ASSESSMENT OF MICROMYCETES VIABILITY FROM NATIONAL COLLECTION OF NON-PATHOGENIC MICROORGANISMS AFTER 15 YEARS OF STORAGE

Tamara SIRBU[*], Cristina MOLDOVAN[*], Olga TURCAN[*]

[*] *Institute of Microbiology and Biotechnology, Chisinau, Republic of Moldova*
Correspondence author: Tamara Sirbu, Institute of Microbiology and Biotechnology, 1 Academiei Str., MD-2028, Chişinau, Republica Moldova, phone: 0037322739609, e-mail: tfsirbu@gmail.com

Abstract. The assessment of the viability and stability of micromycetes stored in NCNM, as potential producers of active substances, for 15 years by different methods: subculturing, under a layer of mineral oil, lyophilized, showed that all are viable and retain their morpho-cultural characteristics according to strain passport. Some cultures kept under mineral oil Vaseline or infected during storage. The safest method of preservation for long-term storage is lyophilization. The viability of strains belonging to the genus *Aspergillus* after 15 years of preservation in lyophilized state varies in the limits of 71.0-87.2%; for the strains of the genus *Penicillium* – 65.2-86.8%, and for those of the genus *Fusarium* – 54.0-70.0%, in comparison with viability established immediately after lyophilization.

Key words: protective media; viability; lyophilization; subculturing; preservation under mineral oil.

INTRODUCTION

Microorganisms are inexhaustible and advantageous sources of bioactive substances (antibiotics, vitamins, proteins, food additives, enzymes, organic acids, biopreparations for agricultural use, etc.), for which they are widely used in biotechnology. The sustainable functioning of national collections of microorganisms and their permanent completion is a key necessity for the further expansion of biotechnologies in all industrially developed countries [21, 22].

The method of storage of microorganisms of industrial interest is important for any microbiological investigation, and their long-term conservation, without obvious modification of morpho-cultural and biochemical characters is a task of major importance for any collection. Conservation and storage conditions can influence a number of characteristics of microorganisms, in particular their bioproductive potential. That is why it is necessary to periodically assess the viability and stability of microorganisms of industrial interest [9, 13, 14, 16]. The preservation of microorganisms by different drying methodologies has been used for decades. Freezing drying, in particular, is the preferred method for transporting and storing large collections of cultures of different microorganism groups [21].

Of the large number of methods used for the long-term storage and preservation of microorganisms, more effective are cryopreservation and lyophilization. These methods are applied in the Collections of Microorganisms from different countries of the world, including in the Republic of Moldova [3, 8-10, 24].

Numerous studies have shown that the viability of microbial cells after lyophilization depends on the culture, protection and regeneration media. Preservation of the viability of lyophilized microorganisms depends on both the protective medium used and the taxonomic features of the strains studied. Various protective media used to lyophilize micromycetes are known [7, 17, 26]. The protective media comprising skimmed milk, glucose and sucrose were selected as optimal for the lyophilization process for micromycetes stored in the National Collection of Non-Pathogenic Microorganisms of Moldova (NCNM) [22]. Both distilled water and some liquid culture media are used to rehydrate and revitalize lyophilized microorganism cultures. Thus, for yeasts and bacteria from NCNM it was proved to be the optimal regeneration medium – distilled water, and for fungi and actinobacteria – Czapek and Dulaney culture media [22, 23, 25].

Based on the above, the purpose of the research was to assess the viability and stability of micromycetes after 15 years of storage in NCNM.

MATERIAL AND METHODS

As object of study served 35 strains of micromycetes stored in NCNM by the methods: subculturing, under a layer of mineral oil Vaseline and lyophilization.

The viability and stability of micromycetes belonging to the genera: *Aspergillus*, *Penicillium*, *Fusarium*, *Mucor* and *Rhizopus* from NCNM after 15 years of conservation were evaluated. These strains were deposited in the NCNM for long-term storage by researchers from various Research Institutions in the Republic of Moldova, as potential producers of bioactive substances.

Protective media in which the micromycetes were lyophilized: skimmed milk + 7% glucose (SM + 7% G) and sucrose 20% (S 20%).

For the examination of the cultures kept in tubes on agarized media, as well as under sterilized mineral oil Vaseline (tº=+4ºC), the strains were grown on malt agar of 6ºBlg (pH=5.5) at 28°C.

Rehydration of lyophilized cultures

The experiment began with the addition of 1 mL of sterile distilled water into each ampoule with lyophilized culture. Rehydration was performed at 28°C for 2 hours. After 2 hours of rehydration, the suspension was inoculated into Petri dishes on malt

agar medium. After 4 days of cultivation at a temperature of 28°C, the micromycete colonies grown on Petri dishes were counted, then after 7-10 days the cultural features of the micromycete colonies after lyophilization were examined and compared with the initial ones according to the strain passport.

Determination of culture viability before and after lyophilization

The viability of micromycete strains before and after lyophilization (expressed in CFU mL^{-1} colony forming units) was determined by the method of counting colonies on malt agar medium on Petri dishes. After performing successive dilutions and inoculating them on agar medium, the colony forming units were calculated after incubation at 28°C.

The number of viable cells was expressed by *log*10 of colony forming units (CFU) in 1.0 mL of suspension. Viability was calculated according to the formula:

$$c\ \% = (lgCFUmL^{-1}_{fin} / lgCFUmL^{-1}_{in}) \times 100\%$$ [15]

where:

- $lgCFU\ mL^{-1}_{in}$ is the logarithm (with base 10) of the number of colony forming units before lyophilization;
- $lgCFU\ mL^{-1}_{fin}$ is the logarithm of the number of colony forming units after lyophilization or storage;
- c - culture viability in percentages.

Determination of the antifungal activity of micromycetes

Antifungal activity was evaluated in 4 micromycetes of the genus *Penicillium*, after 15 years of conservation, which initially showed antagonism to a number of phytopathogens.

The antifungal activity was determined by the method of agar blocks, before and after of their conservation by the mentioned methods [4].

As test cultures, phytopathogens were used: *Aspergillus niger*, *Alternaria alternata*, *Botrytis cinerea*, *Fusarium solani* and *Fusarium oxysporum*.

All experiments were performed in 3 repetitions. The calculation of the statistical indices was performed using the MS Excel 2010 software.

RESULTS

The strains studied are kept in NCNM by 3 methods: 1) subculturing (in tubes inclined on the malt agar medium); 2) under mineral oil Vaseline; 3) in lyophilized state.

To evaluate the viability of micromycete strains stored in NCNM by the method of subcultring and under mineral oil Vaseline, cultures were inoculated on Petri dishes on malt-agar medium. As a result of visual and microscopic examination of the colonies it was found that all strains are viable. Essential changes of the morpho-cultural characteristics during 15 years of conservation by the mentioned methods did not occur. However, in 6 cultures kept under mineral oil Vaseline, the growth and sporulation was observed slower compared to the initial one, even after three consecutive passages (the duration of growth was longer and sporulation was 2 days later). Also 10 strains were infected during storage. This demonstrates the shortcoming of the given method - the risk of infection.

It is known that biochemical characteristics over the long-term storage can be diminished. In order to establish the current state of the biochemical peculiarities of some strains kept for 15 years by subculturing, under mineral oil Vaseline and in lyophilized state methods, the antimicrobial activity of 4 strains of the genus *Penicillium* (*P. funiculosum* CNM FP 01, *P. funiculosum* CNMN FD 11, *P. corylophilum* CNM FP 04 and *P. verrucosum* CNM 02) and compared with their initial activity, before lyophilization. The results are shown in fig. 1, 2 and 3.

According to the obtained results (Fig. 1) it could be seen that the antifungal activity of the studied strains against the tested phytopathogens, after 15 years of storage in NCNM by subculturing method, varies within 80-90% compared to the initial activity.

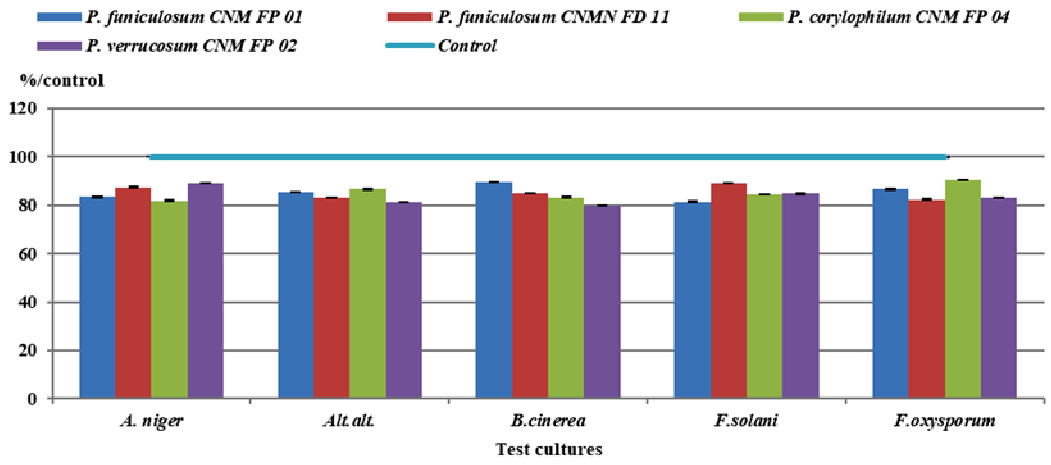

Figure 1. Antifungal activity of *Penicillium* strains after 15 years of storage by subculturing compared to the initial

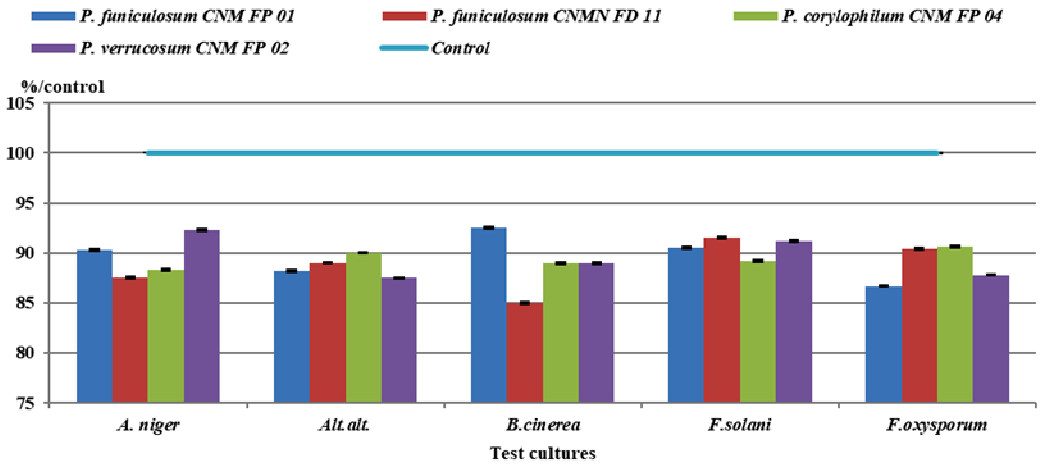

Figure 2. Antifungal activity of *Penicillium* strains after 15 years of storage under mineral oil Vaseline compared to the initial

A decrease in antifungal activity was also established in the strains kept under mineral oil Vaseline (Fig. 2). The antifungal activity of the cultures preserved by this method varies in the limits of 85-93% compared to the initial activity.

According to the results of research on the viability of lyophilized cultures, the viability of strains belonging to the genus *Aspergillus* after 15 years of lyophilized storage, regardless of the protective medium, in comparison with viability immediately after lyophilization decreased (Table 1). Thus, the titer of CFU mL^{-1} after 15 years of storage decreased by 2-3 orders from 10^{11} to 10^{9}-10^{8}. Comparing the viability of the strains according to the protection medium that was used, we can mention that for 6 strains out of the 9 studied, their viability on SM + 7% G is higher in comparison with registered on S 20%.

It was found that the viability of the mentioned strains after 15 years of preservation in lyophilized state is 71.0-87.2% in comparison with initial viability established immediately after lyophilization.

The difference in culture viability depending on the protection medium used can be from 2.0% (*A. niger* CNMN FD 01) to 14.0% (*A. niger* CNMN FD 06).

The strains belonging to the genus *Penicillium*, the viability after 15 years of storage in the lyophilized state decreased more significantly. Thus, the titer of CFU mL^{-1}, detected in ampoules, after 15 years of preservation, decreased from 10^{11}-10^{12} to 10^{8}-10^{7}, so by 3-4 orders (Table 2). Being expressed in percentages, we can mention that their viability varies from 65.2% (*P. vermiculatum*, SM + 7% G) to 86.8% (*P. expansum* CNMN FD 05, SM + 7% G). Comparing the viability of *Penicillium* cultures of the same species (eg *P. funiculosum* CNM FP 01 and *P. funiculosum* CNMN FD 01; *P. viride* CNMN FD 04 and *P. viride* CNMN FD 04) we find that their viability varies, which shows that each strain undergoes the lyophilization process, as well as keeping it in a different lyophilized state (Table 2).

Table 1. Viability of micromycete strains of the genus *Aspergillus* after 15 years of preservation in the lyophilized state

Strain	Protective medium	Viability		
		Immediately after lyophilization Control	After 15 years of preservation	
		Titer, log_{10} CFUmL^{-1}	Titer, log_{10} CFUmL^{-1}	% / Control
***A. alliaceus* CNM FA 01**	SM + 7% G	$1.0 \pm 0.2 \times 10^{11}$	$4.0 \pm 0.2 \times 10^{8}$	78.2 ± 0.4
	S 20%	$1.0 \pm 0.2 \times 10^{11}$	$2.0 \pm 0.6 \times 10^{8}$	75.4 ± 0.5
***A. flavus* BKMF 3292**	SM + 7% G	$3.0 \pm 1.0 \times 10^{11}$	$3.0 \pm 1.1 \times 10^{8}$	73.8 ± 1.7
	S 20%	$1.0 \pm 0.2 \times 10^{11}$	$1.3 \pm 0.3 \times 10^{9}$	82.8 ± 1.6
***A. fumigatus* CNM FA 02**	SM + 7% G	$4.8 \pm 0.9 \times 10^{11}$	$3.0 \pm 1.1 \times 10^{8}$	72.4 ± 1.7
	S 20%	$1.8 \pm 0.6 \times 10^{10}$	$7.0 \pm 1.1 \times 10^{8}$	79.4 ± 2.1
***A. flavus* (B)**	SM + 7% G	$3.0 \pm 1.0 \times 10^{11}$	$2.7 \pm 0.7 \times 10^{8}$	73.4 ± 0.9
	S 20%	$4.5 \pm 1.5 \times 10^{11}$	$2.0 \pm 1.1 \times 10^{9}$	79.5 ± 1.5
A. niger	SM + 7% G	$2.0 \pm 1.0 \times 10^{11}$	$5.8 \pm 0.7 \times 10^{9}$	86.7 ± 2.1
	S 20%	$4.5 \pm 1.5 \times 10^{11}$	$6.3 \pm 0.7 \times 10^{9}$	84.2 ± 1.0
***A. niger* CNMN FA 03**	SM + 7% G	$1.2 \pm 0.4 \times 10^{11}$	$4.4 \pm 0.5 \times 10^{9}$	87.2 ± 0.8
	S 20%	$4.5 \pm 1.5 \times 10^{11}$	$2.2 \pm 0.9 \times 10^{9}$	80.1 ± 0.7
***A. niger* CNMN FD 06**	SM + 7% G	$2.5 \pm 1.0 \times 10^{11}$	$6.0 \pm 0.2 \times 10^{9}$	85.9 ± 1.3
	S 20%	$4.5 \pm 1.5 \times 10^{11}$	$2.0 \pm 1.1 \times 10^{8}$	71.0 ± 1.4
***A. niger* CNMN FD 02**	SM + 7% G	$2.0 \pm 0.5 \times 10^{11}$	$2.4 \pm 0.4 \times 10^{9}$	83.0 ± 1.5
	S 20%	$2.4 \pm 0.6 \times 10^{11}$	$8.0 \pm 1.1 \times 10^{8}$	78.3 ± 0.5
***A. niger* CNMN FD 01**	SM + 7% G	$2.0 \pm 0.2 \times 10^{11}$	$2.3 \pm 0.3 \times 10^{9}$	82.9 ± 0.8
	S 20%	$2.0 \pm 0.6 \times 10^{11}$	$1.5 \pm 0.4 \times 10^{9}$	81.1 ± 1.2

Note: CNM – first name of the NCNM; BKMF – All-Union Collection of Microorganisms; FD – cipher of fungi.

Table 2. Viability of micromycete strains of the genus *Penicillium* after 15 years of preservation in the lyophilized state

Strain	Protective medium	Viability		
		Immediately after lyophilization Control	After 15 years of preservation	
		Titer, log_{10} CFUmL^{-1}	Titer, log_{10} CFUmL^{-1}	% / Control
***P. funiculosum* CNM FP 01**	SM + 7% G	$9.1 \pm 0.5 \times 10^{11}$	$4.0 \pm 0.2 \times 10^{8}$	71.9 ± 0.3
	S 20%	$5.0 \pm 0.3 \times 10^{11}$	$1.0 \pm 0.3 \times 10^{8}$	68.3 ± 1.1
***P. funiculosum* CNMN FD 11**	SM + 7% G	$5.2 \pm 1.4 \times 10^{11}$	$1.1 \pm 0.5 \times 10^{8}$	68.4 ± 2.4
	S 20%	$1.8 \pm 0.2 \times 10^{12}$	$1.1 \pm 0.3 \times 10^{8}$	65.5 ± 0.8
***P. viride* CNMN FD 04**	SM + 7% G	$2.2 \pm 0.5 \times 10^{10}$	$6.0 \pm 1.7 \times 10^{8}$	84.9 ± 1.4
	S 20%	$4.4 \pm 0.7 \times 10^{10}$	$3.0 \pm 1.1 \times 10^{8}$	79.5 ± 1.9
***P. viride* CNMN FD 09**	SM + 7% G	$2.0 \pm 0.6 \times 10^{11}$	$1.0 \pm 0.2 \times 10^{8}$	70.8 ± 0.7
	S 20%	$3.0 \pm 1.3 \times 10^{10}$	$1.0 \pm 0.5 \times 10^{8}$	76.3 ± 0.5
***P. verrucosum* CNM FP 02**	SM + 7% G	$1.0 \pm 0.2 \times 10^{11}$	$4.3 \pm 1.7 \times 10^{8}$	78.4 ± 0.9
	S 20%	$2.0 \pm 0.3 \times 10^{11}$	$5.0 \pm 0.6 \times 10^{8}$	77.0 ± 0.5
***P. verrucosum* (B)**	SM + 7% G	$1.5 \pm 0.3 \times 10^{12}$	$1.6 \pm 0.7 \times 10^{8}$	67.2 ± 2.2
	S 20%	$1.0 \pm 0.3 \times 10^{12}$	$1.1 \pm 0.4 \times 10^{8}$	67.0 ± 2.2
***P. expansum* CNMN FD 05**	SM + 7% G	$4.2 \pm 0.8 \times 10^{10}$	$1.7 \pm 0.8 \times 10^{9}$	86.8 ± 1.4
	S 20%	$6.3 \pm 0.7 \times 10^{10}$	$5.0 \pm 1.1 \times 10^{8}$	80.6 ± 0.7
***P. expansum* (B)**	SM + 7% G	$4.2 \pm 1.2 \times 10^{11}$	$3.0 \pm 0.6 \times 10^{8}$	73.0 ± 1.2
	S 20%	$4.1 \pm 1.0 \times 10^{11}$	$1.3 \pm 0.7 \times 10^{8}$	69.8 ± 2.3
***P. corylophilum* CNM FP 04**	SM + 7% G	$7.2 \pm 0.4 \times 10^{11}$	$3.0 \pm 0.6 \times 10^{8}$	71.5 ± 0.8
	S 20%	$4.1 \pm 1.0 \times 10^{11}$	$2.7 \pm 0.7 \times 10^{9}$	77.0 ± 8.4
P. vermiculatum	SM + 7% G	$5.1 \pm 0.4 \times 10^{11}$	$4.3 \pm 0.7 \times 10^{7}$	65.2 ± 0.6
	S 20%	$3.1 \pm 0.6 \times 10^{11}$	$4.0 \pm 1.1 \times 10^{7}$	66.1 ± 1.1

Note: *P. funiculosum* – synonym *Talaromyces funiculosum*; *P. viride* – synonym *P. veridicatum*.

Comparing the viability of the strains according to the protective medium used for lyophilization, we find that for 7 of the 10 *Penicillium* strains a higher viability was registered in the variant in which as protective medium was used SM + 7% G (with 0.2-6.2% more) in comparison to the protective medium S 20%, and for 3 strains the viability in the sucrose variant was slightly increased in comparison with SM + 7% G variant (more by 0.9-5.5%).

As a result of the evaluation of the antifungal activity of the strains *P. funiculosum* CNM FP 01, *P. funiculosum* CNMN FD 11, *P. corylophilum* CNM FP 04 and *P. verrucosm* CNM 02, compared to some phytopathogens it was found that during 15 years of conservation in lyophilized state the activity of the strains did not change significantly. Thus, the antifungal activity of the strains after 15 years of preservation in lyophilized state constituted 92-95.2% of the initial activity before lyophilization (Fig. 3). More significant decreases in activity were recorded by *P. funiculosum* CNMN FD 11 strain compared *to A. niger* (87.5%) and *B. cinerea* (86.5%), also *P. corylophilum* CNM FP 04 compared to *Alt. alternata* (85.3%).

In the case of strains belonging to the genus *Fusarium*, the viability of the strains, obtained after 15 years of storage in the lyophilized state, is lower in comparison to that obtained for strains of the genera *Aspergillus* and *Penicillium* (Table 3). The titer of CFU mL^{-1} decreased from 10^{9}-10^{10} to 10^{5}-10^{6}, by 3-4 orders. The viability expressed as a percentage after 15 years of storage in the lyophilized state varies within the limits of 54.0-70.0%, in comparison with viability registered immediately after lyophilization. Comparing the viability of cultures according to the protection medium used for freeze-drying, we can see that it had a different influence on the viability of cultures in the process of freeze-drying and preservation. An insignificant stimulation (2.0-6.0%) of viability in most strains of the genus *Fusarium* was obtained in the variant with SM + 7% G protection medium.

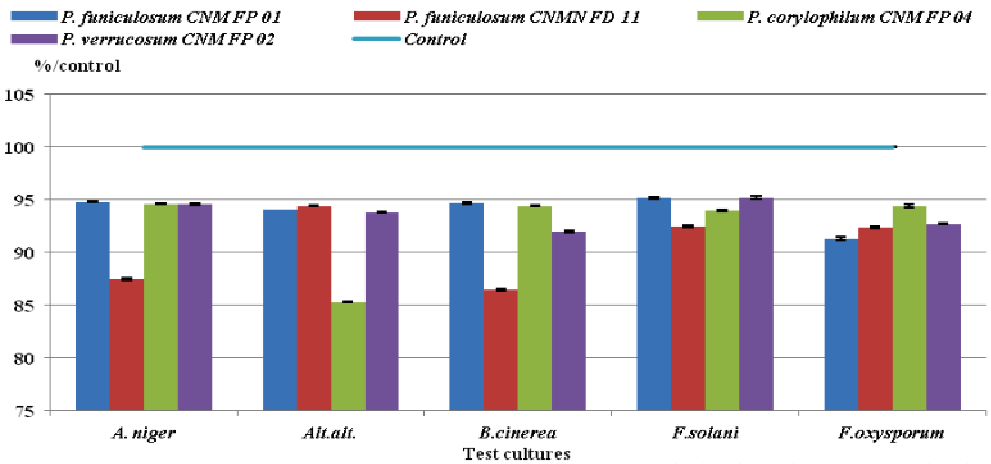

Figure 3. Antifungal activity of *Penicillium* strains after 15 years of preservation in the lyophilized state compared to the initial

Table 3. Viability of micromycete strains of the genus *Fusarium* after 15 years of preservation in the lyophilized state

Strain	Protective medium	Viability		
		Immediately after lyophilization Control	After 15 years of preservation	
		Titer, log_{10} CFUmL^{-1}	Titer, log_{10} CFUmL^{-1}	% / Control
F. nivale	SM + 7% G	$5.0 \pm 1.5 \times 10^{8}$	$1.1 \pm 0.4 \times 10^{6}$	63.0 ± 11.1
	S 20%	$7.2 \pm 1.1 \times 10^{10}$	$8.0 \pm 1.1 \times 10^{6}$	63.6 ± 0.9
F. culmorum	SM + 7% G	$5.0 \pm 1.0 \times 10^{8}$	$3.5 \pm 0.6 \times 10^{5}$	63.6 ± 1.1
	S 20%	$1.5 \pm 0.6 \times 10^{10}$	$1.8 \pm 0.3 \times 10^{6}$	61.6 ± 1.8
F. gibbosum	SM + 7% G	$5.0 \pm 1.0 \times 10^{10}$	$8.0 \pm 2.3 \times 10^{6}$	67.7 ± 2.2
	S 20%	$4.2 \pm 0.8 \times 10^{10}$	$5.7 \pm 0.7 \times 10^{6}$	63.6 ± 0.3
***F. sporotrichiela* 815**	SM + 7% G	$1.3 \pm 0.5 \times 10^{7}$	$3.0 \pm 1.1 \times 10^{5}$	76.8 ± 3.2
	S 20%	$1.7 \pm 0.3 \times 10^{9}$	$3.2 \pm 0.8 \times 10^{6}$	70.4 ± 1.5
***F. sporotrichiela* 846**	SM + 7% G	$8.5 \pm 1.0 \times 10^{7}$	$1.8 \pm 0.3 \times 10^{5}$	66.2 ± 1.1
	S 20%	$2.0 \pm 0.5 \times 10^{9}$	$2.8 \pm 0.3 \times 10^{6}$	69.4 ± 1.0
***F. oxysporum* F4**	SM + 7% G	$1.0 \pm 0.1 \times 10^{8}$	$1.2 \pm 0.2 \times 10^{5}$	63.6 ± 1.2
	S 20%	$4.0 \pm 1.1 \times 10^{9}$	$1.5 \pm 0.3 \times 10^{5}$	54.0 ± 1.4
***F. oxysporum* CL - 1**	SM + 7% G	$1.8 \pm 0.1 \times 10^{10}$	$6.0 \pm 1.1 \times 10^{5}$	56.3 ± 0.9
	S 20%	$4.3 \pm 0.7 \times 10^{9}$	$5.0 \pm 1.1 \times 10^{5}$	59.1 ± 1.2
***F. oxysporum* CL - 2**	SM + 7% G	$1.1 \pm 0.2 \times 10^{11}$	$5.5 \pm 1.0 \times 10^{6}$	61.0 ± 0.6
	S 20%	$4.0 \pm 0.2 \times 10^{9}$	$2.7 \pm 0.7 \times 10^{6}$	66.8 ± 1.2
***F. moniliforme* (B)**	SM + 7% G	$5.0 \pm 2.0 \times 10^{9}$	$2.3 \pm 0.7 \times 10^{6}$	65.7 ± 0.1
	S 20%	$8.0 \pm 2.0 \times 10^{10}$	$3.2 \pm 0.9 \times 10^{6}$	59.6 ± 1.2
***F. moniliforme* F 1**	SM + 7% G	$2.7 \pm 0.7 \times 10^{10}$	$2.3 \pm 0.7 \times 10^{6}$	61.0 ± 1.1
	S 20%	$2.7 \pm 0.7 \times 10^{10}$	$1.0 \pm 0.2 \times 10^{6}$	55.0 ± 0.5
***F. moniliforme* F 136**	SM + 7% G	$1.2 \pm 0.2 \times 10^{8}$	$2.3 \pm 0.7 \times 10^{5}$	66.4 ± 2.1
	S 20%	$3.5 \pm 0.6 \times 10^{11}$	$2.0 \pm 1.1 \times 10^{5}$	45.6 ± 2.5
F. graminearum	SM + 7% G	$5.0 \pm 2.0 \times 10^{9}$	$2.3 \pm 0.7 \times 10^{5}$	55.4 ± 1.8
	S 20%	$1.0 \pm 0.1 \times 10^{9}$	$1.0 \pm 0.1 \times 10^{6}$	66.6 ± 0.5

Note: *F. nivale* – synonym *Microdocium nivale*; *F. sporotrichiela* – synonym *F. sporotrichioides*; *F. moniliforme* – synonym *F. verticillioides.*

Evaluation of the viability and stability of the strains belonging to the genera: *Mucor* (2 strains) and *Rhizopus* (2 strains) demonstrated that they are viable, and changes in morpho-cultural features were not detected.

Evaluation of the viability of strains belonging to the genera: *Mucor* (2 strains) and *Rhizopus* (2 strains), preserved by subculturing, under mineral oil Vaseline and lyophilization, have been shown to be viable. As a result of the examination of the cultures, changes in the morpho-cultural characteristics were not detected. The cultures grow and develop very quickly on the malt agar medium, they sporulate intensely and it was not possible to count the number of colonies on the Petri dishes.

It was found that all strains preserved and kept in NCNM for 15 years by the methods: subculturing, under mineral oil Vaseline and lyophilization are viable and retain their morpho-cultural characteristics according to the strain passport.

The antifungal activity of the strains of the genus *Penicillium* after 15 years of preservation, by the methods of subculturing, under mineral oil Vaseline and lyophilization varies within the limits of 80-95%, compared to the initial one, depending on the method used.

The viability of strains belonging to the genus *Aspergillus* after 15 years of preservation in lyophilized state varies in the limits of 71.0-87.2%; in the strains of the genus *Penicillium* – 65.2-86.8%, and in those of the genus *Fusarium* – 54.0-70.0%, in comparison with viability established immediately after lyophilization.

DISCUSSION

The storage, maintenance of long-term viability and long-term stabilization of taxonomiccharacteristics for microorganisms of scientific and practical interest are the main tasks of all collections in the world [2, 6, 12, 20].

Keeping some strains of actinobacteria under a layer of mineral oil allows the maintenance of biosynthetic activity for up to 14-15 years [1]. Brazilian scientists have established that micromycetes of the genus *Absidia* preserved in dextrose under mineral oil preserve their viability and morphological characteristics for 44 years at room temperature. They found that out of 37 strains, after 44 years of storage - 81% are viable and stable, 5% perished and 15% became infected [19]. The micromycete strains from NCNM kept on the malt agar medium under a layer of mineral oil after 15 years also maintained their viability as well as stability, but some of them were infected, which proves that this method is not so safe for long-term storage.

The results presented in this study are consistent with the data in the literature which states that lyophilization and cryopreservation as preservation methods ensure a long-term storage of the viability and stability of the initial parameters of cultures (25-50 years). Their efficiency is largely determined by the culture subjected to cryopreservation or lyophilization, cryoprotective or lyoprotective media, storage conditions and used revitalization media, selection of freezing temperature and duration, sublimation regime, initial concentration of spores in the protective medium. Also, the viability of lyophilized microbial

cultures differs from genus to genus depending on the initial cultivation conditions, the development phase, the ontogenesis stage, the systematics of the microorganism [5, 9, 11, 18, 22, 23, 25].

Russian scientists, evaluating the viability and stability of lyophilized eukaryotic and prokaryotic microorganisms, using skimmed milk and sugars as lyoprotectants, have established that they have maintained their viability and over 50 years of storage, the titer decreased by 2-3 orders, but the amount of viable cells is sufficient to restore strains of biotechnological interest [10].

The high viability and stability of the morpho-cultural features of the strains studied after 15 years of storage in lyophilized state demonstrated that lyophilization is a safe method of long-term storage of micromycete strains of biotechnological interest. Practically micromycetes whole retain their properties, but at the same time some of them perish. The percentage of micromycete viability after storage in the lyophilized state varies from genus to genus. Thus, according to the results presented, the species of the genera *Aspergillus* and *Penicillium* better withstood the process of lyophilization and storage in the lyophilized state in comparison with representatives of the genus *Fusarium*. Strains of this genus being more sensitive to the process of freezing, sublimation and storage in a state of anabiosis.

Acknowledgements. The work was done based on the project 20.80009.7007.09. (ANCD).

Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

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Received: September 28, 2020
Accepted: March 8, 2021
Published Online: March 12, 2021
Analele Universităţii din Oradea, Fascicula Biologie
http://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433
University of Oradea Publishing House