

STATEMENT

By: **Prof. Svetla Danova, PhD,**

Institute of Microbiology "Stefan Angelov", Bulgarian Academy of Sciences

SUBJECT: Evaluation of a PhD thesis titled:

RESTING STATE IN SACCHAROMYCES CEREVISIAE YEAST – A MODEL FOR STUDYING TOXICOLOGICAL AND STRESS RESPONSE

for the acquisition of an educational and scientific degree "Doctor" in the field of higher education **4. Natural sciences, mathematics and informatics, Professional direction, 4.3**

Biological sciences, doctoral program Microbiology, with author

full-time doctoral student **Polya Galinova Marinovska**

Scientific supervisor: Assoc. Prof. Dr. Ventsislava Petrova

The PhD student Polya Galinova Marinovska is enrolled in regular doctoral studies at the Department of General and Industrial Microbiology at the Faculty of Biology, by order RD 20-1012/16.07.2020 of the Rector of the SU. Kliment Ohridski for the period from 20.07.2020 to 20.07.2023. As a member of the scientific jury, according to order No. RD-38-354/10.07.2023 of the Rector, in accordance with Art. 4 of the Law (ZRASR) and the Regulations to it, I declare that I have no joint publications, projects or conflict of interest of any other nature within the meaning of paragraph 1 items 3 and 5 of the ZRASRD with the doctoral student.

According to procedure for the official defense, by decision of the Faculty Scientific Council (Protocol No. 11 of 04.07.2023) I received for evaluation a set of materials, including the dissertation work, an abstract, the publications related to the dissertation and an autobiography of the doctoral student, which meets the requirements of the ŽRASRB, the Regulations for the implementation of the ŽRASRB and the Regulations for the growth of the academic staff of Biological Faculty - SU "Kliment Ohridski".

1. Relevance of the problem developed in the dissertation work

The thesis submitted for opinion is aimed at solving the serious challenge for the planet's ecology caused by the annual accumulation of over 10,000 synthetic chemicals. The PhD student is looking for new successful approaches to evaluate the effects of these xenobiotics on human and animal health. In this regard, an alternative model based on quiescent *Saccharomyces cerevisiae* cells was developed to serve as an easily reproducible and accurate toxicological assay in higher eukaryotes. This gives me reason to rate the thesis developed as original and up-to-date.

2. Evaluation of the structure and content of the dissertation work

The dissertation is set out in 229 standard pages of text. The generally accepted scheme and the recommended ratios between the separate parts of the work were followed, as follows: *Introduction* - 2 pages, *Literature review* - 45 pages, *Aim and tasks* - 1 p., *Materials and methods* - 15 pages, *Results and discussion* - 83 p., *Conclusions* - 2 p., *Contributions* - 2 p.; *References* – 55 p. The bibliographic reference includes an impressive 575 titles, even for a large (DSc) doctoral thesis. All sources are in Latin and mostly from the last 10 years, which shows an excellent theoretical awareness of the problem developed by the doctoral student.

The dissertation is richly illustrated with 61 figures and 5 tables. The lists of figures and tables added to the table of contents are original elements in the structure of the work, which significantly contributes to the easier perception of this voluminous development. An excellent impression is made by the correct pronunciation, the grammatically sound and tight scientific style used in the writing and the overall layout of the work.

2.1. Literature review

The literature review is specific, well structured, following the logical connection of the information consistently leads to an analytical conclusion that justifies the need for such development. The overview proves the doctoral student's excellent theoretical training and knowledge of the problem she is working on. Concluding with a conclusion, Polly Marinova clearly outlines the potential of *Saccharomyces cerevisiae* as a model for accumulating knowledge about the effect of various toxic compounds, which, in combination with the selected variant - cells in a state of rest and non-proliferative in a stationary phase, gives scope for scientific contributions to the development.

2.2. Purpose and tasks

The objective is specific and follows logically from the overview and especially from the conclusion drawn to it. To achieve this goal, 8 experimental tasks have been defined that predetermine a voluminous research work.

2.3. Materials and methods

The section systematically presents the variety of microbiological, biochemical and molecular genetics, including bioinformatics methods, that cover the different aspects of research. A wide range of classic and modern methods, correctly selected and adequate for the realization of the dissertation work, was used. They are accurately described. I would advise the PhD student to note the elements of personal contribution and adaptation of the protocols to the object of research, which are the fruit of her good theoretical preparation, evidenced by their choice. The only omission is the lack of the method by which ethanol was quantitatively reported in the cultural experiments in order to determine the diauxic transition of the strain *Saccharomyces cerevisiae* BY4741.

I appreciate the selection of a well-studied genetically auxotrophic haploid strain BY4741 from the German collection. As a small technical omission, I also consider the lack of the trademark/company supplier of the evaluated toxic substances.

2.3. Results and discussion

In accordance with the objectives, the Results and Discussion section consists of 3 main sections: (i) Growth dynamics and cell differentiation in *S. cerevisiae* yeast, (ii) Yeast as a model system for environmental impact assessment; (iii) *In silico* analysis of genes from signaling pathways regulating dormancy and stress response. I highly appreciate the combined approach combining multiple biochemical and molecular genetic analyzes to evaluate the toxicological effects of four investigated substances and the molecular mechanisms in response to toxicologically generated cellular stress and dysfunction. The PhD student skillfully combines inductive and deductive approaches, combining biochemical with *in silico* analyzes of genes with a role in determining signaling pathways under stress, to achieve the goal. This proves that Poli Marinova is a built molecular biologist with excellent theoretical and practical preparation. She successfully solves the set tasks, choosing new and original approaches to achieve the goal of the dissertation. The results are presented correctly and analytically and are discussed in the light of the data from recent years. A bit bolder should be to include/present the obtained data from the statistical processing (t-Student test) as proof of their significance. The doctoral student follows a well-structured and methodically secured experimental scheme, as the results of the previous task determine the setting of the subsequent experiments. The final part of the section and the synthetically presented "Functional profile of stress response" in *S. cerevisiae* BY4741 received a very high rating. It once again clearly shows the volume and significance of the scientific research carried out and the scientific contribution in uncovering the mechanisms of stress and the successful model created.

The presented conclusions, although many in number - 15, follow logically from the obtained results and are precisely formulated. Important scientific and applied contributions are outlined, incl. original and methodical ones. They are based entirely on the results obtained.

The abstract fully and faithfully reflects the main results, formulated conclusions and contributions of the dissertation work.

The publication activity of the dissertation student, including two publications on the subject of the dissertation work, published in periodical scientific journals with an Impact Factor, meets the requirements of the Bulgarian law (ZRASRB) and Rules and Regulations of Biological Faculty-SU for obtaining the scientific and educational degree "Doctor". In both, as well as in one oral presentation by report and 4 posters from international and national scientific forums, Polly Marinovska is the first author, which is a clear proof of her personal contribution.

3. CONCLUSION

Polly Marinovska's PhD thesis summarizes the obtained scientific and applied results by demonstrating both in-depth theoretical knowledge of the dissertation student and the ability for

independent, logically constructed and in-depth research. Completed are the set tasks and is realized the goal. The work is up-to-date and complex, combining classic and modern methods in a good methodological base. On the basis of the presented arguments for the topicality of the issues and the original contributions reflected in the dissertation work, I give my high evaluation and recommend to the members of the scientific jury to award the doctoral candidate Polly Marinovska the educational and scientific degree "Doctor" in professional direction 4.3. Biological Sciences, specialty Microbiology.

25.09.2023
Sofia

Prepared the opinion:
(Prof. S. Danova, PhD)