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ISOLATION, IDENTIFICATION, AND CONSERVATION OF BIOTECHNOLOGICALLY RELEVANT BACTERIA FROM THE WATER OF "LA IZVOR" LAKE

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Keywords: *microbial biodiversity, isolation, molecular identification, long-term conservation, sustainable agriculture.*

Introduction. *The identification of biotechnologically relevant bacterial strains allows for their utilization as a basis for the development of sustainable agriculture technologies.* ***Material and methods.*** *From the lakes of "La Izvor" Park, 213 representatives of various genera were isolated, out of which 148 were pathogenic. Each suspension culture was diluted until 10^{-8}, serial dilutions were inoculated on plates with Tryptic Soy Agar, Kings medium B, Salmonella Shigella Agar, Kligler's Iron Agar, Endo agar. For the non-pathogenic ones, enzymatic properties were determined, antimicrobial activity was assessed, and they were identified using the Polymerase Chain Reaction (PCR) method. Selected strains were preserved through lyophilization for an extended period.* ***Results.*** *The study revealed significant microbial biodiversity in the lakes of "La Izvor" Park, from which 213 bacterial strains were isolated in pure culture, with 65 selected for further research. The purpose of the research was to isolate and identify non-pathogenic biotechnologically relevant bacteria with various properties that could be applied in the development of sustainable agriculture. Nineteen strains with enhanced enzymatic properties and high antifungal activity were highlighted. These strains were molecularly identified and deposited for inclusion in the National Collection of Non-pathogenic Microorganisms (CNMN).* ***Conclusions.*** *The results of our study have shown that microorganisms can be used as safe biological control agents without the use of chemical compounds, which can prevent soil and water contamination. Lyophilization has confirmed that these strains have tolerance to osmotic and thermal shocks while maintaining viability of over 75%. Based on these properties, the strains are ideal candidates for application in sustainable agriculture.*

Cuvinte-cheie:*. biodiversitate microbiană, izolare, identificare moleculară, conservare îndelungată, agricultură durabilă.*

IZOLAREA, IDENTIFICAREA ŞI CONSERVAREA BACTERIILOR DE INTERES BIOTEHNOLOGIC DIN APA LACURILOR DIN PARCUL „LA IZVOR"

Introducere. *Identificarea tulpinilor de bacterii de interes biotehnologic permite valorificarea lor ca suport pentru dezvoltarea tehnologiilor agriculturii durabile.* ***Material şi metode.*** *Din lacurile parcului „La Izvor" au fost izolaţi 213 reprezentanţi ai diferitor genuri, dintre care – 148 de tulpine patogene. La fiecare cultură a fost obţinută o serie de diluţii zecimale până la 10^{-8}, cu inoculare pe medii agarizate Tryptic Soy Agar, Kings medium B, Salmonella Shigella Agar, Kligler's Iron Agar, Endo agar. La cele nepatogene au fost determinate proprietăţile enzimatice, activitatea antimicrobiană, au fost identificate prin metoda Polymerase Chain Reaction (PCR) şi tulpini selectate au fost conservate prin liofilizare pentru o perioadă îndelungată.* ***Rezultate.*** *Studiul a relevat o biodiversitate microbiană semnificativă în lacurile parcului „La Izvor", de unde au fost izolate în cultură pură 213 tulpini bacteriene, dintre care 65 au fost selectate pentru cercetări ulterioare. Scopul studiului a constat în izolarea şi identificarea bacteriilor nepatogene de interes biotehnologic, cu diferite proprietăţi, care ar putea fi aplicate în dezvoltarea agriculturii durabile. Au fost evidenţiate 19 tulpini cu proprietăţi enzimatice sporite şi cu activitate antifungică înaltă. Aceste tulpini au fost identificate prin metode biologice moleculare şi au fost depuse pentru completarea Colecţiei Naţionale de Microorganisme Nepatogene (CNMN).*

Concluzii. *Rezultatele studiului nostru au relevat că microorganismele pot fi utilizate ca agenţi de control biologic de siguranţă, fără utilizarea compuşilor chimici, în acest mod prevenindu-se contaminarea solurilor şi a apelor. Liofilizarea a confirmat că aceste tulpini au toleranţă la şocurile osmotice şi termice, cu păstrarea viabilităţii de peste 75%. Conform acestor proprietăţi, tulpinile sunt candidaţi ideali pentru aplicare în agricultura durabilă.*

INTRODUCTION

Conserving biodiversity involves maintaining, increasing, or actively managing the abundance and variety of all species worldwide, regardless of classification, ecosystems, or genetic diversity (1, 2, 3).

Biodiversity is defined as the variety of all life on Earth: ecosystems and their living organisms, including animals, plants, habitats, microorganisms, and their genes. Biodiversity assessment is the first step in the process of defining biodiversity management objectives and conservation measures for a particular area. The goal of assessment is to gather, analyse, and evaluate the most relevant information to inform decision-making and provide recommendations for the future (1, 2, 3).

For the conservation of biodiversity, the focus is often placed on the most important species, which can be grouped based on certain indicators, as biodiversity holds multifaceted significance for humans. It plays a crucial role in our lives because we depend on the products it provides (3).

Various species with their characteristics and roles form one of the most important foundations for sustaining human life. It's important to remember that, although their role has not yet been scientifically proven, each species is important and has a well-defined role in the Earth's web of life. Microorganisms, due to their rich and diverse enzymatic equipment, can metabolize a wide variety of organic and inorganic compounds (1, 2, 3). Biodiversity conservation can be *ex-situ* (outside their natural habitats, as mentioned in Article 9 of the CBD) or *in-situ*, referring to the protection of species in their natural habitats (3).

Ex-situ conservation involves methods for removing individuals of various species from their natural environment for purposes such as reproduction, storage, cloning, or rescue, especially in cases where their habitats can no longer support their existence or when they can be used for research and public awareness materials. The purpose of *ex-situ* conservation of species is diverse and includes scientific research activities, the production of individuals for *in-situ* reintroduction programs, the maintenance of genetic diversity, and the establishment of self-sustaining captive populations (auto-conservation), so that no individuals of the species are taken from the wild.

Thus, *ex-situ* conservation programs should be considered as a last resort (1, 2, 3).

Within the National Collection of Non-pathogenic Microorganisms (CNMN), the method of lyophilization is widely used for the long-term conservation and preservation of microorganisms. Lyophilization is a common and safe method for maintaining the biological properties of microorganisms. It allows for the long-term maintenance of cultures without the need for continuous subculturing, which is a clear advantage, as repeated subculturing can lead to changes in biological properties. To date, there has been extensive experience in applying the lyophilization method for preserving microbial cultures. The successful preservation in a dry state relies on strict adherence to all the details of the lyophilization process and the conditions for storing lyophilized cultures. Maintaining the viability and purity of strains while preserving their valuable properties is an important task for every microbial collection, from scientific research to practical implementation (4 – 8).

Our research has focused on isolating, identifying, and conserving aquatic microbial biodiversity from the "La Izvor" lakes in Chisinau municipality. The aim was to select biotechnologically relevant strains based on their enzymatic and antimicrobial properties to contribute to the development of sustainable agricultural and technological advancements (9).

To achieve this *aim*, a screening of aquatic biodiversity was conducted, leading to the isolation of various species of microorganisms such as bacteria (including cyanobacteria and actinobacteria), fungi (both mycelial and yeast), and microalgae. Among all the groups of isolated microorganisms, the study of bacterial strain diversity is of particular interest, as they represent a wide variety and can be both pathogenic and non-pathogenic.

MATERIAL AND METHODS

The study included 213 types of bacteria isolated as a result of assessing the aquatic biodiversity from the water, mud, and biofilms formed on the lakes in "La Izvor" Park. The samples were collected from various sectors within the park.

For the isolation, cultivation, and subculturing of bacterial cultures, the following culture media

were used: nutrient agar, agarized meat peptone medium, meat peptone broth, King A, and King B (dilutions 10^{-5} and 10^{-6}) (10, 11, 12). The isolation of bacterial cultures was carried out through successive dilutions. Subsequently, as a result of these successive dilutions, they were inoculated onto Petri dishes with various culture media.

To identify strains of pathogenic enterobacteria, the following culture media were used: Salmonella Shigella Agar, Endo Agar, Kligler Iron Agar (KIA), and Triple Sugar Iron Agar (TSI) (13 – 16).

For species identification, molecular biological methods were employed, involving the amplification and sequencing of the 16S rRNA (17).

For conservation, through the lyophilization method, 19 strains of biotechnologically relevant bacteria were selected based on their high antimicrobial properties against mycelial fungi and phytopathogenic bacteria. These strains are now stored in the National Collection of Non-pathogenic Microorganisms (CNMN) at the Institute of Microbiology and Biotechnology of the Technical University of Moldova: *Bacillus velezensis* CNMN-BB-12, *Bacillus velezensis* CNMN-BB-13, *Bacillus velezensis* CNMN-BB-14, *Bacillus velezensis* CNMN-BB-15, *Bacillus velezensis* CNMN-BB-16, *Bacillus velezensis* CNMN-BB-17, *Bacillus velezensis* CNMN-BB-18, *Micrococcus yunnanensis* CNMN-BM-19, *Micrococcus yunnanensis* CNMN-BM-20, *Paenibacillus pabuli* CNMN-BP-21, *Planococcus ruber* CNMN-BP-22, *Peribacillus simplex* CNMN-BP-23, *Planococcus chinensis* CNMN-BP-24, *Bacillus safensis* CNMN-BB-25, *Bacillus safensis* CNMN-BB-26, *Bacillus safensis* CNMN-BB-27, *Peribacillus simplex* CNMN-BP-28, *Bacillus rugosus* CNMN-BB-29, *Micrococcus aloeverae* CNMN-BM-30.

As a nutritional medium for the cultivation and growth of the mentioned strains, nutrient agar was used. Cultivation was carried out at a temperature of +30±1°C for a period of 24-48 hours.

As a stabilizing medium with a protective effect against osmotic and thermal shocks for lyophilization, 7% sucrose in skim milk was used. After resuspending the strains in the protective medium and distributing them into vials with 1 mL each, the samples were frozen at -80°C in the *ARCTICO ULTF 80* freezer, then lyophilized using the *Free Zone Plus* lyophilized. After lyophilization, they were sealed and stored at +4°C.

The subculturing of microorganism cultures after lyophilization was carried out on nutrient agar medium, following the standard method. This involved rehydrating them with distilled water and incubating them at a temperature of +30±1°C for 3 hours to facilitate their revival (10, 11, 12, 18).

The determination of the viability of microorganism cultures was performed using the method proposed by Donev in 2002 (1). The method involves performing successive dilutions, inoculating onto Petri dishes, and counting the colony-forming units, following a specific formula:

$$c\ \% = (\lg UFC ml^{-1}_{fin} / \lg UFC ml^{-1}_{in}) \times 100\%$$

in which:

lgUFC ml$^{-1}_{in}$ represents the logarithm with base 10 of the number of colony-forming units before lyophilization;
lgUFC ml$^{-1}_{fin}$ represents the logarithm of the number of colony-forming units after lyophilization or storage;
c – the viability of cultures in percentages.

Statistical analysis was performed using Microsoft Excel 2010, by logarithmizing the data and determining the percentage of viability. The statistical significance threshold was set at p=0.05.

RESULTS

In order to study the aquatic biodiversity of the lakes in "La Izvor" Park, 11 water samples, 11 mud samples, and 11 biofilms were examined. From these samples, 213 types of bacterial colonies were isolated. Subsequently, for the purpose of selecting pathogenic isolates of enterobacteriaceae, they were cultivated on characteristic media including SS agar, Endo, KIA, and TSI. As a result of the analysis of bacterial growth on these characteristic media, 148 samples of enteropathogenic bacteria were identified, including Escherichia coli, Salmonella, Shigella, Enterobacter, Klebsiella, and other strains from the Enterobacteriaceae family (9).

The remaining 65 isolates in the study were tested for various parameters to identify the most active ones, which are biotechnologically relevant to us. Consequently, enzymatic properties such as amylolytic activity (detected in 27 strains), catalase activity (in 42 strains), lipase activity (in 21 strains), cellulase activity (in 18 strains) were determined. Additionally, their antifungal and antibacterial activities were assessed. As a result of

the screening, the most active 19 strains were selected. These selected strains were later identified using molecular biological methods (amplification and sequencing of 16S rRNA) in collaboration with the Institute of Biology in Bucharest, Romania, and the Mother and Child Institute in Chisinau, Moldova (17).

Using molecular biology techniques, it was determined that the majority of the isolated strains were identified as *Bacillus velezensis*, with 16S rRNA sequences showing a similarity of over 99.6% (and a 99.4% similarity with *Bacillus amyloliquefaciens* MPA 1034). Additionally, strains of *Micrococcus yunnanensis* were identified, where the 16S rRNA sequences exhibited a similarity of over 99.7% (and a 99.5% similarity with *Micrococcus luteus* DSM 200030) (17).

Following microscopic examination, the selected bacterial strains are both Gram-positive and Gram-negative. On nutrient agar medium, they form colonies that can be white or yellow, matte or shiny, with a smooth and wavy edge. Some may be of the "S" type, while others are "R." Microscopically, the cells have a spherical cocci shape and do not form spores (as seen in *M. yunnanensis, Planococcus ruber/Planococcus massiliensis*), while others have an elongated rod-shaped (bacilli) form (as seen in *B. velezensis, Paenibacillus pabuli*), which can form endospores (9).

The selected strains with biotechnological potential were described according to the deposition passport at CNMN and deposited with the issuance of a microorganism storage certificate. After deposition at CNMN, these microorganism strains are preserved using various methods: through periodic subculturing (though this method is not efficient for long-term preservation, as there is a risk of culture loss or changes in the initial morpho-cultural characteristics), under a layer of mineral oil, or by lyophilization (which is the safest method) (4, 5, 6, 19). Thus, as a result of preserving bacterial strains through the lyophilization method, it can be observed that the cell viability after lyophilization varies between 75.91% to 93.08%, depending on the studied strain (tab. 1).

The analysis of the data in the table above also shows that, for some strains, the titer decreases by 3 units compared to the data prior to lyophilization, while for others, it only decreases by 1 unit. This could be associated with their resistance to the osmotic and thermal shocks they were subjected to. Thus, for those with a sudden drop in titer of up to 3 units, the post-lyophilization viability is lower and ranges between 75.91% and 79.84%. Meanwhile, for those where the titer drops by one unit, the viability is higher, ranging between 85.17% and 93.08%. The highest viability, reaching 93.08%, is observed in the *Bacillus velezensis* CNMN-BB-16 and *Peribacillus simplex* CNMN-BP-23 strains.

All bacterial strains revived from lyophilized state have achieved viability values of over 75.91%, indicating a very good index for cultures preserved for 5 to 19 years. The results obtained confirm the preservation of biotechnological properties, morpho-cultural characteristics, and the enhanced regenerative capacity of the described strains, as observed by other researchers (4, 5, 20).

DISCUSSIONS

The conservation of biological diversity at the level of ecosystems, species, populations, and genes is one of the main concerns of humanity in the 21st century (the third millennium). The problem lies in the fact that, with the advancement of technological progress and the intensive use of natural resources, the anthropogenic impact on biological diversity has significantly increased, leading to a substantial reduction in the number of species and varieties of living organisms that inhabit the Earth (1, 2, 3).

To identify microorganisms down to the genus or species level, a series of tests are necessary. These tests include both morphological and cultural characteristics, as well as a series of biochemical tests for microorganism determination. This is because microorganisms utilize various chemical substances from the culture medium as a source of energy or as building material for growth and reproduction, resulting in changes to the initial environment (the disappearance of certain substances from the medium, the appearance of new products, alterations in pH, gas production, etc.) (21, 22). These modifications reflect the nature of the enzymatic equipment and, indirectly, the genetic characteristics of the respective microorganisms. Therefore, biochemical properties are of particular importance for the identification and classification of microorganisms. Some characteristic biochemical tests include agglutination tests, catalase tests, oxidase tests, coagulation tests, and others.

Table 1. The viability of bacterial strains isolated from the water of "La Izvor" Lake, both before and after lyophilization.

No	Strain name	Colony-Forming Units (CFU), mL-1		Viability, %
		Before lyophilization	After lyophilization	
1	***Bacillus velezensis* CNMN-BB-12**	$1.2 \times 10^{12} \pm 0.1$	$2.3 \times 10^{9} \pm 0.4$	77.41±0.7
2	***Bacillus velezensis* CNMN-BB-13**	$1.8 \times 10^{12} \pm 0.2$	$3.9 \times 10^{9} \pm 0.2$	78.31±0.3
3	***Bacillus velezensis* CNMN-BB-14**	$9.3 \times 10^{11} \pm 0.7$	$1.2 \times 10^{9} \pm 0.3$	75.91±1.0
4	***Bacillus velezensis* CNMN-BB-15**	$1.7 \times 10^{12} \pm 0.2$	$2.9 \times 10^{9} \pm 0.5$	77.31±0.5
5	***Bacillus velezensis* CNMN-BB-16**	**$7.3 \times 10^{8} \pm 1.7$**	**$1.8 \times 10^{8} \pm 0.2$**	**93.08±0.6**
6	***Bacillus velezensis* CNMN-BB-17**	$1.1 \times 10^{12} \pm 0.1$	$1.6 \times 10^{9} \pm 0.3$	76.39±0.9
7	***Bacillus velezensis* CNMN-BB-18**	$1.3 \times 10^{12} \pm 0.1$	$1.8 \times 10^{9} \pm 0.3$	76.52±0.5
8	***Micrococcus yunnanensis* CNMN-BM-19**	$1.5 \times 10^{12} \pm 0.1$	$2.5 \times 10^{9} \pm 0.5$	76.05±0.9
9	***Micrococcus yunnanensis* CNMN-BM-20**	$1.8 \times 10^{12} \pm 0.1$	$3.1 \times 10^{9} \pm 0.5$	76.33±0.3
10	***Paenibacillus pabuli* CNMN-BP-21**	$2.2 \times 10^{12} \pm 0.1$	$7.2 \times 10^{9} \pm 0.4$	79.84±0.3
11	***Planococcus ruber* CNMN-BP-22**	$2.1 \times 10^{12} \pm 0.1$	$6.3 \times 10^{9} \pm 0.7$	79.51±0.4
12	***Peribacillus simplex* CNMN-BP-23**	**$1.5 \times 10^{9} \pm 0.2$**	**$3.5 \times 10^{8} \pm 1.1$**	**93.08±1.9**
13	***Planococcus chinensis* CNMN-BP-24**	$1.4 \times 10^{9} \pm 0.1$	$1.6 \times 10^{8} \pm 0.3$	89.75±0.7
14	***Bacillus safensis* CNMN-BB-25**	$8.8 \times 10^{9} \pm 0,7$	$5.1 \times 10^{8} \pm 0.3$	87.59±0.6
15	***Bacillus safensis* CNMN-BB-26**	$9.2 \times 10^{9} \pm 0.4$	$6.2 \times 10^{8} \pm 0.4$	88.23±0.1
16	***Bacillus safensis* CNMN-BB-27**	$1.1 \times 10^{10} \pm 0.02$	$7.6 \times 10^{8} \pm 1.0$	88.56±0.6
17	***Peribacillus simplex* CNMN-BP-28**	$6.5 \times 10^{9} \pm 0.8$	$2.3 \times 10^{8} \pm 0.5$	85.17±1.2
18	***Bacillus rugosus* CNMN-BB-29**	$1.3 \times 10^{10} \pm 0.1$	$1.0 \times 10^{9} \pm 0.01$	89.13±0.2
19	***Micrococcus aloeverae* CNMN-BM-30**	$1.2 \times 10^{10} \pm 0.1$	$1.1 \times 10^{9} \pm 0.1$	89.90±0.2

**p=0,05*

For the selection of pathogenic isolates of Enterobacteriaceae, seeding on Endo agar allows for the isolation and identification of *Escherichia coli* strains. These will manifest as colonies with a dark pink to light red color, displaying a greenish metallic sheen, and the medium may exhibit marked reddening. On SS Agar, according to the data from specialized literature, *Salmonella* will not ferment lactose but will produce hydrogen sulfide (H_2S), thus the bacterial colonies formed will appear colorless with black centers. *Shigella* strains neither ferment lactose nor produce H_2S, resulting in colorless colonies as well (13 – 16).

Coliform bacteria, such as *E. coli*, can also be differentiated in this medium. E. coli will ferment lactose in the medium, resulting in bacterial growth with a pink color and no hydrogen sulfide production. *Enterobacter* and *Klebsiella* typically appear larger than *E. coli*, with colonies that are creamy mucoid, pale, opaque to pink (13, 14).

Another specific medium used for the isolation and selection of pathogenic cultures is KIA and TSI medium, which are widely employed in identifying Gram-negative bacteria, particularly from the *Enterobacteriaceae* family. These media are identical except for the fact that TSI contains an overdose of dextrose and lactose compared to what is found in KIA (13 – 16). The media are poured into slant tubes and inoculated with a stab followed by a streak on the slant surface. As a result, bacteria are exposed to both anaerobic conditions (at the bottom) and aerobic conditions (on the slant). Phenol red is present as an indicator. If the bacteria are non-fermentative, such as *Pseudomonas*, they can grow on slant tubes by aerobic degradation of proteinaceous components in the medium. This test is particularly valuable in the initial identification of the *Enterobacteriaceae* family (13 – 16).

For fermentative organisms, glucose is the first catabolized sugar. If glucose is consumed, the bacteria take a detour in their metabolic pathway. However, if glucose is limited, and the organism does not produce the necessary enzymes to catabolize lactose, the organism can use the protein from the medium. After inoculation on these given media, the culture is incubated for 24 hours at a temperature of +37°C. Time is crucial in reading the KIA results. An early reading could reveal yellow throughout the medium, leading to the conclusion that the organism is a lactose fermenter when it may simply not have exhausted the glucose yet. A reading after lactose has been exhausted could mean that the organism may be just a glucose fermenter (13, 14, 15).

Thus, the practical importance of biochemical properties has led to a diversification of working techniques and, in some cases, their standardization. Biochemical tests are performed only with the help of pure cultures, using control cultures that produce positive and negative reactions (18, 23).

The author Huda isolated and characterized the carotenoid pigment from *Micrococcus luteus*, which exhibited antibacterial and antifungal activity, along with the ability to absorb UV rays in the range of 300 to 500 nm (24). It is well known that aerobic bacteria of the *Bacillus* genus exhibit high antagonistic activity (22), and biological preparations based on them offer significant advantages, such as safety for humans and animals, resistance to adverse environmental factors. This makes them effective for use against plant diseases caused by phytopathogens (5, 19, 25). Devi S. and colleagues mentioned that *B. velezensis* FZB42 is the most extensively researched biofertilizer and biocontrol agent based on Bacillus, commercially used in agriculture (26). Moreover, researchers Li-Ting Wang and Fan have proposed a heterotypic synonym for *Bacillus velezensis*, with the subsequent name *Bacillus amyloliquefaciens*. This proposal has been confirmed through conducted studies and may be considered for further research (27, 28, 29).

The bacterial species *Bacillus amyloliquefaciens, Bacillus siamensis, Bacillus velezensis*, and *Bacillus nakamurai*, belonging to the operational group *Bacillus amyloliquefaciens* (OGBa), are all Gram-positive, spore-forming organisms. They are widely distributed in various niches, including soil, plants, food, and water. Members of the OGBa group are known as Plant Growth-Promoting Bacteria (PGPB) due to their abilities to fix nitrogen, solubilize phosphate, produce siderophores, phytohormones, antimicrobial compounds, and various enzymes (such as amylase, protease, lipase, cellulase, xylanase, pectinase, aminotransferase, barnase, peroxidase) (27, 28).

After identifying and determining strains of biotechnologically relevant bacteria, their conservation is of paramount importance due to the long-term preservation of their characteristic morphocultural and biochemical properties. The determination of the number of microbial cells immediately after lyophilization is a crucial parameter, allowing us to estimate the viability of the studied strains. A high number of viable cells after lyophilization can ensure the preservation of strain viability for an extended period. During the storage period, the viability of lyophilized cells tends to decrease relatively slowly (4, 6).

CONCLUSIONS

1. 213 types of bacterial colonies were isolated from the lakes in "La Izvor" Park, out of which 148 were enteropathogenic, such as *Escherichia coli, Salmonella, Shigella, Enterobacter*, and *Klebsiella*, as well as other strains from the *Enterobacteriaceae* family.
2. Nineteen strains of bacteria with enhanced enzymatic and antifungal properties were identified. Subsequently, they were characterized through molecular biological methods, described morphologically, and stored in the National Collection of Non-pathogenic Microorganisms.
3. The selected strains of biotechnologically relevant bacteria were preserved using the lyophilization method, which enabled a viability of over 75.91%. This high viability ensures their long-term preservation.
4. The strains *Bacillus velezensis* CNMN-BB-16 and *Peribacillus simplex* CNMN-BP-23 demonstrated remarkable resilience to the stresses they were subjected to, achieving the highest viability, reaching up to 93.08%.

CONFLICT OF INTEREST

There are no conflicts of interest.

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ETHICAL APPROVAL

The article does not have ethical approval.

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