REVIEW

by Prof. Dr. Petya Koycheva Hristova
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PhD Thesis title: "Quiescent state in yeast *Saccharomyces cerevisiae* - a model for studing toxicological and stress response" for awarding the educational and scientific degree "Doctor" in Professional field 4.3. Biological Sciences (Microbiology)
PhD Thesis author: Polya Galinova Marinovska

Scientific supervisor: Assoc. Prof. Vencislava Yankova Petrova, PhD
By order of the Rector of SU No. RD 38-354/10.07.2023, I have been designated as a Member of the Scientific Jury for conducting a procedure for the defense of the dissertation of Polya Galinova Marinovska. The submitted documents and materials comply with the minimum requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for its implementation.

1. Brief data on the doctoral student

Doctoral candidate Polya Marinovska has a master's degree in Microbiology and Microbiological Control at the Faculty of Biology, at the University of St. Kliment Ohridski". From 2020, she is a full-time doctoral student in the Department of General and Industrial Microbiology, and in 2021 she will start working as a microbiologist on a permanent employment contract.

2. Relevance and significance of the dissertation topic

The dissertation proposed for review focuses on the yeast species *Saccharomyces cerevisiae* as one of the most used model organisms in molecular biology, biotechnology for the study of a number of processes related to human health and disease.

With their unique genetic characteristics and high degree of conservatism with higher eukaryotic organisms, these microorganisms represent a suitable model system for studying the mechanisms of chemical toxicity. Moreover, their quiescent G_0 state, where metabolic fluctuations are greatly reduced and resembles mammalian cells, is an advantage for studying the toxic action of various chemicals, for characterizing the main elements of the toxicological response, developing strategies for their neutralization, and for evaluating of environmental risk. Knowledge of the yeast genome also provides a valuable basis for modeling the gene expression of metabolites that play a key role in the toxicological response.

In this context, the dissertation is aimed at developing an alternative model based on quiescent *S. cerevisiae* cells to serve for more accurate and sensitive toxicological analysis in higher eukaryotes.

The presented facts clearly motivate the choice of the topic, its relevance and practical importance. The content of the dissertation fully covers the announced nomenclature specialty in professional field 4.3 Biological sciences, scientific specialty "Microbiology" for the award of the ONS "doctor".

3. Evaluation of the structure of the dissertation work

The dissertation is written on 230 standard pages - A4 format, the results are summarized and presented in 5 tables and 60 figures. It is structured according to the classical scheme and is balanced in terms of the volume of each section: Introduction (1 p.), Literature review (45 p.), Aim and tasks (2 p.), Materials and methods (15 p.), Results and discussion (91 pages), Conclusions (2 pages), Contributions (1 page) and References (54 pages). A very good impression is made by the attached lists of figures, tables and abbreviations used.

The dissertation is written in a good scientific style, with accurate use of terminology, which shows that the doctoral student has a thorough knowledge of the subject. It should be noted that the presented literature reference (574 publications on the subject) offers a serious analysis of the state of the problem. The dissertation student has become very familiar with the literature data and skilfully uses it in interpreting the results.

3.1. Literature review

The literature review is structured correctly with direct reference to the purpose of the dissertation and the tasks set. The thesis overview covers the main aspects of yeast as a model organism. Conditionally, several main topics are distinguished, which, however, are closely related to each other. In the first part, the doctoral student presents the yeast cell cycle and pays particular

attention to the characteristics of the G_0 state. The second part is aimed at studying the mechanisms of toxic action of various medicinal preparations. The third topic reveals the influence of physical parameters on the survival of yeast cells - temperature, pH, osmotic pressure, ultraviolet rays, ultrasound, gravitational force and mechanical force. In the fourth topic, the doctoral student presents the stress response in yeast. In the fifth topic, bioinformatic approaches to studying the resting state are discussed in detail. The conclusion of the literature review clearly summarizes the knowledge achieved to date, outlines the need for a new integrative approach to study how cells interact with their environment.

3.2. Purpose and tasks

The aim of the dissertation is clearly formulated and includes studying the applicability of *Saccharomyces cerevisiae* cells in different phases of their life cycle as a eukaryotic model for cellular response to various toxic and stress agents.

In order to achieve the formulated goal, the doctoral student sets herself eight main tasks - developing a model scheme for obtaining and isolating yeast cells from different phases of the cell cycle - logarithmic (Log), quiescent cells (Q) and stationary non-proliferative cells (NQ), research of the oxidation-reduction status of the isolated yeast populations, study of the effect of various medicinal (zeocin, ibuprofen) and toxic (H_2O_2, menadione) preparations on the survival of the studied yeast populations, evaluation of the cytotoxic effect of the tested chemical agents on Log, Q and NQ yeast cells, analysis of the genotoxic effect of the selected chemicals zeocin and menadione on the three yeast cell populations, study of the role of different physical stress factors for the survival of yeast cells isolated at different stages of the cell cycle, application of in silico analysis of evolutionary strategies to enter quiescent yeast cells and create a stress response model "Functional Profile" of S. cerevisiae cells in different phases of the cell cycle.

3.3. Materials and methods

This is an important part of the dissertation work, which shows the level of scientific research carried out. Modern microbiological, biochemical, molecular, physical and microscopic methods are presented, so their development and successful application is already an indisputable success and shows the serious methodical preparation of the doctoral student. The multidisciplinary nature of the research allowed the doctoral student to acquire new methodological skills.

3.4. Results, discussion and conclusions

The main results are presented according to the set tasks and the selected methodical approaches. The experimental part is made very precisely and presented reliably. All results are presented in a comparative plan, well analyzed and compared with the literature data.

The research quite logically begins with studying the dynamics of growth and cell differentiation of the S. cerevisiae strain selected for the study. After that, the toxicological and genotoxic effects of selected chemical agents such as hydrogen peroxide, ibuprofen and zeocin were evaluated in a sequential plan. The impact of various physical factors and an In silico analysis of genes from signaling pathways regulating the entry into the resting state and the stress response were evaluated.

As a result of the conducted analyses, it was established that the entry of the cell into the G_0 cell cycle is provoked under conditions of limitation of one or several of the main biogenic elements in the medium and a high percentage of resting cells is reached when cultured for 168 hours on YPD medium. Also, the non-latent NQ yeast population appears to be heterogeneous and consists of viable and reproductively competent cells and cells that show characteristic features of apoptotic and/or necrotic states. The experimentally toxic doses (LD_{50}) for the chemical agents used were determined, and the NQ yeast cell population was found to be the most sensitive. Of the four chemical agents tested, zeocin had the strongest toxic effect on the yeast populations tested, inhibiting 100% growth of NQ and Log cells and 95% - of yeast in the G_0 state. Ibuprofen had little effect on the cell viability of the *S. cerevisiae* BY4741 yeast populations tested. It was observed that the effect of some of the applied toxic agents depends on the specific phase of the life cycle – H_2O_2 more strongly affects cells in logarithmic phase, and menadione cells in rest (G_0).

Of particular interest are the studies showing that the stress response in G_0 cells of *Saccharomyces cerevisiae* BY4741 to various toxic agents is significantly different from that of actively proliferating and non-latent stationary cells. It depends not only on the cell cycle phase (logarithmic, G_0 and/or stationary) but also on the specific mechanism of cytotoxic action of the compound. The highest percentage of intracellular damage upon exposure to various medicinal (zeocin, ibuprofen) and toxic (H_2O_2, menadione) preparations is observed in actively dividing cells. Quiescent G_0 cells are the most resistant to chemical insults and show the lowest levels of intracellular damage (< 25 %). A lower DNA sensitivity of G_0 cells was found compared to that of cells in the logarithmic growth phase, but the highest repair capacity was found in the logarithmically growing cells. Cells in the resting phase are significantly more resistant to physical effects and show many times higher survival in conditions of low and high temperatures, UV radiation and hyperosmotic stress. Bioinformatic approaches have shown that genes and proteins that play key roles in the entry and exit of the G_0 cell cycle in yeast have corresponding homologues in humans. And the metabolic plasticity of yeast is due to the targeting of the core regulatory proteins TORp, PKAp, PKCp and Snf1p to different intracellular compartments.

4. Scientific and applied contributions

Both theoretical and original applied contributions can be outlined in the research carried out, which prove the importance of scientific development.

I accept the doctoral student's contributions to the dissertation. It is essential to establish a model to predict the toxicological response in eukaryotes and to generate physiological profiles as a test system to study the toxic action at the molecular level.

5. Participation of the doctoral student in the development of the dissertation

The contacts made with the doctoral student when discussing issues from the dissertation work give me reason to consider that the implementation of the dissertation work is entirely her work.

6. Publications related to the dissertation

The PhD student submitted a list of two scientific publications (Q2, total IF 2022 = 2.334) related to the dissertation. The results of the dissertation were reported with poster participation in 5 international and national scientific forums and 1 sectional report. Participation in one research project related to the topic of the dissertation and in three more projects with other topics is presented.

7. Abstract

The abstract is prepared in accordance with the requirements and faithfully reflects the results of the dissertation, as the most important elements from all sections are presented in abbreviated form (without the literature review).

8. Recommendations, comments and questions

There are no significant errors in the dissertation regarding the approaches used and the presentation of the results, so I have no recommendations or remarks.

I have the following question for the doctoral student:

a) How would you explain the difference in the survival of cells from different life cycle phases when treated with the different toxic compounds?

9. Acquired competence and compliance with the requirements of the educational and scientific degree "doctor"

In the course of the implementation of the experimental work and the shaping of the dissertation work, the doctoral student Polya Galinova Marinovska has acquired competencies and skills that can be grouped as follows:

- she has expanded her theoretical competence in a specific area of microbiology,

- she acquired skills for working with scientific literature, analyzing and summarizing scientific information.

- she has enriched her methodical skills, especially in the part of applying modern methods

- acquired skills to independently shape and discuss the results obtained during the development of the dissertation, as well as draw conclusions based on them.

10. Conclusion

The presented dissertation work is a completed study that provides valuable scientific information and reveals opportunities for practical application. The very good theoretical training has given the doctoral student the opportunity to select and combine a set of methods that ensure the fulfillment of the set tasks and the achievement of the goal.

On the basis of the stated arguments, I positively evaluate the thesis proposed for review, which meets the requirements of the Law for the development of the academic staff in the Republic of Bulgaria, the Regulations for its application and the Regulations of the SU, and I propose to the members of the Scientific Jury to award Polya Galinova Marinovska the educational degree "Doctor".

18.09.2023 Reviewer:

prof. Petya Hristova