

# REVIEW

for holding the academic position of Professor  
presented to the scientific jury appointed by Order No ПД-38-570 / 03.12.2020 of the  
Rector of Sofia University "St. Kliment Ohridski"

**Re:** competition for the academic position "Professor" in the professional area 4.3. Biological Sciences (Microbiology - General and Food Microbiology), announced in SG No 88 / 13.10.2020 for the needs of the Faculty of Biology of Sofia University - Department of General and Industrial Microbiology.

**Prepared by:** Prof. Dr. Veneta Ivanova Groudeva, Faculty of Biology, Sofia University "St. Kliment Ohridski", a retiree

An application for participation in the announced competition was submitted by a single applicant – Assoc. Prof. Dr. Petya Koycheva Hristova, Head of the Department of General and Industrial Microbiology at the Faculty of Biology. All documents for the competition are prepared precisely and correctly, and are presented according to the requirements set out in the Regulations on the terms and conditions for obtaining scientific degrees and holding academic positions at Sofia University "St. Kliment Ohridski".

## 1. General information on the professional career of the applicant

Assoc. Prof. Dr. Petya Koycheva Hristova graduated from Sofia University "St. Kliment Ohridski", Faculty of Biology, speciality "Molecular and Functional Biology", specialization "General and Industrial Microbiology" as a biologist in Molecular and Functional Biology, with specialization General and Industrial Microbiology in 1987. From 1988 to 1991 she was a full-time PhD student at the Department "General and Industrial Microbiology" and after successfully defending in December 1995 a PhD thesis on "Study of the collagenolytic activity of some soil streptomycetes" was awarded the degree of Candidate of Biological Sciences (Doctor, diploma No 24148 / 03.04.1996).

From 1997 to 2000 she held the position Assistant Professor in Microbiology at the department, from 2000 to 2012 – the positions of Senior and Chief Assistant, and from 2012 to the present - the positions of Associate Professor at the Department of General and Industrial Microbiology.

For the period 1994 - 2004 she conducted several specializations abroad, at the University of Nantes through the French Cultural Institute in Bulgaria and the Tempus program in cooperation with Faculty of Biology and ENITIAA-Nantes, France.

From 1998 to 2000 he completed a successful postdoctoral specialization at ENITIAA Nantes, France on "Development of a new diagnostic algorithm for molecular identification of species of the genus *Carnobacterium* based on polymorphism in the 16S-23S rDNA region of the ribosomal operon".

Along her education and professional development, Assoc. Prof. Dr. Petya Hristova acquired professional experience and skills in two main areas: research and teaching. The professional realization of the applicant so far, fits the competition requirements in respect to the

scientific topic and reflects current and promising areas of microbiology, in particular molecular biology of microorganisms.

Assoc. Prof. Dr. Petya Hristova is performing her scientific and teaching activities entirely at the Faculty of Biology and actively participates in the development of the Faculty and the Department of General and Industrial Microbiology. In 2016 she was elected as a head of the Department and currently holds this position. From 2016 - 2020 she was a Vice-Dean of the Bachelor's Degree of Education at the Faculty of Biology, Head of the MSc Program "Food Quality and Safety", speciality "Agrobiotechnology", of which she is the founder.

## 2. Analysis of the materials submitted for participation in the competition

A comparative analysis of the materials submitted for participation in the competition, in accordance with the requirements of the Act for the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its application, and the additional requirements in accordance with the Regulations of Sofia University "St. Kliment Ohridski" is presented in the table below.

<b>Indicators group</b>	<b>Content</b>	<b>Requirements for "Professor" position</b>	<b>Applicant data</b>
<b>A</b>	Indicator 1	<b>50</b>	<b>50</b>
<b>B</b>	Indicator 2	<b>100</b>	<b>0</b>
<b>C</b>	Indicators 3 or 4	<b>100</b>	<b>100</b>
<b>D</b>	Indicators 5 to10	<b>200</b>	<b>207</b>
<b>E</b>	Indicator 11	<b>100</b>	<b>566</b>
<b>F</b>	Indicator 12	<b>150</b>	<b>220</b>

The applicant has a total of 65 research papers, 35 of which are in journals with impact factor and SJR, which are included in the *Scopus* database, while the remaining 30 are in proceedings from scientific forums, chapters from a book published in a foreign publishing house, printed materials in international educational programs and non-refereed journals, one monograph, two textbooks.

Among these papers, 22 are published after acquiring the academic position of "Associate Professor". The total impact factor of the applicant is 30.712, and the *Scopus* h-index is 9.

Reference in the *Scopus*, *Web of science* and others databases is given on the citations of the applicant's publications (529), among which 283 are after the habilitation for Associate Professor.

The applicant has participated in 33 international and national forums with oral presentations and posters, 19 of which are presented in the current competition.

A detailed reference on the participation in 32 projects is presented; Assoc. Prof. Dr. Petya Hristova was a coordinator of three of them and a member of the scientific teams of the others. Among these projects, 20 are research and 11 international educational ones. Evidence for participation in 9 research and 3 international educational projects is presented in the current competition.

The scientific supervision of Assoc. Prof. Dr. Petya Hristova includes 35 graduates, 22 of whom are after her election as an Associate Professor (for the period 2013-2020).

She is a supervisor of 5 full-time PhD students, 3 successfully graduated in 2015 and 2016, and 3 currently studying.

### **Conclusion on item 2:**

*The applicant fully meets the criteria of the Act for the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for its application for the academic position of Professor and in indicators D, E and F significantly exceeds its requirements.*

### **3. Analysis of the research activity and contributions of the applicant**

The research papers submitted for review are in the research area of the competition for the academic position of "Professor" in General and Food Microbiology and can be structured in the following areas: **Molecular taxonomy of microorganisms, Biological hazards in food, Microbiological control of probiotics, Antibiotic resistance to opportunistic pathogens, Obtaining of biologically active substances with antimicrobial activity.**

Part of the research studies in these areas, the applicant began before the habilitation and subsequently expands and deepens.

In this competition, the applicant submits 22 research papers as follows:

1 monograph, 14 research papers in peer-reviewed and indexed journals, 7 research papers in journals without impact factor, other publications and educational projects, two textbooks for students, which are also related to the subject of the competition.

The monograph "Cross-pathogens - new biological hazards in plant foods", of which the applicant is the sole author, is a particularly relevant topic in recent years, to clarify the origin and role of cross-pathogens as new biological hazards in foods of plant origin. The main contributions are related to the in-depth analysis of the published data and own research on the molecular mechanisms of cross-pathogenicity, establishing the presence of specific gene profiles and microbial structures of cross-pathogens associated with plant colonization. The preconditions for the emergence of new cross-pathogens and the creation of evolutionary models that would explain the emergence of new relationships are clarified. The analyzed information on these issues is an important basis to improve the existing standards in the production of plant foods and to expand the research of their microbial composition. As a result, the author proposes the creation of a common coordination network, unifying different food safety specialists and the development of new strategies for minimizing the risk of microbial contamination in food. The monographic work is of high scientific and practical value.

In the above areas of the applicant's activity, the most significant scientific contributions are as follows:

Antibiotic resistance studies of opportunistic pathogens (**research papers No 44, 50, 52, 54**) have identified the most effective rapid tests to detect producers of broad-spectrum beta-lactamases, carbapenemases and metallo-beta-lactamases. Their quick and accurate identification in opportunistic bacteria is essential for the selection of appropriate antibiotic treatment. The necessity of using more than one phenotypic method for reliability of the obtained results has been proved. ESBL-producing strains have been shown to express the lowest degree of carbapenem resistance. Carbapenem-resistant strains have a high degree of resistance to ampicillin-sulbactam (95.5%), ciprofloxacin (87.8%) and cefepime (83.3%). Very rarely, (0.9%) carbapenem-resistant strains are producers of metallo-beta-lactamases. Many of these opportunistic pathogens have been shown to have the potential for cross-pathogenicity, which increases the importance of the studies performed.

The applicant's contributions in the field of Molecular identification and typing of microorganisms in food (**research papers No 48, 49, 57, 61**) are mainly related to the creation and validation of a common diagnostic algorithm applicable for rapid and accurate identification of microorganisms in dynamic microbial communities. A polyphasic-taxonomic approach has been

developed, combining phenotypic and genotypic methods (PCR amplification of the 16S rDNA gene with restriction analysis) for analysis of mixed microbial communities of different nature. A complex approach of culture-independent molecular methods (ARDRA with *HinfI* and *Csp6I* endonucleases), cluster analysis and phenotypic culture methods for analysis of the intestinal microflora of snails has been developed and validated. An effective diagnostic algorithm for rapid typing of bacteria in foods, based on multiplex PCR, PCR with species-specific primers and sequencing has been created and its effectiveness in assessing the dynamics of lactic acid bacteria in rye dough, intestinal tract of *Cornu aspersum* and *Apis mellifera* has been proved.

An original contribution is the isolation and identification for the first time in Bulgaria from rye dough of the species *Lactobacillus spicheri*, *Lactobacillus paralimentarius*, *Lactobacillus kimchii* and *Lactobacillus sanfranciscensis*. A significant contribution is the demonstration for the first time of a complex lactic acid component in the intestinal tract of edible snails at different physiological stages of their life cycle. For the first time, a molecular analysis was performed for genus- and species-specific typing of the intestinal lactic acid bacteria in *Cornu aspersum*.

An original contribution of fundamental and applied significance is the study of the composition and functional activity of the lactic acid microflora of the intestinal tract of *Apis mellifera* in Bulgaria. A significant contribution is the finding that representatives of the species *Lactobacillus plantarum*, *L. pentosus*, *L. iwatensis*, *L. kunkeei* and *Weissella confusa* are the main components of the intestinal microbiota of Bulgarian bees.

One of the most significant studies of the applicant is related to the molecular identification and typing of several groups of phytopathogenic bacteria on plants of the species *Solanum lycopersicum* L. (tomatoes) and *Capsicum annuum* L. (pepper) (**research papers No 47, 51, 55, 58**).

Potential pathogens caused by members of the genus *Xanthomonas*, *Pseudomonas* or *Erwinia* were analyzed. Rapid molecular analysis was developed by amplification with species-specific primers to identify the three species of the genus *Xanthomonas*: *Xanthomonas vesicatoria*, *Xanthomonas euvesicatoria* and *Xanthomonas gardneri*. It has been proven that in the studies from 2015, the dominant species for Bulgaria is *Xanthomonas vesicatoria*, which infects only *Solanum lycopersicum* L., while *Xanthomonas gardneri* infects to a lesser extent *Solanum lycopersicum* L., and *X. euvesicatoria* mainly affects *Capsicum annuum* L.

The molecular approach developed by the applicant on the basis of PCR with subsequent RFLP-analysis for identification and differentiation of the causes of bacterial scab has been validated on 262 Bulgarian and Macedonian strains. Restriction maps of the species *Xanthomonas euvesicatoria* (132 strains), *Xanthomonas vesicatoria* (115 strains) and *Xanthomonas gardneri* (15 strains) have been developed, which help to quickly identify the causative agents and detect penetration routes. For complete discriminative molecular typing of the genotype of phytopathogenic species of the genus *Xanthomonas*, the method of pulse electrophoresis (PFGE) was applied and the genetic diversity of 100 strains isolated between 1985 and 2012 from different varieties of *Solanum lycopersicum* L. and weeds from 11 geographical region in Bulgaria was characterized. Two new haplotypes for *Xanthomonas vesicatoria* and one haplotype for *Xanthomonas gardneri* strains have been identified for the first time. The isolation of *X. euvesicatoria* as a pathogen causing bacterial scab on tomatoes in Bulgaria was established for the first time. The species affiliation of 27 strains isolated from different regions was determined by species-specific PCR amplification and the BIOLOG system. In addition to their fundamental value, these results implicate significant practical contributions related to the possibility of analyzing the disease dynamics and penetration ways, and forecasting the disease distribution in economically important crops.

In the field of Microbiological control of probiotic products (**research papers No 57, 59**) a procedure for quality control of probiotic food supplements has been developed and proposed. Significant deviations from the information provided on the labels regarding the strain composition of the products and the physiological and probiotic properties of the cultures used were found. A strategy has been proposed for the molecular identification of food additives from lactic acid bacteria at the species level, which would actually prove the quality of the additives and probiotic products in general.

The antimicrobial potential of hemocyanin from *Eriphia verrucosa* to obtain biologically active substances with antimicrobial activity was studied (**research paper 53**), and it was found that native hemocyanin (EvH) has no biological activity, unlike its five structural glycosylated units (SU), which show differentiated antibacterial activity. The results that the degree of hemocyanin glycosylation plays an important role in the manifestation of its functional antibacterial properties are of fundamental and practical importance.

The results presented in the research papers, proceedings and scientific forums have a wide repercussion in the international scientific community (529 citations). The applicant has significant scientific contributions of an original nature, scientific and theoretical contributions of a confirmatory nature, as well as of methodological one. It is obvious that the applicant has established herself as a good researcher and expert in biology of food pathogens, expert in microbiological techniques for isolation, cultivation and phenotypic characterization of microorganisms, practicing techniques for isolation, purification and characterization of enzymes and peptides, electrophoretic and hybridization techniques. analysis of proteins, peptides and nucleic acids, PCR techniques - classical, multiplex, nested, RAPD, real-time PCR, restriction analysis - macrorestriction (PFGE) and microrestriction (RFLP), cloning of DNA fragments / PCR products, construction of genomic libraries, bioinformatics.

The applicant introduced the research filed of food microbiology and cross-pathogens in plant foods at the Department of General and Industrial Microbiology - a promising opportunity for basic research and application in food control and human health.

#### **4. Analysis of the educational activity of the applicant**

The applicant has performed significant teaching activity as follows:

**In BSc degree programs** - Lectures in Microbiology for “Molecular Biology”, Lectures in Microbiology and Virology for “Pharmacy”, Faculty of Chemistry and Pharmacy, Sofia University, Lectures in Pathogenic Microorganisms for “Molecular Biology”.

**In MSc degree programs** - Lectures in Molecular Biology of Prokaryotes and Eukaryotes for "Microbiology and Microbiological Control", Lectures in Food Microbiology for "Microbiology and Microbiological Control", Lectures in Microbiological Control of Food and Food Products for “Food quality and safety”, Lectures in Biological hazards in food for “Food quality and safety”, Lectures in Cellular Pathogens for “Cellular Biology and Pathology”.

**Average auditorium workload: 448 hours, Average total workload: 711 hours** (documented by the Training Department of Faculty of Biology).

In general, this is a huge workload, significantly exceeding the regulatory requirements. Unfortunately, this activity has not yet found a proper place as a criterion in the requirements for the development of the academic potential of the HEI.

All lecture courses that the applicant is performing are within the scope of the competition. It is obvious that she has a defined affinity for teaching, presents to students, especially from the MSc degree, the latest achievements in the relevant fields, introduces innovative teaching methods, strengthens the discussion element in the presentation of the problems, which leads to increased

interest and high appreciation of her teaching activities. Undoubtedly, the experience as a lecturer gained during the specializations abroad, where she has lectured in Molecular Biology, Food Microbiology and Food Hygiene, also contributes to this.

Assoc. Prof. Dr. Petya Hristova was the scientific supervisor of 35 graduates and 5 PhD students.

She is the creator of several new courses in the MSc program in Food Quality and Safety. For the competition she presents two textbooks, one co-authored and one as a sole author. The textbook on Microbiology (co-authored with Prof. S. Vlahov) is a completely revised edition of a textbook on microbiology, published in 2006. It presents the latest advances in molecular microbiology, providing information on basic processes in the prokaryotic cell (biosynthesis of cell wall, glycocalyx components, spore formation, motility, etc.). It is designed for students of all specialities at Sofia University "St. Kliment Ohridski", as well as for students from other Universities in the country where microbiology is studied.

The textbook on Pathogenic Microorganisms reveals for the first time the role of microorganisms in the infectious process amalgamated with the protective responses of their hosts; it presents the mechanisms of action of the major groups of toxins and factors of pathogenicity and virulence of selected, most common human pathogens. The textbook is intended for students of biological specialities who have interests in studying the pathogenic potential of microorganisms.

Both textbooks are a significant contribution to the academic education of biologists in various specialities.

In conclusion, it could be summarized that Assoc. Prof. Dr. Petya Hristova is a highly erudite teacher of microbiology, actively and responsibly engaged in teaching, with an innovative spirit and competencies in the field of microbiology and molecular biology.

#### **4. Personal impressions of the applicant**

I have known Assoc. Prof. Dr. Petya Hristova since she was a student at the Faculty of Biology. As a PhD student, and later a participant in the TEMPUS training programs, I had the opportunity to follow her development and to establish her potential for research work. I want to share a fact that is little known, namely that, thanks to her excellent exam performance at the French Cultural Institute, one of the French examiners showed interest in the department, visited it and thus formed a team to work on the TEMPUS program (the first TEMPUS project for Faculty of Biology and Sofia University).

These impressions were strengthened during her work with students as a lecturer in the Department of General and Industrial Microbiology. Thanks to her participation in various educational projects of the department she received a high qualification as a scientist, a bearer of a new spirit in the study of biology of microorganisms, the introduction of molecular methods in taxonomy, their application in both research and teaching activity. For several years she has been leading an elective course in Pathogenic bacteria, which is quite popular among the students – annually, more than 75% of the students in Molecular Biology choose this course. The same is valid for the MSc program in Food Quality and Safety, which is introduced by her, in which successful performance and leadership she has put a lot of efforts. There are a significant number of applicants for this MSc program every year and a strong students' selection.

Analyzing the materials for this competition, I considered my assessment of the applicant's abilities for research and training fully correct.

I am satisfied that the applicant manages the department, organizes modern training in microbiology, contributes to the significant improvement of the material and technical resources.

Projects have been implemented and sponsors have been attracted to create the first microbiological digitalized laboratories for student training. This is very important to me as a former lecturer and head of the department.

### **Conclusion**

In terms of volume, content and quality, the presented scientific production and the active teaching activity of the only applicant in the announced competition for the academic position "Professor", Assoc. Prof. Dr. Petya Hristova fully meets the requirements of the Act for the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its application, and the additional requirements of Sofia University "St. Kliment Ohridski". The complex evaluation of the submitted materials, as well as the overall activity of the applicant allows me to convincingly propose to the scientific jury and the esteemed Faculty Council of the Faculty of Biology at Sofia University to elect Assoc. Prof. Dr Petya Hristova as a PROFESSOR in professional area 4.3. Biological Sciences (Microbiology - General and Food Microbiology), launched for the needs of the Department of General and Industrial Microbiology of the Faculty of Biology at Sofia University.

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Sofia