## **OPINION**

Regarding the acquisition of the academic position "professor", in a professional field 4.3. Biological sciences /Microbiology - genomics and regulation of gene expression in prokaryotes/ for the needs of the Department of General Microbiology, Laboratory of Microbial Genetics

Candidate: Assoc. prof. Penka Petrova, DSc

**Opinion was prepared by:** Assoc. prof. Lyudmila Kabaivanova, PhD, IMiKB "Stephan Angeloff" – BAS

The candidate Penka Petrova is currently an associate professor at the Stephan Angeloff Institute of Microbiology - BAS and Head of the General Microbiology Department, and since 2020 she has been the Director of the same Institute. She has been a Doctor of Science since 2020.

Her research activities are in the field of microbiology, biotechnology, molecular biology, as well as genetic and cell engineering and bioinformatics. Assoc. prof. Petrova's scientific qualification covers isolation, taxonomy and identification of bacteria, morphological, biochemical, physiological, genetic studies of microorganisms, metagenomic studies of communities, sequencing of prokaryotic genomes, as well as isolation of new and development of recombinant strains-producers with target enzymatic activities and their application in biotechnological processes. The topics she develops are indisputably relevant due to the fact that in addition to gaining knowledge about the taxonomy, morphology, physiology and biochemistry of microorganisms, today an important task is to clarify the relationship between microorganisms and humans, deepen the work on improving quality of life, health and longevity through microbiome control. Assoc. prof. Petrova has significant results and achievements in the application of new molecular approaches in three main areas: demonstration of new enzymes through genomic, transcriptional and enzymological approaches; sequencing of genomes and metagenomes and application of genetic engineering methods to construct new bacterial producers of acids and fuels. Last but not least is the elucidation of the molecular mechanisms of decomposition and conversion of substrates, of plant biomass from microorganisms into valuable products, which makes possible to change the attitude of mankind to waste substrates. Assoc. Prof. Petrova's scientific activity can be evaluated highly, based on her high scientometric indicators: Total number of publications - 68, cited 731 times.

According to the submitted report for fulfillment of the minimum requirements for the academic position "Professor" on the basis of the Regulations for application of the Law for Development of the Academic Staff of the Republic of Bulgaria and the Bulgarian Academy of Sciences, as well as according to the Additional Requirements of IMicB, it can be seen that the candidate fully meets all the requirements - she has submitted materials corresponding to 1375 points out of the required 600.

I cannot miss to mention some important contributions reflected in the published scientific papers:

- 1. 1. For the first time, the genes responsible for the hydrolysis of  $\alpha$ -glucans in certain species of lactic acid bacteria (LAB) have been identified. The world's first amylolytic representatives of the species *Lactobacillus sakei* and the genus Enterococcus have been isolated. Transcription analysis was performed and the regulation of gene expression of starch-modifying enzymes was studied.
- 2. 2. A new cgt gene and the enzyme cyclodextrin glucanotransferase have been identified in *Bacillus pseudalcalophilus*, and the amino acid sequence homology with the enzymes known so far is less than 87%. For the first time, a recombinant enzyme cyclodextrin glucanotransferase was immobilized in magnetically modified carriers to produce cyclodextrins. The reuse of CGT-ase magnetic biocatalyst shows the possibility of obtaining three to four times more CD in 120 minutes compared to the yield of single-use enzyme preparations.
- 3. The complete genome of *Bacillus velezensis* 5RB was sequenced, and gene analysis revealed the ability of the strain to convert lignocellulosic substrates into valuable products. For the first time, the synthesis of enzymes hydrolyzing cellulose and hemicellulose has been demonstrated in the species *B. safensis*, *B. toyonensis* and *B. Velezensis in vivo*.
- 4. The relationship between the hydrophobicity of the cell surface of lactic acid bacteria and their resistance to organic solvents has been proven for the first time. An adaptive mechanism has been demonstrated by which *L. plantarum* and *L. helveticus* cells reduce the amount of hydrophobic SLP proteins on their cell surface under butanol stress.
- 5. For the first time a detailed molecular-biological study of neuraminidase from nontoxigenic strain *Vibio cholerae* was performed. Sequencing and characterization of the nanH gene and the enzyme encoded by it allow the development of safe sialidase production.
- 6. For the first time, a recombinant strain of *Klebsiella pneumoniae* G31 A was constructed with the  $\alpha$ -amylase gene from *Bacillus licheniformis* 44MB82 / G introduced. The recombinant strain is able to fully convert highly concentrated starch solutions to 2,3-

butanediol. The highest concentration of 2,3-butanediol (53.8 g / L) was achieved in the metabolism of 200 g / L of starch, and the obtained concentration and productivity are 14 and 3 times higher than those achieved in the world so far.

- 7. For the first time it was proved that the Bulgarian strains *L. delbrueckii* subsp. bulgaricus synthesize galacto-oligosaccharides (GOS) in milk and lactose medium due to the rarely found transferase activity of the enzyme  $\beta$ -galactosidase.
- 8. The extracellular α-amylase gene was first introduced in *Zymomonas mobilis* DSM 424. The *Bacillus licheniformis* gene was cloned into shuttle vectors pZT1, pZT2 and pZT3, under the control of the Plac (*Escherichia coli*) and SacC (*Zymomonas mobilis*) promoters, and its heterologous expression was demonstrated.
- 9. For the first time, heterologous expression of  $\beta$ -glucuronidase in diploid strains of yeast *Ogataea polymorpha* was performed and their potential as hosts was demonstrated.
- 10. A new biotechnological process for microbial production of fructose from inulin has been developed.

I know Assoc. prof. Petrova from our joint work at the Institute of Microbiology as an established scientist and specialist. The candidate's activities in the field of modern molecular biological methods, which are the basis of genetic analysis, identification of microbial species and genes responsible for specific microbial response and biosynthesis, the study of their expression and regulation, application in molecular taxonomy, including proof of new microbial species are impressive.

## CONCLUSION

Based on my acquaintance with the submitted documents, scientometric data and works, after assessing their importance, the scientific and applied contributions contained in them, as well as the education and experience of the candidate, I find it reasonable to state that my opinion on the acquisition of the academic position "Professor" in the Professional field: 4.3. Biological sciences/ Microbiology - genomics and regulation of gene expression in prokaryotes/ by Assoc. prof. Penka Petrova is positive.

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