REVIEW

of PhD thesis titled:

"CATALASE FROM ANTARCTIC MUSHROOMS: ROLE IN ANTIOXIDANT DEFENSE, REGULATION AND PROPERTIES"

Presented by **Vladislava Georgieva Dishliyska,**

doctoral student of independent preparation for the award of the educational and scientific degree "Doctor" in a professional direction in the field of higher education 4. *Natural sciences, mathematics and informatics, professional direction*, 4.3. Biological Sciences, Doctoral Program Microbiology

Research supervisor: Assoc. Dr. Ekaterina Krumova

Reviewer: Prof. Svetla Danova, PhD,DSc

Institute of Microbiology "Stefan Angelov" (IMicB), BAS

ABOUT THE PROCEDURE:

The current procedure for the acquisition of ONS "Doctor" is conducted on the basis of Art. 29 al. 3 of the Regulations on the terms and conditions for acquiring scientific degrees and holding academic positions at the Institute of Microbiology BAS, decision of the Scientific Council of the IMicB BAS (protocol No. 7 of 30.07.2024 r.). Pursuant to the Order (No. I-107/31.07.2024) of the Director of the Institute, I have been selected as a member of the Scientific Jury for the above competition and I have been designated as a reviewer at its first meeting. In my capacity as a re-examiner, I declare that there is no conflict of interest within the meaning of § 1, item 2a of the additional provisions of ZRASRB between me and the candidate for the ONS "Doctor" procedure, and I am not subject to the restrictions under Art. 33 of ZRASRB.

The absence of plagiarism in the candidate's scientific works has been proven according to the law (ZRASRB).

RELEVANCE AND SIGNIFICANCE OF THE DEVELOPED PROBLEM

The dissertation work, presented for review, is dedicated to psychrophilic representatives of the mushroom kingdom, isolated from poorly studied Antarctic ecosystems. Original is not only the object of research - taxonomically diverse fungi, but also the study of mechanisms of ecological selection, as well as evolutionary adaptation, which ensure their survival in extreme conditions. The impact of low temperatures and manifestations of oxidative stress attracts the interest not only of the scientists. The PhD thesis search data on the cellular response against cold stress and on the adaptation of fungi to survive such extreme low temperatures. In this regard, the ability of previously poorly studied psychrophiles to produce temperature-sensitive (cold-active, CA) enzymes, including catalase as a first line of antioxidant defense, is

characterized. The work studies CA enzymes, which have important applications in the food, textile and pharmaceutical industries, in bioremediation technology, as an indicator of the degradation of hydrocarbons in crude oil-contaminated soil, etc. Understanding their potential as therapeutic agents for diseases caused by elevated free radicals (eg, diabetes, Alzheimer's disease, Parkinson's disease, vitiligo, and acatalasemia), as well as in cryopreservation in reproductive medicine, is very important. All this gives me reason to emphasize the innovativeness and relevance of the developed dissertation work.

SCOPE AND STRUCTURE OF THE DISSERTATION

The PhD thesis is constructed in the generally accepted form, according to the Regulations of ImicB-BAS for the application of ZRASRB. It is written in 125 standard computer pages (at 1.5 pt), which include 90 pages of text and 27 pages of references. The requirements for the form, content and ratio of the individual sections have been met as follows: *Introduction - 2 pages, Literature overview - 28 pages*; *Purpose and tasks - 1 page*; *Materials and methods - 11 p., Results and discussion - 43 p., Conclusions - 2 p., Contributions - 1 p. References - 22 p.*; *Publications - 1 page.* Abbreviations used are duly indicated. The arrangement, the logical sequence in the presentation of the obtained results and their illustration with 30 figures and 12 tables make an excellent impression. The material is presented in a tight scientific style. I would like to note the purposeful introduction, which in a concentrated form presents both the unsolved problems and the originality of the development.

LITERARY AWARENESS AND STATEMENT OF GOAL AND OBJECTIVES

The literature review is very well structured and logically discusses the scientific information needed to present the research conducted as follows:

(1) *"Antarctica - a habitat for filamentous fungi adapted to extremely cold environmental conditions"* - analytically considered as a poorly studied habitat, with a microflora whose research is associated with a number of scientific challenges and from which the studied fungi were isolated; (2) *"Oxidative stress induced by low temperatures and adaptation strategies"* - the author moves from the biotope to the biocenosis and metabolic features in extreme conditions. Current information on climate change and oxidative stress is summarized; Biomarkers of oxidative stress induced by low temperatures and antioxidant defense system of cells; (3) *"Relation between cold stress and oxidative stress"* was sought and (4) Adaptation of microorganisms to oxidative stress caused by low environmental temperatures; (5) Role of CAT in antioxidant defense against low-temperature oxidative stress, adding evidence for (6) Filamentous fungi, as an experimental model. Data from the last 20

years are presented, which clearly shows the excellent theoretical awareness of the doctoral student. This is also evident from the structuring of the review, purposeful and specific, including all aspects of the study. The well-argued and literature-supported rationale regarding the necessity and innovativeness of the scientific development stands out clearly.

An excellent impression is made by the final critical summary of the unsolved problems on the subject, which leads logically to the goal: *"To investigate the involvement of the antioxidant enzyme catalase in the mechanisms of adaptation to low-temperature stress in filamentous fungi isolated from extremely cold habitats (Antarctica)".*

For its implementation, 7 tasks with 3 sub-tasks are clearly formulated, which requires not only theoretical knowledge, but also experimental skills.

EVALUATION OF MATERIALS AND METHODS USED

The Materials and Methods section demonstrates a wide range of routine and modern molecular and analytical methods tailored to the specific requirements of the tasks at hand. I highly appreciate the large number - 61 strains of molds isolated from soil samples from different regions of Antarctica, as an experimental group, to solve the tasks and to select model strains in the subsequent stages. Classic microbiological tests have been carefully selected to characterize the growth and morphology of fungal cells and cell differentiation under conditions of temperature stress. They are complemented in a logical sequence with the classical analytical methods and with molecular genetic methods for the isolation and sequencing of genes and gene expression of the catalase genes. The strategy for the assessment of antioxidant enzymes - SOD and CAT, associated with cold and oxidative stress in normoxic conditions and in long-term and short-term low-temperature stress, allows an objective assessment of the sought mechanisms of cellular response, in this type of psychrophilic mycoorganisms. A panel of biochemical methods was correctly selected and skillfully applied, including the changes in various biomarkers, such *as the content of oxidatively damaged proteins; amount of spare carbohydrates, Lipid peroxidation*, determination of the level of generated free oxy-radicals to reach the selection of a model strain and the isolation, purification and characterization of CAT. The PhD student chooses highly discriminatory molecular genetic methods to characterize the genetic bases of the response to oxidative stress under low-temperature conditions, in the model strain. In addition to finding catalase genes, their gene expression was also proven during cultivation in normoxic conditions and under conditions of low temperature stress.

EVALUATION OF THE RESULTS OBTAINED

The PhD thesis of Vladislava Dishliyska is a thorough and large-scale scientific study on the mechanisms of adaptation to low-temperature stress in filamentous fungi, choosing an object from one of the coldest habitats, unexplored so far - Antarctica. Their choice complements the scientific data on the adaptation of unstudied eukaryotic microorganisms to survive at temperatures close to negative. The doctoral student is looking for the connection between the low-temperature impact and the manifestations of oxidative stress, as well as the participation of antioxidant enzymes in the survival of organisms in such extreme conditions. The foundation is a group of 61 mold strains isolated from Antarctic soil samples, which were studied for growth and development at different temperatures and classified to the respective thermal classes according to 3 different temperature parameters and biomass accumulation. The logical sequence of the development deserves attention. Each stage includes convincingly proven material, which is a starting point for the next direction of the research. The classification, according to the well-chosen physiological-biochemical indicators, is the starting point for the subsequent characterization of the isolated fungi regarding the synthesis of intracellular and extracellular catalase. V. Dishliyska evaluated the distribution of extracellular CAT in strains from different taxonomic groups, which has an important practical application. The choice of model strains is also scientifically justified with a comparative analysis of CAT synthesis data in the different strains. All this gives the dissertation a characteristic of completeness based on scientific knowledge and theoretical preparation. It is confirmed by the choice of methodological approaches and the inclusion of modern molecular-genetic methods, building on the established classical ones. An original approach was applied by the PhD student, evaluating the role of catalases in the cellular response of model strains to stress caused by low temperatures, in the search for catalase genes and the transcriptional analysis of their expression. The role of the expressed cat1 gene at low temperature has been competently discussed. Tasks resolution were generally achieved by comparative multifactorial analysis based on the assessment of different biomarkers of oxidative stress. I highly appreciate the variety of methods used and their combination to solve the experimental tasks. Undisputed quality of the developed work is the proof of the genetic basis for the obtained empirical data on the evaluation of the key enzyme catalase and the response of the correctly selected model strains in normooxic conditions and in conditions of low temperature stress.

To the merits of the dissertation work are added the newly obtained data on optimizing the cultivation conditions of strain *P. griseofulvum* P29 to increase the synthesis of the enzyme catalase (KAT), its biochemical purification and enzymatic characterization. In this chapter of the dissertation, the PhD student has successively developed the parameters for cultivating the

producer strain and optimized those to obtain the desired enzyme. With the protocol for enzymatic purification of CAT, 12 times higher activity was achieved, and with an uncomplicated and effective procedure. This section of the work undoubtedly has author's solutions and new approaches, but this is not reflected in the discussion. I would recommend that in this part and in the remaining experimental results sections, the original contribution finds expression in the discussion of the obtained results. This is also important due to the fact that information on efficient production of temperature-sensitive KAT is scarce. Among a large number of microorganisms capable of producing intracellular enzymes, including CAT, filamentous fungi are particularly interesting due to their ease of cultivation and biomass accumulation. These results are well illustrated with 22 figures and 8 tables.

The scientific experiments are correctly set up, in repetition and proven statistical reliability, which confirms the excellent preparation of the doctoral student and the ability to choose and combine methodical approaches and conduct experiments. This leads to the successful solution of specific experimental tasks, evident from the presented publications on the subject of the dissertation. The 2 publications presented are in authoritative international journals and have a common IF-5.532. The doctoral student is the first author of one of them.

The conclusions are a logical consequence of the experimental data. They accurately represent the performance of the assigned tasks. They are written in a tight style and concise statement, which presents a difficulty in summarizing the results of such a voluminous experimental work. Therefore, their number corresponds to the number of formulated tasks and subtasks. It is clear from them that the goal has been achieved.

Four contributions have been formulated, which I accept as such, of an original and confirmatory nature. Their precise formulation with a clearly defined scientific and methodical contribution to the research of little-studied psychrophilic molds in the conditions of low-temperature effects and oxidative stress is also impressive.

ABSTRACT, CRITICAL NOTES AND QUESTIONS

The autoa-bstract as well as the entire dissertation work are very well designed and I have no critical remarks. I would like to make a recommendation that the accumulated previous experience of the research team on the developed problems should be reflected either in the literature review or in the discussion during the comparative analysis of the results. This will even more clearly outline the personal and scientific contribution to the development of this current topic:

In this regard, I would also like to ask the doctoral student one question: In view of the original results for the obtained and characterized temperature-sensitive enzyme, how do you see the research development of and perspectives, and may we expect a practical application.

CONCLUSION

In conclusion, I would like to emphasize that the material is dissertable, the topic is current, the doctoral student has mastered a wide range of modern methods, the experiments are set methodically correctly, the results obtained are credible and are a solid basis for further scientific and applied developments, original scientific and applied contributions.

Based on the objective analysis of the dissertation work and bearing in mind the relevance and level of the work, I confidently propose to the respected members of the Scientific Jury to award the doctoral student **Vladislava Georgieva Dishliyska** with the educational and scientific degree **"Doctor"** in the field of higher education *4. Natural sciences, mathematics and informatics, professional direction, 4.3. Biological Sciences, PhD Program: Microbiology.*

26.09.2024 **Reviewer:.....**

(Prof. S. Danova, PhD)