OPINION

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Member of the Scientific Jury, confirmed by Order No. I-44 / 31.03.2023 of the Director of the "Stephan Angeloff" Institute of Microbiology, Prof. Penka Petrova, PhD, DSc.

Subject: PhD thesis for acquiring the educational and scientific degree "Doctor" to Nikolina Atanasova Atanasova, an independent PhD student at the Laboratory "Extremophillic bacteria, Department "General Microbiology" **on the topic „Degradation of plastics by thermophilic and halophilic bacteria isolated from Bulgarian extreme niches"** Professional direction: 4.3 Biological sciences (specialty Microbiology)

Relevance of research

The scientific research in Nikolina Atanasova's dissertation is aimed at investigating the potential of thermophilic and halophilic bacteria, isolated from Bulgarian extreme niches, to degrade plastics. The discovery of the advantages of these synthetic polymers by manufacturing goods companies leads to their entry into various fields of production, which is associated with the generation of increasing amounts of garbage. Plastic pollution has already been documented in all major ocean basins, coastlines, rivers, lakes, terrestrial habitats and even in remote locations such as the Arctic and Antarctic. In 2019, the global release of macro- and microplastics into the environment was estimated at 22 Mt, with this figure projected to double by 2060. Plastic pollution has a major negative impact on human health and climate change, due to its significant carbon footprint and the release of huge amounts of greenhouse gases into the atmosphere. These facts highlight the urgent need to implement sustainable, environmentally friendly solutions for waste management while seeking new approaches for efficient biodegradation of plastics. With her experiments, Nikolina Atanasova contributes to solving this global problem by offering the possibility of developing a new efficient process for the biodegradation of plastics with the participation of extremophilic microorganisms and their enzymes. Until now, the number of described enzymes from extremophilic plastic-degrading bacteria is scarce. In response, the PhD student Atanasova screened 96 thermophilic and halophilic strains from the laboratory's Extremophillic Bacteria collection for their ability to degrade different types of plastics as a sole

carbon source. In parallel, she uses the microbial resource of the Bulgarian extreme niches in search of effective degraders of plastics. For this purpose, Atanasova isolated 13 microbial communities from samples from hot springs (5) and salt pans (7) and studied their growth and esterase activity as well as the pure bacterial cultures isolated from them. Until now, no similar extensive study of the Bulgarian extreme niches has been conducted, regarding the microbial capacity to degrade different types of plastics, determining the relevance and scientific significance of the presented research. The dissertation contains new results regarding the growth and esterase activity of the newly isolated thermophilic and halophilic communities in the presence of 4 different types of plastics (polycaprolactone PCL, polystyrene PS, polypropylene PP and polyvinyl alcohol PVA). New data were obtained regarding the microbial diversity in two plastic-active communities and the influence of PCL and PS on the species composition was proven. A promising thermophilic *Brevibacillus thermoruber* strain 7, producing lipase and efficiently degrading polycaprolactone at 55°C, was isolated. A technological scheme for the purification of the enzyme was developed and the properties of the pure enzyme preparation were characterized. This is the first reported thermostable enzyme capable of degrading polycaprolactone at high temperatures and enabling the creation of an efficient plastic biodegradation process. The presented study provides new information on the dynamics of the biodegradation process of polycaprolactone by a microbial community and by *Brevibacillus thermoruber* strain 7, the achieved degradation rate of 4 weeks being among the highest reported so far for a plastic substrate.

The presented dissertation is written on 180 pages, including 38 figures and 23 tables. The material is structured in the standard sections for a dissertation, namely: Introduction (2 pages), Literature review (68 pages), Aim and Tasks (2 pages), Materials and Methods (13 pages), Results and Discussion (66 pages), Conclusions (2 pages), Contributions (2 pages) and References (26 pages). The literature reference on the developed problem and interpretation of the results is based on the use of 187 literary sources, all in Latin ьха most are from the last 10 years.

Introduction and Literature Review

The introduction is written in a short and concise manner and emphasizes the problem of plastic pollution and the possibilities of creating new approaches for their biodegradation, using extremophilic microorganisms and their enzymes.

The literature review presents very well-summarized and analyzed information presented in precise scientific language. A large number of used literary sources shows the good awareness and

preparation of the PhD student to perform the tasks related to the developed problem. The literature review logically includes a description of the types of plastics and their areas of application, the mechanisms of biodegradation and the role of microorganisms from extreme niches, as effective biodegraders of various types of plastics. The mesophilic and thermophilic enzymes described so far, which participate in plastic biodegradation processes, are thoroughly described, emphasizing the advantage of extremozymes, in particular thermostable lipases, in the development of various biotechnological processes.

Aim and tasks

The aim of the dissertation thesis and the 10 research tasks formulated for its implementation derive from the working hypothesis of the unexplored potential of thermophilic and halophilic microorganisms to degrade plastics, leading to possibilities for isolating effective biodegraders from Bulgarian extreme niches. The main tasks are logically selected and arranged.

Materials and Methods

The experimental work was carried out as a result of the application of various methods, including microbiological and biochemical methods, metagenomic and chromatographic analysis, electrophoretic methods and scanning electron microscopy. The PhD student has mastered a large number of laboratory methods, which she has described precisely and comprehensibly in the Materials and Methods section.

Results and Discussion

The obtained results are described in a logical sequence, according to the order of the tasks. The screening data for the degradation of different types of plastic from the laboratory`s collection and newly isolated extremophillic strains and microbial communities are presented in tabular form, which greatly facilitates their perception. As a result of the conducted research, a total of 13 thermophilic and halophilic bacterial communities were isolated from Bulgarian extreme niches and their potential to degrade 4 types of plastics was studied (esterase activity analysis). Two of the studied thermophilic and halophilic communities stand out with a significant ability to degrade plastic. For the Marikostinovo community MK, the highest reported temperature for the degradation of polycaprolactone is reported - 55°C. In the thermophilic and halophilic communities, a relationship between the number and type of taxonomic groups and the presence of polycaprolactone in the environment was proven. The performed phylogenetic analysis of the microbial communities provides valuable information about the taxa that actively participate in the

degradation of plastic waste. As a next direction, the results of the isolation and identification of thermophilic and halophilic bacteria are presented and the potential of the pure cultures for plastic degradation is evaluated. Esterase activity against polycaprolactone was found in 12 isolates, the highest being *Brevibacillus thermoruber* strain 7. In the case of halophilic bacteria, 6 esterase-producing strains stand out among the total of 25 studied. Within 4 weeks, the biodegradation process of polycaprolactone was monitored in terms of loss of PCL gravimetric weight and obtained final products from the degradation of the polymer. The obtained results are presented in a comparative aspect for community MK, *Brevibacillus thermoruber* strain 7 and co-culture of strain 7 and strain 2. For community MK and strain 7, the results are complemented by an analysis of changes in the surface of the polymer made by scanning microscopy.

As the next direction in the chapter, the experimental results of the study of the thermostable lipase from *Brevibacillus thermoruber* strain 7 are presented. They include data from the purification of the enzyme, determination of its molecular mass, as well as detailed characterization of the properties of the pure lipase. Significant changes on the surface of PCL beads treated with the pure enzyme were demonstrated.

The presented results are thoroughly interpreted and compared with results from similar studies.

Conclusions and contributions

Based on the data from the conducted complex study, 12 conclusions were formulated and 7 contributions were outlined, which emphasize the originality of the conducted study and reveal the importance of scientific results in developing effective processes for bioremediation of plastic-contaminated sites.

Administrative documents

The PhD student has presented four publications on the topic of the dissertation, in which she is the first author. They have been published in international scientific journals with high quartile and SJR. With the collection of 287 points due to the fulfillment of all criteria of the curriculum for the entire course of study, Nikolina Atanasova significantly exceeds the required 200 points for the defence of a dissertation.

Summary

The summary attached to the dissertation has a volume of 51 pages and is prepared as a synthesized version of the dissertation, which fully reflects all its main sections and is very well structured and illustrated with figures and tables.

Notes and questions

I have two minor technical remarks about the doctoral student:

- in the description of Table. 7, page 85 of the Results and Discussion chapter, medium № 7 appears, which is not described in the Materials and Methods section.
- 10 tasks are formulated, but their numbering causes confusion that their number is 12.

Question:

What characteristics of halophilic bacteria and their enzymes make them promising for use in plastic degradation processes?

Conclusion

In conclusion, I would like to summarize that the submitted dissertation and accompanying documents fully meet the legal criteria for obtaining the educational and scientific degree "Doctor". In view of the large-scale and in-depth research conducted and the contributions derived from it, the use of various modern methods, and the importance of the obtained original results of a fundamental and applied nature, I give my positive assessment and recommend the Scientific jury to award the scientific and educational degree "Doctor" to Nikolina Atanasova.

25.05.2023 г.

Sofia

Member of the Scientific jury:

/Assoc. Prof. Anna Tomova/

Anna Tomova

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