SUMMARY

Clonal microbial populations influenced by environmental and genetic factors can naturally diversify into distinct phenotypes. Consequently, individual cells within these populations can exhibit variations in gene expression patterns, physiological states, and metabolic activities. This phenotypic heterogeneity can provide significant evolutionary advantages since subpopulations can respond to selection pressures in diverse ways. Therefore, heterogeneous populations are generally better adapted to unpredictable environmental changes.

In response to starvation signals, a cell can enter a reversible growth-arrested state known as quiescence. In fact, most microorganisms spend the majority of their life in the non-proliferating, quiescent state, which is crucial for surviving long periods of starvation and restarting cell division once conditions improve. For example, *Saccharomyces cerevisiae* cells grown in a batch culture are known to enter quiescence when they sense first signals of glucose exhaustion. However, cells do not respond uniformly to the same starvation signal, in particular, starvation signals typically induce a transition to a quiescent state in a fraction of cells (Q) while others remain non-quiescent (NQ). These phenotypically distinct subpopulations can be isolated by density-based centrifugation: Q cells are gathered in the lower fraction and NQ cells are in the upper fraction. This fractionation procedure is one of the most frequently used method to study phenotypic heterogeneity in the context of quiescence on the population level.

CHAPTER 1 of this dissertation employs systematic review methodology to comprehensively analyse the approaches used to study quiescence in *S. cerevisiae*. We analyse whether all cells in the stationary phase population are treated as quiescent, or whether any phenotypic heterogeneity is considered (i.e. if the authors distinguish distinct subpopulations in the stationary phase). The other investigated traits include: chronological age of the populations, laboratory methods used to induce quiescence, and the metabolic profile of strains used in the research (prototrophic or auxotrophic). By combining all of these factors, we identify the most and the least frequently applied experimental setups for studying quiescence. On the one hand, our work highlights the lack of standardization in reporting the experimental details of quiescent cells and populations, which can be confusing and misleading when drawing general conclusions. On the other hand, the diversity of methodological approaches used in the published studies may be useful for higher-level comparisons that could be made using meta-analyses. Our catalogue of studies in each category could be very useful for this purpose. The publication concludes by proposing guidelines for including crucial information regarding experimental setup to study quiescent populations in research articles.

CHAPTER 2 focuses on the evolutionary significance of phenotypic heterogeneity observed in a population exposed to starvation. We analysed the regrowth of starved monocultures composed of only one cellular phenotype (i.e. quiescent (Q) or non-quiescent (NQ) cells), compared to that of mixed, heterogeneous cultures (Q and NQ cells) from simple (sterile water) and complex (spent YPD) starvation

environments. Through experiments and mathematical modelling, we confirm that Q monocultures exhibit better survival during long starvation periods and have shorter lag phases upon resupply of nutrients. However, when the starvation period is very short, NQ monocultures outperform Q and mixed cultures due to shorter lag phase. Additionally, the relative disadvantage of NQ monocultures is smaller in complex than in simple starvation environment. We argue this effect is due to nutrient recycling which is possible in complex but not in simple environment. These findings demonstrate that both Q and NQ cells can provide fitness advantage and that the Q:NQ cells ratio can be an important population feature. We suggest that phenotypic heterogeneity, *i.e.* the presence of Q and NQ cells in starved populations, may represent adaptive *bet-hedging* strategy, which is particularly important when environmental factors vary over time.

CHAPTER 3 focuses on the SPS amino acid sensing pathway and SIR complex and it explores the genetic aspect of the Q:NQ cells ratio in the context of these genes. We test several SNVs identified in genes which can play important role in the transition to the quiescent state. These genes belong mostly to the SPS amino acid sensing pathway and the SIR complex. We analyse Q:NQ cells ratio and amino acid content in strains holding different mutations in SPS amino acid sensing pathway and SIR complex. We demonstrate the significant role of *SSY1*, the primary receptor component of the SPS sensor, in the transition to the quiescent state. Analysed mutations in the *SSY1* gene increase yeast sensitivity to amino acid presence, resulting in a decreased quiescent cell fraction and an increase in total amino acid content in starved populations. External amino acid sensing via the SPS pathway is discussed as one of the mechanisms influencing the transition to quiescence.

CHAPTER 4 focuses on the methods used to assess quiescence exit, specifically the duration of the lag phase. The lag phase refers to the temporary non-replicative period when a microbial population is introduced to a new nutrient-rich environment. Its duration significantly affects population fitness and is often measured in laboratory conditions. However, estimating the length of the lag phase can be challenging and is dependent on selected method and parameters. This publication discusses various methods of calculating the lag phase duration commonly used in both experimental and theoretical studies, highlighting inconsistencies between the methods. Using experimental and simulated data we study the performance of the lag duration estimation methods depending on the frequency of population size measurements, and parameters determining the growth curve shape, such as growth rate. Based on our results, we propose a decision tree to choose a method most suited to one's data. Additionally, we have developed a web tool where the lag phase duration can be calculated with user-specified growth curve data, methods, parameters, and data pre-processing techniques.

In conclusion, this dissertation explores various aspects of yeast phenotypic heterogeneity with the focus on the quiescent state. Quiescence is a crucial cellular state with implications for fundamental biological research and clinical studies. I believe that the research presented in this dissertation provides valuable insights to the study of the quiescent state and its significance.