

EXAMINER'S ASSESSMENT

by Prof. Dr. Velizar Kostadinov Gochev,

Head of the Department of Biochemistry and Microbiology, Plovdiv University "Paisii Hilendarski",

of a thesis for the award of the educational and scientific degree "Doctor" (PhD) in the field of Higher education 4. Natural Sciences, Mathematics and Informatics, Professional field 4.3 Biological sciences, doctoral program (Microbiology)

Candidate: Nikoleta Ivanova Boteva

Title: "Diversity and biotechnological potential of thermophilic microorganisms from Bulgarian hot springs."

1. General presentation of the procedure and the candidate

By decision of the Scientific board of The "S. Angeloff" Institute of Microbiology at Bulgarian Academy of sciences Protocol № 17/22.04.2021 I was appointed as a member of the scientific jury of a thesis entitled "**Diversity and biotechnological potential of thermophilic microorganisms from Bulgarian hot springs**" for the award of the educational and scientific degree "Doctor " (PhD) in the field of Higher education 4. Natural Sciences, Mathematics and Informatics, Professional field 4.3 Biological sciences (Microbiology) by Nikoleta Ivanova Boteva, who is full-time PhD student at department General microbiology, laboratory Extremophiles at The "S. Angeloff" Institute of Microbiology.

The set of digital materials presented by Nikoleta Boteva is in accordance with Academic Staff Development Act in the Republic of Bulgaria and Regulations of the Institute of Microbiology BAS on the Implementation of the Academic Staff Development. Based on the documents enclosed it is clear that formal requirements for reaching educational and scientific degree "Doctor" have been fulfilled, which allows me to determine the procedure as lawful and to proceed to a detailed evaluation of the PhD thesis and the contributions presented by Nikoleta Boteva.

2. Actuality of the subject, aim and research tasks

Limitations caused by classical cultural methods for isolation and cultivation of microorganisms negatively reflect on the knowledge about microbial diversity at extreme ecological niches. On the other hand constant interest on the possibilities for application of enzymes into various industrial branches such as food and flavor industry, chemical and pharmaceutical industries at extreme conditions (high temperature, acidic and alkaline pH, etc.) is registered.

Isolation of thermophilic microorganisms and evaluation of their potential to produce extra-cellular enzymes is a base for development of biotechnologies for production of thermostable enzymes. In this way the limitations of industrial application of enzymes should be overcome. For these reasons the actuality of the presented PhD thesis is undoubted both from theoretical and practical points of view.

The major goal of the present thesis is clear, but its scope is too wide for so called “PhD research” and unnecessarily increase the number of the research tasks to eight.

3. Familiarity of the problem

The literature review is based on 404 scientific publications, pointing the major achievements in the studied problem, which allows me to determine the literature review as detailed and comprehensive. The scope and the way of organization and analytical discussion on the literature information allows me to conclude that Nikoleta Boteva is very well acquainted with worldwide achievements on the research problem in details.

4. Research methodology

All of the used materials and the applied methods are precisely listed. Experimental conditions for carrying out the separate stages of metagenome studies, the used software products, the applied enrichment procedures, isolation and cultivation procedures are explained in details, which allow reaching correct and reproducible results. The whole experimental scheme is correctly assembled, separate stages of the study are carried out in a logical sequence and in this way it is a prerequisite for successful reaching of the major research goal. Nikoleta Boteva is acquainted and applies wide range of classical and modern microbiological, molecular, spectral and biotechnological methods and in this way the educational goal of the PhD is successfully realized.

5. Characteristics and evaluation of the PhD thesis and contributions

The thesis is structured in the accepted order and includes: *Introduction, Literature review, Goal and tasks, Methodology, Results and discussions, Conclusions, Contributions and Reference list*. Ratios among separate chapters are optimal. The *Introduction* fulfills its goal to formulate the research problem focusing on the limited knowledge about biotechnological potential of thermophilic microorganisms and in this way successfully motivates the necessity of the current study. As already mentioned the *Literature review* is very well structured and the information is presented analytically, but the connection between the last part of the review (point 7) and the rest of the review is not clear enough. Probably this point 7 has to be placed at the beginning of the literature review, but not at the end. The *Methodology* section fulfills its purpose, but it has to follow the *Aim and tasks* section, because usually the selection of one or

another method depends on the aim and research tasks, not the opposite as it is in the thesis. Probably it is a technical misunderstanding because in the Summary of the thesis (autoreferate) the *Methodology* section follows the *Aim and scope* section as it has to be. The major part of the experimental work is focused on the evaluation of microbial diversity and the connections in microbial communities in six Bulgarian geothermal hot springs (Rupi I, II and III, Vlasa, Misinka and Levunovo). By different approaches for metagenome analyses of sediments and water samples from Rupi I, II and Vlasa taxonomic and functional status of thermophilic bacteria and archaea is clarified. Based on the metagenomic data several draft genomes of the enriched thermophilic cultures, including non cultivable, are reconstructed. From the reconstructed genome of bacterium *Candidatus calescibacterium* (originated spring Rupi II) a gene encoded thermostable lipase is selected, cloned and expressed. The produced lipase demonstrates increased temperature optimum and increased thermostability in comparison with other thermostable lipases published in the literature and it is comparable with archaeal lipases. The second major part of the PhD research is focused on the biotechnological potential of thermophilic bacteria to produce extracellular enzymes. By application of Huntage technique for anaerobic cultivation a prospective polygalacturonase, xylanase and arabinoxylanase producing strain *Caldicellulosiruptor sp.* 11.4 is isolated. A laboratory scheme for partial purification of polygalacturonase is realized. The partially purified enzyme demonstrates high temperature optimum and wider range of pH stability in comparison with polygalacturonase produced by *Caldicellulosiruptor bescii*.

Number of conclusions can definitely be reduced and some of the conclusions have to be revised and rewrite, but I am deeply convinced that this section of the PhD thesis is the most difficult for the PhD students and we have to be the most tolerant to this part of their work. I accept the contributions formulated and most of them could be categorized as original. I strongly appreciate the fact that the results obtained are a solid base for further studies in the research area of thermophilic bacteria.

I have to highlight that the evaluation of bacterial and arhaeal diversity is not an end in itself, but it is strongly motivated by the desire to develop biotechnology for production of thermostable enzymes. I am not surprised by this fact, because all of the research projects and scientific publications carried out by Prof. Kamburova , who is a supervisor of Nikoleta Boteva, successfully transferee fundamental scientific results to real biotechnologies and important practical applications.

6. Evaluation of the publications

Two scientific publications, which are published in *Comptes rendus de l'Académie bulgare des Sciences* (Q2) and one book chapter, published by Springer are applied. Nikoleta Boteva is the leading author of these materials, which demonstrate her key role in their realization.

CONCLUSION

The evaluated PhD thesis contains scientific and applied results, which represent an original contribution to science and meet the requirements of Academic Staff Development Act in the Republic of Bulgaria and Regulations of the “S. Angeloff” Institute of Microbiology BAS on the Implementation of the Academic Staff Development. The PhD thesis shows that Nikoleta Ivanova Boteva has theoretical knowledge and professional skills in the scientific specialty Microbiology. Due to the above, I give my positive assessment of the research presented in the PhD thesis, summary, results and contributions, and I propose to the esteemed jury to award the educational and scientific degree “Doctor” (PhD) to Nikoleta Ivanova Boteva 4. Natural Sciences, Mathematics and Informatics, Professional field 4.3 Biological sciences, doctoral program (Microbiology).

31.05.2021

Prepared by:.....

(Prof. Dr. V. Gochev)

..