

REVIEW

by **Prof. Dr. Velizar Kostadinov Gotchev,**
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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: Assistant professor Nikolina Atanasova Atanasova

Title: “Degradation of plastics by thermophilic and halophilic bacteria isolated from Bulgarian extreme niches”

1. General presentation of the procedure and the candidate

By Order № I-44 dated 31.03.2023 of the Head of The “Stefan Angeloff” Institute of Microbiology at Bulgarian Academy of Sciences (IM-BAS) I was appointed as a member of the scientific jury of a thesis entitled “*Degradation of plastics by thermophilic and halophilic bacteria isolated from Bulgarian extreme niches*” 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 by Nikoleta Atanasova Atanasova – PhD student at department “General microbiology”, laboratory Extremophiles at IM-BAS, scientific supervisor Prof. Margarita Kaburova, DSc.

The set of digital materials submitted by Asst. Prof. Nikolina Atanasova complies with Academic Staff Development Act in the Republic of Bulgaria (ASDARB) and Academic staff development requirements and procedures act at IM-BAS (ASDRPA-IM-BAS) and includes the following documents: application form to the Head of IM-BAS; CV in European format; Master’s degree of higher education; orders; examination protocols and certificates; reference for the fulfilment of the minimum national requirements stipulated in ASDARB; protocol by National seminar of “General microbiology”, PhD-thesis, summary of the thesis in Bulgarian and English; list of scientific publications and citations; declaration of originality and absence of plagiarism.

2. Brief Biographical Information About the Candidate

In 2005, PhD-student Nikolina Atanasova obtained a Master's degree in Biotechnology from the Faculty of Biology at “St. Kliment Ohridski” University of Sofia, qualification Master in industrial biotechnologies. In the period 2005-2008 she was a specialist – biotechnologist at IM-BAS and since 2008 she has been Asst. Prof. at the same institute.

3. Actuality of the subject, aim and research tasks

Ecological problem, reflecting the accumulation of large amounts of xenobiotics of various types into environment, is one of the major problems of modern society, which effects the quality of life. Plastics are large group of waste chemical pollutants, which are too stable in the environment and are almost undegradable. Development of effective and efficient eco-friendly methods for biodegradation of plastics is extremely important research problem from both practical and fundamental points of view. For this reasons the submitted PhD thesis is in the center of the most actual research topics in a sphere of microbial eco-biotechnologies.

4. Familiarity of the problem

Начинът на оформяне на обзора и неговата изчерпателност показват, че докторантката е много добре запозната с разработвания изследователски проблем.

The literature review is based on 187 scientific publications, pointing the major achievements in the studied problem. The review presents the classification of the various types of plastics, their chemical structure, composition and possible applications. Different mechanisms for plastics biodegradation by microorganisms and methods for evaluation of microbial biodegradation potential are explained. The major focus is pointed on the possibilities of extremophiles, such as thermophiles, psychrophiles, alkalophiles and halophiles for plastic biodegradation. The literature review is detailed and comprehensive and the final conclusion of the review is focused on the still unsolved research problems, so called “white spots”, in the field of the studied problem and in this way Asst. Atanasova motivates the needs of the current research. The scope and the way of organization of the literature review allows me to conclude that Nikolina Atanasova is very well acquainted with worldwide achievements on the research problem in details.

5. Research methodology

To reach the major scientific goal of the study and to evaluate the research hypothesis wide range experimental scheme is realized. The applied experimental scheme highly exceeds the purposed of PhD thesis. Wide range of classical and modern microbiological, biochemical,

instrumental methods and microscope techniques are applied. The way the methods are explained ensure accuracy and reproducibility of the results.

6. Characteristics and evaluation of the dissertation

PhD thesis is structured in accepted order, including: Introduction (2 pages), Literature review (58 pages), Goal and tasks (2 pages), Methodology (13 pages), Results and discussions (65 pages), Conclusions (3 pages), Contributions (2 pages), Reference list (26 pages). The introduction section categorically points the importance of the studied problem and clearly defines negative effects of plastic wastes on the environment and society. As I already mention, the literature review is comprehensive but its volume could be reduced and the style of the review could be more analytical. A high state of fragmentation of the information is marked and a lot of subsection numbers (from 1 to 11). Some of the subsections could be gathered. The aim and scope and research tasks are clearly stated but a large number of research tasks are listed. Some of the tasks could be gathered, because in a current state it looks like a working program. Based on the analysis of plastic waste composition in five locations – Rupi, Levunovo, Marikostinovo, Simitli and Dolno Osenovo, Nikolina Atanasova selects four type of plastic wastes – polivinyle alcohol (PVA), polycaprolactone (PKL), polypropylene (PP) and polystyrene (PS). Experimental scheme starts by screening of thermophile bacteria from laboratory culture collection for plastic biodegradation potential. Eighty strains belonging to 8 genera are tested. The strains belonging to species *Geobacillus thermodenitrificans* and *Geobacillus stearothermophilus* demonstrate high esterase activity on PS and PP. Biofilm forming capacity of the strains is also determined, but no one of the studied strains did not demonstrate the ability to form biofilm, which explains the low rates of biodegradation process. For this reason the research activity directs to isolation of new strains from extreme ecological niches – five geothermal springs, temperature range form 46 °C to 72°C. Following the enrichment procedure mixed microbial cultures are tested for esterase activity and biodegradation ability against PVA, PKL, PP and PS. Microbial culture isolated form Marikostinovo demonstrates the highest growth rate and biodegradation potential. The phylogenic diversity of the isolated mixed microbial culture, cultured without and with PKL and PS is determined by metagenomic analysis. The dominant strains belong to genera *Meiothermus* and *Brevibacillus*. Eighteen strains of the mixed culture are isolated as pure cultures and identified to species level by sequencing of 16S rRNA.

The strain *Brevibacillus thermoruber* is screened based on the highest esterase activity 290 E/ml for further experiments and it is deposited in NBIMCC and WDCM. It is determined that co-culture of *Brevibacillus thermoruber* and *Aneuribacillus thermoaerophilus* demonstrates increased level of enzyme activity. For the first time in science thermophile strain *Brevibacillus thermoruber*, capable to degrade PKL is published. Conditions for cultivation of pure culture, co-culture and mixed culture are optimized. By mixed microbial culture, isolated form Marikostinovo spring total biodegradation of PKL are reached for 4 weeks. Extracellular production of lipase of *Brevibacillus thermoruber* is proved. Chromatographic analyses of the degradation products, released by sole culture *Brevibacillus thermoruber*, co-culture of *Brevibacillus thermoruber* and *Aneuribacillus thermoaerophilus* and mixed culture were carried out. The surface of plastics, treated by sole culture *Brevibacillus thermoruber*, co-culture of *Brevibacillus thermoruber* and *Aneuribacillus thermoaerophilus* and mixed culture were analyzed by SEM and the formation of microbial biofilm was evaluated. Experimental scheme continues by development of laboratory scheme for isolation and purification of lipase. Temperature-optimum and pH-optimum of the enzyme, thermal and pH stability range, influence of metal ions and EDTA on the enzyme activity, Vmax and substrate specificity were also determined. Experimental work continues following the analogical experimental scheme focused on halophilic bacteria. Sixteen halophilic strains belonging to 7 different genera from laboratory microbial culture collection are screened for ability for biodegradation of PVA, PKL, PP and PS. Mixed microbial cultures are isolated from lye, mud and water from Pomorie and Burgas salt pans. The phylogenetic diversity of the isolated mixed microbial culture, cultured without and with PKL is determined by metagenomic analysis. In a presence of PKL 1 phylogenetic groups are detected. They belong to five classes and *Halomonadaceae* is the dominant. Six strains are isolated as pure cultures and identified to species level by sequencing 16S rRNA. Two of the species belong to genus *Virginibacillus* and one species belongs to genus *Oceanobacillus*.

The scale and consistency of the experimental scheme, the results obtained and their interpretation are impressive. Based on the results 12 important conclusions and 8 original fundamental contributions with a high level originality were formulate.

7. Evaluation of the publications and the personal contribution of the candidat

The results of the PhD thesis are presented in 4 scientific papers, which are published in scientific journals indexed in Scopus and Web of Science as follows: one in Q₁, two in Q₂ and

one in Q₄. For a short period, less than two years, publications are cited 31 times, which is indisputable certificate of a high quality and originality of the results.

8. Evaluation of the Personal Contributions of the Candidate

I believe that the personal contribution of Asst. Nikolina Atanasova to the realisation of the experimental work, the discussion and publications submitted is relevant to the level of her competence and I would like to emphasize that in each of the listed element clearly show the high erudition and style of her supervisor Prof. Margarita Kmaburowa, DSc.

9. Summary of the dissertation

The summary of the dissertation fulfills of the formal and adequately reflects its essence and achievements.

10. Recommendations for future use of dissertation contributions and results

I have no significant critical remarks on the dissertation and the quality of the results obtained.

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 Nikolina Atanasova Atanasova has theoretical knowledge and professional skills in the scientific specialty Microbiology. Due to the above, I give my positive assessment of the research pre-sented 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).

21.05.2023

Reviewer:.....

(Prof. Dr. V. Gochev)

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