INFLUENCE OF MANNOPROTEIN-BASED REACTIVATION MEDIA ON THE POST-LYOPHILIZATION VIABILITY OF *RHODOTORULA MUCILAGINOSA* CNMN-YS-10

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ABSTRACT
Preserving the long-term viability of microorganisms is a fundamental objective of the National Collection of Nonpathogenic Microorganisms (NCNM). The aim of the study was to evaluate the influence of mannoprotein preparations from *Lager* beer yeast sediments (MPB) and *Merlot* red wine (MPV) at concentrations of 1%, 5% and 10% on the cell viability of the *Rhodotorula mucilaginosa* CNMN-YS-10 strain after long-term preservation by lyophilization. Reactivation with MPB at concentrations of 1% and 10% reduced cell viability to 78.14 ± 2.8%, whereas at the intermediate concentration, viability remained comparable to the control range. Reactivating the culture with MPV, even at the minimum concentration, reduced viability to 78.44%, whereas at higher concentrations, viability remained comparable to the control.
Keywords: *Rhodotorula mucilaginosa*, mannoproteins, viability, rehydratation

1. INTRODUCTION

The preservation of cell viability during long storage is essential to ensure the genetic and functional integrity of strains, as the decline in viability can irreversibly compromise biological resources of significant value. Preservation of viability, morpho-cultural and biochemical characteristics of microorganisms involves the use of effective methods and continuous monitoring of their effectiveness [1, 2].
Pigmented yeasts of the genus *Rhodotorula*, and especially *Rhodotorula mucilaginosa*, are of particular interest due to their ability to synthesize significant carotenoids and to adapt to diverse environmental conditions, which places them among the most valuable microbial sourse of natural pigments [9]. This species demonstrates a remarkable capacity to accumulate lipids, carotenoids and possesses diverse enzymatic activity, including lipase and protease activity, properties that recommend it for biotechnological applications on an industrial scale [12, 13].
The most effective long-term preservation method used within the CNMN is lyophilization, which involves dehydrating the culture by sublimation under vacuum conditions and low temperatures, significantly reducing metabolic activity and allowing for long-term preservation of the strains [1]. However, the lyophilization process exposes cells to intense thermal and osmotic stress, which can affect the integrity of cell membranes and reduce viability after reactivation. The quality of the reactivation medium is a determining factor for the recovery of viability and biosynthetic potential of strains [8, 11].
Mannoproteins are the major protein components of the external cell wall of yeasts, consisting of a central protein structure heavily glycosylated with mannose chains, the glycosidic fraction exceeding 90% of the total molecular mass [12]. They are released into the environment following cell autolysis and can be recovered from the sediments remaining after beer or wine fermentation [4]. Their unique structure endows them with excellent biological activities, such as: antioxidant activity, immunostimulatory properties, cell membrane

stabilization capacity and cryoprotective potential [11]. Research has demonstrated that mannoproteins isolated from conventional and unconventional yeasts exhibit antimicrobial activity and probiotic effects [6], and their inclusion in microbial inoculation systems contributes to improving microbiological stability [13].
Considering the importance of mannoproteins as biopreparations with antioxidant properties and cryoprotective potential, the aim of this study is to evaluate the bioprotective potential of mannoproteins from brewer's yeast (MPB) and wine yeast (MPV) at concentrations of 1%, 5% and 10% on the viability of the *R. mucilaginosa* strain CNMN-YS-10 after long-term preservation.

2. MATERIALS AND METHODS

The pigmented yeast strain *Rhodotorula mucilaginosa* CNMN-YS-10, preserved in the National Collection of Non-pathogenic Microorganisms at the Institute of Microbiology and Biotechnology of the Technical University of Moldova, was used as the study object.
Mannoprotein extracts were obtained in the Laboratory of Fungal Biotechnology from yeast sediments remaining after production of Lager beer (MPB) and fermentation of Merlot red wine (MPV) [10]. Both extracts were standardized to 25 mg/mL dry matter.
Culture reactivation after lyophilization was performed with 1 mL distilled water (control) or MPB and MPV extracts at 1%, 5%, and 10% (v/v) for 16 h at 28 ± 1°C.
Viability was determined by cultivating reactivated strains on YPD agar at 28 ± 1°C for 5 days, followed by counting colony-forming units (CFU mL^{-1}) according to Uzunova-Doneva and Donev. Viability was calculated as c% = (lg CFU mL^{-1}fin / lg CFU mL^{-1}in) × 100%, where lg CFU mL^{-1}in represents the control culture and lg CFU mL^{-1}fin the culture reactivated with MPB or MPV [15].
Statistical analysis was performed using Microsoft Excel and Statistica 9.0. Results are presented as mean ± standard deviation of three biological replicates. Differences were considered significant at $p \leq 0.05$.

3. RESULTS

The results obtained demonstrated that the *Rhodotorula mucilaginosa* CNMN-YS-10 strain remains viable after prolonged storage in a lyophilized state and can be recovered for further research, confirming the efficiency of the lyophilization as a long-term preservation method [12].
The effect of mannoprotein preparations in the reactivation medium revealed a concentration-dependent behavior, distinct depending on the source of the mannoproteins. The viability results are illustrated in Fig. 1.
The use of MPB extract at a concentration of 1% and 10% led to a reduction in cell viability compared to the control, recording a value of 78.14 ± 2.8% (Fig. 1). At a 5% concentration, MPB did not affect cell viability, with survival rates remaining comparable to the control group. This profile, with the maximum at 5%, suggests the existence of an optimal concentration for MPB extract at which the bioprotective effect of beer mannoproteins is maximized.
Mannoprotein extract from wine yeast (MPV) reduces the viability of the *Rhodotorula mucilaginosa* CNMN-YS-10 strain to 78.14 ± 2.8% even at a concentration of just 1%, while at the higher concentrations studied (5% and 10%), it maintains viability at the level of the control sample.

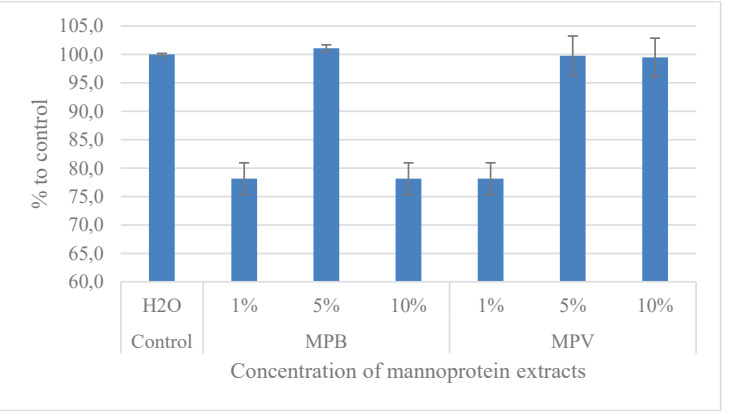

Fig. 1. Viability of *Rhodotorula mucilaginosa* CNMN-YS-10 strain after reactivation with mannoprotein extracts

4. DISCUSSION

The effects observed for the MPB extract may be explained by the antioxidant properties of mannoproteins, which at moderate concentrations protect cell membranes from oxidative stress accumulated during long-term storage [11]. At higher concentrations, the inhibitory effect may result from changes in the osmotic pressure of the reactivation medium or non-specific interactions with cellular components, as reported for yeast extracts used as bioprotective agents [4, 8].

The different behavior of MPV and MPB at higher concentrations is likely related to their distinct biochemical composition and structure. *Saccharomyces cerevisiae* strains are adapted to wine fermentation conditions through specific cell wall composition. Mannoproteins from different yeast sources vary in glycosylation patterns, affecting their biological properties and interactions with target cells [6, 13]. In addition, wine yeast sediments differ from beer sediments in protein and polysaccharide content [10]. A comparative analysis of the two preparations showed that MPB mannoproteins at 5% maintained cell viability at a level comparable to the control. Likewise, MPV at 5% and 10% effectively preserved viability without statistically significant changes. These findings are consistent with the functional properties of yeast mannoproteins reported in the literature [9, 14].

Previous studies on *Rhodotorula gracilis* CNMN-YS-03 and *Rhodotorula glutinis* CNMN-YS-08 showed that, although mannoproteins did not significantly affect cell viability, they markedly influenced biosynthetic activity, increasing the synthesis of protective compounds such as carotenoids and altering the accumulation of reserve carbohydrates [3].This metabolic reorientation can be explained by stress response mechanisms which involve the regulation of gene expression and enzymatic activity to enhance cellular resistance to external factors. Thus, even without directly affecting cell viability, mannoproteins can indirectly enhance cellular adaptability and metabolic performance, with significant implications for biotechnological processes and the optimization of cultivation conditions. A limitation of this study is that the exact molecular mechanism by which mannoproteins confers protection was not investigated, representing an important direction for future research.

The obtained data open important perspectives for further research on the effect of MPB and MPV preparations on the carotenoid synthesis potential of the *Rhodotorula mucilaginosa* CNMN-YS-10 strain in the regeneration process after long-term preservation.

5. CONCLUSIONS

The results obtained confirm that the *Rhodotorula mucilaginosa* strain CNMN-YS-10 retains a high level of viability after long-term preservation by lyophilization, confirming the effectiveness of this method for yeast preservation of biotechnological importance. At the same time, the study highlights the crucial role of the reactivation medium's composition in restoring cellular capacity after storage.

Among the tested concentrations of mannoprotein extract from beer (MPB), 5% demonstrated the highest efficacy, with a cell viability of 101.10 ± 0.6%, slightly above the control. At concentrations of 1% and 10%, viability decreased to 78.14 ± 2.8%, indicating the existence of an optimal range of action. Mannoprotein extract from wine (MPV) showed a more stable behavior, concentrations of 5% and 10% maintained viability at levels comparable to the control, 99.76 ± 3.5% and 99.48 ± 3.4%, respectively, without significant inhibitory effects.

The experimental data obtained support the inclusion of the mannoprotein preparations MPB and MPV in the composition of the reactivation media for yeasts of the genus *Rhodotorula*, the optimal concentrations - 5% for MPB and 5–10% for MPV were validated as essential parameters for the efficient recovery of cell viability after lyophilization.

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DECLARATIONS

Conflict of interest: The authors declare that they have no conflict of interest.

Author Contributions: B.L. designed the study; B.L. and B.G.N. performed experiments; B.L. analyzed data; T.E. and R.A. writing—original draft preparation; B.G.N. writing – review & editing; all authors reviewed and approved the final version.

*Data Availability Statement***:** The datasets generated during the current study are available from the corresponding author upon reasonable request.

Originality Statement: The authors confirm that this manuscript is original, has not been published previously, and is not under consideration elsewhere.

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