

Review

by prof. Dr. Petya Koycheva Hristova,
Faculty of Biology, Sofia University "St. Kliment Ohridski"

Regarding: The materials presented in a competition for the academic position "ASSOCIATE PROFESSOR" in a Professional area 4.3. Biological sciences (Microbiology - New Functional Foods).

The competition for the academic position of "Associate Professor" has been launched for the needs of the Department of General Microbiology in the higher education area 4.3. Biological Sciences (Microbiology - New Functional Foods) and published in State gazette issue 29/12.04.2022. This review is presented to a scientific jury formed by Order № I-70/30.05.2022 of the Director of the Institute of Microbiology, BAS.

According the deadline regulated by the HEL, an application for participation in this competition was submitted by only applicant - assist. prof. Galina Dinkova Stoyancheva, Ph.D., who currently works on a permanent employment contract in the Department "General Microbiology", "Microbial Genetics" laboratory, IMcB, BAS.

In accordance with the meaning of para 1, p. 3 and p. 5 of ZRASRB I declare that as a member of this jury I have no common publications with the evaluated candidate in the competition.

I. General presentation of the procedure and the applicant

For participation in the competition assist. prof. Galina Stoyancheva presented the documents that are in compliance with the requirements of the Act for the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its implementation, and the Regulations for the conditions and the order for acquiring scientific degrees and holding academic positions in Institute of Microbiology "Stefan Angelov" . The documentation for the competition is well structured and contains all the data necessary for the evaluation of the scientific-applied and pedagogical activity of the applicant.

Assistant Galina Stoyancheva graduated from the Faculty of Biology of the University of St. Kliment Ohridski" in 1999 as a master's degree in Biotechnological processes with a specialization in Gene and Cell Engineering. In 2006, she defended her doctoral dissertation on "*Combined approach for molecular - taxonomic characterization of lactobacilli*" (diploma No. 31275/12.04.2007) to obtaining the educational and scientific degree "PhD".

Dr. Stoyancheva's scientific career in the field of microbiology began in 1998 as a specialist at the IMikB, BAS in the "Microbial Genetics" laboratory. From 2000 to the present, she has successively held the positions of research associate III degree and assistant professor. The total work experience of the candidate in the specialty is more than 20 years.

During the period 2007-2008, the candidate participated in a specialization at the University of Verona, Italy, Department of Science and Technology, Laboratory of General and Food Microbiology.

Galina Stoyancheva is a member of the Union of Scientists in Bulgaria, Microbiology Section and of FEMS. In 2006, the candidate received the Diploma of the VAK, SUB and FNS for achievements of doctoral students. In 2007, she received a scholarship from FEMS and the Annual Award of the „Stefan Angelov“ Foundation for the best work of a young microbiologist.

The candidate's scientific interests are in the field of molecular microbiology related to the problems of antibiotic resistance, the search for new therapeutic agents and the development of new functional foods with probiotic characteristics beneficial to human health.

II. General assessment of the applicant activity

1. Assessment of educational-pedagogical activity

The teaching activity of Ass. Professor Galina Stoyancheva is involved in training students under the "Student Practices" Program. There are no data on the number of students being trained.

2. Assessment of scientific and applied research activities

2.1. *Scientific papers*

The scientific works of Galina Stoyancheva has a total of 30 scientific publications, nine of which she is the first author. Of the articles, 24 were published in IF journals (total IF 27.49), 6 were reports in peer-reviewed publications, book chapters, proceedings of international forums (published in full text), which were not referenced and indexed in WoS/Scopus .

Dr. Stoyancheva's scientific publications have been cited 303 times and form a total h-index of 7. According to Scopus data, 24 publications have been referenced, which have been cited 198 times and form an h-index of 5. Dr. Stoyancheva reports her results to 30 national and international scientific forums. Her scientific output can be found in renowned scientific journals, most of which have a high IF such as Archives of microbiology, Polar Biology, Journal of basic microbiology, Antonie van Leeuwenhoek, Starch-Starke, Engineering in Life Sciences, which is proof of the high scientific value of the results.

For participation in the competition for the academic position " Associate Professor " Assistant Professor Galina Stoyancheva, PhD, presents the relevant report on the implementation of the minimum national requirements under Art. 2b of 3PACPE, which fully meets the established criteria.

According to the Reference on Indicators, the data are as follows:

- Indicators from group A - dissertation for "PhD" - **50** points;
- Indicators from group B - 6 scientific articles in IF journals and with rank Q1 (1), Q2 (3); Q4 (2), which thematically correspond to the competition - total 109- **100** points.
- Indicators from group C - 17 scientific articles with IF and rank Q1 (1) Q2 (7), Q3 (3), 2 book chapters; - a total of **288** points.
- Indicators from group D - citations - **600** points.

The indicators from group E are not obligatory for the academic position "associate professor", but the candidate presents a significant activity. Information for participation in 17 projects is presented, five of which are international. She was the head of 4 projects.

These data show that the report on the fulfilment of the minimum requirements for the academic position "Associate Professor" of Dr. Galina Stoyancheva *covers and exceeds* the required points for the individual indicators, gaining a total of **1047** points (instead of the required 450).

The candidate also meets all the additional requirements defined in the Regulations of Institute of Microbiology, BAS. She presents 28 publications, of which she is the first in 8 and corresponding author in one. It meets and exceeds requirements for project participation, impact factor, h-index and citations.

2.2. Scientific and applied contributions

Dr. Stoyancheva's scientific publications fully correspond to the theme of the current competition (Microbiology - new functional foods) and can be systematized in three main area.

2.2.1. Isolation and molecular characterization of lactic acid bacteria.

The intensive research work of the applicant, aimed at to analysing the genome of active lactobacilli strains have been carried out with the aim of identifying and sequencing bacteriocin genes, characterizing peptides with antibacterial activities produced by lactic acid isolates from the human microbiome; a probiotic evaluation of certain strains of lactic acid bacteria was performed.

The presented results form several contributions of theoretical and applied nature are the following:

- Original strains of *Lactobacillus*, isolated from clinical samples, with pronounced antimicrobial activity against pathogenic microorganisms were selected. The protein nature of the active substances has been established and the genes that determine them have been characterized.
- New specific primers were designed for the amplification of two bacteriocin operons after sequencing the genomes of three *Lactobacillus crispatus* strains. An operon responsible for the synthesis of the bacteriocin "gasericin A" has been identified for the first time in the genome of *Lactobacillus crispatus*.
- An original strain of *Lactococcus lactis* HV219 was isolated, producing bacteriocin HV219, which has antimicrobial activity against Gram-positive and Gram-negative bacteria. The producer's growth conditions, media composition, and cultivation conditions necessary for optimal bacteriocin production are optimized
- The probiotic capacity of ten strains of lactobacilli isolated from clinical samples was evaluated. The production of antimicrobial compounds (lactic acid, ethanol and hydrogen peroxide) and biomass accumulation in the studied strains under continuous cultivation conditions were studied.

2.2.1. Biodiversity of microorganisms in different ecosystems. Species identification of bacteria, yeasts and fungi.

In this field, species identification of microorganisms that are pollutants in a number of

industries or producers of biologically active substances was carried out. The main contributions have a scientific and applied nature. The most significant of these are:

➤ New strains of algae have been researched to produce high value molecules that may find applications in medicine, pharmacy, agriculture and cosmetics.

➤ A bacterial strain was isolated, assigned after sequencing the 16S rRNA gene to the species *Microbacterium* sp., which inhibits the growth of Antarctic microalgae (cyanobacteria *Synechocystis salina* and green eukaryotic microalgae *Choricistis minor*). This strain may find application as a regulator of algal blooms in water bodies.

➤ The microbial content of domestic and commercial lactic acid products (yogurt, cheese and yellow cheese) was studied. Twenty-one pure cultures of *Lactobacillus* genera (*Lactobacillus delbrueckii*, *Lactobacillus helveticus* and *Lactobacillus plantarum*) and six yeast strains (*Kluyveromyces*, *Rhodotorula* and *Candida*) were isolated.

➤ Amylolytic lactic acid bacteria of the genera *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Pediococcus*, *Carnobacterium* and *Weissella* have been isolated. Amino acid sequences corresponding to ALAB amylase enzymes were compared and some features of gene expression were analyzed. The possible application of ALAB strains for direct production of lactic acid from starch is discussed.

➤ For the first time, research has been conducted to study the biodiversity of fungi inhabiting historical monuments in Egypt, part of the world cultural heritage.

➤ Through sequence analysis, more than 250 strains of filamentous fungi isolated from Magura cave, from soils, numerous strains producing different enzymes were identified.

➤ The cellular response of two *Aspergillus fumigatus* strains isolated from highly contaminated soils against treatment with several metal ions (Cd, Cu, Ni and Zn) was studied. A link between oxidative stress and heavy metal toxicity has been established.

2.2.2. Studies on genes related to enzyme production.

In this field, catalase enzymes, which are key antioxidants in aerobic organisms, were studied. They reduce oxidative stress by detoxifying cellular hydrogen peroxide. Filamentous fungi are the subject of the study, which are known to possess several catalases, but data on temperature-sensitive enzymes are scarce. Studies in this direction have contributions of both fundamental and applied nature.

➤ For the first time, an in-depth molecular biological study of catalases from the Antarctic strain *Penicillium griseofulvum* P29 was carried out. After sequencing, the characterization of five catalase genes was achieved, enabling the development of new approaches in the production of temperature-sensitive catalase. The complete sequences of the five genes, which are between 2467 bp - 1501 bp in size, have been deposited in the NCBI GenBank database under accession numbers MW618002 to MW618006.

➤ New specific primers for catalase genes in the *Penicillium griseofulvum* species were created, through which the expression levels of the five genes at different temperature regimes were investigated with reverse-transcription Quantitative Real-Time PCR (RT-PCR).

➤ The influence of temperature as a factor in the regulation of the expression of catalase genes in filamentous fungi was investigated for the first time. Four of the catalase genes were found to be induced by low temperature.

➤ Two fungal strains producing ligninolytic enzymes were isolated from Bulgarian forest soil. They were identified as *Trametes trogii* and *T. hirsuta*, which were evaluated for their enzyme activity, laccase, lignin peroxidase and Mn-dependent peroxidase. *T. trogii* was selected as a better producer of ligninolytic enzymes. The production process has been improved by optimizing a number of parameters that allow increasing yields.

III. Assessment of the personal contribution of the candidate

The evaluation of all submitted documentation gives me the reason to assume that all scientific works are the work of the candidate.

IV. Conclusion

In conclusion, it can be stated that the Assistant Professor Galina Stoyancheva, applicant in this competition, meets all scientometric indicators requirements of the LDASRB, the Regulations to it, as well as the Regulations of Institute of Microbiology concerning the academic position "Associate Professor". The documentation for the competition presents convincing evidence of its scientific, scientific-applied and pedagogical activities.

In view of all mentioned above, I give confidently my positive assessment and strongly recommend to the members of the Scientific Jury formed by order № I-70/30.05.2022 of the Director of the Institute of Microbiology, BAS., to prepare a proposal to the Scientific Council of

the IMkB for election of Assistant professor Galina Dinkova Stoyancheva PhD, to the academic position of "ASSOCIATE PROFESSOR" in the professional area 4.3. Biological sciences (Microbiology - New Functional Foods).

15.08.2022

Reviewer:

Prof. Petya Hristova