

**THE VIABILITY OF *BACILLUS*, *PSEUDOMONAS* AND
LACTIC ACID BACTERIA STRAINS AFTER 15 YEARS OF STORAGE****Bogdan-Golubi N., Slanina V.***Institute of Microbiology and Biotechnology, Republic of Moldova*

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<https://doi.org/10.52757/imb22.14>

The National Collection of Nonpathogenic Microorganisms (NCNM) contains bacterial species like *Rhizobium* sp., *Pseudomonas* sp., *Bacillus* sp., which are known for their antimicrobial activity, plant stimulation effects, and exometabolites that can be used for plant protection. Some can be used for the insect and plant disease controls.

The Collection also contains the lactic acid bacteria isolated from naturally fermented homemade dairy foods, that can be used for obtaining sour cream, fresh cheese, yoghurt, soy milk, brined cheese. These bacteria permit to create a better taste, flavor and texture of the fermented foods, and to ensure manufacturing dairy products enriched with beneficial microorganisms, with an extended shelf-life and enhanced food safety of food products (due to the production of the lactic acid as an antimicrobial substance).

Cell viability during storage is of a great importance for the cultures used in the food and/or agriculture industries. Freeze-drying (lyophilization) provides a higher cell viability and is used for the long-term preservation. Depending on the resistance to the freeze-drying process there are three groups of bacteria: the resistant, the moderately resistant and the sensitive ones. According to this classification, bacteria from the *Pseudomonas* and *Bacillus* genus have been either resistant or moderately resistant to the lyophilization process.

For the NCNM just like for any other similar collection conservation and long-term storage of valuable microbial strains (fungi, yeasts, actinomycetes, bacteria, cyanobacteria, microalgae) is of a special importance.

The aim of the research was to check the viability and stability of the pure strains of *Bacillus* sp., *Pseudomonas* sp. and lactic acid bacteria strains a 15-year storage in the NCNM. Lactic acid bacteria included *Lactococcus* sp. and *Streptococcus thermophilus*.

The number of viable cells was determined as the colony forming units per ml (CFU/ml), and the survival rate was calculated as CFU/ml after freeze-drying divided by CFU/ml before freeze-drying.

The *Bacillus* sp. and *Pseudomonas* sp. strains were found to be viable and their titer ranged from 6,8 to 7,6 \log_{10} UFCml⁻¹ and from 7,9 to 8,1 \log_{10} UFCml⁻¹ respectively. It is known that the *Pseudomonas* and *Bacillus* bacteria can be stored for over 30 years in freeze-dried form with no changes in the high level cell viability at 6-7 \log_{10} UFCml⁻¹. Lactic acid bacteria strains after 15 years of storage in freeze-dried form had a survival rate of 80% with the titer ranged from 6,2 to 8,3 \log_{10} UFCml⁻¹. According to other studies the minimal viability of different species of *Streptococcus*, *Staphylococcus*, *Brevibacterium*, *Pseudomonas*, *Corynebacterium*, *Lactobacillus*, *Salmonella*, *Bacillus* after freeze-drying could reach 70%. Thus, the number of viable cells remaining in the tested ampoules was sufficient to maintain the culture.

Microscopic examination confirmed the purity of the cultures. *Bacillus* sp. Was represented by rod-shaped Gram-positive cells, and *Pseudomonas* sp - by Gram-negative. Lactic acid bacteria were present as cocci in short or long chains. All their strains were able to cause active milk coagulation, producing dense consistence, without gas eruption, and, therefore, respected the technological requirements for the lactic acid bacteria species.

The obtained results confirmed the effectiveness of freeze-drying for the tested strains

The research was funded by NARD within project 20.80009.7007.09