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 factors of cell differentiation in colonies of the yeast *Saccharomyces cerevisiae*

Thesis Supervisor: Dr hab Dominika Wloch-Salamon

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

Reviewer: Dr Daniel Rozen (Institute of Biology, Leiden University, The Netherlands)

THESIS EVALUATION

1. **Scientific merit of the thesis**

a. Originality of the research *(25-200 words):*

Microbial populations are increasingly recognized as highly phenotypically heterogenous. This can be due to a variety of factors, including age, transcriptional and translational noise, among others, and have diverse consequences for population growth and viability. The work presented in this thesis focuses on population heterogeneity in the transition between growth and non-growth in yeast, focusing specifically on two co-existing sub-populations, Q (Quiescent) and NQ (non-Quiescent). Quiescence is important for population fitness through its effects on cellular survival and the time required to initiate growth under different resource conditions. This thesis sheds light on conceptual issues concerning how quiescence is defined and studied, the role of Q and NQ cells in population fitness, the molecular mechanisms underlying the ratio of Q:NQ cells, and technical aspects of the lag phase. The thesis is very well-written and the results are novel and interesting, particularly with respect to the role of heterogenous populations in microbes and the evidence that the Q:NQ ratio is a form of bet-hedging. Both the technical and conceptual advances are highly original and will pave the way to more detailed follow-up studies.

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

The thesis is presented as four research chapters (three of which are published) together with a General Introduction and Conclusion. The General Introduction provides a clear overview of the yeast model system. Quiescence is defined and its importance placed within a broader context of similar phenomena in other eukaryotic systems. It also presents quiescence as a form of heterogeneity by introducing Q and NQ populations. Chapter 1, a published systematic review, elaborates on these themes and identifies technical and conceptual issues that bear on how quiescence is studied in yeast. After reviewing the history of quiescence and stationary phase research in yeast, the chapter identifies several factors, including age, induction conditions, and proto/auxotrophy that influence how Q:NQ is understood and measured. This follows with specific recommendations for work in the field. Chapter 2, also published, combines experiments and theory to understand the evolutionary role of Q and NQ cells and the ratio of Q:NQ cells. Using a novel method to separate and study Q and NQ cells, the chapter concludes that while Q cells survive better during long starvation, NQ cells facilitate more rapid regrowth after brief starvation which is facilitated by the ability to scavenge nutrients in complex media. The balance of Q:NQ is described as a type of bet-hedging that may offer evolutionary benefits in fluctuating environments. Chapter 3, also published, focuses on the mechanisms of Q and NQ cells

(primarily NQ) by exploiting strains from an earlier experimental evolution study. The chapter examines the role of specific mutations on the Q:NQ ratio and identifies a key role of SSY1. The study shows that mutations in this gene (and others in the same pathway) lead to amino acid hypersensitivity, thereby resulting in a higher fraction of NQ cells as well as increased accumulation of intracellular amino acids. Finally, chapter 4, provides an overview and guidelines to measuring the microbial lag phase, together with a web-tool to facilitate lag measurements. The guidelines and web-tool will be a significant value to the field given the importance of lag estimates in several aspects of microbiology.

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 thesis is very well-written and addresses a topic of general importance. The transition between growth and non-growth is central to all cells and the thesis does a nice job of placing the work within this broader context. Similarly, I was impressed at the depth of the discussion on the cause and consequences of population heterogeneity during starvation. The Q:NQ ratio is dynamic through time and can be understood as an unavoidable by-product of the ways in which cells sense starvation. Instead, the thesis convincingly argues that the Q:NQ ratio can be moulded by natural selection in response to changing environmental conditions as a form of bet-hedging. In addition, the thesis identifies an intuitive mechanism that underlies the fraction of NQ cells that is associated with cellular sensitivity of amino acids. This result has fundamental merit, but also opens avenues to manipulate cellular protein content in yeast used as feed supplements. The systematic review and lag chapters have merit in providing clear definitions and guidelines/tools for future work in yeast and other microbial systems.

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

The presentation, in terms of writing and figures, is very good throughout. Three of four research chapters are already published, while the final chapter is submission ready. Figures are clear and well presented, in terms of aesthetics and content. The General Introduction and Conclusion do a good job of outlining the objectives of the thesis while placing the study in a broader context and also offering suggestions for further study. There are some small grammatical errors in the General Introduction, but these do not at all detract from and otherwise strongly written thesis.

4. **Critical notes**

Given the fact that three of four chapters are already published, there is little room for further comment. These notes therefore only reflect my interest in the subject, and perhaps could be interesting for further discussion during the thesis defence.

Although quiescence in yeast is considered generally, I was curious about mechanistic and evolutionary parallels between yeast and bacteria. GASP mutants are mentioned but could be further elaborated upon, especially the potentially convergent role (and mechanisms) of nutrient scavenging during long-term starvation.

I was curious about methods to study the Q:NQ ratio, and factors that drive this, in natural yeast populations. Are there recognizable factors in nature that would push the Q:NQ ratio in different directions and could these factors be explicitly tested in the lab?

Separation of Q:NQ is very original and neat. I am curious if these studies, based in part on independent assays of Q and NQ, could be supplemented with direct competition assays between differentially labelled Q and NQ cells? This may help to establish conditions of co-existence and be used to test model predictions across fluctuating environments.

Results on amino acid hypersensitivity are particularly neat and provide an intuitive mechanism driving NQ cells. Is it possible to examine heterogeneity in this trait in otherwise homogenous populations? Are there mechanisms, e.g. transcriptional noise, cell age/cycle, that could underlie the Q:NQ ratio in clonal populations?

For the discussion of lag in Ch4, it might be useful to include a brief note on other methods (flow cytometry and plating) in the Discussion since these are also frequently used to examine lag.

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

Overall, this thesis represents an important and novel contribution to the understanding of the evolution and mechanisms of microbial heterogeneity, specifically focusing on yeast quiescence. The merit of the work is reflected in three published papers together with a manuscript that is ready for submission. There is little question that the thesis fully satisfies the criteria for a PhD and demonstrates that the candidate, Monika Opałek, has gained sufficient scientific quality and skill during her studies.

I, hereby, declare that the reviewed PhD thesis by Monika 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 Monika Opałek for further stages of doctoral proceedings in the field of exact and biological sciences, in the discipline of biological sciences

YES

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

I am impressed with the quality and scope of this PhD thesis. It uses a range of approaches to address a fundamental question with potential applications. The merit of the work is reflected in three published papers together with a manuscript that is ready for submission.

YES

26 August 2023

Daniel Rozen

INFORMATION FOR THE REVIEWER:

A digital copy should be sent to:
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A duly signed original should be sent to:

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