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Microbial Preservation Efficacy of Dacron Swabs: A Study on Freeze-Drying vs. Conventional Drying for Bacterial Viability

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Abstract

In general, microbiological and biotechnological applications depend significantly on microbial preservation. Researchers and medical professionals have used swabs to transfer and preserve clinical specimens for many years. Studies have demonstrated that the synthetic fiber Dacron has a high sample release rate, which is essential for the effectiveness of bacteriological and molecular tests, including the collection of bacterial samples. This study evaluated the recovery of five commonly referenced bacteria: *Lacticaseibacillus casei* IBRC-M 10711, *Bacillus subtilis* subsp. *subtilis* IBRC-M 10997, *Salmonella enterica* subsp. *enterica* IBRC-M 10707, *Staphylococcus aureus* subsp. *aureus* IBRC-M 10917, and *Streptococcus mutans* IBRC-M 10682 after preservation on Dacron swabs using standard microbiological assays. The ability of Dacron swabs to retain viable bacteria following two drying methods, freeze-drying and conventional drying methods, and the bacteria recovery was monitored for one year. The results showed that all five bacteria maintained their viability for three months after freeze-drying the bacterial cells on Dacron swabs and another conventional carrier, with no discernible differences in their viability percentages. *B. subtilis* was recovered after one year when preserved by both drying methods. In contrast, *L. casei* sustained viability only with the freeze-drying swabs process, and only *B. subtilis* and *L. casei* were effectively recovered from the Dacron swabs maintained at 4 °C after one year. However, only freeze-dried swabs containing *L. casei* were recovered. So, for medium-term bacterial preservation (viability of about three months), conventional drying methods are a cost-effective and straightforward approach for preserving bacteria on Dacron swabs. The results for one-year preservation showed that the type of strain may have a major impact on how effectively bacteria can be preserved using this method. Although the freeze-drying method may be more effective in terms of *L. casei* preservation, more strains need to be assessed before an ultimate decision on the method's suitability for long-term preservation can be made.

1. Introduction

For decades, various preservation techniques and microbial drying have been employed. The two most efficient and long-lasting techniques for preserving microorganisms are lyophilization and cryopreservation (Criste et al., 2014). A microorganism that is preserved effectively is kept

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in a viable state free from contamination or genetic mutations and has to be easily recovered without changing its original characteristics, either genotypically or phenotypically (Tedeschi & De Paoli, 2010). Preserving and transferring microbial strains to research and industry establishments is of significant concern. National Microbial Culture Collections actively work to provide these strains promptly and cost-effectively for research and industrial applications. In Iran, the extensive geographical area coupled with various climates poses notable logistical challenges in delivering microbial samples while maintaining their integrity. Such challenges necessitate innovative preservation and transportation strategies to ensure that viable samples reach customers. The Microorganisms Bank of the Iranian Biological Resource Centre (IBRC) has developed methods to overcome the logistical challenges of delivering viable microbial samples. Unlike most microbial culture collections that provide strains primarily in culture or freeze-dried powder (ampoule) formats, the IBRC also seeks alternatives due to the limitations associated with active cultivation, such as rapid deterioration with a short shelf life. Freeze-drying, a commonly used method for bacterial preservation, necessitates special facilities, including vacuum freeze-dryers. Given these requirements, some research centers, as discussed in the works of Prakash et al. (2020) and Vitorino and Bessa (2017), have explored swabs for storing and transporting bacterial isolates. Drawing from clinical practices where swabs are the standard for sample collection, transport, and storage, this approach has been adapted for bacterial preservation in research settings (Kumar et al., 2024). The use of buccal swabs for long-term preservation of genetic material has been the subject of two recent studies, specifically the use of various swabs in the preservation of microbes and genetic material (Martin et al., 2024; Sciuto et al., 2024).

Dacron swabs have emerged as a preferred medium due to their durability and compatibility with various bacteria, offering an efficient alternative to more resource-intensive methods. For microbial sampling procedures, swabs are often classified based on the material composing the swab tip. While cotton swabs are less expensive, they may not be as effective as synthetic alternatives for specific testing applications. Dacron, a type of synthetic fiber, has been shown to exhibit a high sample release rate, which is paramount for the efficacy of molecular and bacteriological assays, including the collection of bacterial samples (Jansson et al., 2020). Dacron's superior release rate can significantly enhance sample quality compared to cotton. Villa et al. (2020) utilized cotton swabs for the preservation of certain fastidious bacteria requiring strict growth conditions, such as *Neisseria* spp. and *Haemophilus* spp. While they maintained these bacteria at temperatures as low as -80 °C, there remains a need for preservation methods that maintain bacterial viability at various temperatures. Therefore, alternative materials and drying methods must be explored for standard bacterial preservation, particularly in resource-limited settings where ultra-low temperatures are not feasible.

This study examined the cultural recovery of five bacterial species preserved on Dacron swabs to assess their viability post-storage. The selected species represent common targets in clinical and microbiological research, reflecting the relevance of the findings to both applied and fundamental scientific domains.

2. Materials and Methods

2.1. Bacterial strains and culture conditions.

Five reference strains from Gram-positive and Gram-negative bacteria, namely *Staphylococcus aureus* IBRC-M 10917 (ATCC 12600), *Streptococcus mutans* IBRC-M 10682 (ATCC 35668), *Bacillus subtilis* subsp. *subtilis* IBRC-M 10997 (ATCC 6051), *Salmonella enterica* subsp. *enterica* IBRC-M 10707 (ATCC 13076), and *Lacticaseibacillus casei* IBRC-M 10711 (ATCC 393), were acquired from the Iranian Biological Resource Center in Tehran, Iran. Culture medium and incubation condition for *B. subtilis*, *S. enterica*, and *S. aureus* were Tryptic Soy Agar (Merck) at 37 °C aerobically; for *L. casei*, MRS agar (Merck) medium under 5% CO_2 at 37 °C; and for *S. mutans*, Blood Agar (blood agar base

medium Merck+ 5% sheep blood) medium, under 5 % CO_2 at 37 °C.

2.2. Preparation of bacterial suspensions for preservation

Fifty-milliliter volumes of the appropriate media were prepared for each bacterial strain, inoculated, and incubated overnight. Bacterial suspensions (5 mL) were prepared by combining 1 mL of centrifuged bacterial cells from the overnight culture with 3.5 mL of 20% skim milk (Merck) and 0.5 ml of a sucrose/sodium glutamate solution (containing 25 mg sucrose and 5 mg sodium glutamate; Sigma). The bacterial concentration was adjusted to achieve a final turbidity close to 2.0 McFarland standard (6×10^8 CFU/mL) in suspension. Subsequently, thirty-six sterile Dacron swabs (Expilab) were immersed in the bacterial suspensions for 20 seconds. After the swabs had fully absorbed the suspension, they were used for the next step.

2.2.1 Freeze-drying method

The swabs were dried using a freeze-drying instrument (Christ, Germany). The swabs were cooled at 4 °C for 15 minutes to allow for temperature stabilization, then at -20 °C for 30 minutes. The swabs were frozen at -50 °C for 30 minutes, then at 0.14 mbar and -10 °C for 2 hours. The samples underwent an additional drying step at 0.001 mbar and 30 °C for 2 hours to ensure the removal of any bound water. The swabs were immediately transferred into sterile, dry packaging under aseptic conditions.

2.2.2 Conventional drying method

The swabs were dried in a laminar airflow cabinet at room temperature and airflow stream for 30 minutes, and then packaged aseptically in the swab container.

2.3. Storage conditions and viability assessment

Six Dacron swabs from every strain were stored at a temperature of 37 °C for one week (W1) and two weeks (W2) to predict long-term preservation time. Twelve Dacron swabs were stored at 4 °C for one day (D1), one month (M1), three months (M3), and one year (Y1). Each experiment was performed three times.

2.4. Bacteria recovery

Each swab was transferred into 1 mL of a specified liquid medium (Tryptic Soy broth for *B. subtilis*, *S. enterica*, *S. aureus*, and *S. mutans*, and MRS broth for *L. casei*) and agitated for 20 seconds to dislodge the bacteria, followed by preparing serial dilutions. One mL of the dilutions was spread on a specified solid medium and incubated under the conditions mentioned above. The bacterial colony count (CFU/mL) was performed as described in Celik and O'Sullivan (2013) and Jansson et al. (2020). The viability of these five strains using both storage methods was calculated by comparing the colony count at the beginning of storage (P) with the colony count at each time interval (D1, W1, W2, M1, M3, and Y1).

3. Results and Discussion

The study of the viability of strains preserved in microbial resource centers, such as the American Type Culture Collection (ATCC) and German Collection of Microorganisms and Cell Cultures (DSMZ), is of significant importance. Freeze-drying and cryopreservation, two prevalent methods for microbial strain preservation, have been extensively evaluated for their effectiveness. For example, Heckly (1978) investigated the survival of various microbial groups through freeze-drying. However, most studies examining swabs have traditionally focused on collecting microbiological samples from various surfaces. Despite the longstanding use of swabs, few studies have evaluated the stability of microorganisms using these materials (Ball et al., 2020). Alongside historical data, recent advances indicate an evolution in the preservation efficacy due to improvements in methodologies and materials. Research has primarily focused on short-term storage and transport systems, with fastidious bacteria in the medical field receiving attention in

some studies. For instance, DeMarco et al. (2017) evaluated the viability of 17 vaginal microorganisms preserved on swabs using three different transport systems at room temperature and 4 °C for periods lasting up to 96 hours. They determined that storage at 4 °C yielded higher survival rates than room temperature storage. Celik and O'Sullivan (2013) assessed the appropriateness of swabs specifically for bacterial preservation. In 2020, Villa et al. (2020) examined the storage potential of fastidious, nutritionally demanding bacteria on Dacron swabs at -80 °C. However, no research has described the mode of drying swabs for storage, making this study the first to cover this subject. The scope of previous studies does not extend to the evaluation of freeze-dried swabs for long-term storage. Therefore, this study contributes novel data on freeze-drying as a viable preservation method for bacterial strains on swabs, an area not previously addressed in the literature at the time of the manuscript's writing.

3.1. Viable cell count recovery post-preservation

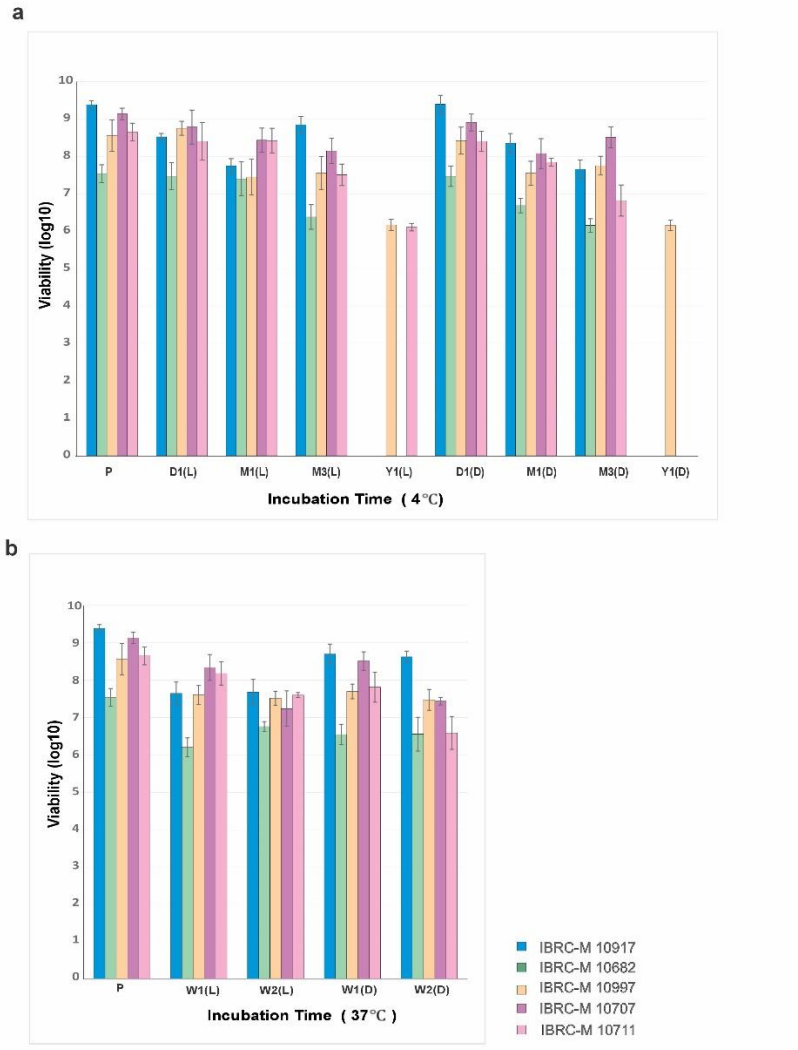
We compared the viable cell count of the bacterial strains immediately after preservation at the beginning of the process (P) to their post-preservation count. To check survival rates, we used two different temperatures: refrigerator temperature (4℃) and 37℃ for predicting long-term preservation (Iino & Suzuki, 2006). Survival rate at 4℃ was checked at day 1 (D1), month 1 (M1), month 3 (M3) and year 1 (Y1) (Fig.1 a), and long-term preservation predictions were checked after one week (W1) and two weeks (W2) incubation at 37 ℃ (Fig.1 b). Comparing the results of the two drying methods throughout the entire process showed significant differences between day 1 (D1), month 1 (M1), month 3 (M3), and one year (Y1). Among the bacteria evaluated, *B. subtilis* subsp. *subtilis* and *S. enterica* subsp. *enterica* demonstrated a superior survival rate compared to the other bacteria. The quantitative analysis indicates that the recovery of these two strains was higher, providing insight into their potential robustness under the applied preservation conditions. This differential recovery underscores the need for a tailored approach to bacterial preservation that accounts for strain-specific responses to drying methods. Our findings indicate that for *B. subtilis* IBRC M-10997, there was no significant difference in viability in Dacron swabs that were dried using freeze-drying compared to simple drying methods, such as air-drying. One exception was observed in the long-term preservation of *L. casei*, which warrants further investigation. Overall, the robustness of freeze-dried swabs as a preservation strategy for microorganisms was not distinctly superior to the other drying techniques in our assays for the majority of tested bacteria.

As shown in Fig. 1, after three months of preservation, Dacron swabs carried at least one million (1.0×10^6) microbial cells for each strain in both methods, suggesting their potential utility for defined short-term storage tasks (in this context, a duration of up to three months). Despite a decrease in microbial cell counts over this storage period, the results indicate adequacy for certain short-duration applications. Among the strains, the survival rate of *S. mutans* was significantly low, demonstrating remarkable sensitivity to the drying procedures. This sensitivity can be attributed to factors not yet fully understood but may be related to the strain's physiological traits. Understanding these survival differences is crucial for developing effective preservation strategies for bacteria with high desiccation sensitivity.

After one year of refrigeration at 4 °C, only *B. subtilis* and *L. casei* were successfully recovered from the Dacron swabs. Notably, *L. casei* was exclusively recovered from swabs that had undergone freeze-drying, highlighting freeze-drying as a better preservation method for this species. In contrast, *B. subtilis* showed no significant difference in recovery between swabs preserved through freeze-drying and those preserved using the conventional swab drying method. The robustness of *B. subtilis* to different drying methodologies and the exclusive recovery of *L. casei* from freeze-dried swabs provide valuable insights for developing storage protocols tailored to bacterial strains with varying desiccation sensitivities. These findings suggest

that less resource-intensive methods may suffice for preserving certain bacterial strains. Also, since this process used a temperature of 37 ℃ to predict long-term storage for several years, the one-year results demonstrate that this method cannot be used to predict the storage of strains on swabs because the results at week 1 and week 2 of storage at this temperature differed significantly from the one-year results for the majority of strains (Fig.1).

Figure 1. The Viability of the Bacterial Strains Preserved on Dacron Swabs Over Time



Note. a: at refrigerator temperature (4 ℃). b: 37 ℃ for long-term prediction. P: in the beginning of process, D1: day 1, M1: month 1, M3: month 3, Y1: year 1; W1: week 1, W2: week 2. L: lyophilization method. D: drying method. IBRC-M 10917: *S. aureus*, IBRC-M 10682: *S. mutans*, IBRC-M 10997: *B. subtilis* subsp. *subtilis*, IBRC-M 10707: *S. enterica* subsp. *enterica*, IBRC-M 10711: *L. casei*. The experiments were performed in triplicate.

However, the unique recoverability of specific organisms underscores the importance of tailoring preservation methods to the microbial species in question. A key benefit of freeze-drying is the reduction of oxygen levels and the establishment of a vacuum state, both of which are critical for long-term preservation of bacterial viability. It is hypothesized that partial moisture absorption could also play an important role, as the dried biomass and the surrounding air are not completely separated, potentially affecting shelf life. Also, compromised vacuum integrity during swab packaging may have contributed to the observed decrease in bacterial viability with freeze-drying. To address this, adjustments to the packaging process, such as ensuring swabs are packaged under a maintained vacuum, could potentially improve bacterial stability over extended periods. Future studies should explore the direct impact of vacuum packaging quality on the long-term viability of various bacterial species following freeze-drying.

Also, the stability of bacterial strains during freeze-drying preservation can be affected by several factors. Different strains of bacteria can react differently to freeze-drying. Some strains may survive better than others due to inherent genetic variations and different physiology, such as the cell walls, which influence their resilience to dehydration and freezing stresses (Farfan Pajuelo et al., 2023; Jalali et al., 2012). For example, due to spore formation, *Bacillus subtilis* can tolerate harsh conditions and has a longer shelf life than non-spore-forming bacteria (Li et al., 2022). Also, factors such as drying protectant, storage conditions, freeze-drying process, moisture content, medium, and growth conditions can influence the viability of strains.

4. Conclusion

The findings of this research suggest that the defined drying protocol using Dacron swabs offers a cost-effective preservation strategy, potentially reducing financial overhead for laboratories by simplifying the maintenance of microbial strains. For medium-term storage (up to three months), preservation on Dacron swabs may serve as a viable alternative to the traditional method of maintaining live cultures on agar plates. However, more research may be needed to fully quantify the cost savings and verify the long-term viability of microbes preserved by this method compared to plate cultures. The present study investigated five reference strains using Dacron swabs for short-term storage of microorganisms. However, future research should continue exploring the comparative advantages and limitations of swab-based preservation under various conditions to determine if the method is useful and practical. Factors optimizing bacterial preservation on swabs, such as adjusting the lyophilization protocol and storage conditions (suspension concentration, dry protectant, temperature, and time duration) should be considered to achieve more comprehensive results. Also, selecting more microbial strains from diverse microbial groups, such as *Actinomycetes*, *Enterobacteriaceae*, lactic acid bacteria, and spore-forming bacilli, is recommended. Overall, this research provides new insights into the preservation of bacterial strains on Dacron swabs, offering actionable information for both industrial and research sectors engaged in the maintenance of microorganisms over the studied preservation durations.

Conflict of interest

The authors declare that they have no competing interests.

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Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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The authors declare no conflict of interest.

Authors' Contributions

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