REVIEW

BY

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REGARDING: competition for the academic position "Associate Professor" in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3 Biological Sciences (specialty Microbiology) from the Law on the State Agency for Biological Sciences of the Republic of Bulgaria, announced in the State Gazette No. 30 of 08.04.2025 for the needs of the Department of Biotechnology, Laboratory "Extremophilic Microorganisms" IMicB - BAS**.**

This review was prepared in accordance with Order No. I-77/25.05.2025 of the Director of the Institute of Microbiology "Stefan Angelov" - BAS (IMikB) by decision of the Scientific Council of IMikB, Protocol No. 7 of 23.05.2025, according to which I am included in the scientific jury of a competition announced in the State Gazette, SG, No. 30 of 08.04.2025.

CANDIDATE(S): Senior Assistant Professor Ivanka Petrova Boyadzhieva, PhD – sole candidate

The review of the documents shows that the procedure for opening and announcing the competition has been complied with. The submitted set of materials is in accordance with the requirements of Chapter IV, Section III of the Terms and Conditions for Occupying the Academic Position "Associate Professor" of the Regulations on the Terms and Conditions for Acquiring Scientific Degrees and Occupying Academic Positions at the Stefan Angelov Institute of Microbiology at the Bulgarian Academy of Sciences and includes all necessary documents.

I know the candidate for the competition, I have excellent impressions of her, we have no common publications, and I am not in a conflict of interest, according to the law.

BRIEF BIOGRAPHICAL DATA OF THE CANDIDATE

The only participant in the announced competition – Senior Assistant Professor Dr. Ivanka Petrova Boyadzhieva – is a researcher at the Institute of Microbiology - BAS on a permanent employment contract since 2000. Her work experience is 25 years, and three months were spent at the IMIcB in the Laboratory "Extremophilic Microorganisms". The presented CV shows that she has formed and grown as a scientist, passing through all the steps of the professional ladder. In the period 2003 - 2008 she was a doctoral student, followed by a period of motherhood, and in 2011 she defended her dissertation on the topic "Biosynthesis and properties of superoxide dismutase from thermophilic bacteria isolated from Bulgarian hot springs", with her scientific supervisor Assoc. Prof. Dr. Elka Emanuilova. From 2011 to 2015 she was an assistant, and from 2015 to the present, she holds the position of Chief Assistant.

Ivanka Petrova Boyadzhieva was born on May 8, 1975. In 1999, she obtained a master's degree in biotechnology from Sofia University "St. Kliment Ohridski" with a specialization in Virology. She is fluent in English and Russian at an intermediate level.

EVALUATION OF THE SUBMITTED MATERIALS IN THE COMPETITION

In this competition, Dr. Boyadzhieva presents a list of 22 scientific papers, and the total number of publications is 27, after acquiring the ONS "Doctor".

The presented publications are distributed by groups of indicators, according to the requirements of the ZRASRB in the relevant professional field, as follows: 22 publications and reports published in scientific journals, referenced and indexed in world-renowned databases with scientific information, of which 5 by indicator B and 17 by indicator D. The table under indicator D also includes one useful model, of which Dr. Boyadzhieva is the first author. The total impact factor for the entire scientific career is 35.835, H-index 6.

The scientific indicators of Senior Asst. Ivanka Boyadzhieva according to the point system, compared with the minimum requirements according to the Law on the State of the Republic of Bulgaria on the State of the Republic of Bulgaria, are reflected in the short table. I have established that when presenting the points according to indicator D 7, a technical inaccuracy was made. According to my calculations, the points are 232, and 220 were given, so the total number for participation in the competition is 750, instead of 738.

Indicator group	Evidence presented	Requirements for ASSOCIATE PROFESSOR	Number of points achieved by the candidate
А.	Dissertation on the topic of ONS Doctor	50	50
В.	Habilitation thesis / 5 scientific publications in journals that are referenced and indexed in WoS/Scopu	100	100
Г.	Scientific publications in refereed journals and indexed in world-renowned databases Web of Science/Scopus – 13 articles; Articles outside Web of Science/Scopus - 1; Published book chapter or collective monograph – 3; Utility model - 1	220	**312 + 12=324**
Д.	Citations in scientific publications, as required	60	326
TOTALLY			**738 +12 = 750**

With the scientometric indicators indicated above, Dr. Boyadzhieva meets the so-called additional criteria for the growth of the academic staff at the Institute of Microbiology - BAS to occupy the academic position of ASSOCIATE PROFESSOR.

MAIN SCIENTIFIC CONTRIBUTIONS

The general impression of the documentation presented for the competition is that several characteristics can be brought to the forefront before the detailed analysis:

- Focus on the specific scientific topic;
- Depth in the development of a priority biotechnological topic;
- Combination of experimental approaches and functional-mechanistic justification;
- Original, interesting and innovative, but pragmatic elements of the developments.
- There is an excellent integration and correspondence of the topic of the laboratory and the department for which the current competition for the position of associate professor is announced.

The scientific topics that the candidate develops are systematized into four scientific areas:

1. Biodiversity of microbial communities in extreme niches;
2. Biosynthetic potential of extremophiles from Bulgarian extreme niches;

3. Microbial enzymes and exopolysaccharides of biotechnological importance;
4. Participation in joint research with teams of scientists working in other scientific areas.

Dr. Boyadzhieva's main interests are in the field of new microbial enzymes that degrade various industrially important substrates. The available information from studies on microbial phytases is systematized and summarized, with emphasis on bacterial enzymes of extremophilic origin that retain their activity under various adverse conditions.

Isolated, purified and characterized a new phytase from *Cobetia marina* strain 439 with valuable application in the feed industry and 439 shows higher resistance to gastric proteases. Modern methods for 17-fold purification with 35% yield were used. The pH and temperature range of stability and the specific action with only the substrate phytate, and not other phosphorus-containing compounds, were characterized.

For the first time, it has been documented that the bacterium *Virgibacillus salarius* strain 434 produces an extracellular haloalkalophilic pectinase with very high enzymatic activity. The enzyme was found to be alkaliphilic (with maximum activity at pH 9.0) and thermostable, making it suitable for industrial use. Its physicochemical properties, including molecular weight and substrate specificity (polygalacturonase), have been determined. Studies have shown that the active site of the enzyme probably contains sulfhydryl functional groups, since its activity is suppressed by heavy metals and inhibitors.

For the first time, a pectinase from the species *Anoxybacillus gonensis* has been studied, purified and characterized. The optimal conditions for culturing the strain have been determined and it has been found that the enzyme is thermostable (retains 100% activity at 60°C for 1 hour) and alkaliphilic (optimal activity at pH 8.5). The enzyme has been shown to be a polygalacturonase, as it exhibits the highest activity towards unmethylated pectin structures.

A new thermophilic strain of the genus *Bacillus* (UG-5B) has been classified and deposited in the National Bank for Industrial Microorganisms and Cell Cultures. It has been proven that the strain produces nitrilase, the activity of which is manifested only in the presence of a substrate, which indicates that the enzyme is inducible. It has been established that immobilization of cells in agar gel significantly increases the thermostability of the enzyme, making it more resistant to high temperatures. Due to its thermophilicity and high resistance up to 90°C, the enzyme is ideal for detoxification of high-temperature industrial wastewater containing toxic nitrile compounds. Immobilization of cells as an approach allows their repeated use, extends the operating time of the enzyme and provides high efficiency, which makes the technology economical and environmentally applicable. It has been established that Bulgarian corn starch is the most suitable substrate for the production of cyclodextrins (CDs), achieving a good yield of the sought-after γ-CD. The addition of Mg^{2+} further stabilizes the enzyme and slightly increases the production of γ-CD.

I consider it a record-breaking discovery and highly appreciate the identification of the bacterial community that degrades the plastic ε-polycaprolactone (PCL) at the highest temperature reported so far of 55°C. A new strain, *Brevibacillus thermoruber* strain 7, was isolated, which is a representative of an important biotechnological group and shows the ability to degrade PCL. Metagenomic analysis shows that the species *Meiothermus* and *Brevibacillus* have a key role in the biodegradation process. The discovery offers a new, high-temperature method for the biodegradation of plastic waste, which has great potential for environmental applications in the fight against pollution.

Lipase from *Brevibacillus thermoruber* strain 7 is the first thermostable enzyme active on ε-polycaprolactone (PCL) to be isolated, purified and characterized. The enzyme was found to act as a lipase with an exogenous mechanism, with the main hydrolysis product being the monomer ε-caprolactone. Due to its high temperature optimum, exceptional thermostability and wide pH range, the enzyme is highly suitable for

recycling and degrading PCL waste at high temperatures, even close to the melting point of the polymer. This allows *in situ* treatment of plastic waste.

Thermophilic microorganisms synthesize exopolysaccharides (EPS) as a mechanism for adaptation and protection against stressful conditions, such as high temperatures, UV rays and osmotic stress. Thermophilic EPS have great potential for biotechnological applications due to their thermostability, viscosity and emulsifying properties. They can be used in the food and cosmetic industries as emulsifiers and stabilizers. They are also suitable as flocculants for wastewater treatment. Production processes with thermophiles are economically advantageous, as they are characterized by short fermentation times and low risk of contamination.

A new and efficient producer of exopolymeric substances (*Chromohalobacter canadensis* 28) has been identified, which is the first report of a halophilic bacterium synthesizing a polymer with γ-PGA (polyglutamic acid). The exopolymer contains a unique combination of γ-PGA and various sugars, which has not been reported for other halophilic bacteria. The high hydrophilicity, water retention capacity, foaming and emulsifying activity of the biopolymer have been proven.

The optimal conditions for culturing the bacterium *Chromohalobacter canadensis* 28 in a bioreactor (stirring rate, aeration) were determined, which is the first such study for this species. An EPZ yield of 3.085 mg/mL was achieved, which is three times higher than the average for marine bacteria and places this strain among the most productive. The exopolymer shows significantly better emulsion stability than commercial hydrocolloids and can be mixed with other polymers to achieve 100% stability.

Study of the production of EPZ by the halophilic bacterium *C. canadensis* 28 under continuous culture conditions. *C. canadensis* 28 is the only known halophilic bacterial strain that produces γ-PGA (polyglutamic acid) - a non-toxic, non-immunogenic and biocompatible polyamino acid that forms hydrophilic gels suitable for drug delivery, bioadhesive materials, cryoprotection, especially in cosmetics. EPZ from *C. canadensis* 28, containing γ-polyglutamic acid (72%) and EPZ-fraction (14.3%) has high biocompatibility towards human skin cells. When treating human dermal fibroblasts (PCS-201-012) with unpurified and purified EPZ, higher cell viability was observed compared to treatment with γ-PGA alone at doses of 100 and 500 μg/mL. EPZs produced by *C. canadensis* 28 have significant potential for application in cosmetics, especially in products with moisturizing, regenerating and anti-aging effects. Due to its natural origin and safety profile, this biopolymer is suitable for the development of cosmetic formulas with high tolerance and efficacy.

The exopolysaccharide produced by *Chromohalobacter canadensis* strain 28 lowers the surface tension of water, with the critical concentration for micelle formation being about 0.05%. At a concentration of 1.5%, EPZ achieves 100% emulsifying stability, without separation of the oil phase even after centrifugation or prolonged standing. The highest foaming ability (up to 140%) is achieved at a concentration of 1%, which is comparable to that of egg white. The high foaming ability of EPZ makes it suitable for use in the production of foamy foods, cosmetics and cleaning products. Emulsions formed with EPZ are stable for a long period of time, which is evidence of long-term effectiveness without the need for preservatives.

The immobilization of *Bacillus* sp. UG-5B in sol-gel matrices offers biocatalysis, with possible applied importance in industrial and environmental processes. The study of the gut microbiota in lizard species in Western Bulgaria provides new data on the role of the microbiome and shows links between host physiology and microbial composition. The development of bacterial nanocellulose membranes, using food waste and symbiotic cultures for supercapacitors, shows the possibility of use in green energy and the creation of highly efficient, biodegradable and stable materials for modern energy systems.

It has been shown that *Bacillus* sp. UG-5B cells can be successfully encapsulated in sol-gel hybrid matrices. It has been found that the addition of organic components such as polyethylene glycol (PEG) and glycerol to the matrix improves the viability and enzymatic activity of the immobilized cells.

Another very positive highlight in my opinion is that part of the research and scientific products were done in teams with diverse cross-disciplinary skills. This only confirms the valuable research qualities of Dr. Boyadzhieva – to be integrated into a scientific team and play an important role in it. An original and innovative study was conducted, which provides data on the fecal microbiota of five species of lizards in Bulgaria. It was found that the intestinal microbial communities of different species of lizards differ significantly, despite inhabiting the same area. A significant amount of the *Cyanobacteria* type was found in only one of the species, which is unusual and cannot be explained by the diet alone. The study shows that the similarity in diet is not directly related to the similarity in microbiota, which suggests that factors such as the genotype and physiology of the host also play an important role.

As an important contribution, I consider the development of hybrid membranes by impregnating bacterial nanocellulose (BNC) with poly(benzimidazole) (PBI) for use as separators in supercapacitor cells. It has been shown that alkaline treatment improves the hydrophilicity and electrolyte absorption of BNC, leading to better performance. A new type of ecological separator obtained by fermentation of food waste has been created. BNC membranes show high resistance and conductivity in alkaline electrolytes, making them suitable for alkaline batteries and other energy devices. The study confirms the potential of BNC as a functional material for energy systems, combining biological origin with high technological efficiency, which is a concept of "green electrochemistry".

Overall, Dr. Boyadzhieva's work demonstrates a strong commitment to innovative biotechnological solutions and has made significant contributions to the field of microbiology. Through research on extremophile microorganisms, she has discovered new species and enzymes with unique properties. These discoveries have broad potential for biotechnological applications, including in the production of biodegradable materials, pharmaceuticals, and cosmetics. Her work contributes both to fundamental scientific knowledge and to the development of innovative and sustainable solutions for industry. Her contributions represent an important step forward in the use of natural resources to address environmental and technological challenges.

DIRECTIONS FOR ITS FUTURE WORK are also duly described. They are a natural continuation of the research work carried out so far and the prospects are their deepening.

- Research into new extreme niches: Research on thermophilic and halophilic bacteria will continue, expanding to new ecological niches such as psychrophiles (microorganisms inhabiting cold environments).
- Modern molecular biological, metagenomic and bioinformatics analyses will be used to identify uncultivable microorganisms and to search for genes for new enzymes and bioactive compounds.
- Based on the experience gained, research into the ability of extremophiles (thermophiles, halophiles, psychrophiles) to degrade plastics will be deepened, including through genetic engineering to improve the properties of biodegradable polymers.
- A new direction for biological "green" synthesis of nanoparticles (e.g. silver and copper) from extremophile bacteria will be developed. The goal is to create environmentally friendly and biocompatible materials with potential for application in medicine, ecology and energy.
- Work will be done to attract and train young scientists, as well as to secure funding through national and international programs to ensure the successful implementation of research goals.

OVERALL ASSESSMENT OF THE CANDIDATE'S COMPLIANCE WITH THE MANDATORY REQUIREMENTS OF THE ZASRRB

Dr. Boyadzhieva's publication activity fully meets the requirements for the growth of the academic staff. It is also worth noting the fact that she has publications in international journals positioned in Q1 with high impact, such as Molecules, Fermentation, Appl Microbiology and Biotechnology, etc. 163 citations have been noted in Scopus.
It has been presented 11 participations in national and international scientific forums in 9 scientific projects, of which 5 are international, as the leader of 1 of them.
According to the attached certificate for fulfilling the minimum requirements of the ZRASRB and those of the IMicB - BAS, Senior Asst. Dr. Ivanka Petrova Boyadzhieva meets and exceeds the indicators for holding the academic position of "Associate Professor".

CRITICAL NOTES – some technical inaccuracies are noticeable, which I do not find significant.

CONCLUSION
The analysis of the scientific and research activities of Senior Asst. Prof. Dr. Ivanka Petrova Boyadzhieva shows that her expertise is entirely in the field of the announced competition. The results obtained are original, up-to-date and of public importance. The contributions have a significant fundamental nature, as well as a clearly outlined practical focus - they reveal opportunities and prospects for the development of new technologies.
The candidate's scientific and research activities cover and exceed the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Implementation of ZRASRB, the Regulations of the Bulgarian Academy of Sciences, as well as the Institute of Microbiology "Stefan Angelov" - Bulgarian Academy of Sciences. In view of all the above, I can conclude that Senior Asst. Prof. Dr. Ivanka Petrova Boyadzhieva is a well-established researcher with a bright scientific profile.

As a member of the scientific jury for the announced competition, **I give a positive assessment of the scientific contributions of Senior Assistant Professor Dr. Ivanka Petrova Boyadzhieva and I strongly recommend that the members of the esteemed jury vote for the election of the academic position "Associate Professor" in the professional field 4. Natural Sciences, Mathematics and Informatics, 4.3 Biological Sciences (specialty Microbiology) from the ZRASRB, announced in for the needs of the Department of Biotechnology, Laboratory "Extremophilic Microorganisms" at the Institute of Microbiology "Stefan Angelov" - BAS.**

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