CZU: 579.6:579.86

BACTERIAL VIABILITY AFTER 15 YEARS STORAGE

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Lyophilization, or freeze-drying, is a process is a widely applied effective method for the preservation of microorganisms. The National Collection of Nonpathogenic Microorganisms (NCNM) contains *Pseudomonas* sp., *Bacillus* sp. which are known for its antimicrobial activity, also lactic acid bacteria strains with important biotechnological properties for the dairy industry and prospective for starter cultures composition. It conservation and preservation for long-term storage present the purpose for the collection.

The aim of the research was to check the viability and stability of pure strains *Bacillus* sp. CNMN-BB-01, CNMN-BB-02, *Pseudomonas* sp. CNMN-PFB-01, CNMN-PsB-03 and lactic acid bacteria strains after 15 years storage in NCNM. Lactic acid bacteria pure cultures used in this study comprised *Lactococcus* sp. from CNMN-01 to CNMN-14 strains, *Streptococcus thermophilus* CNMN-15 and CNMN-16 strains.

The number of viable cells was determined as colony forming units per ml (CFU/ml). The survival rate was calculated as CFU/ml after freeze-drying divided by CFU/ml before freeze-drying. Therefore, the results of this work show that *Bacillus* sp. and *Pseudomonas* sp. strains are viable and their titer was ranged from 6,8 to 7,6 $\log_{10}$UFCml^{-1} for *Bacillus* and from 7,9 to 8,1 $\log_{10}$UFCml^{-1} for *Pseudomonas*. It is known that *Pseudomonas* and *Bacillus* bacteria can be stored for over 30 years in freeze-dried form with no changes of high level cell viability at 6-7 $\log_{10}$UFCml$^{-1.}$

Similar research results on lactic acid bacteria strains after 15 years of storage in freeze-dried form demonstrated viability more than 80% with titer ranged from 6,2 to 8,3 $\log_{10}$UFCml^{-1}. According to other studies viability of species *Streptococcus, Staphylococcus, Brevibacterium, Pseudomonas, Corynebacterium, Lactobacillus, Salmonella, Bacillus* after freeze-drying amount to min. 70%. Thus, the numbers of viable cells remaining in the ampoules are sufficient to maintain the culture.

Microscopic smear appearance of strains investigated confirmed the purity of cultures, cell cultures represented rod-shaped cells typical of Gram-positive *Bacillus* sp. and Gram-negative *Pseudomonas* sp. Lactic acid bacteria cells present cocci and diplococci in the medium and long chains. Morphological and cultural properties correspond to the technological requirements for lactic acid bacteria species. All strains were able to active milk coagulation, formed firm, gel dense consistency, without eliminating of whey, without gas eruption.

Freeze-drying provides higher cell viability, is used for the long-term preservation, also it plays a fundamental role in scientific and practical fields. The obtained results confirm effectiveness of freeze-drying as a method of conservation through high ability to strains regeneration.

Acknowledgments: The research was funded out within the project 20.80009.7007.09 (ANCD).

Keywords: bacterial viability, lyophilization, national collection, nonpathogenic microorganisms, long-term preservation.