

Scientific opinion

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Dissertation topic: "Degradation of plastics by thermophilic and halophilic bacteria isolated from Bulgarian extreme niches" for the award of the educational and scientific degree "Doctor" in professional field 4.3. Biological Sciences (Microbiology).

Author of the dissertation: Nikolina Atanasova Atanasova

Scientific supervisor: Prof. Margarita Kamburova, PhD

By order of the Director of the Institute of Microbiology at the BAS No. I-44/31.03.2023, I have been appointed as a Member of the Scientific Jury for conducting a procedure for the defense of Nikolina Atanasova Atanasova's dissertation. The submitted documents and materials meet the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LAD) and the Rules for its implementation.

1. Brief data on the doctoral student

Nicolina Atanasova Atanasova holds a bachelor's degree in "Biology" and a master's degree in "Biotechnology" at the Faculty of Biology of the University of St. Kliment Ohridski". He began his career as a specialist - biotechnologist in 2005 at the Institute of Microbiology at the BAS, and since 2008 he has been an assistant in the laboratory of "Extremophilic microorganisms".

2. Relevance and significance of the dissertation topic

The continuous increase in the use of plastic products is one of the main reasons for the increasingly significant accumulation of ecologically polluting waste, which leads to environmental degradation and climate change, a decrease in biodiversity and an increase in human carcinogenic diseases.

Therefore, the search for new opportunities for their biodegradation with the participation of microorganisms gives many hopes for effective recycling. In the literature, however, information on the discovery of efficient producers of enzymes capable of degrading polystyrenes, polyurethanes or polycaprolactones is scarce. Therefore, directing research to a special group of microorganisms - extremophiles is a new approach to solving the problem. The

unusual properties of thermophiles and halophiles, as well as their metabolic characteristics, offer a new approach for the bioremediation of contaminated extreme habitats or the development of new composting processes. Developments in this area can significantly contribute to solving one of the biggest societal problems, namely plastic pollution.

The presented facts clearly motivate the choice of the topic, its relevance and practical importance. The content of the dissertation fully covers the announced nomenclature specialty in professional field 4.3 Biological sciences, scientific specialty "Microbiology" for the award of the ONS "doctor".

3. Evaluation of the structure of the dissertation work

The dissertation is written on 166 standard pages - A4 format, the results are summarized and presented in 23 tables and 39 figures. It is structured according to the classical scheme and is balanced in terms of the volume of each section: Introduction (1 p.), Literature review (50 p.), Aim and tasks (1 p.), Materials and methods (11 p.), Results and discussion (66 pages), Conclusions (2 pages), Contributions (2 pages) and References (25 pages).

The dissertation is written in a good scientific style, with accurate use of terminology, which shows that the doctoral student has a professional knowledge of the subject matter. It is necessary to note that the presented literature review (190 publications on the subject) offers a thorough analysis of the state of the problem. The dissertation student has familiarized herself very well with the literature data and skillfully uses them in interpreting the results.

3.1. Literature review

The literature review is properly structured with direct reference to the purpose of the dissertation and the tasks set. Three thematic axes are presented in the overview of the dissertation work: one presents plastics as an inevitable part of our daily life with its positive and negative effects, as well as introduces the types of plastics. Moreover, the structure of different types of polymers is described in detail. The second thematic axis examines the mechanisms of biodegradation with the participation of mesophilic microorganisms. The third topic reveals the nature of extremophilic bacteria and their abilities to participate in the biodegradation of plastics, and a promising role of their lipases and esterases for biodegradation processes is clearly outlined. A very good solution is to conclude the overview with an outline of the real problems in the technical cultivation of extremophiles and the difficulties that may arise in industrial production.

3.2. Purpose and tasks

The aim of the dissertation is clearly and precisely formulated as it includes the study of the ability of extremophilic bacteria isolated from Bulgarian extreme niches to effectively

degrade various types of plastics. The working hypothesis is based on the understanding that the unexplored capacity of extremophilic microorganisms from Bulgarian hot springs and salt lakes to degrade plastics will lead to the isolation of microorganisms carrying out effective biodegradation. In this way, it will contribute to the development of new, more efficient processes to solve an important world problem. To achieve the goal, 12 tasks have been formulated, which include a consistent study from the isolation of producers, through their taxonomic characterization to obtaining an efficient purified enzyme.

3.3. Materials and methods

This is one important part of the dissertation work that shows the level of scientific research. Presented are modern microbiological, biochemical, molecular, chromatographic and microscopic methods. The very development and successful implementation of them is already a clear success in the training of the doctoral student. The multidisciplinary nature of the research allowed the doctoral student to acquire new methodological skills.

3.4. Results and discussion

The main results are presented according to the tasks set and the methodological approaches chosen, and in their nature are a thorough characterization of the potential of the extreme community to degrade plastics. The experimental part is made very precisely and presented reliably. For the purpose of the study, four types of plastics were selected: polypropylene (PP), polystyrene (PS), polycaprolactone (PCL) and polyvinyl alcohol (PVA). The research started with the screening of strains from the laboratory's collection of "Extremophilic microorganisms", but due to their weaker ability to form biofilms, the PhD student turned to isolating new producers from natural habitats. From the investigated samples of five hot springs, 18 pure strains were isolated and identified by molecular methods. One strain was isolated from the hard-to-degrade PP and PS, two strains were isolated from a medium with PVA, one from Rupi and one from Levunovo. The large number of isolates from the sample from Marikostinovo cultivated in a medium with PKL, as well as the high esterase activity, led the doctoral student to conduct a complete metagenomic analysis of this microbial community. One strain of the species *Brevibacillus thermoruber*, which produces an extremophilic esterase capable of degrading polycaprolactone, was selected from the studied group. In the next stages of the work, the parameters of the biodegradation of plastics, the production of purified lipase, and the mechanisms of PCL degradation in in vitro conditions were studied. The properties of the purified lipase were determined. Electron microscopic analysis shows the significant deformation of plastic during the degradation process with the participation of the microbial community and *B. thermoruber* strain 7. The doctoral student

does not limit herself to the search for active producers only from thermophilic communities, but also studies the potential of the halophilic microflora from the lye and water mud of Atanasovsko Lake, the Burgas salt pans and the Pomorie salt pans. The investigated halophilic communities show enormous phylogenetic diversity and have real potential for further studies as producers of various enzymes.

3.5. Conclusions and recommendations

The doctoral student summarizes the enormous experimental work in 12 conclusions that correspond to each stage of the research done. The conclusions correctly summarize the obtained results. It may be noted that output 5 needs reformulation for more clarity of the information provided.

4. Scientific and applied contributions

Both theoretical and original applied contributions can be outlined in the research carried out, which prove the significance of the scientific development. The contributions that I regard as particularly significant and of great application are as follows:

- For the first time, a bacterial community was isolated, degrading the plastic ϵ -polycaprolactone at 55°C.
- For the first time, a strong selective influence on the biodiversity of thermophilic and halophilic communities has been established under the action of the PCL plastic used as the only carbon source.
- A thermophilic *Brevibacillus thermoruber* strain 7 capable of degrading polycaprolactone was isolated.
- A degradation rate was achieved that was among the highest reported for a plastic substrate.
- Purified lipase is the first reported thermostable enzyme capable of degrading PCL.

5. Publications related to the dissertation

The doctoral student has submitted a list of four scientific publications (Q1 -1; Q2 -2; Q4 -1) related to the dissertation that fully meet the requirements.

6. Author's abstract

The abstract is prepared in accordance with the requirements and faithfully reflects the results of the dissertation, as the most important elements from all sections are presented in abbreviated form (without the literature review).

7. Recommendations, comments and questions

There are no significant errors in the dissertation regarding the approaches used and presentation of the results, therefore I have no recommendations or remarks.

I have the following **question for the doctoral student**:

a) What information about the studied community do operational taxonomic units bring in metagenomic analysis?

8. Acquired competence and compliance with the requirements of the educational and scientific degree "doctor"

In the course of the implementation of the experimental work and the design of the dissertation work, the doctoral student Nikolina Atanasova acquired competencies and skills that can be grouped as follows:

- expanded her theoretical competence; - acquired skills for working with scientific literature, analyzing and summarizing scientific information;

- she has enriched her methodical skills, especially in the part of applying modern methods;

- acquired skills to independently shape and discuss the results obtained during the development of the dissertation, as well as draw conclusions based on them.

All of this gives me reason to believe that the doctoral student has acquired the necessary competencies and skills to acquire the educational and scientific degree "Doctor".

9. Conclusion

The presented dissertation work is a completed study that provides valuable scientific information and reveals opportunities for practical application. The very good theoretical training has given the doctoral student the opportunity to select and combine a set of methods that ensure the fulfillment of the set tasks and the achievement of the goal. I believe that the volume of this work is impressive, many analyzes have been made, valuable results have been obtained, important conclusions and recommendations have been made, and the research has significant scientific and applied contributions. The abstract is made according to the requirements and faithfully reflects the main results and conclusions of the dissertation work.

Based on the analysis made and the proven growth of the doctoral student, I propose to the respected members of the scientific jury, formed by order of the Director of the Institute of Microbiology at the BAS No. I-44/31.03.2023, to award Nikolina Atanasova Atanasova the educational and scientific degree "Doctor" in professional field 4.3 Biological Sciences (Microbiology).

22.05.2023

Reviewer:

Prof. Dr. Petya Hristova