VIABILITY OF FUNGAL AND BACTERIAL STRAINS AFTER LYOPHILIZATION IN AN OPTIMAL PROTECTIVE MEDIUM

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https://doi.org/10.52757/bsd26.14

ABSTRACT:
In recent decades, microorganisms have increasingly influenced all human activity, which requires special attention to studies of estimating the biodiversity of microorganisms and their application in various biotechnological processes. Storage, maintaining the viability and long-term stability of microorganisms of scientific and practical interest are the main tasks of all collections in the world. Lyophilization is one of the main methods of preserving microorganisms and long-term storage. Maintaining the viability of lyophilized microorganisms depends on various factors: taxonomic peculiarities of the strains, the usedprotective medium, the revitalization medium, etc.
In this study, the viability of fungal and bacterial strains after lyophilization on optimized protective media containing skimmed milk supplemented with carbohydrates and ascorbic acid as an antioxidant was evaluated. The results obtained demonstrated a high viability of 92.7 - 98%, of fungal strains from the genus *Penicillium* and *Trichoderma* and of 75.5 - 91.2% of bacteria from the genera *Bacillus*, *Pseudomonas*, *Micrococcus*.

Keywords: fungi, bacteria, conservation, lyophilization, viability.

1. INTRODUCTION

For long-term preservation of most bacteria, yeasts and fungi, lyophilization is used as a safe method of preservation with conservation of the initial productive properties and a long storage period. Lyophilization is based on a biological principle – anabiosis and on two physical processes: freezing and sublimation. Freezing allows to keep intact the physiological state from the initial moment, as a result of stopping any enzymatic activity. The efficiency of the method of preserving microorganisms in a lyophilized state is determined by several factors, such as: the culture subjected to lyophilization, the phases of cell growth and concentration, the composition of the protective medium, the rehydration of lyophilized cells and the packaging and storage conditions. The use of inappropriate protective media in the lyophilization process leads to significant changes in microorganisms after lyophilization: of morpho-cultural and biosynthetic properties, in the cell membrane, the secondary protein structure, DNA damage and even their death (Sîrbu T., 2005; Грачева И.В., 2016; Guowei Shu 2017; Xiao-min Li, 2024).
Cryopreservation and lyophilization are currently considered the safest methods for storing active cells, which would allow for greater shelf-life stability at ambient temperatures and facilitate easier transportation and storage (Morgan, 2006).
During freeze-drying, drying (primary and secondary), also the revitalization step, the risk of potential cell damage arises influenced by various physicochemical factors, including the operational parameters during freezing, sublimation, thawing and the type of cryoprotectants and used reconstitution medium. These factors play a crucial role in determining both the survival and metabolic activity of microorganisms during freezing and lyophilization

processes. To prevent these harmful processes, a wide range of protective excipients have emerged, which can be classified, depending on their chemical affiliation, into sugars, macromolecules, polyols, antioxidants and chelating agents (Егинчибаева, А.Д., 2015; Ute Rockingera, 2021; Wang, J., 2025).

Among the soluble substances in protective media, sugars and protein products are used. Carbohydrates are usually used in combination with colloids, less often as simple protective media. Various concentrations from 1 to 10% or more are used. However, the effect of the carbohydrate suspension added to the protective medium varies depending on the culture subjected to lyophilization (ОхапкинаВ.Ю., 2009). Very often, skimmed milk is used as a protective in combination with carbohydrates (XymenaPołomska, 2012; Peiren J., 2015; Valdez-Tenezaca, A., 2025).

The objectives of this study consisted in evaluating the viability of filamentous fungal strains and bacterial strains after lyophilization in the optimal protective medium.

2. MATERIALS AND METHODS

The objects of study were 10 strains of filamentous fungi and 14 strains of bacteria, isolated from aquatic reservoirs in Chisinau municipality, Republic of Moldova. The fungal strains were represented by the genera *Penicillium* (4 strains) and *Trichoderma* (6 strains), and the bacterial strains by the genera: *Bacillus* (9 strains); *Peribacillus* (1 strain), *Pseudomonas* (2 strains) and *Micrococcus* (2 strains).

As a protective medium for lyophilization of fungi, the medium with the composition: skimmed milk with 5% glucose, 5% sucrose and 0.01% ascorbic acid was used, and for lyophilization of bacteria, the medium containing: skimmed milk with 5% glucose; 5% glycerin; 0.01% ascorbic acid. The titer of the spore suspensions before lyophilization and the viability of the strains after lyophilization were determined.

The dried samples after lyophilization were rehydrated with 1 ml of sterile distilled water for 2 hours at a temperature of 28-30°C, after appropriate dilutions they were plated on nutrient agar (bacteria) and acidified agar (fungi). The plates with fungi were incubated at 28-30°C for 96 hours, and those with bacteria at 36°C for 24-48 hours. After cultivation, the colony forming units (CFU/ml) were counted. The results were expressed as a percentage of viable cells compared to the initial number of cells in the suspensions before lyophilization.

The number of viable cells was expressed as *lg*10 colony forming units (CFU) in 1.0 ml of suspension. Viability was calculated according to the formula BSR = (*lg*BL/*lg*AL) × 100, where BSR is the viability of the strain in %, *lg*BL – the logarithm of the CFU number before lyophilization and *lg*AL – the logarithm of the CFU number after lyophilization or storage (Muñoz-Rojas J. et al., 2006).

3. RESULTS AND DISCUSSION

Preservation of microorganisms by lyophilization still appears to be a science based on empirical tests rather than on tested facts and theories. The literature describes different methodologies for different species and even different strains of the same species, often being strain-specific and dependent on the induction of lyophilization tolerance in microbial cells (Morgan 2006; Guowei Shu, 2017).

The results obtained after lyophilization of the fungal strains in the usedprotective medium (skimmed milk with 5% glucose, 5% sucrose and 0.01% ascorbic acid), demonstrated a very high viability in all studiedstrains (Fig. 1). The viability of fungal strains of the genus *Penicillium* after lyophilization varied within the limits of 92.7 - 98%, compared to the initial titer of the suspension subjected to lyophilization. The highest viability of 98% was recorded in the strain *Penicillium* sp. 8. In strains of the genus *Trichoderma*, a high viability was also recorded after lyophilization, which constituted 94 - 97.5% compared to the initial titer. We

can consider that the high viability of fungal strains after lyophilization is due to the optimal protective medium as well as the tolerance of the strains to the lyophilization process (freezing and sublimation), but also to rehydration.

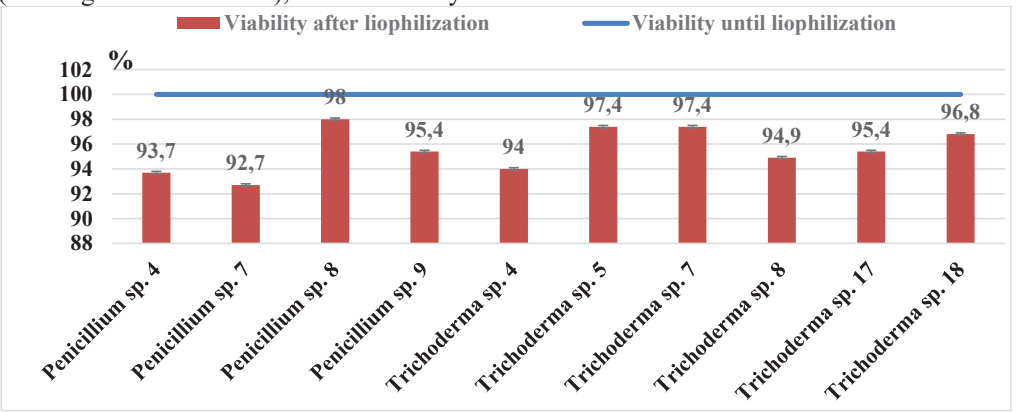

Figure 1. Viability of filamentous fungal strains after lyophilization

The bacterial strains studied reacted differently to the lyophilization process regardless of genus (Fig. 2; Fig. 3), thus demonstrating the different tolerance of the cultures to the freezing and vacuum sublimation process.

Out of the 14 strains lyophilized in the protective medium: skimmed milk with 5% glucose, 5% glycerin and 0.01% ascorbic acid, the viability of 6 strains varied within the limits of 89.1 - 91.2% (Tab. 2). Thus, demonstrating a high viability of bacterial cultures from the genera *Bacillus*, *Pseudomonas* and *Micrococcus*.

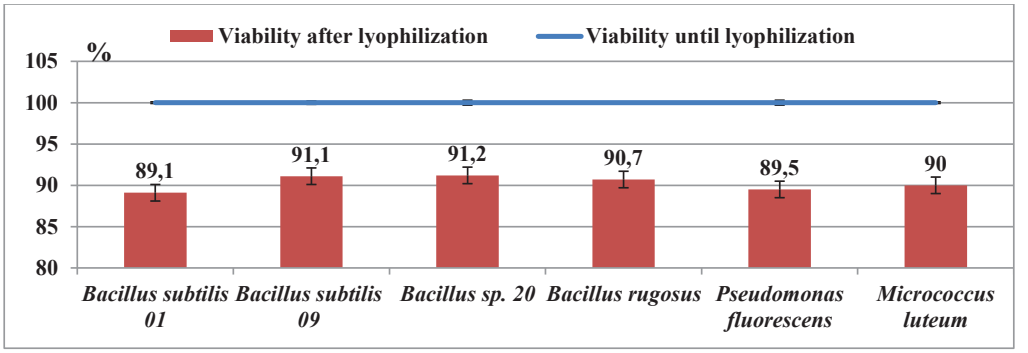

Figure 2. Viability of bacterial strains after lyophilization

In 8 strains of bacteria (Fig. 3), which belong to the same genera (*Bacillus*, *Pseudomonas*, *Micrococcus*) viability was lower after lyophilization, their value varying within the limits of 75.5 - 83.9%. These strains tolerate the lyophilization process less.

Bacteria of the genus *Bacillus* recorded a different viability. Viability in 3 strains out of 5 exceeded 80%, and in 2 strains the recorded viability value was 77.8% and 79.3%.

The viability value of strains of the genera *Pseudomonas* and *Micrococcus* was lower than 78% and 78.4% compared to 89.5% and 90%, respectively compared to the strains in Tab. 2.

The lowest viability after lyophilization of 75.5%, compared to the initial titer, was recorded in the strain *Peribacillus simplex*.

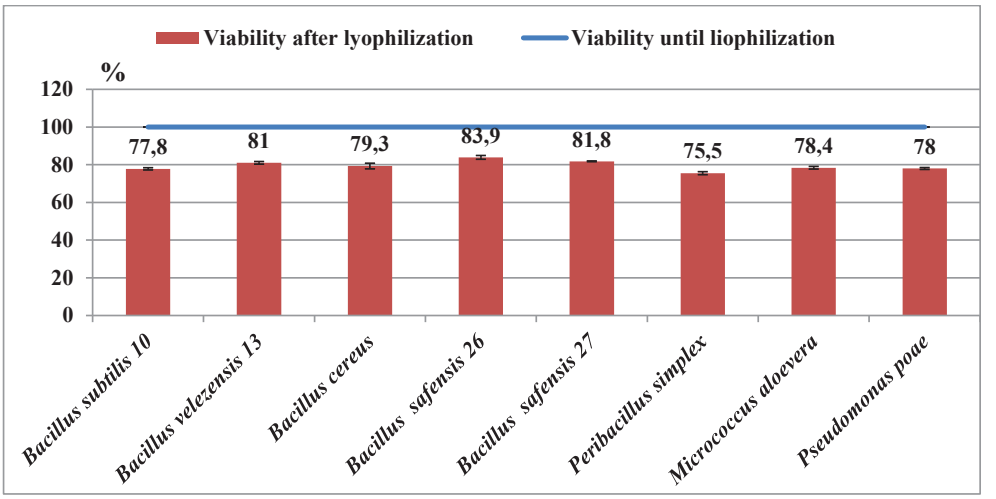

Figure 3. Viability of some bacterial strains after the lyophilization process

The results obtained are consistent with data from the specialized literature, which mentions that microorganisms suffer varying degrees of damage during dehydration and the stabilizing effects of excipients (increased interaction of membrane lipids, accompanied by leakage of intracellular components, replacement of water with small polar substances, etc.) which induces loss of cellular viability and stability (Егинчибаева, А.Д.,2015;Li, X.-M., 2024).

4. CONCLUSIONS

The viability of lyophilized microbial cultures varies not only between different genera but also among strains within the same genus.

The results of this study demonstrated that fungal cultures are more tolerant to the lyophilization process than bacterial ones. The viability of filamentous fungal strains ranged between 92.7–98%, while bacterial viability ranged between 75.5–91.2%, relative to the initial titer. These viability levels indicate that the applied preservation conditions are suitable for maintaining microbial cultures over long-term storage.

DECLARATIONS

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

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

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

ACKNOWLEDGMENTS: This research was funded by the project 25.80012.7007.32SE NARD (Innovative protective media for the preservation of aquatic microorganisms)

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