

# STATEMENT

**From:** Associated Professor Milen Ivanov Georgiev, PhD  
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on thesis for awarding the degree Doctor of Sciences (DSc)

**Thesis Author:** Associated Professor Penka Mladenova Petrova, PhD  
**Title of the thesis:** Molecular biological studies on new bacterial glycoside hydrolases with commercial significance  
**Professional area:** 4.3. Biological Science

The present statement is prepared in agreement with an order (I-128 from December 23, 2019) of the Head of Stephan Angeloff Institute of Microbiology – BAS (IMicB), following a decision of the Scientific Council of the IMicB with Protocol numbers 12 from November 28, 2019 and 13 from December 17, 2019.

## **General description of the procedure and the candidate**

The set of documents agrees with the formal requirements of Section III. Terms and orders for acquisition of scientific degree “Doctor of Sciences” from Rules for the terms and orders for acquisition of scientific degrees and occupation of academic positions of the Stephan Angeloff Institute of Microbiology at Bulgarian Academy of Sciences and contains all necessary documents for evaluation.

The thesis is presented at the National seminar of General microbiology on November 18, 2019 and further addressed for initiating a procedure of its official defense.

Penka M. Petrova joined IMicB-BAS as a PhD student, at 1997. After successful defense (PhD diploma from 2004) she went through all levels of Research Scientist (III – I), while at 2011 she was elected as an Associated Professor at IMicB-BAS, a position that she occupies at present. My personal impression of Assoc. Prof. Petrova, PhD is that she is respected erudite scientist with considerable competency in the field of contemporary microbiology, molecular biology and biotechnology.

## **Relevance of the topics, performance and evaluation of the DSc thesis**

The consumption of enzymes and enzyme preparation from microbial sources is continuously growing worldwide. The studies of enzyme diversity (*i.e.*, hydrolases) and their encoding genes, significantly contributes to the knowledge of adaptive and evolutionary mechanisms (at gene’s level), as well as phylogenetic networks among different microbial groups. Moreover, glycoside hydrolases appear important industrial enzymes with diverse applications in food industry, pharmacy, medicine and biotechnology. Here, I strongly believe the DSc thesis of Assoc. Prof. Petrova, PhD is an up-to-date solid and sound work with both scientific and applied nature, as sequencing of genome(s), identification of novel genes, heterologous expression of proteins with catalytic activity, and isolation, purification and immobilization of enzymes, for instance.

The DSc thesis of Assoc. Prof. Petrova, PhD is written on 358 pages, with an adequate structure, following widely acceptable standards. The References section covers 625 sources, while only 3 appears in Cyrillic. It makes a good impression that significant portion of the

cited sources is published in the last couple of years, which is another testimonial for the up-to-date nature of the present DSc thesis.

The detailed knowledge of the contemporary tendency in biotechnology of bacteria's glycoside hydrolases producers is revealed mostly from the literature survey, which is well structured and provides a good overview. The literature survey ends with a summary, aims of the thesis along with an adequate formulation of the main tasks and working hypotheses.

Methods used: again, it makes a good impression, that Assoc. Prof. Petrova, PhD uses contemporary assays with wide-spectrum of application, as biochemical, molecular biological (PCR, RP-PCR, RAPD, MLST, PFGE, DNA-, RNA-hybridization) as well as analytical (HPLC, LC-MS).

The Section Results and Discussion is divided into four sub-sections, covering mass workload on molecular biological study of glycoside hydrolases, produced by lactic acid bacteria (sub-section 1) as well as *Bacillus* genus bacteria (sub-section 2), besides the thorough studies on enzymes with sialic acid as substrate (sub-section 3) and on characterization of  $\beta$ -glucuronidase (sub-section 4). Conclusions section summarizes the work done by Assoc. Prof. Petrova, PhD, aims achieved, as well as outlines the possibilities for further research and applications, eventually.

**The contributions** of the DSc thesis are divided into two main groups, labelled as scientific ones (6) and applied (6). Presented as such, contributions of the DSc thesis reflect its nature and at the same time do not exaggerate the thesis findings.

Scientific contributions:

1. Genes responsible for the hydrolysis of  $\alpha$ -glucans in *Lactococcus lactis* and *Lactobacillus paracasei* species have been identified for the first time. The world's first amyolytic representatives of the species *Lactobacillus sakei* and the genus *Enterococcus* have been isolated.
2. For the first time, the cell-wall associated fructan- $\beta$ -fructosidases of *Lactobacillus paracasei* have been purified. The nucleotide sequence of their coding genes proves that the enzymes are novel, with amino acid replacement adjacent to the catalytic triad of the active center.
3. A novel *cgt* gene and new cyclodextrin glucanotransferase enzyme from *Bacillus pseudocalophilus* have been identified. The homology of the amino acid sequence of CGTase with the known enzymes is less than 87%.
4. Heterologous expression of  $\beta$ -glucuronidase in diploid strains of *Ogataea polymorpha* yeast has been performed for the first time.
5. The complete genome of *Bacillus velezensis* 5RB has been sequenced, and gene analysis revealed the strain's ability to convert lignocellulosic substrates into valuable products.
6. The relationship between cell surface hydrophobicity and resistance to organic solvents has been demonstrated for the first time.

Applied contributions:

1. A method for rapid transcriptional analysis of genes responsible for the digestion of amyolytic lactic acid bacteria has been established.
2. A recombinant enzyme cyclodextrin glucanotransferase has been immobilized in magnetically modified carriers for the first time. Re-use of the CGT-magnetic magnetic biocatalyst indicates the possibility of producing three to four times the amount of cyclodextrin in 120 minutes compared to the yield of single-use enzyme preparations.
3. For the first time, a detailed molecular biological study of neuraminidase from a non-toxigenic strain of *Vibrio cholerae* has been performed. Sequencing and

characterization of the *nanH* gene and its encoded enzyme enable the development of safe production of sialidase.

4. The *in vivo* synthesis of enzymes hydrolyzing (hemi)cellulose in the species *B. safensis*, *B. toyonensis* and *B. velezensis* has been demonstrated for the first time.
5. A new PCR-based method for the detection of short heat-shock genes in *Streptomyces thermophilus* has been developed, which enables rapid selection of strains for use in starter cultures.
6. The content of the prebiotic carbohydrates galactooligosaccharide, indole-3-propionic acid and cyclic antimicrobial peptides in Bulgarian yogurt has been demonstrated for the first time.

### **Evaluation of the publications' record and author's contribution**

The results of the present DSc thesis are summarized in 33 scientific papers (*while 20 are mandatory, excluding these included within the PhD thesis*), *i.a.*, 18 in an impact factor journals (total IF of 25.762), 1 in an SJR journal, 2 book chapters, 6 proceedings from conferences and seminars and 6 others in different journals.

Protocols, for distribution of the data generated among co-authors, are thoroughly supplied.

In total 281 citations, on papers related to the thesis, are observed (*while 150 are mandatory*). These numbers, however, should not be absolutized, as major part of the thesis papers is published within 2017-2019, hence yet to be appreciated and cited by the scientific community.

The DSc thesis results are presented at 37 conferences in Bulgaria and abroad. This fact along with the relatively high citations record clearly indicate that the work of Assoc. Prof. Petrova, PhD has received a reasonable international resonance.

I, personally, don't have major critical remarks to Assoc. Prof. Petrova's, PhD thesis. Some linguistic flaws and typos are observed within the thesis body, which are however considered as minor; hence don't diminish the sounds of the thesis. My personal recommendation to Assoc. Prof. Petrova, PhD would be to try to combine the present methodology used with the last of so-called omics approaches, namely metabolomics, towards studying microbial enzyme producers at systems level ("systems biology").

### **Author's summary of the thesis**

The author's summary of the DSc thesis is written on 118 pages and contains 80 figures and 26 tables. The content of the DSc thesis summary is acceptable and in full agreement with the main results of the thesis. I believe, the thesis would only benefit from a summary in English provided.

### **CONCLUSION**

The thesis contains both sounds scientific and applied results, which significantly contribute to the knowledge on the field and fully corresponds to the requirements of the Act for the Development of the Academic Staff in the Republic of Bulgaria (ADAS), the Rules for ADAS application, as well as specific Rules of BAS and the Institute of Microbiology – BAS. The presented set of documents and reported results are in agreement with the specific requirements of the Institute of Microbiology – BAS's Rules.

The thesis shows, that Assoc. Prof. Penka Petrova, PhD possesses exhaustive theoretical knowledge, scientific refinement and professional skills in the respective field.

Taking all above into account, I confidently express my positive assessment on the above-mentioned studies (hereby presented by the thoroughly evaluated DSc thesis, author's summary of the thesis, and supplementary documents) and **suggest to the esteemed scien-**

**tific jury to award Doctor of Sciences degree to Penka Mladenova Petrova on professional area 4.3 Biological science.**

March 4, 2020  
Plovdiv, Bulgaria

**Prepared by:** .....  
/Assoc. Prof. Milen I. Georgiev, PhD/