EXPERT OPINION

By: Assoc. Prof. Dr. Trayana Nedeva, Department of General and Industrial Microbiology, Faculty of Biology, Sofia University “St. Kliment Ohridski”

Re: PhD thesis presented for defence to a scientific jury for acquiring the educational and scientific degree "Doctor"

PhD Thesis author: POLYA GALINOVA MARINOVSKA

PhD Thesis title: „Quiescent state in yeast *Saccharomyces cerevisiae* – a model for studying toxicological and stress response”

Saccharomyces cerevisiae yeasts have been used for bread, wine, and beer making for millennia. The well-known and routine procedures for their manipulation, the annotated genome, the broad set of molecular genetic instruments for working with them, and the remarkable degree of conservatism of the essential life processes with higher eukaryotes define them as the principal model of eukaryotic cell biology and genetics, including the study of the chemical toxicity mechanisms. It is well-known that complex metabolic processes, organized in a dynamic, continuously changing metabolic network, occur in metabolically active cultures. In contrast, the cells at rest (so-called G_0 cells) that are metabolically not active and resemble those of higher eukaryotic species (mammals) offer an alternative to studying the potential action of chemical toxicants and identifying the main components of the toxicological response, the development of strategies for their elimination, and assessment of the environmental risk. Namely, such strategy development is the subject of the Ph.D. thesis of Polya Marinovska. Her studies focus on creating a model for higher eukaryotes toxicological analysis using quiescent Saccharomyces cerevisiae yeast cells (G_0). This model is two-dimensional: On the one hand, it guides the use of G_0 yeast cells as a system for assessing a toxicological response (screening and forecast) to higher eukaryotes, and on the other - it introduces additional valuable information on the molecular mechanisms of the cell cycle, and in particular, the G_0 state, which is essential for various basic processes, such as tissue homeostasis, development and aging, but is still incompletely understood. In light of these facts, the research activity of Ms. Marinovska reflects the current trends in the G_0 state investigation and demonstrates the scientific potential of the Ph.D. thesis subjected to evaluation. The general objective and the eight specific tasks of the work fully support this activity.

The Ph.D. thesis is written in a precise scientific style; the experiments' description and the presentation of the results are logical, clear, and consistent, and the experimental data discussion - comprehensive, coherent, and creative in the context of known scientific information.

The references review is structured to fully reflect the Ph.D. thesis topic with an internal balance between its eight parts. It is in-depth and, at the same time – focused on defined problems, which shows the skills acquired by the Ph.D. student to handle analytically scientific information, make scientific conclusions, and formulate work hypotheses. It is commendable that the references review ends with a final part that focuses on the unresolved problems and smoothly but accurately directs the reader to the use of *S. cerevisiae* G_0 cells as a model for studying the toxicological response of the eukaryotic cell and its resistance mechanisms.

As a Ph.D. student, Ms. Marinovska conducted an impressive experimental work logically related to the continuum: cultivation of the model yeast strain and isolation of various cellular populations (proliferative, quiescent, and non-quiescent cells), study of the various toxic agents' impact on the individual populations' viability, revealing the influence of eight different physical factors on these populations survival; assessment of the damaging effect of four chemical agents at the biochemical and genetic level, bioinformatics analysis.

As a result of this combinatory approach, valuable quantitative and qualitative characteristics were accumulated of the toxicological effects (cytotoxicity and genotoxicity) of various chemical agents. Different physical factors that impact the model strain growth were evaluated. *In silico* analysis of the G_0 phenomenon at the genomic level was performed. The G_0 cells' response was studied to different abiotic stressors. These multidirectional studies, the scientific data they generate, and their analysis and interpretation allow Ph.D. student Marinovska to propose a model of physiological profiling of *S. cerevisiae* populations from different cell cycle stages and subjections to various abiotic stressors. The creation of such a model is justifiable since its principles are set based on a representative number of experimental data, logically arranged and interpreted in a multi-factor context, not only in terms of the stressors used but also in terms of the biomarkers for evaluating the effect of these stressors and its consequences at the morphological, metabolic and genomic level.

The results are presented in correctly designed and well-crafted figures (60 pcs.) and tables (5 pcs.). They illustrate the results convincingly. Generally, the Ph.D. thesis is written skillfully, with precision and professionalism.

The formulated conclusions follow the logical sequence of the major topics in the thesis. They correspond to the scope of the studies and their importance in a fundamental and applied aspect. A total of 15 conclusions actually reflects the work achievements. For a better presentation of the most significant achievements, probably some of them can be unified (e.g., No. 4 and 5, 8 and 9, 10 and 12).

The presented for review Ph.D. thesis has convincing contributions. Those possessing defined originality can be listed as follows:

1. The principal significance of the Ph.D. thesis is the complex approach used to establish a system for screening and forecasting toxicological responses in higher eukaryotes - a problem with fundamental and applied importance.

2. The created model for generating physiological profiles, whose characteristics I commented above, is also original.

3. The proven resistance of the G_0 population to zeocin is also an original contribution, especially to the clinical practice, since the metabolically inactive eukaryotic cells are less susceptible to the antitumor antibiotics action.

4. From a methodological point of view, the created test system for examining the mechanisms of toxic action at the molecular level and the functional profiling of the genetic requirements for chemical tolerance are also original.

The Ph.D. thesis abstract fully corresponds to the thesis content and fits the official state requirements for this type of document.

These facts allow me to evaluate the Ph.D. thesis of Ms. Poly Marinovska as an up-to-date achievement dedicated to an essential fundamental problem and supported by results and contributions that can trigger new research ideas and attainments.

The Ph.D. thesis meets the criteria for obtaining an educational and scientific degree Doctor. The Ph.D. student has acquired knowledge and skills in a specialized scientific field: creating a model for toxicological analysis in higher eukaryotes using *S. cerevisiae* G_0 cells. She has mastered combined scientific approach application to study these problems at a population, physiological, biochemical, and molecular level. By applying specialized techniques for the research results analysis and interpretation, Polya Marinovska revealed new scientific data and confirmed established tendencies while creating a system of physiological profiles for S. cerevisiae response to physiological stress evaluation and forecasting. Concerning the qualification descriptor autonomy and responsibility, Ms. Marinovska shows a defined interest in modern scientific ideas, independence and creativity in their development, good theoretical training and methodical skills, and high potential to fulfil various obligations in high academic quality.

Based on the above arguments, I appreciate positively the Ph.D. thesis, which fully meets the requirements of the Academic Staff Development Act in the Republic of Bulgaria, the Rules for its application, and the rules of Sofia University. Hence, I propose the respected members of the scientific jury, assigned by the Order of the Rector of the Sofia University No. RD 38-354 / 10.07.2023 to award POLYA GALINOVA MARINOVSKA the educational and scientific degree Doctor.

Sofia Assoc. Prof. Dr. Trayana Nedeva:

08.09.2023