C4-4, C4-5, D7-6, D7-9, D7-12, D7-13, D7-14, D7-15, D7-18, D8-23). The candidate is a co-author of research on the purification and characterization of enzymes and EPS from extremophile bacteria with proven application in practice.

The most important contributions in this section are as follows:

1. A halophilic strain *Cobetia marina* 439 has been identified as a phytase producer. The enzyme has been isolated and characterized as halophilic, with a wide range of temperature and pH stability, clearly defined substrate specificity, and high resistance to the action of proteases. The new enzyme is suitable for application in the feed industry.

2. For the first time, a pectinase producer has been identified from the species *Virgibacillus salaries*. The strain has been isolated from Bulgarian salt pans, and it synthesizes an enzyme with high activity. An electrophoretically homogeneous enzyme preparation has been obtained, which was characterized as a haloalkalophilic polygalacturonase. The enzyme is promising for application in the pretreatment of wastewater from the textile and paper industries.

3. A highly efficient pectinase producer from the species *Anoxybacillus gonensis*, isolated from a hot mineral spring in the village of Varvara, has been selected. A purified alkaliphilic enzyme with high thermostability and a temperature optimum at 65°C has been obtained. Due to these properties, this enzyme can be applied in the textile and paper industry, the food industry, and in processes related to thermal treatment.

4. It has been proven that a new thermophilic strain of the genus *Bacillus* (*Bacillus* sp. UG-5B) produces a highly thermostable nitrilase. The broad substrate specificity of the nitrilase has been proven to degrade aromatic nitriles (benzonitrile), heterocyclic compounds (4-cyanopyridine), alkenes, and dialkyl nitriles, including those with chlorine substituents. The immobilized enzyme showed increased thermostability and resistance to toxic compounds. The new nitrilase is promising for application in the detoxification of industrial wastewaters at high temperatures.

5. A new enzyme, cyclodextrin-glucanotransferase (CGTase), has been obtained from the alkaliphilic strain *Bacillus pseudalcaliphilus*. The purified enzyme showed high pH and thermostability, as well as resistance to industrial inhibitors. This makes it suitable for industrial processes at high temperature and alkaline pH. The newly isolated CGTase can be used in the production of industrially preferred β- and γ-cyclodextrins, necessary for the needs of the pharmaceutical and food industries.

6. An extracellular thermostable lipase produced by the thermophilic bacterial strain *Brevibacillus thermoruber* strain 7, active on Ɛ-polycaprolactone, has been isolated and purified. This is the first thermostable enzyme active on PCL. The enzyme has high thermostability and substrate specificity, which refers to the group of esterases in the subgroup of lipases. The main product of enzyme degradation is the monomer Ɛ-caprolactone. 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.

7. A purified EPS preparation synthesized by *Chromohalobacter canadensis* 28 was obtained, which has been characterized in terms of its composition. It has been proven that EPS has high hydrophilicity and water-holding capacity, good foaming activity, and excellent emulsifying and stabilizing efficiency in oil-in-water dispersion systems. An optimized method for obtaining EPS

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

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

The fourth area reflects the candidate's activity as a sought-after partner in multidisciplinary research (3 journal articles - G7-7, G7-8, G7-16). Dr. Boyadzhieva is involved in developments to create immobilized matrices for incorporating whole cells under gentle conditions and high operational stability. Her qualification has been used in the study of the intestinal microbiota in lizard species in Western Bulgaria, which provides new data on the role of the microbiome and shows links between host physiology and microbial composition. She also participates in the development of bacterial nanocellulose membranes using food waste and symbiotic cultures for supercapacitors.

The contributions in this direction are as follows:

1. Sol-gel hybrid matrices have been developed for the immobilization of cells of *Bacillus* sp. UG-5B, a nitrilase producer. The immobilized system preserves biological activity and improves the viability and enzymatic activity of the immobilized cells compared to pure silicon matrices.

2. Bacterial profiles of five species of lizards have been established. By molecular genetic methods, 6 types, 12 classes, 21 orders, 43 families, and 106 genera of bacteria have been identified.

3. Nanocellulose has been obtained by fermenting a symbiotic culture isolated from a kombucha drink. Hybrid membranes impregnated with poly-benzimidazole have been created from this nanocellulose as an ecological and effective separator for supercapacitor cells.

4. PARTICIPATION IN PROJECTS

The applicant presents information about participation in 9 research projects, 3 of which are nationally funded and 6 are funded by international institutions. Dr. Boyadzhieva is the head of one project and a participant in the remaining 8. They are distributed as follows

- Scientific Research Fund at the Ministry of Education and Science – 3 pcs.
- International projects under the bilateral cooperation program of the Bulgarian Academy of Sciences (BAS) – 6 pcs., respectively with Italy, Turkey, Russia, and Romania.

5. CRITICAL COMMENTS AND RECOMMENDATIONS

As a recommendation, I would like to note that the author's reference should reflect the achievements of the candidate's scientific and research activities more clearly and concisely.

6. PERSONAL IMPRESSIONS

I have known Dr. Boyadzhieva since the beginning of her scientific career, and I was a reviewer of her PhD dissertation. For me, she is a qualified scientist in the field of microbiology, specifically extremophile bacteria. The topics of her publications are contemporary and very relevant, at the center of important problems of the 21st century. Her participation in collective developments characterizes her as a researcher with good teamwork skills.

I would like to point out the candidate's activity as a reviewer of scientific articles. She demonstrates skill and responsibility, as well as a desire to contribute to a competent assessment.

7. CONCLUSIONS

The documents and materials presented by Chief Asst. Prof. Dr. Ivanka Petrova Boyadzhieva meets the requirements of the LDASRB, the Regulations for its implementation, as well as the Regulations of the SAIM-BAS. The candidate has a sufficient number of scientific papers for the competition, published after the materials used in the defense of the education and scientific degree "doctor". The achieved results in scientific research activities fully comply with the minimum national requirements and the additional requirements of SAIM-BAS, adopted in connection with the implementation of the LDASRB.

The presented scientific papers define Dr. Ivanka Boyadzhieva as a professionally competent specialist. They have been published in reputable publications and have become known to our and the international scientific community. The formulated scientific and applied contributions are the basis for further developments. She is a sought-after partner in the development of scientific projects and an active member of the teams she works with.

After reviewing the materials and scientific papers presented in the competition, after analyzing their significance and the scientific and applied contributions contained therein, I give my positive assessment and strongly recommend to the Scientific Jury to prepare a report-proposal to the Scientific Council of the Stephan Angeloff Institute of Microbiology at the Bulgarian Academy of Sciences for the election of Chief Assistant Professor Dr. **IVANKA PETROVA BOYADZHIEVA** to the academic position of "**ASSOCIATE PROFESSOR**" at the SAIM-BAS in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3. Biological Sciences, Scientific specialty Microbiology.

August 12, 2025
Sofia

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