R E V I E W

of the Ph.D. Thesis of Assistant Professor Nikolina Atanasova Atanasova

Topic: "Degradation of plastics by thermophilic and halophilic bacteria isolated from Bulgarian extreme niches",

Scientific supervisor: Prof. Dr. Margarita Stoyanova Kamburova

Reviewer: **Prof. Penka Petrova, DSci**, head of the General Microbiology Department, Stephan Angeloff Institute of Microbiology - BAS

1. General description of the presented materials

By decision of the Supreme Council of the Institute of Microbiology "S. Angeloff" at the BAS Protocol No. 44/30.03.2023 (Order No. I-44/31.03.2023) I have been designated as a member of the Scientific Jury for assessment of the Ph.D. thesis, topic "Degradation of plastics by thermophilic and halophilic bacteria, isolated from Bulgarian extreme niches" for the acquisition of the educational and scientific degree "doctor" in the field of higher education 4. Natural sciences, mathematics, and informatics, professional direction 4.3 Biological sciences, doctoral program Microbiology. The author of the dissertation is assistant professor Nikolina Atanasova Atanasova – a doctoral student independent form of education at the Department of General Microbiology, laboratory "Extremophilic microorganisms" with scientific supervisor Prof. Margarita Kamburova, Ph.D. The set of materials presented by Nikolina Atanasova is in accordance with the requirements of the Law for the Development of the Academic Staff, and the Regulations for the Implementation of this Law.

2. Brief biographical data

Nikolina Atanasova Atanasova was born on 16.07.1979, she graduated from the Faculty of Biology at the Sofia University "St. Kl. Ohridski" and obtained a master's degree in Biotechnology at the same Faculty. Since 2005, she has been working at the Institute of Microbiology in the

"Extremophilic Microorganisms" laboratory, as part of the team of Prof. Dr. Margarita Kamburova. Over the years, Assistant Professor Nikolina Atanasova has gained enormous experience in the isolation and characterization of strains with different extremophile needs: thermophiles and halophiles. To date, she has co-authored eleven publications referenced in Scopus, cited 159 times in the same database, and has an H-index of 7. Four of the collective's recent papers are directly related to the results of the Ph.D. thesis and were published in 2021 in famous journals Biomolecules and the International Journal of Molecular Sciences.

3. Relevance of the topic of the dissertation

Industrialization, urbanization, and modern lifestyles lead to the production and accumulation of millions of tons of plastic every year. Different in their composition and qualities, plastic solid waste makes up one-third of all solid household waste. Unfortunately, less than 10% of plastic produced is recycled, a quarter of it is incinerated, and more than half is simply thrown into nature. This leads to massive and uncontrollable environmental pollution with an adverse impact on a number of ecosystems and to a particularly large extent, the world's oceans. It is well known that plastic pollution, in addition to endangering the environment, seriously harms human and animal health.

The scientific development of Assistant Professor Atanasova represents a new look at solving the problem of destroying plastic waste, aiming to find microorganisms capable of dealing with their degradation. The topic is new for Bulgaria and the world. Until recently, no microbial species had been found to degrade plastics. Recently, single strains have been described that are possibly capable of degrading polystyrene, polyurethane, or polycaprolactone, but the authors have not shown the degradation efficiency.

4. Assessment of the PhD Thesis

The dissertation is written on 180 pages with the following sections: 57 pages of literature review with conclusions from the literature review, 2 pages "Aim and tasks", 13 pages "Materials and methods", and 66 pages "Results and discussion", 3 pp. "Conclusions" and 2 pp. "Contributions". There are about 187 cited sources, most of them from the last ten years, one-third - from the last five years. The material is illustrated with 38 figures and 23 tables.

The review of the literature shows an excellent awareness of the subject and an analytical approach, in the spirit of modern "systematic" reviews. The literature of recent years is reviewed and the latest statistical information is extracted from the sites of Global Market, Plastics Europe, and the World Economic Forum 2021. The role of plastics as environmental pollutants is examined in detail. The structure of different types of polymers is described. The possibilities of microorganisms as producers of enzymes that contribute to the degradation of different types of plastic are discussed. A separate chapter is dedicated to extremophilic enzymes and their features. It is emphasized that the use of extremophilic microorganisms and their lipase enzymes from the esterase group initially showed promise in biodegradation processes. The literature review is written with desire and skill and ends with a section “Unsolved problem”, after which the goals and tasks of the work appear logically, and in focus.

The section "Aim and tasks" clearly outlines the purpose of the work: to study the ability of extremophilic bacteria isolated from Bulgarian extreme niches to efficiently degrade different types of plastics and to characterize these processes. The main hypotheses and stages in the work process are outlined. The formulated 12 tasks are too detailed, but they guide the reader on what future results to expect and imply a conscientious implementation of a preliminary work plan.

The section "Materials and methods" thoroughly describes both the classical methods for the cultivation of extremophilic microorganisms, biochemical and enzymological methods, as well as high-precision analytical methods such as gel-permeation chromatography for the analysis of degradation products of polymers, scanning electron microscopy (SEM) and etc.

In the "Results and Discussion" section, the steps to achieve the aim of the dissertation are clearly and systematically described. The results include a variety of experiments demonstrating the degradation of plastics by microbial communities, by pure strains, and in the co-cultivation of two strains. The extremophile isolates in the available laboratory collection (thermophiles and halophiles) were screened for growth in nutrient media with different types of plastics as the sole carbon source. Specific habitats were selected to isolate extremophilic bacteria, with microbial communities from hot springs and salt pans cultivated in environments with different types of plastic. Metagenomic analysis of the communities was performed. 18 strains of thermophilic and

strains of halophilic bacteria from seven different natural samples were isolated and identified to species. The esterase activity of the newly isolated strains was quantified, as it was found to be key for the degradation of the target polymer polycaprolactone. In addition to the enzymatic activity, the analysis of the degradation products of polycaprolactone, as well as impressive electron-microscopic pictures, is proof of the destructive effect of the strains on the plastic. One of the investigated strains belonging to the species Brevibacillus thermoruber showed particularly high activity, was defined as a primary degrading microorganism, and contained the first extremophilic esterase with proven capabilities to degrade polycaprolactone. As the next stage in the work, the parameters for the degradation of plastics from the communities, the pure strains, and the co-cultures were optimized, the temperature and pH optimum of the enzymes, the optimum concentration of the substrate, the molecular weight of the substrate, etc. were determined. A major merit of the thesis is the isolation and purification of a lipase enzyme from B. thermoruber strain 7. The enzyme successfully degrades PCL in vitro, being shown to attack the main chain of the polymer and not side branches. As for halophilic communities, they show enormous phylogenetic diversity and have great potential for future research. 16 strains of halophiles from the laboratory's microbial collection were studied, but since they did not prove to be promising, the doctoral student turned to research new natural samples. From them, representatives of the genus Virgibacillus and the genus Oceanobacillus, capable of degrading polyvinyl alcohol, polycaprolactone, and polystyrene, were isolated.

The dissertation ends with 12 conclusions and 8 contributions. I fully accept both the conclusions and the contributions, with one caveat about conclusion 5, where the comparison remains a bit unclear. In the conclusions, some denizens could be avoided, e.g. "demonstrated possibility", but in general, the dissertation is beautifully written, in an excellent scientific style, and at the same time - completely understandable even for specialists in related fields. The complete absence of spelling, stylistic, or punctuation errors makes a very good impression.

Among the most significant achievements of the Ph.D. thesis are the following:

- For the first time, a bacterial community degrading the polycaprolactone plastic at 55 °C was isolated, which allows the combining of the physical and biological factors of degradation of this plastic.

- For the first time, selective pressure was applied to the development of a microbial community under the influence of plastics as the sole carbon source.

- Strains unique in their qualities have been isolated, for example, Brevibacillus thermoruber strain 7, etc., capable of actively degrading polycaprolactone at considerable speed.

- The mechanisms of PCL degradation by thermophilic communities are explained.

- The purified lipase is the first reported thermostable enzyme capable of degrading PCL, which can be directly applied in plastic biodegradation processes.

5. Evaluation of publications on the Ph.D. Thesis

In connection with the dissertation, four scientific publications, referenced and indexed in the world, are presented the main databases: in the journal Biomolecules (Q2, IF 6.0), BioTech (Q2), IJMS (Q1, IF 6.2) and Ecologia Balkanica (Q4). All these journals are unquestionably authoritative in the field of PhD Thesis, and Nikolina Atanasova is the first author of all these articles, which shows her key contribution in their preparation and publication. The topic has a wide horizon in front of it and suggests continuation and expansion in future research, as proof of this are the citations - only up to April of this year - 31 in number.

6. CONCLUSION

The PhD Thesis of Assistant Professor Nikolina Atanasova is dedicated to a current topic of great importance for the quality of life of humanity. The obtained results are new, significant and open the way for the development of a whole new direction in the field of extremophilic microorganisms. The dissertation contains scientific and scientific-applied results that meet all the requirements of the Law, the Regulations for the implementation of the Law in the BAS, and the requirements of the Institute of Microbiology. Ph.D. student Nikolina Atanasova possesses

rich theoretical knowledge and enviable professional skills in the scientific specialty of Microbiology.

I give my positive assessment of the Ph.D. Thesis, the abstract, and the contributions of the dissertation, and I strongly support the awarding of the educational and scientific degree "doctor" to assistant Nikolina Atanasova Atanasova in field 4. Natural sciences, mathematics and informatics, professional direction 4.3 Biological sciences, doctoral program Microbiology.

05/28/2023 Reviewer:

(Prof. Dr. Penka Petrova)