

SCIENTIFIC OPINION

From Prof. Nelly Vladova Georgieva, PhD, Department „Biotechnology“, University of Chemical Technology and Metallurgy - Sofia

on a dissertation submitted to a Scientific Jury, formed by order № I-107/ 31.07.2024 of the Director of the Stefan Angeloff Institute of Microbiology at BAS for obtaining the educational and scientific degree "Doctor" in Professional field: 4.3. Biological Sciences (Microbiology)

Author of the dissertation: **Vladislava Georgieva Dishliyska**

Topic of the dissertation: **Catalase from Antarctic fungi: role in antioxidant defense, regulation and properties**

Supervisor: **Assoc. Prof. Ekaterina Krumova, PhD**

1. Relevance and significance of the dissertation topic

Microorganisms from extreme habitats show multiple and different mechanisms of adaptation to these extreme conditions. In recent years, many researchers have focused their research on microorganisms adapted to extreme environmental conditions and, in particular, low temperatures. It is of interest to clarify the role of the cellular response against oxidative stress in the mechanism of survival of microorganisms under these conditions, and in particular the antioxidant defense system, where enzymes with catalase activity play a major role. The doctoral student's research is focused on the role of enzymes with catalase activity in the mechanisms of adaptation to low-temperature stress in strains of filamentous fungi isolated from extremely cold habitats. Therefore, I consider that the topic of the dissertation work is relevant.

2. Dissertation structure and assessment of contributions

The dissertation is written on 125 standard A4 pages of text, following the generally accepted scheme as follows: Introduction, Literature review, Aim and tasks, Materials and methods, Results and discussion, Conclusions, Contributions, Literature. The recommended ratios between the individual parts of the work have been observed.

The concise scientific style and technical layout of the dissertation make a very good impression. The work is richly illustrated with 30 figures and 12 tables summarizing the obtained results. The literature review is structured correctly, following the logical connection of the information on the problem that the doctoral student is developing. Oxidative stress induced by low temperatures and adaptation strategies are elucidated. Special attention is paid to the role of CAT in the antioxidant defense against low-temperature oxidative stress and in

particular to filamentous fungi as an experimental model. The list of cited literature numbers an impressive 305 titles. This is evidence of excellent theoretical awareness of the doctoral student and is a prerequisite for subsequent successful development.

The goal and the 7 experimental tasks related to its achievement are clearly formulated. The materials used, as well as the methods related to the cultivation of the studied strains, molecular genetic analyses, electron microscopic (TEM and SEM) analyzes as well as analytical methods for determining the specific enzyme activity of antioxidant enzymes, polymerase chain reaction (PCR), quantitative PT PCR amplification, DNA sequencing analysis, etc., are precisely described. The methods used are modern and adequate for the realization of the dissertation work, and the necessary details are accurately presented.

In the "Results and discussion" section, the results obtained by the doctoral student are presented and discussed in the order of the assigned tasks. The influence of different temperatures on the growth of 61 strains of Antarctic filamentous fungi and their ability to synthesize intracellular and extracellular catalase was monitored. Based on the obtained data on changes in growth, development, oxidatively damaged protein molecules, Vladislava Dyshliyska determined strain *Penicillium griseofulvum* P29 to be a very good producer of catalase and a potential producer of thermosensitive enzymes, as well as a lower degree of oxidative damage to proteins and lipids. The following analyzes in the thesis are focused on this strain. The comparative analysis of the obtained complete genome sequence of strain *P. griseofulvum* P29 demonstrated the presence of 5 different catalase genes, identified, sequenced and characterized in terms of the functionality of their encoded proteins. A rather large volume of experimental work was carried out at a very good methodical level, and the experiments carried out were in the required number of repetitions. Based on the experimental work, 9 conclusions were formulated, which logically follow from the obtained results. The contributions of the dissertation have a fundamental and applied nature, and for the first time an effective laboratory method for obtaining and purifying a temperature-sensitive CAT was developed. The new enzyme isolated from *Penicillium griseofulvum* P29 has a temperature optimum of 20⁰C and a pH optimum of 6.0. The prevalence of intracellular CAT in Antarctic fungi from different thermal classes has been demonstrated. The presence of extracellular CAT was detected, which is a rare phenomenon. 5 nucleotide sequences corresponding to the 5 genes encoding CAT synthesis in *P. griseofulvum* strain P29 have been deposited in a database.

3. Assessment of dissertation publications

Vladislava Dishliyska has reflected the results of her dissertation work in 2 publications in prestigious journals with an impact factor and Q3. Citations of already published articles are also presented. In one of the articles, the doctoral student is the first author, which is also evidence of her personal contribution to the dissertation.

4. Opinions, recommendations and notes

In the course of the implementation of the experimental work on the dissertation by Vladislava Dishliyska, it is clearly evident that she has acquired competence in terms of knowledge in the specific scientific field, skills in applying a complex methodological approach to solving the set tasks. She has mastered a variety of methodological approaches – microbiological, biochemical, molecular genetic, analytical, used in biological research, as well as competence for analysis and evaluation of the obtained results. She has the skills to work with scientific literature, to analyze and summarize scientific information. I have no comments or recommendations.

5. Conclusion

I positively evaluate the dissertation work developed by Vladislava Dishliyska. The precisely performed experiments, the good layout and illustration, as well as the thoroughly interpreted results give me reason to believe that during her doctoral studies, Vladislava Dishliyska developed herself as a young scientist and researcher with good knowledge in the field of microbiology.

The presented dissertation work in terms of relevance, volume of research, achieved scientific contributions, as well as publication activity, fully meets the requirements of PRASRB and the additional requirements of the Institute of Microbiology, BAS for the acquisition of the educational and scientific degree "Doctor". I consider it reasonable to suggest that Vladislava Georgieva Dishliyska acquire the educational and scientific degree "Doctor" in the scientific specialty of Microbiology.

18.09.2024

Sofia

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/Prof. Nelly Georgieva, PhD/