

## **R E V I E W**

on PhD Thesis in professional field 4.3. Biological sciences (microbiology)

Author: Nikoleta Ivanova Boteva, Title: Diversity and biotechnological potential of thermophilic microorganisms from Bulgarian hot springs

Reviewer: Prof. Kaloyan Petrov, DSc

### 1. Relevance of the topic of the dissertation

The presented dissertation aims to determine the bacterial and archaeological content of Bulgarian hot springs and to identify potential producers of thermostable enzymes for use in industry. Although the isolation of extremophilic microorganisms and their applications as producers of biologically active substances have been known for half a century, the topic remains relevant as new generation sequencing techniques (NGS) take knowledge of the phylogenetics of natural habitats to a new level. The application of NGS for metagenomic analysis reveals the huge variety of non-cultivated microorganisms (95% of the presumed species), enriches the taxonomy with candidates for new species and provides a hitherto unused genomic natural resource. On the other hand, this technology is boosting functional genomics and sequence-based approaches to the study of gene expression, which is a prerequisite for promising new data on the metabolic potential of hitherto unexplored classes of microorganisms and the successful isolation of enzymes with wide applications.

### 2. Data about the PhD thesis

The dissertation is constructed in the traditional form, written on 171 standard pages, with the relevant sections: "Literary Review" - 30 pages, "Materials and methods" - 15 pages, "Results and discussion" - 95 pages, conclusions, contributions and cited literature - 20 pages.

### 3. Sections, content, methodology and quality of the evidence

The literature review provides information on extremophilic microorganisms with a focus on thermophiles. Their biodiversity and metabolic strategies for survival in extreme conditions are considered, as well as the molecular basis of the thermophilicity and thermal stability of their enzymes. The metagenomic taxonomic and functional profiling of microbial communities, the metagenomic approaches for the discovery of new biocatalysts and the respective biotechnological significance of thermophiles as producers of industrial enzymes are described in detail. The review completes the theory of the origin of life on Earth from ancient hyper-thermophiles. The section covers 404 literature sources (all in Latin), most of them from the last 10 years. The author knows the topic in detail, the review is written fascinatingly and attention is paid to the achievements in various fields that determine the current level of research.

The selected methodologies fully correspond to the set goal and tasks of the dissertation. Three main groups of methods were used: new-generation sequencing and subsequent bioinformatics analysis, recombinant DNA cloning approaches, and enzymological methods. All of them are modern, with high accuracy and reliability and are perfectly selected to perform the tasks.

The aim of the dissertation is clearly stated and since it is quite broad, eight tasks are dedicated to its achievement, covering culturally dependent and culturally independent research approaches.

The results are illustrated in 19 tables and 54 figures (4 of the figures are in the literature review). The physico-chemical characteristics of the studied springs are described. A metagenomic analysis of sediment samples from three hot springs was performed and the presence of lithotrophic and heterotrophic thermophilic bacteria, representatives of the genera *Aquifex*, *Hydrogenobacter* and *Thermus*, family *Aquificaceae*, *Thermotoga*, *Fervidobacterium*, *Thermosiphon*, *Kosmotoga* and *Kosmotoga*, was proved. Among the most interesting representatives of the archaea are the discovered anaerobes *Methanosphaerula* and *Methanoregula* of the order *Methanomicrobiales*, as well as the hyperthermophilic heterotrophs *Pyrococcus* and *Thermococcus*. Metabolic reconstruction of biochemical pathways by annotation of coding sequences allows for functional analysis of the obtained data. A model of energy metabolism and the probable nitrogen cycle carried out by thermophilic species in the studied ecological niches was made. Thus, it is logical to move on to the second part of the dissertation: cloning and expression of a gene encoding an enzyme for lipase from an uncultured bacterium. Although the gene is synthetic, the recombinant enzyme is functional and its biochemical relationships have been determined.

In the study of the biotechnological potential of the cultivated thermophiles, the qualities of 17 aerobic and 5 anaerobic enzyme producers were traced. A strain of *Caldicellulosiruptor* as an extracellular polygalacturonase producer has been studied in detail.

The discussion of the obtained results once again emphasizes the originality of the research, as historical data are skillfully intertwined with the latest innovations, the Bulgarian contribution on the topic in the purification of thermostable lipase from *Bacillus stearothermophilus* and the application of thermophiles for the synthesis of polysaccharides is also emphasized.

#### 4. Contributions to the dissertation

The dissertation has several contributions. The most important of these are the following:

(1) For the first time in Bulgaria the microbial diversity in Bulgarian hot springs has been characterized using new-generation sequencing, for the first time the diversity in enriched anaerobic thermophilic cultures from Bulgarian hot springs has been characterized and the ratios between different taxa have been compared.

(2) For the first time in Bulgaria a taxonomic and functional analysis of non-assembled and assembled metagenomes with MG Rast has been made, the open reading frames in the metagenomes from different sources have been identified and referred to a certain biochemical pathway.

(3) A large number of metabolic pathways have been reconstructed and the role of the various thermophilic representatives in these ecological niches has been determined.

(4) For the first time anaerobic hyperthermophilic microorganisms have been isolated from Bulgarian hot springs, a new species of the genus *Caldicellulosiruptor* has been discovered.

(5) For the first time in Bulgaria a lipase has been cloned and expressed from a non-culturable thermophilic anaerobic bacterium.

(6) In the analysis of non-assembled metagenomes, 22% to 49% of sequences encoding proteins with unknown functions were detected.

The results of the dissertation are described in 3 publications - 2 scientific articles and one chapter of a book. In the three publications, the dissertation is the first author. The abstract meets the requirements and is an accurate reflection of the dissertation, showing synthesized the most important results and conclusions. Some of the figures have been adapted and prepared especially for the abstract, which makes a very good impression.

5. Opinions, recommendations and notes

I have no remarks on the dissertation.

6. Conclusion

In conclusion, the dissertation presented is a remarkable study and contains significant scientific and applied contributions. 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. Based on the analysis I give a completely positive assessment of the developed dissertation, I support the acquisition of the educational and scientific degree "Doctor of philosophy" by Nikoleta Ivanova Boteva, in scientific direction 4.3. Biological sciences, scientific speciality Microbiology.

Date: June 14, 2021

Signature:  
(Prof. Kaloyan Petrov, DSc)