

R E P O R T

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Subject: evaluation of a PhD thesis for an educational and scientific degree "Doctor" in the field of Higher education 4. Natural sciences, mathematics and informatics, professional field, 4.3 Biological sciences, doctoral program Microbiology

Author of the dissertation: **Nikoleta Ivanova Boteva**

Title of the PhD thesis: **Diversity and biotechnological potential of thermophilic microorganisms from Bulgarian hot springs**

Supervisor: Prof. Margarita Kamburova

Nikoleta Boteva was appointed as a PhD student in full-time education at the Institute of Microbiology (IMicB), BAS, Laboratory "Extremophilic Bacteria" under my supervision on January 1, 2016. I declare that I am a co-author of her three publications and I have been elected as a member of the Scientific Jury according to the Law for the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB) acting in 2016. I was included in the Scientific Jury for an evaluation of the dissertation "Diversity and biotechnological potential of thermophilic microorganisms from Bulgarian hot springs" by an order I-41 / 23.04.2021 of the Director of the Institute of Microbiology (IMicB), BAS.

1. Actuality of the problem developed in the thesis

Culturable microorganisms represent a very small part of the biological species presented in natural ecosystems (not more than 5%). Their share in samples from extreme niches is especially low due to difficulties in reproducing the extreme conditions in the laboratory. Thermophilic microorganisms have an important place in both, basic and applied science. Poor knowledge of the inhabitants of hot niches determines difficult use of their biotechnological potential for a

synthesis of various biomolecules. The enzymes from extreme and hyperthermophilic microorganisms are desirable catalysts in a number of new or improved biotechnological processes. The scarce information on the diversity of extreme and hyper thermophiles worldwide and the lack of such research in Bulgaria, as well as the unused natural reservoir of extremozymes determine the actuality of the thesis.

2. Description and evaluation of the submitted materials

As a member of the Scientific Jury I have received a set of materials, including the thesis, thesis summary, publications related to the dissertation and CV of the PhD student, which meets the requirements of ZRASRB, Rules for application of ZRASRB and Career Rules of IMicB. The dissertation is written in a volume of 171 pages and is constructed according to the classical scheme for this type of work - Literary Review, Aim and Tasks, Materials and Methods, Results and Discussion, Conclusion, Implications, Contributions, Literature. It contains 19 tables and 58 figures. The bibliography includes 404 titles, more than half of them are from the last ten years. The literature review is comprehensive and shows the student's knowledge of the current literature in the field of the possibilities of the metagenomic approach to reveal the diversity of non-cultivated microorganisms and functional metagenomics, phylogenetic diversity of thermophilic microorganisms and the enzymes synthesized by them. The aim of the dissertation is clearly formulated, and the tasks arising from the aim are well defined. The Materials and Methods section describes the variety of methods, including both, those used in the Laboratory of Extremophilic Bacteria and those adopted in the two international laboratories in which she has worked during her doctoral studies, namely six months at the University of Bergen, Norway and one month at the Institute of Biomolecular Chemistry, CNR, Naples, Italy. She has described in details the Hungate technique for an anaerobic cultivation that she introduced in the laboratory, as well as several software programs for metagenomic analysis.

In accordance with the assigned tasks, the Results and Discussion section consists of six main parts: Metagenomic analysis of microorganisms presented in samples from three Bulgarian hyperthermal springs and reconstruction of draft genomes from these metagenomes; Identification of ORF, a significant part of them unknown proteins; Metagenomic analysis of target-enriched cultures from two springs in the presence of desired substrates; Cloning, expression and characterization of a thermophilic enzyme (lipase) from a non-culturable microorganism; Isolation and characterization of extreme thermophilic anaerobes; Synthesis,

purification and characterization of thermostable pectinase synthesized by a newly isolated microorganism. The discussion of the obtained results was conducted in a comparative aspect with the results of other authors working in the same field with a clear highlighting of the Bulgarian contribution among these studies.

The presented implications follow logically from the obtained results. As main contributions of the present thesis I define the accumulated for the first time knowledge about the reach microbial phylogenetic and functional diversity in Bulgarian hyperthermal springs with the use of metagenomic approach; identification of a significant number of unknown taxa and unknown proteins in thermal springs' metagenomes; the successful expression of lipase from a methagenome of a Bulgarian hot spring; isolation of an unknown extremely thermophilic anaerobic strain; the isolation of a producer of one of the most stable pectinases described in the literature.

The thesis summary fully and reliably reflects the main results, formulated conclusions and contributions of the thesis.

The PhD student's publishing activity includes two publications on the topic of the dissertation, published in periodical scientific journals with Impact Factor and one book chapter in an international edition (Springer) and meets the requirements of ZRASRB and IMicB for the scientific degree „Doctor”.

CONCLUSION

The PhD thesis of Nikoleta Boteva summarizes received important results with an impact for science and practice. It demonstrates a profound theoretical knowledge of the PhD student as well as an ability for independent, logically constructed and in-depth research, which achieves the tasks and achieves the goal. The laboratory work is profound and complex performed by classical and modern methods in a good methodological base. Based on the materials presented, the arguments for the actuality of the investigation and the original contributions reflected in the thesis, I confidently support my high assessment of the thesis and recommend to the members of the Scientific Jury to award the educational and scientific degree "Doctor" to Boteva in professional field 4.3. Biological sciences, specialty Microbiology.

June 1, 2021
Sofia

Report by:
(Prof. M. Kamburova, DSc)