

STATEMENT

by Assoc. prof. Maria Gerginova
Department of General Microbiology, Laboratory of Microbial Genetic - The Stephan Angeloff
Institute of Microbiology, Bulgarian Academy of Sciences
of a PhD thesis for awarding the educational and scientific degree "Doctor" in the Scientific field
4.3. Biological Sciences, doctoral program Microbiology.

Title: CATALASE FROM ANTARCTIC MICROMYCETES: ROLE IN ANTIOXIDANT DEFENSE, REGULATION AND PROPERTIES

Author: Vladislava Georgieva Dishliyska

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In recent years, research on microorganisms adapted to Antarctica's cold conditions has intensified and become a topical issue. Fungi are a major force in Antarctic population dynamics and ecology. Substantial evidence suggests that they are well adapted to exist in ecosystems with extremely low temperatures compared to other microorganisms. Psychrophilic and psychrotolerant fungi are specialized in the production of extracellular and intracellular enzymes, attracting interest due to their potential benefits in various industrial fields. Studies of bioactive molecules, such as catalases, in microorganisms isolated from habitats with low positive temperatures provide new knowledge and concepts for antioxidant protection under hypothermic stress that microorganisms have to cope with.

The present thesis of the PhD student Vladislava Dishliyska is dedicated to the study of filamentous fungi isolated from Antarctica and the activation of their antioxidant protection under low temperature conditions. Novelties here are not only the physiological and molecular genetic characterization of catalase enzymes from fungi but also the presented laboratory method for the preparation and purification of a cold-active catalase from *Penicillium griseofulvum* P29.

The PhD thesis is organized according to the model adopted in Bulgaria on 118 pages, with 30 figures, 12 tables, and 206 references. The presented thesis has balanced individual sections, which shows the good knowledge of Vladislava Dishliyska but also her ability to analyze and systematize a wide range of data. The literature review is written in detail and shows a full knowledge of the literature sources related to the topic. A substantial part of it contains new and interesting information on the topic under consideration.

Based on the analysis in the literature review, the main scientific research purpose of this PhD thesis is presented. It is clear, well formulated, and covers all areas of experimental work. Following the clearly formulated aim of the thesis, there are 7 main tasks that are interrelated and outline the focus of the dissertation work.

The PhD student starts by using the potential of classical screening of fungi and establishes the presence of intracellular catalase and the rare phenomenon of the presence of extracellular catalase. Two model strains characterized by high catalase activity were selected: *Penicillium griseofulvum* P29, a representative of psychrotolerant Antarctic fungi, and *Penicillium*

chrysogenum P27, a representative of mesophilic Antarctic fungi. Vladislava Dishlijska continues with in-depth and detailed studies of the growth dynamics of both strains under different temperatures of cultivation, and the synthesis of intracellular and extracellular catalase followed. A new approach to induce oxidative stress was used. The PhD student applied short-term temperature stress and studied the change in the activity of antioxidant protection. She investigated physiological changes as a result of the survival response under extreme conditions in filamentous fungi: presence of lipid peroxidation, carbonylated proteins, and accumulation of reserve carbohydrates trehalose and glycogen. The presented studies of the ultrastructural changes in the cells of fungal strains subjected to cold stress are interesting.

Filamentous fungi are known to have more than one catalase enzyme, and they exhibit great diversity in their structure, location, and function. Gene expression studies of 5 catalase genes, identified in *Penicillium griseofulvum* strain P29, induced at low cultivation temperature, were carried out. The results showed that four of the genes had statistically significant increased expressions. The most pronounced increase was in the expression of the *cat1* gene encoding the enzyme catalase-peroxidase. The PhD student hypothesised that the enzyme with catalase-peroxidase activity is one of the most important ones, associated with the growth of *P. griseofulvum* P29 under conditions of oxidative stress induced by low temperature.

A very wide range of methods was used in the experimental work. Without neglecting the basic ones, the author has mastered and applied microbiological, biochemical, molecular biological, and bioinformatic methods. From the presented methodological part, it can be concluded that PhD student has acquired a number of methodological skills that have established her as a qualified specialist for conducting experiments in the field of modern microbiological research.

The conclusions and contributions are a logical consequence of the experimental data and provide the necessary information on the value of the research conducted.

With appropriately selected research, implemented at a modern level, PhD student Vladislava Dishlijska has developed a model that makes it possible to evaluate the participation of the antioxidant enzyme catalase in the strategy of microbial cells in adapting to environmental conditions. The expression of selected genes of potential interest represents a promising tool and expands the understanding of the role of antioxidant enzymes in the physiology of filamentous fungi. The isolation of cold active enzymes is an optimal alternative to their mesophilic equivalents and is considered an object with a future potential for application in medicine and biotechnology.

The peer-reviewed thesis contains the quality and scope requirements for the education degree of "Doctor". Experimental and analytical work was carried out at a high scientific level. The role of PhD student Vladislava Dishlijska is undeniable. The minimum national requirements for the PhD degree are covered. Vladislava Dishlijska participated in writing 2 articles, which were printed in refereed journals with high scientific indicators.

A complete set of the necessary documents has been submitted, according to the requirements of the Act for the Development of Academic Staff in the Republic of Bulgaria (ADAS), the Rules for ADAS application, as well as the specific Rules of BAS and the Stephan Angeloff Institute of Microbiology – BAS.

On the basis of the presented analysis, I express my positive assessment regarding the prepared PhD thesis and I propose to the scientific jury to award the educational and scientific degree “Doctor” (PhD) to Vladyslava Dishliyska in the Field of Higher Education 4. Natural Sciences, Mathematics and Informatics, Scientific field 4.3. Biological Sciences, specialty Microbiology.

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Sofia, Bulgaria

Prepared by: ...

**На основание
чл. 2 от ЗЗЛД**

Assoc. Prof. Maria Gerginova, PhD