EFFECT OF PRE-FREEZING REGIMES AND REACTIVATION CONDITIONS ON THE VIABILITY OF LYOPHILIZED *SACCHAROMYCES CEREVISIAE* STRAINS

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Background: Long-term preservation of *Saccharomyces cerevisiae* by lyophilization is highly important for scientific research and for the biotechnological and food industries. Maintaining cell viability and genetic stability is essential for reproducible results and consistent product quality. Lyophilization includes rapid freezing of microbial cultures followed by water removal through sublimation under vacuum. Therefore, pre-freezing temperature is a key factor affecting post-lyophilization survival, as it influences ice crystal formation, membrane integrity, and intracellular stability. Higher pre-freezing temperatures cause slower freezing and larger ice crystals, leading to irreversible cell damage and reduced viability. Lower temperatures ensure faster freezing, smaller and more uniform ice crystals, less mechanical injury, and higher survival rates.
The aim of this study was to evaluate the influence of pre-freezing temperature and reactivation time on the survival capacity of *Saccharomyces cerevisiae* yeasts after 13 years of storage in a lyophilized state.
Materials and methods: The study included 11 strains of *Saccharomyces cerevisiae* from the National Collection of Nonpathogenic Microorganisms of the Institute of Microbiology and Biotechnology, Technical University of Moldova, lyophilized in 2013 using skimmed milk as a protective agent. Prior freezing was performed at two temperature regimes: −50°C and −80°C. Cultures were reactivated with 1 mL of distilled water for 4 and 16 hours, and viability was assessed by colony-forming unit (CFU) counts, followed by logarithmic transformation of the data.
Results: The results demonstrated that rapid freezing at −80°C led to higher cell viability, ranging from 38.2% to 67.8%, depending on the strain analyzed. The use of a higher pre-freezing temperature (−50°C) was associated with a slightly reduced survival rate, with values between 33.0% and 67.1%. Additionally, standard reactivation (4 hours) resulted in low titers of 2.1–3.7 $\log_{10}$ CFU·mL^{-1}. Under these conditions, optimization of reactivation parameters became necessary; extending the reactivation time to 16 hours led to a significant increase in yeast titers, reaching up to 6.8 $\log_{10}$ CFU·mL^{-1}.
Conclusions: This study demonstrates significant long-term survival of lyophilized yeast strains after 13 years of storage. Selecting an optimal pre-freezing temperature, such as −80°C, minimizes the negative effects associated with ice crystal formation, osmotic stress, and molecular instability, thereby enhancing the survival capacity of *Saccharomyces cerevisiae* strains. Furthermore, extended reactivation time significantly improves viability parameters, highlighting the importance of post-lyophilization recovery conditions.
Keywords: yeasts, viability, freezing

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