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LONG-TERM PRESERVATION AND BIOLOGICAL PROPERTIES OF *BACILLUS THURINGIENSIS* STRAINS MAINTAINED IN A MICROORGANISM CULTURE COLLECTION

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Introduction

Spore-forming bacteria belonging to the genus *Bacillus* have been widely studied because of their physiological diversity, environmental adaptability, and broad biotechnological applications. Among them, *Bacillus thuringiensis* (Bt) is particularly important in sustainable agriculture as a biological control agent against insect pests and is also known for its antifungal and plant growth-promoting activities. During sporulation, Bt produces parasporal crystal proteins (Cry and Cyt toxins) that exhibit specific toxicity against target insect groups.

The long-term preservation of industrially and scientifically valuable bacterial strains while maintaining their phenotypic stability is a fundamental task of microbial culture collections. An effective preservation method should ensure high cell viability while retaining essential physiological and biological characteristics for future research and practical applications. However, prolonged storage may lead to physiological changes or reduced metabolic activity, depending on the preservation conditions and strain-specific characteristics. Therefore, evaluating the functional stability of long-term preserved strains is essential for selecting appropriate preservation methods. This study aimed to evaluate the viability, morphological stability, halotolerance, and antagonistic activity of *B. thuringiensis* strains maintained in a microbial culture collection for 15 years using different preservation methods.

Materials and Methods

Five *Bacillus thuringiensis* strains (Bt 62-5, Bt 70-5, Bt LM 18, Bt LM 24, and Bt LM 3) maintained in the Microorganism Culture Collection were examined after 15 years of storage using three preservation methods: lyophilization, mineral oil overlay, and storage in the dry spore state under ambient conditions. Strain viability (CFU/mL), cultural and morphological characteristics, halotolerance at NaCl concentrations ranging from 0 to 15%, and antagonistic activity against three phytopathogenic fungi (*Verticillium dahliae*, *Fusarium oxysporum*, and *Fusarium solani*) were evaluated.

Results and Discussion

The results demonstrated that the preservation method substantially influenced the viability of *B. thuringiensis* strains after 15 years of storage. Among the methods evaluated, lyophilization was the most effective in maintaining viability. Strain Bt 62-5 exhibited 98.0% viability, with a reported viable cell titre of 56×10^8, whereas strain Bt 70-5 retained 95.0% viability, with a viable cell titre of 49×10^8. These findings indicate that lyophilization effectively maintained the viability of the investigated strains over the extended storage period.

The strains preserved under mineral oil exhibited lower viability, at 88.0% and 72.6%, respectively. Their viable cell titres also decreased by approximately one to two orders of magnitude

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compared with the initial level of 10^9. The lowest viable counts were observed in strains stored as dry spores under ambient conditions, ranging from 22 to 75 × 10^5 CFU after 15 years. These differences highlight the importance of preservation conditions in maintaining the viability of spore-forming bacteria.

The cultural and morphological characteristics of the strains were generally retained during long-term storage. On meat-peptone agar, the investigated strains formed round, grey or white colonies with slightly wrinkled surfaces, approximately 2–3 mm in diameter. The cells were Gram-positive, while growth in liquid medium was characterized by sediment formation and the development of a surface pellicle. Sporulation and parasporal crystal formation on solid medium were observed within 3–5 days. However, strains preserved under mineral oil required two to three successive subcultures to restore their typical cellular morphology. This finding suggests that the recovery period following preservation may depend on the storage method.

Halotolerance varied among the investigated strains. At 10% NaCl, the highest optical density (OD) values were recorded for Bt 62-5 (0.98 ± 0.03) and Bt 70-5 (0.95 ± 0.07). At 15% NaCl, Bt 70-5 exhibited the highest OD (0.83 ± 0.05), whereas Bt LM 3 showed no detectable growth. The growth of Bt LM 18 and Bt LM 24 also decreased as salinity increased. These results indicate that Bt 70-5 exhibited the highest salt tolerance among the tested strains under the conditions examined.

After 15 years of storage, Bt 70-5 demonstrated the highest antagonistic activity against the tested phytopathogenic fungi, with inhibition zones ranging from 32 ± 0.4 to 35 ± 2.7 mm. For Bt 62-5, the inhibition zones ranged from 20 ± 1.2 to 30 ± 1.5 mm. Bt 70-5 inhibited the growth of *V. dahliae*, *F. oxysporum*, and *F. solani*, indicating that its antagonistic activity was retained after long-term preservation.

Conclusion

Lyophilization was the most effective method for maintaining the long-term viability and phenotypic stability of *B. thuringiensis* strains over a 15-year storage period. The retention of high halotolerance and antagonistic activity against phytopathogenic fungi highlights the biotechnological potential of strains Bt 70-5 and Bt 62-5 for agricultural biological control applications.

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