

## OPINION

by Prof. Todor Kantarjiev, DSc

Regarding the competition for the academic position of "Associate professor", under direction 4.3. Biological Sciences (Microbiology - microbial degradation of toxic environmental pollutants), announced for the needs of the Department of General Microbiology, Laboratory "Microbial Genetics", Institute of Microbiology "StefanAngelov", BAS

The only candidate in the announced in the State Gazette, issue 29/12.04.2022 is Dr. Maria Gerginova Gerginova, in the position of Assist. Professor at the Institute of Microbiology, BAS. The documentation submitted for participation in the competition is regular and prepared precisely in accordance with the requirements of the Law on the development of the academic staff in the Republic of Bulgaria (ZRASRB) and the Regulations for occupying academic positions at Institute of Microbiology, BAS.

### **General characteristics of the candidate's scientific activity**

In the competition for associate professor, Dr. Gerginova has presented 20 publications, which are referenced and indexed in world known databases with scientific information on the indicators of ZRASRB. She is the first author of 5 and second author of 8 of the reviewed publications, which demonstrates the active and scientific participation.

The analysis of the data relating to the indicators under the National Minimum Requirements from the Regulations for the Application of the ZRASRB shows the following: 50 points are set for the possession of PhD. According to group B indicators, 5 publications are presented: 1 with Q1; 3 - with Q2; and 1 - with Q3, which gives a total of 100 points. According to indicators from group D, within indicator 7, 13 publications are presented, of which 1 - with Q1; 8 publications, with Q2; 2 publications with Q3 and 2 publications with Q3. These data give a total of 239 points. From the indicator for citations of the publications in world-famous databases with scientific information (Web of Science and Scopus) or in total for indicators D, 289 citations are presented without self-citations, with which 578 points are collected. The total number of points from the data filled in the Table for the minimum national requirements is 967 with a minimum required of 430 points.

The conclusion is that Dr. Gerginova fulfils the requirements for each of the presented groups of indicators, necessary for occupying the scientific position "Associate Professor" and, in general, scores significantly more than the sum of points from the mandatory national minimum.

Regarding "Additional criteria for the growth of the academic staff at IMikB", it can be noted, as well that the criteria have been exceeded. For the competition, Dr. Maria Gerginova has submitted a total of 26 scientific papers (post-doctoral) in 7 of which she is the first author. 18 of the presented scientific publications and 4 book chapters are referenced and indexed in world-known scientific information databases, as well as 4 book chapters from international forums published in full text. A general list of 41 scientific papers is presented, illustrating the candidate's overall scientific activity. The presented list of citations contains 289 sources; The Hirsch index, according to Scopus (without self-citations of all co-authors) is 9. The impact factor of the publications submitted for this competition is 23.454.

Dr. Maria Gerginova has led 1 and participated in the elaboration of 11 research projects, of which 10 are national projects, 1 international and 1 under the Operative Program "Science and Education for Intelligent Growth", co-financed by the European Union. It can be

emphasized that although they are not required in the competition for "Associate professor", the candidate also has 100 points in indicators from group E, which she has not indicated.

A list of participations in 14 international and 15 national forums with poster reports is presented, in 11 of which Dr. Gerginova is the first author.

Dr. Gerginova's scientific production shows her deep interest in the field of microbial degradation of toxic organic environmental pollutants, with the focus being mainly on the utilization of aromatic and polyaromatic compounds. To achieve results in this area, Dr. Gerginova researches the abilities of a number of microbial species, including bacteria, yeasts and molds.

The most researches were reported in the direction "Biodegradation of toxic chemical compounds by bacteria, yeasts and filamentous fungi". A significant part of these studies is devoted to the yeast strain *Trichosporon cutaneum* R57. Original results were obtained that convincingly demonstrate that, in addition to phenol, the strain is capable of degrading and assimilating as a sole carbon source a wide range of aromatic and polyaromatic compounds, as well as binary mixtures of phenol with its chloro-, hydroxy-, methyl-, and nitro -derivatives [3,6,7,12]. The degradation of highly toxic and extremely difficult to degrade industrial pollutants from the oil refining industry, such as 2,6-dinitrophenol,  $\alpha$ -methylstyrene and acetophenone, has also been demonstrated [6]. These characteristics of the strain place it among the described microorganisms with a high potential for application in the development of biotechnological schemes for the treatment of industrially polluted waters.

One of the most interesting original results is demonstrated by the studies with newly isolated, Antarctic strains of filamentous fungi. Based on the obtained results, it can be concluded that the strains of filamentous fungi studied by Dr. Gerginova show high degradation activity in relation to phenol, the studied phenolic derivatives, and low molecular weight polyaromatic compounds (PAH) – naphthalene, anthracene and phenanthrene. A number of strains of *Aspergillus fumigatus* capable of degrading phenol in significant concentrations (from 0.3 to 1 g/l) have been found. For the first time, the degradative activity with respect to aromatic compounds and PAH of representatives of *Aspergillus glaucus* and *Alternaria maritima* species was established [8, 11, 19, 22, 24, 26, 34, 36, 38, ].

Studies were also conducted with bacteria from the genera Dietzia, Arthrobacter, Rhodococcus and Gordonia, isolated from an oil-refining region of Kazakhstan. Strains were selected that demonstrated high degradation activity and degraded phenol, hydroxylated phenols, cresols, naphthalene, anthracene and phenanthrene [14].

In order to mathematically describe, evaluate and compare the studied biodegradation processes, biokinetic models have also been created [2, 3, 5, 6, 7, 20].

The main research in the direction "Analysis of enzymes directly involved in the degradation of aromatic and polyaromatic compounds" is focused on the specificity of the first two enzymes of the  $\beta$ -ketoacid pathway (ortho) pathway with a key role in phenol metabolism - phenol hydroxylase and catechol 1,2 -dioxygenase. The studies were carried out on all strains tested. Among all the original achievements, the data on the broad substrate specificity of the enzyme phenol hydroxylase [EC 1.14.13.7] in cells of strain *T. cutaneum* R57 [5] should be highlighted; The analysis of the activity of the enzymes phenol hydroxylase and catechol 1,2-dioxygenase in cells of strain *A. glaucus* AL1 [26]; The demonstrated presence of high phenol hydroxylase activity in the cultivation of *A. glaucus* strain AL1 and *Alternaria maritima* strain AL10 in media including naphthalene, anthracene and phenanthrene as sole carbon sources [19,26,38]; The ability of *A. fumigatus* strains to degrade phenol simultaneously by the classical pathway of ortho-cleavage through catechol and by hydroxylation of phenol in the para-position to hydroquinone has been demonstrated [11]. The obtained results confirm the thesis that flavoprotein mono-oxygenases are highly adaptable with respect to the type of oxidation reactions they catalyze and to the range of substrate molecules.

The enzyme studies dedicated to the biodegradation of phenol in the conditions of a constant electric field in *Pseudomonas putida* and *Bradyrhizobium japonicum* 273 strains are also original in nature. It was found that the applied anodic potential of the electric field significantly affected the activity of the two studied enzymes. In a constant electric field with an anodic potential of 0.8 V/S.H.E., in strain *B. japonicum* 273, a phenomenon of coexistence of the two main phenol degradation mechanisms (ortho - and meta -) was observed or the presence of catechol-2,3- dioxygenase activity along with catechol-1,2-dioxygenase activity [18].

In the heading "Identification of microorganisms and genes encoding enzymes with catabolic activity in the degradation of aromatic xenobiotics" are presented results concerning the taxonomic affiliation of 21 bacterial and 2 fungal active strains from the laboratory collection, established by PCR and nucleotide sequence analysis. Genes encoding proteins with phenol hydroxylase and catechol 1,2-dioxygenase activities are identified and partially sequenced in strains of *A. glaucus* and *A. fumigatus* [11,19,26,37,38]. The resulting oligonucleotide sequences are registered in the NCBI GeneBank.

In addition, the metagenomic analyzes presented in the "Other" section, performed to establish the presence of microorganisms associated with sulfate reduction processes from three different carbon sources (glucose, lactate, and ethanol) can be highly appreciated, as well [39]. Oligonucleotide primer pairs for PCR and nucleotide sequence analysis of Cu/Zn-superoxide dismutase in *A. glaucus* 363 have also been designed.

### **Main scientific contributions of the candidate**

In the candidate's documents, 10 scientific contributions are formulated, which correspond to the results of the scientific activity of Dr. Maria Gerginova. I would point out the following as the most significant among them:

The established capabilities of *Trichosporon cutaneum* R57 and *Trametes versicolor* 1 strains to degrade and assimilate mixtures of phenol and phenolic derivatives prove their high potential for purification of aromatic compounds-contaminated soils and waters.

For the first time, a degradative activity regarding aromatic compounds has been established and the presence of key enzymes of the ortho-mechanism for the degradation of phenolic compounds is proved in representatives of the species *A. glaucus*, *A. maritima* and *T. versicolor*.

For the first time, catabolic genes encoding enzymes with a key role in the degradation of highly toxic aromatic compounds have been identified and sequenced in a representative of the *Aspergillus glaucus* species.

### **Conclusion**

The scientific activity of Dr. Maria Gerginova fully complies with the requirements of the RAS RB and the Regulations for its application, as well as covering all the scientometric criteria required for the occupation of the scientific position "Associate professor", laid down in the Regulations for the acquisition of scientific degrees and occupation of academic positions at the "Stefan Angelov" Institute of Microbiology, BAS. The elaborations presented by Dr. Gerginova are topical, significant and directly related to improving the quality of life. In her experimental work, Dr. Gerginova uses a rich arsenal of classical and modern analytical, microbiological and molecular genetic methods. She stands out as a thorough and active researcher in the field of biochemical and molecular aspects of microbial biodegradation processes. The obtained results reveal opportunities and prospects for new research on current problems in the field of ecological biotechnology, which are indicated in Dr. Gerginova's plans for future research activities.

Assistant Professor Dr. Gerginova has over 27 years of experience as a researcher at Institute of Microbiology - BAS, which shows that the scientific career of the candidate is entirely related to the institution and the Laboratory "Microbial Genetics" that declared the need for a qualified person.

Based on the contributions noted above, the overall documentation of the competition and the undoubted qualities of the elaborations submitted for the competition, I give a positive assessment of Dr. Maria Gerginova's participation in the competition and I strongly support and propose to the scientific jury and the Scientific Council of the Institute of Microbiology - BAS to vote for the awarding of the academic position of Associate Professor to Dr. Maria Gerginova.

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Wrote the opinion:

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