

OPINION

**by Assoc. Prof. Tsvetelina Sashkova Paunova-Krasteva
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Subject: evaluation of for acquiring the educational and science degree “Doctor“ in the professional field 4.3. Biological sciences, Scientific specialty: Microbiology

Doctoral program: Microbiology at the Department of General and Industrial Microbiology

Author of the dissertation: Polya Galinova Marinovska

Title of the dissertation: "Quiescent state in yeast *Saccharomyces cerevisiae* – a model for studying toxicological and stress response"

Research supervisor: Assoc. Ventsislava Petrova

Relevance and significance of the PhD thesis

Environmental pollution with various types of toxic materials, xenobiotics, medicinal substances, synthetic chemicals, and others is a serious problem for human health and ecosystems. However, studies related to the toxicological response at the cellular and molecular level due to the exposure of cells to toxic stress are scarce. In recent years, interest in this direction has been directed to the yeast model *S. cerevisiae* for studies following the genotoxic and cytotoxic effects of various chemical compounds and medicinal preparations. The unique genetic and metabolic characteristics of this eukaryotic model make it suitable for studying a number of processes such as gene expression, signal transduction, cell cycle, etc. Of particular interest are the developments related to the study of the cellular response to stress effects, either chemical or physical. The scarce information about the cellular response as a consequence of toxic and stressful effects determines the relevance of the research, namely to study the applicability of *S. cerevisiae* cells in different phases of the life cycle, as a eukaryotic model for cellular response to toxic and stressful agents. From here, it should be concluded that the developed topic embedded in the dissertation has a current and significant scientific aspect.

Structure and evaluation of the PhD thesis

The dissertation is constructed in 229 standard pages. Its structure complies with the accepted arrangement for this category of scientific works, containing all the necessary sections: Title page, Table of Contents (5 pages), Introduction (2 pages), Literature review (45 pages), Conclusion (1 page) Aim and objectives (1 p.), Materials and methods (15 p.), Results and discussion (90 p.), Conclusions (2 p.), References (55 p.), Contributions (1 p.), illustrated with 5 tables and 61 black and white and color figures.

The **literature review** includes a detailed analysis of the yeast model - cell cycle, resting state, application in pharmacological studies, mechanism of toxic action, influence of physical parameters and stress response of yeast and bioinformatics approaches applied to resting cells. The in-depth knowledge of the state of the researched problem reflects the good awareness and competence of the doctoral student in the field not only of microbiology. In addition, the figures and diagrams available in the review visually summarize and structure its overall volume.

The objective and related experimental tasks are clearly and precisely defined, in accordance with the unsolved problems found in the literature review, which speaks of promising perspective and significance.

The materials and methods section includes a wide range, both classic microbiological, biochemical, molecular, genetic, and modern microscopic, bioinformatics and sequencing, etc. methods of analysis, selected in accordance with the implementation of the set tasks. 1 figure is included a number of formulas for precise calculations are applied, which allows for high accuracy and reproducibility of the obtained data.

The "Results and Discussion" part fully corresponds to the tasks set. The logically selected methods begin with a description of the data obtained from the cell parameters of growth and differentiation of the applied haploid, model strain *Saccharomyces cerevisiae* BY4741. Detailed analysis and assessment of survival, cytotoxicity, and genotoxicity were made after treatment with various chemical or medicinal agents, and the levels of oxidized proteins, lipids, and glutathione were monitored. The experimental scheme was continued by tracking the effect of various physical factors on the growth and survival of the yeast population. Particularly impressive is the modern approach for in silico analysis of genes regulating resting state and cellular stress response. The presented results are visualized with a

significant number of figures and tables, which allows reproducibility and good visualization of the obtained results. Experimental data are precisely and comprehensibly interpreted. The logically placed 15 conclusions are precise and clearly formulated, as a consequence of the conducted experiments. I note as extremely valuable the derived contributions of a scientific nature - new information was provided on the adaptive response of the *S. cerevisiae* model system at different phases of the cell cycle. The genotoxic and DNA-damaging potential of toxic medicinal preparations was determined, and the intracellular levels of ROS were characterized. Through innovative bioinformatics approaches, the homology of yeast genes and proteins with corresponding homologs in humans is reflected. For the first time, "functional stress response profiles" have been developed in *S. cerevisiae* strain BY4741, in different phases of the cell cycle. Scientific-applied contributions include a large-scale methodological approach for obtaining and isolating resting model cells from the *S. cerevisiae* BY4741 strain. A new test system for investigating molecular mechanisms of toxic action and functional profiling for chemical tolerance is proposed. Valuable medico-biological information is provided on the resistance of yeast cells to the action of zeocin, which makes them less susceptible to the action of antitumor antibiotics. Moreover, their reduced metabolic activity in the G0 state determines their resistance to physical, chemical, and toxic agents, which makes them a convenient model system for studying toxicity mechanisms and increases their potential for industrial applications.

The sources cited in the dissertation work are a total of 575, most of which are from the last five years, which shows an analytical attitude to the literature review.

The results of the conducted scientific research were reported at 6 scientific conferences. The PhD student participated in a total of 4 projects, two international and two with national funding.

Based on a certificate provided by the Faculty of Biology, Sofia University, "St. Kliment Ohridski", it is clear that Polyana Marinovska has passed all the required exams, which proves that the set curriculum from the individual study plan is fully covered.

Evaluation of publications on the PhD thesis

The results of the dissertation work are presented in 2 publications in the scientific journal *BioRisk*. Each of the articles has an impact factor of 1.167 and quartile Q2. The PhD student is first author on both publications, indicating her significant contribution to the development. At the time of drafting the opinion, three citations from 2023 were found.

Evaluation of the abstracts of PhD thesis

The abstract for the dissertation contains 59 pages, is formatted according to generally accepted requirements and reflects the main results achieved in the dissertation.

Fulfillment of minimum national requirements

- Group of indicators A – 50 points out of 50 required
- Group of indicators D – 40 points out of the required 30

Conclusion

Polya Marinovska's dissertation summarizes scientific and scientific-applied results with an original contribution and novelty in science, meets all the requirements for obtaining the educational and scientific degree "Doctor", defined in the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its implementation, as well as the Regulations for the terms and conditions for acquiring scientific degrees and holding Academic positions at Sofia University "St. Kliment Ohridski". Developing the dissertation work, the doctoral student gained theoretical knowledge and professional experience in the field of microbiology and acquired practical skills to competently analyze data and use specific terminology.

Based on the above analysis, I confidently give my positive assessment of the submitted dissertation and abstract and recommend to the respected members of the Scientific Jury to vote positively for awarding the Educational and Scientific Degree "Doctor" to Polya Galinova Marinovska in Professional Direction 4.3 Biological Sciences (Microbiology).

19.09.2023

Reviewer:

/Assoc. Prof. Tsvetelina Paunova-Krasteva/