PhD Thesis Acceptance Report
Research Discipline Council of Biological Sciences
Jagiellonian University in Kraków

Candidate's name and surname: Monika Opałek

PhD Thesis Title: Environmental and Genetic Aspects of *Saccharomyces cerevisiae* Yeast Cells' differentiation

Thesis Supervisor: dr hab. Dominika Włoch-Salamon

Assistant Supervisor / Second Supervisor/ Co-supervisor (if applicable): dr Bogna Smug

Reviewer: dr hab. Renata Zadrąg-Tęcza, prof. UR

THESIS EVALUATION

1. **Scientific merit of the thesis**
   a. Originality of the research *(25-200 words):*

   The subject of the PhD thesis concerns the environmental and genetic aspects of Saccharomyces cerevisiae yeast cells' differentiation, with a particular emphasis on the issue of cell quiescence. Quiescence is a state of reversible proliferative arrest when cells do not divide but retain the ability to re-enter the cell cycle upon receiving an appropriate stimulus. This state is quite common and affects the cells of both unicellular and multicellular organisms. Understanding the mechanism that controls the transition between proliferative and quiescent states as well as the consequences of cellular quiescence is critical to understanding many biological processes. Knowledge about cellular quiescence, although constantly developed, is still insufficient to fully understand the biological processes and molecular mechanisms underlying this phenomenon. Cellular quiescence is studied experimentally in multiple model systems including the yeast *Saccharomyces cerevisiae*. This is a widely used model for studying quiescence due to its wide range of experimental tools but also the evolutionary conservation of the pathways and processes that control this process in different organisms.

   The research subject and the experimental approach used in this thesis are original and provide not only valuable knowledge but also tools helpful for researchers using yeast as a model of studying cell quiescence.

   b. Scientific merit of the chapters/articles *(25-200 words):*

   The dissertation has unquestionable scientific value and consists of a thematically coherent series of articles providing valuable scientific data. Chapter 1 provides a comprehensive literature overview in terms of terminology and methodological aspects of the study of yeast cell quiescence. The main value of this chapter is the development of a set of guidelines, that can be very valuable in planning experiments. It is also an important step toward the standardization of the definitions, cell characteristics, and analytical methods used in studies of yeast quiescence.

   Chapter 2 addresses the issue of phenotypic heterogeneity manifested by the presence of quiescent (Q) and non-quiescent (NQ) cells in starved clonal populations, analyzed both in the context of genetic sources and environmental factors. The construction of appropriate monocultures of Q and NQ cells, and Q/NQ cells showed that monocultures of Q cells had a higher survival rate during long periods of starvation and a shorter lag phase when re-supplied with a rich medium. Moreover, NQ cells are not a byproduct of an imperfect quiescent state, as their presence may be beneficial. These results

suggest the adaptive nature of phenotypic heterogeneity in starving populations, especially when environmental determinants (e.g. length of the starvation period) change over time.

The research presented in Chapter 3 showed that the *SSY1* gene, which is the main component of the receptor of the SPS amino acid sensing pathway, may play an important role in the transition of yeast cells to a quiescent state. Therefore, hypersensitivity to the presence of amino acids may be one of the regulatory mechanisms of the transition to the quiescent state.

Chapter 4 highlights the importance of the lag phase duration, which can be used as an indicator of adaptation to the new environment. Based on the analysis performed on both empirical and simulated data, a decision tree was developed to facilitate the selection of the Lag phase duration estimation method best suited to the data set and experimental conditions. In addition, an online tool was developed - Microbial Lag Phase Calculator - enabling comparison of the lag phase duration estimated by different algorithms.

2. **Substantial merit of the thesis**
   (ability to introduce the research topic and clarity of research hypotheses, the choice of research methods and statistical tools for data analysis, presentation and critical analysis of the research data, the ability to discuss research data and the theoretical background, clarity and quality of the conclusions) (25-200 words):

The research topic of the PhD thesis is important, and the substantial merit of the dissertation is definitely satisfactory. The thematically coherent series of articles, which is the basis of the dissertation, was planned with great care and knowledge of the methodological techniques. A well-written introduction presents all the main issues discussed in the series of articles. My critical remark concerns the aim of the thesis, which was presented in the form of a very detailed description of the research tasks carried out. The aim of the research was formulated in individual articles, but it would be appropriate to formulate the aim that would integrate the research presented in individual chapters (articles). The methodology used in individual articles does not raise any doubts, it is very precisely described and precisely adapted to the research tasks carried out. The statistical tools used allowed for a precise analysis of the results. The research was conducted based on various research methods and methodological approaches. The results were presented in a transparent form using a series of diagrams, and charts as well as a rich database of supplementary material. The obtained results constitute an important contribution to the development of knowledge about cellular quiescence and a better understanding of the mechanisms regulating this phenomenon. Conclusions are formulated very carefully, without over-interpretation and too broad interpolation to other groups of organisms, which proves the scientific maturity and ability to critically approach research results.

3. **Layout and register**
   (layout, register and the clarity of the language, the quality of the visual material etc.)
   (25-200 words):

The dissertation was prepared as a thematically coherent series of four scientific articles, which imposes a slightly different layout of individual chapters. In this form, the dissertation includes a summary (in English and Polish), a general introduction, and the aims and content of the dissertation followed by four articles with supplementary data identified as chapters I to IV. The further part of the dissertation is a general discussion, which includes subsections such as key conclusions relating to individual parts of the work (scientific articles), the impact on the discipline development, and future directions of research. I would like to emphasize that this format of discussion is very transparent and constitutes a specific framework for the presented research and the conclusions drawn on its basis. Citations are consistently provided and are accurate. The last part is a collection of the co-author's statements, specifying their participation in each of the articles. The dissertation is well-written in terms of language. The individual chapters have been prepared very carefully and with great attention

to editorial details. The layout has a logical, easily understandable sequence. Additionally, I would like to emphasize the very well-thought-out concept of the order of the articles constituting the basis of the dissertation, which proves very good knowledge of the subject. Illustrations, diagrams, and figures in individual articles have been carefully prepared and clearly described. Overall the dissertation is well edited with adequate attention to editorial details.

4.   **Critical notes**

The individual scientific articles constituting the basis of the PhD dissertation have undergone a rigorous review process before publication. Therefore, my comments are in the form of supplementary questions or issues that, in my opinion, may be related to further research.

(1) Chapter 1:

Figure 3 shows the number of publications concerning cellular quiescence for the years 2000-2022. Data from years 2021 and 2022 indicate that basically half of the publications are those where cells from the stationary phase are treated as a homogeneous population of quiescent cells. Within that context, I would like to ask what may be the explanation of such proportions. How do the population heterogeneity and the presence of NQ cells affect the interpretation of the results? Which type of analysis and its results can be particularly "sensitive" if, upon the interpretation of the results, the stationary phase will be treated as a homogeneous population of quiescent cells or as a heterogeneous population?

(2) Chapter 2:

– One of the methods of obtaining Q and NQ cells is density gradient fractionation. Does the procedure include checking the homogeneity of the fractions obtained?

– The monocultures of fractionated Q and NQ cells were stored at -70°C and after thawing, the survival rate was measured using a FUN LIVE/DEAD yeast viability kit. Did the results show differences between Q and NQ cells, and did this have an impact on preparing the cell population for further analysis?

– Why for the "long-starvation experiment" the starting point was OD=0.8, in turn for the "short-starvation experiment" the starting point was OD=0.4

(3) Chapter 3:

Quiescence can be induced by nutrient starvation including carbon, nitrogen, phosphorus, and sulfur. Does population heterogeneity and the proportion of Q and NQ cells depend on the type of nutrient starvation?

(4) Quiescent cells have many distinct features compared to actively proliferating cells. However, these features do not give the opportunity to distinguish in a heterogeneous stationary phase culture, a fraction of quiescent and non-quiescent cells using e.g. flow cytometry or microscopy techniques. Are there any suggestions for molecular markers that might be helpful in solving this problem?

(5) Whether the time that a cell spends in a quiescent state depends on the overall level of protection against long-term cellular stress? Is there any evidence that the mechanism that controls the maintenance and depth of quiescence is evolutionarily conserved? How helpful would be the analysis of the SBF (the yeast homolog of E2F in mammalian cells) transcription factor that activates gene expression during the G1/S transition of the cell cycle?

5. **Final grade** (justification *25-200 words):*

This PhD dissertation provides new and valuable insight into the understanding of the quiescent state and its importance, as well as the genetic and environmental aspects of the yeast *Saccharomyces cerevisiae* phenotypic heterogeneity. The PhD student showed great knowledge of the research topic as well as the ability to plan, conduct experiments, and formulate conclusions, which is the essence of solving scientific problems. Moreover, the obtained results also became an inspiration to development an online tool (Microbial Lag Phase Calculator) enabling comparison of the lag phase duration estimated by different algorithms.

The research presents a high substantive level, and therefore the thesis meets the criteria for a PhD thesis. For that reason, the PhD student may be admitted to further stages of the doctoral proceedings.

I, hereby, declare that the reviewed PhD thesis by **Moniki Opałek** meets the criteria pursuant to art. 187 of Act of 20 July 2018 The Law on Higher Education and Science (Journal of Laws of 2018, item 1668, as amended) and request that the Research Discipline Council of Biological Sciences of the Jagiellonian University in Kraków accepts **Moniki Opałek** for further stages of doctoral proceedings in the field of exact and biological sciences, in the discipline of biological sciences

YES/~~NO~~

Niniejsza rozprawa doktorska stanowi istotny wkład w zrozumienie czym jest i jakie jest znaczenie stanu spoczynku komórek oraz dostarcza nowych informacji odnośnie genetycznych i środowiskowych aspektów heterogeniczności fenotypowej drożdży *Saccharomyces cerevisiae*. Doktorantka wykazała się bardzo dobrą znajomością tematu badań oraz umiejętnością planowania, przeprowadzania eksperymentów i formułowania wniosków, co jest istotą rozwiązywania problemów naukowych. Ponadto uzyskane wyniki stały się także inspiracją do opracowania narzędzia internetowego (Microbial Lag Phase Calculator) umożliwiającego porównywanie czasu trwania fazy opóźnienia, szacowanego za pomocą różnych algorytmów.

Badania prezentują wysoki poziom merytoryczny, dzięki czemu praca spełnia kryteria rozprawy doktorskiej. Z tego powodu doktorant może być dopuszczony do dalszych etapów przewodu doktorskiego.

Ja, niżej podpisany stwierdzam, że recenzowana rozprawa doktorska **Moniki Opałek** spełnia warunki określone w art. 187 Ustawy z dnia 20 lipca 2018 r. Prawo o szkolnictwie wyższym i nauce (Dz. U. z 2018 r. poz. 1668 z późn. zm.) i wnioskuję do Rady Dyscypliny Nauki biologiczne Uniwersytetu Jagiellońskiego w Krakowie o dopuszczenie **Moniki Opałek** do dalszych etapów postępowania ws. nadania stopnia doktora w dziedzinie nauk ścisłych i przyrodniczych w dyscyplinie nauki biologiczne

TAK/~~NIE~~

I, hereby, request that the thesis is accepted with distinctions. Justification *(25-200 words)*

YES/~~NO~~

In my opinion, the presented doctoral dissertation deserves a distinction. First of all, due to the comprehensiveness of the conducted analyses and their very wide scope. It is also worth emphasizing that the conducted research allowed not only to enrich this thematic area with new and

original data but also to influence the scientific community in a different way by proposing specific tools that may be helpful to researchers dealing with similar topics. Despite the fact that the articles constituting the basis of the dissertation were written in co-authorship, the presented statements show that the participation of the doctoral student was significant both in terms of the concept of the research as well as the methodology, analysis, and preparation of the manuscript.

..11.09.2023............
date

Renata Zaolrng-Tęcza
Reviewer's signature