REVIEW

By Prof. Nelly Vladova Georgieva, PhD, Department of Biotechnology, University of Chemical Technology and Metallurgy, Sofia

Regarding: The materials submitted for participation in a competition for the academic position „Associate Professor" at the Institute of Microbiology, BAS for the needs of the Laboratory "Microbial Genetics", Department of General Microbiology, in Professional area 4.3. Biological sciences, scientific specialty "Microbiology – microbial biodegradation of toxic pollutants in environmental", announced in SG ed. 29 on 12.04.2022 with the only candidate being **Assist. Prof. Maria Gerginova Gerginova, PhD.**

General presentation of the submitted documents

The present review is prepared on the basis of Order No I-69/30.05.2022 of the Director of the Institute of Microbiology, BAS and the decision of the scientific jury session from 22.06.2022.

Assist. prof. Maria Gerginova is the only applicant who has submitted documents for this competition within the deadline regulated by the law. She has worked at the same Institute in the Department of General Microbiology, Laboratory "Microbial Genetics" on a full-time position since 1993.

The scientific interests and presented materials of the candidate are in the field of Microbiology, the subject of the competition. The submitted documents are in compliance with the requirements of the Act for the Development of the Academic Staff in 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 the Institute of Microbiology, BAS. They also meet the recommended criteria for academic position of „Associate Professor" in Professional area 4.3. Biological sciences. All submitted documents are structured in a way that fully reflects the scientific research activities of the applicant.

Biographical information about the applicant

Assist. prof. Maria Gerginova was born on 07 October 1970 in Samokov. In 1988 she enrolled as a student in the Biological Faculty of Sofia University "St. Kliment Ohridski" and graduated in 1993 with a Master's degree in "Biotechnological processes". After graduation,

she started working at the Institute of Microbiology, BAS Laboratory "Biosynthesis of organic acids". She worked as an assistant from 1996 until 2011 and has worked as a assistant professor in the Laboratory "Microbial Genetics", IMicB since. During the period 1999-2002 she was a part-time doctoral student at the IMicB, where she developed and defended her dissertation in the specialty "Microbiology" on the "Investigation of phenol biodegradation by yeast *Trichosporon cutaneum* R57", supervised by assoc. prof. Zlatka Alexieva.

Assist. prof. Maria Gerginova has participated in 12 research projects – 10 national and 2 international projects, funded by various organizations. She was the head-manager of one national project, and one project, funded by NSF and European investment fund, Operational programme "Science and Education for Smart Growth". She was also co-supervisor of two doctoral students. The research activity of Dr. Gerginova is in the field of Microbiology and she uses a variety of microbiological, biochemical and physiological methods of investigation as well as enzymes and molecular analytical methods. All this has developed her as a scientist with professional experience.

Main scientific indicators

Assist. Prof. Maria Gerginova has presented a total of 26 scientific papers, which do not include those for the acquisition of the "PhD" degree. The articles are published in referred and indexed journals with IF or SJR - 18 papers, chapters in books - 8, 4 chapters from them are in referred and indexed journals. The total impact factor of the candidate is 23.454, the H – index is 9. The distribution of papers in ranking journals is as follows: in Q1 – 2 papers, in Q2 – 11 papers, Q3 – 3 papers, Q4 – 2 papers. The results of the research activity were reported at many international and national scientific forums. According to the indicators, the evaluation determined by the Law for the Development of the Academic Staff in the RB by which assist. prof. Maria Gerginova participates in the competition, convincingly shows that she meets and exceeds the requirements for position of "Associate Professor". The points of the scientific papers of the candidate for the contest are:

Indicators of A: Dissertation thesis 50 p. (min. 50)

Indicators of B: habilitation paper 100 p. (min. 100)

Indicators of G: scientific papers 239 p. (min. 220)

Indicators of D: cited papers 578 p. (min. 60)

The scientific publications have been cited 289 times, mainly in journals referred in Web of Science and Scopus – 578 p. Regarding "Additional criteria for the growth of the academic staff in IMicB" it can be also noted that the criteria are overfilled.

Scientific contributions

The main scientific contributions from the research of Dr. M. Gerginova are presented in detailed summary and provide well-systematized information about the scientific research activity. They are grouped into three research areas, fully consistent with the topic of the competition, as follows: biodegradation of toxic compounds by bacteria, yeast and filamentous yeast, characterization and analyses of enzymes, involved in degradation of polyaromatic compounds, identification of genes and microorganisms, encoding catabolic enzymes, related with xenobiotics degradation. The scientific contributions of Dr. M. Gerginova can be summarized as follows:

1. **Biodegradation of toxic compounds using bacteria, yeast and filamentous yeast**

The scientific studies and publications in this area are a topical continuation of the doctor thesis of Dr. Gerginova, namely investigation of strain *T. cutaneum* R57 and its ability to assimilate variety of phenolic compounds, as well as polyaromatic compounds as a sole carbon and energy sources. The variety of compounds are as follows: hydroxyphenols (1.6 g/l resorcinol, 1.3 g/l catechol и 1.2 g/l hydroquinone), methylphenols (0.1 g/l *m*- и 0.2 g/l *p*-cresol), 0.1 g/l *o*- and 0.1 g/l *m*- nitrophenols with an ability for degradation of the strain using 0.3 g/l naphtalene, anthracene and phenanthrene being reported for the first time.

Biodegradation has been proven for highly toxic industrial pollutants from oil refinery industries such as 0.7 g/l 2,6-dinitrophenol, 0.5 g/l α-methylstirene and 0.5 g/l acetophenone, Simultaneous biodegradation of phenol mixtures with chloro-, hydroxy-, methyl- and nitro-derivatives has been also proven by *T. cutaneum* R57, important for biodegradation of polluted ecosystems.

The obtained results have both theoretical and applied importance for biodegradation of phenol and phenol derivatives, using filamentous fungi as *Aspergillus awamori* NRRL3112, which possesses high phenol biodegradational activity including catechol, 2,4-dichlorophenol, 2,6-dimethoxyphenol and *Trametes versicolor* 1, degradating 0.5 g/l phenol.

Phenol biodegradation ability (from 0.3 to 1 g/l) has also been proven regarding newly isolated antarctic filamentous fungi strains. These strains have been tested for their ability for

degradation of hydroxylated and methylated phenol derivatives and polyaromatic substances in 23˚C (mesophilic) and 10˚C (psychrophilic) conditions.

Degradation ability of aromatic compounds – phenol, catechol, hydroquinone, *o*-, *m*-, *p*-cresol has been established for the first time regarding *Aspergillus glaucus* AL1 which can partly degradate 0.3 g/l naphthalene and anthracene.

The abilities of strain *A. fumigatus* AL3 to degradate and assimilate catechol, *o*-cresol, to grow in culture media with phenol, hydroquinon or *m*-cresol and partly degradate naphthalene and anthracene in mesophilic conditions have been proven. An ability for high degradation using 0.3 g/l phenol and catechol at 23°C and 10°C for strain *A. fumigatus* AL15 has also been established.

Degradation ability of aromatic compounds has been established for the first time regarding strain *Alternaria maritima* AL10 – using assimilation of catechol and PAH as the sole carbon and energy sources.

The intermediate products – salicilic acid, catechol, adipic acid from degradation of naphthalen by *A. glaucus* AL1 have been established using GC-MS analysis. The intermediate catabolic products from anthracene biodegradation are 2-hydroxy-1-naphthoic acid, o-phthalic acid and protocatechuic acid.

High degradational activity has been established regarding the tested filamentous yeast when it comes to phenol, phenol derivatives and low molecular PAH – naphthalene, anthracene, phenenthrene, which have been added in culture media as sole the carbon and energy sources.

For bacterial strains *Rhodococcus erythropolis* 14/1 and *Gordonia* sp. 12/5, isolated from region Kumkol, Kazahstan, high phenol degradation activity has been also reported

New type *Haldane* kinetic models have been established, corresponding to the degradation rates of phenol, hydroxy- substitute phenols, 2,6-dinitrophenol, α-metylstyrene, acetophenone. A strategy for the management of toxic compound degradation has been proposed, based on the model Fuzzy, leading to improvement of process control and supervision.

2. Analysis of enzymes involved in the biodegradational process of aromatic and polyaromatic compounds.

Novel data has been established for key enzymes (phenol hydroxylase and catechol 1,2-dioxygenase) from the *ortho*-pathway of 3-oxoadipate pathway of phenol assimilation regarding yeast and filamentous fungi. Regarding the enzyme phenol hydroxylase [EC

1.14.13.7], broad substrate specificity is established in the cells of *T. cutaneum* R57, cultivated in media with phenol (0.5 g/l), with high phenol hydroxylase activity reported using hydoquinone as a substrate - 1.2 U/mg P.

Substrate specificity of enzymes phenol hydroxylase and catechol 1,2-dioxygenase is established for strain *A. glaucus* AL1, the activity of phenol hydroxylase is higher - 0.254 U/mg P in the presence of phenol, while in the presence of hydroquinone or catechol, the enzyme activity is 0.125 U/mg P and 0.179 U/mg P, respectively. The activity of catechol 1,2-dioxygenase is higher regarding degradation of phenol or catechol, while in the case of degradation of hydoquinone, *o*-, *m*-, *p*- cresol, it is significantly decreased.

High activity of phenol hydroxylase for fungi, strain *A. glaucus* AL1, cultivated in the presence of naphthelene and anthracene as the sole carbon and energy sources has been reported for the first time. The higher phenol hydroxylase activity (1.48 U/mg P) is established in cells of strain *A. glaucus* AL1, cultivated in the presence of naphthalene, as the sole carbon and energy sources. High activity of the same enzyme is also established for strain *Alternaria maritima* AL10, cultivated in media with 0.3 g/l anthracene or phenenthrene. High catechol 1,2-dioxygenase activity is established regarding the cultivation of strain *A. fumigatus* AL15 in the presence of catehol.

Biodegradation of phenol using the two main pathways is established for filamentous yeast strains *A. fumigatus* (AMA1102, AL8, AL9). The typical pathway *ortho*-cleavage is through catechol, but also through pathway, by which phenol is converted into hydroquinone via hydroxylation in *para*-position. The next step is hydroxylation of hydroquinone using enzyme hydroquinone hydroxylase. The activity of three intracellular key enzymes, involved in phenol catabolisms was estimated - phenol hydroxylase, hydroquinone hydroxylase [EC 1.14.13.x], and catehol 1,2-dioxygenase [EC 1.13.11.1], with their activity varying by strains. The obtained results confirmed that flavoprotein monooxygenase are highly adaptable to the type of oxidation reactions they catalyze and to the range of substrate molecules.

High activity of phenol hydroxylase is established for lignolytic Basidiomycota fungi *T. versicolor* 1 - 0.333 U/mgP. A third enzyme from *ortho*-catabolic pathway - *cis,cis*-muconate cyclase with activity of 0.409 U/mgP – has also been established in regards to this strain.

Novel data estimations regarding enzyme analysis of tested strains have been developed. Combined with discoveries of growth and biodegradation abilities, there has been a demonstration of high degradation potential in regards to the removal of a variety of

aromatic substances in mesophilic conditions as well as ones in psychrophilic 10°C conditions.

The absence of extracellular laccase is established in regards to all strains except *T. versicolor* 1, which means that degradation of aromatic compounds for this strain is due to intracellular enzymes, involved in phenol catabolisms.

3. Identification of microorganisms and genes, that encode catabolic active enzymes, involved in aromatic xenobiotic degradation

Molecular taxonomic analysis is performed for 21 bacterial strains and 2 mould strains, the obtained oligonucleotides sequences are registered in NCBI GeneBank by strains: *Arthrobacter* sp.(MF188995.1), *Dietzia* sp. (12/7MF188990.1), *Dietzia* sp. 13/4 (MF188991.1), *Gordonia* sp. 12/5 (MF188989.1), *Rhodococcus erythropolis* 14/1 (MF188993.1), *Rh.* sp. 1D/1 (MF188988.1), *Rh.* sp. 14/3 (MF188994.1), *Tessaracoccus* sp. 13/8 (MF188992.1), *Aeromonas* sp. (MH394445.1), *Azoarcus* sp. RS4 (MH394448.1), *Clostridium* sp. 4C (MH394443.1), *Burkholderia* sp. phk1 (EU118563.1), *Bacillus* sp. AM3 (MF996775.1), *Porphyromonadaceae bacterium* 3S (MH394442.1), *Pseudomonas* sp. KM1 (MG002174.1), *Ps.* sp. 2C (MH394441.1), *Ps.* sp. 4S (MH394444.1), *Ps.* sp. RS2 (MH394446.1, *Ps.* sp. RS3 (MH394447.1), *Ps.* sp. RS5 (MH394449.1), *Ps.* sp. RS6(MH394450.1); and for moulds strains - *A. fumigatus* strain AL9 (JQ639072.1), *A. fumigatus* AL3 (KT781127.1).

Original oligonucleotides primers, suitable for PCR amplification of genes, that encode enzymes with phenol hydroxylase and catehol 1,2 dioxygenase activity in filamentous fungi have been established. Genes encoding proteins with phenol hydroxylase activity were identified and sequenced in strains *A. glaucus* AL1 (KM360482.1), *A. fumigatus* AL3 (KT781125.1), *A. fumigatus* AL8 (JQ639073.1), *A. fumigatus* AL9 (JQ639074.1) и *A. fumigatus* AL15 (KT371934.1).

Genes encoding enzymes with catehol 1,2 dioxygenase activity were sequenced in the strains *A. glaucus* AL1 (KM360483.1), *A.fumigatus* AL3 (KT781126.1), *A. fumigatus* AL9 (MK598849.1) and *A. glaucus* AL15 (KT371935.1).

The presence of DNA fragments similar to genes encoding the enzymes phenol hydroxylase and cis, cis - muconate cyclase is established in various studied strains capable of degrading aromatic compounds by an *ortho*-mechanism using dot blot hybridization analysis.

The oligonucleotide probe “OligoZA1” is developed for hybridization analysis of genes, that encode proteins with *cis*-*cis*-muconate cyclase activity regarding microorganisms.

Conclusion

The works included for evaluation in this competition are distinguished by scientific accuracy as well as originality, and fully correspond to the scientific field of this competition. The obtained results reveal opportunities and perspectives for new research on current issues. All formal requirements specified in 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 obtaining academic positions in the Institute of Micobiology, BAS, have been fulfilled. The scientific research, achievements and activities of **assist. prof. Maria Gerginova Gerginova** allow me to strongly recommend to the esteemed Scientific Jury and the Scientific Council at the Institute to fully support the acquisition of the academic position of Assosiate Professor by **assist. prof. Maria Gerginova Gerginova** in the Professional field 4.3 Biological sciences, scientific specialty "Microbiology", IMicB, BAS.

15.07.2022 Signature:…….

/prof. Nelly Georgieva, PhD/