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VIABILITY AND ANTIMICROBIAL ACTIVITY OF *STREPTOMYCES* STRAINS FROM NCNM AFTER LYOPHILIZATION

Oleg CHISELIŢA, Svetlana BURŢEVA, Maxim BÎRSA, Valentina BULIMAGA*, *, Anastasia VASILICIUC

Institute of Microbiology and Biotechnology,
Academy of Science of Moldova
**State University of Moldova*

The article deals with the aspects related to lyophilization of streptomycetes strains, preserved in the National Collection of Nonpathogenic Microorganisms (NCNM). Was determined that lyophilization do not significantly modify the antimicrobial activity of streptomycetes. Maximum viability of strains of genus *Streptomyces* (83,2-90,2%) is ensured after lyophilization at initial titer by 9-11 log_{10}UFC ml^{-1} in protective medium (gelatin 2,5% + glucose 7,5%) by rehydration with distillate water.

Keywords: *Streptomyces, lyophilization, cryoprotectants, viability, antimicrobial activity.*

VIABILITATEA ŞI ACTIVITATEA ANTIMICROBIANĂ A TULPINELOR DE *STREPTOMYCES* DIN CNMN DUPĂ LIOFILIZARE

Acest articol prezintă aspecte legate de liofilizarea tulpinilor de streptomicete, depozitate în Colecţia Naţională de Microorganisme Nepatogene (CNMN). A fost stabilit că liofilizarea nu modifică esenţial activitatea antimicrobiană a streptomicetelor. Viabilitatea maximă a tulpinilor genului *Streptomyces* (83,2-90,2%) este asigurată după liofilizarea la titrul iniţial 9-11 log_{10}UFC ml^{-1} în mediu protectiv (gelatină 2,5% + glucosă 7,5%) şi la rehidratarea cu apă distilată.

Cuvinte-cheie: *Streptomyces, liofilizare, crioprotectori, viabilitate, activitate antimicrobiană.*

Introduction

Streptomycetes are widely distributed in nature: in the air, water reservoirs, in various animal and vegetable residues, but many of them are in the soil [1-5]. Representatives of the largest group among the actinomycetes are streptomycetes. Recent years, they are considered not only as producers of antibiotics of different chemical nature, but also of other bioactive substances as enzymes, vitamins, amino acids, lipids, vaccines against human and animal infectious diseases, various drugs of controlling insects and rodents, substances with a phytohormonal activity, which influence the growth and development of plants, stimulate seed germination, increase crop yields [6-11]. In modern medicine are used more than a hundred medicines drugs created by using soil actinomycetes [12,13].

The great importance in the detection of new strains – producers of biologically active substances has preserving their viability, biological activity and properties of valuable properties that is essential from the initial study to their use in the production of different biological products.

There are many methods of preservation of microorganisms related to short (up to several years): storage in soil, periodic subculturing, under a layer of paraffin oil, in distilled water, in dry form on substrates of different nature et al. [14-17], but the most comfortable and effective from the point of view of the majority microbiologists, is cryopreservation in liquid nitrogen and lyophilization, that allows to store microorganisms in a viable state more than 30 years and reduce the risk of various contaminants and genetic mutations to a minimum [18-24]. Effective conservation with unmodified genomes and populations is a problem, especially considering physiological diversity of microorganisms, as well as the fact that the ability to survive in certain conditions is not only connected with the genus and species of the microorganism, but often with his race [25,26]. It is believed that from all of the groups of microorganisms, the bacterial forms better supports lyophilization [17,27,28].

Preservation of the viability and other properties of actinomycetes species is very important for their practical use, because these members of the microbial world a high level of genetic instability [29,30].

The problem of long-term "conservation" of microorganisms is associated (paired) to the solution of some problems such as the selection of optimum media and conditions to protect microbial cells from damage that occur at low temperatures and subsequent storage. Success of the lyophilization also depends on the quality of used cells, on conditions in which they are viable and were grown [28,31].

Usually, the cultures supposed at long preservation in lyophilized condition or by other methods, are tested for survival and preservation properties, in particular, antimicrobial properties, pigmentation, biomass productivity and its amino acid composition [30,32,33].

In addition, the determination of direct correlation of viability of conserved culture and biological activity many authors believe as an important moment for long-term storage of strains – producers of biologically active substances [7,33,34].

In general, the practice of preservation by freezing developed a number of methods, corresponding to the cell immersion mechanisms in anabiotical state that have been identified (and continue to be identified) in the study of the formation and germination of dormant microorganism cells. The cells before the lyophilization were suspended in protective medium solutions. American Type Culture Collection succeeded in long-term storage of various bacteria by using as cryoprotectants of 20% skimmed milk, 12% solution of sucrose, 10% solution of dextran, 10% solution of glycerol and others [35,36].

In microbiology, sucrose-gelatin protective medium (sucrose 10% + gelatin 1%) and its various modifications that are suitable for bacteria and for actinomycetes is used for many years for the lyophilization [31]. The authors are unanimous in the opinion that it is best to preserve the ability of microorganisms to synthesize biologically active substances of different chemical nature, providing the freeze-drying of spores in sucrose-gelatin medium [18,37,38]. However, due to the huge variety of naturally occurring microorganisms, one cryoprotectant in practice is insufficient. Therefore, for preservation of new strains should at first to study the effect of various types of cryoprotectants and select a suitable for the type of microorganisms, as well as conditions for their subsequent storage. For example, the medium comprising skimmed milk supplemented with sucrose, sodium glutamate, polyvinylpyrrolidone, peptone, medium Gause or regulated medium CP-15 containing soybean flour, corn steep liquor, molasses, potato starch, inorganic salts and with addition of 1% carbamide are used in the lyophilization of strain *Streptomyces aureofaciens* [39].

Other protective media, including, for example, peptone (0,1-10%), sucrose (10%), glucose (5-15%), lactose (10%), trehalose (10%), skimed milk (10-20%), sodium glutamate (5%), casein hydrolysate and other many materials successfully are used for the preservation of the genera *Bacillus*, *Pantoea*, *Serratia*, *Erwinia*, *Lactobacillus*, *Acetobacter*, *Streptococcus* and others [17,26,28,31].

Since it is believed that carbohydrates have less pronounced cryoprotective properties than glycerol or dimethyl sulfoxide, they are recommended to be used in combination with other protectors. As a protective media successfully are used the culture media containing peptone, tryptone, yeast extract.

Using of the special media with cryoprotectants for preservation allows to reduce the number of cryodestructed cells and to increase the number of living, structurally and functionally intact cells. Most of the hypotheses of the protective action of traditional cryoprotectants are based on the properties to reduce the amount of ice, change the size and structure of crystals and decreasing of the freezing point of the solution [26,40].

Viability of the microorganisms after lyophilization depends on storage conditions and particularly conditions of rehydration [41,42]. Typically, researchers have used sterile distilled and tap water for rehydration [29, 43-45]. Rehydrated media containing skimmed milk, sucrose, saline solutions, sodium glutamate, peptone extract or water, solutions containing peptone, yeast extract, and manganese sulfate, various substances with antioxidant activity, can be used also [14,46].

Thus, based on the above, the research goal is to assess the viability of different streptomycetes on protective and rehydration media and antimicrobial activity after lyophilization.

Materials and methods

As **objects** of our studies were served nine strains of actinomycetes of the genus *Streptomyces* kept in the National Collection of Microorganisms Nonpathogenic (NCNM). The strains were grown under aerobic in static conditions, in glass tubes on Czapek agarized medium with glucose and oatmeal agarized medium, incubation time - 7-14 days, at t°= 27°C [29,47,48].

Lyophilization. Cultures in stationary phase were suspended in protective media and frozen at t°= -50°C, drying of frozen mass is achieved at -88 ... -94°C of condenser and vacuum - 5-8 Pa.

Cryoprotective media (CrM): Skimmed milk (SM); Skimmed milk + glucose 7% (SM+G7%); Gelatin 1,0% + sucrose 10,0% (Gel1%+S10%); Gelatin 2,5% + sucrose 7,5 % (Gel2,5%+Z7,5%); Gelatin 2,5% + glucose 7,5 % (Gel2,5%+G7,5%) [18,29,46].

After lyophilization strains have been generated with the different rehydration media at room temperature for 1 hour.

Rehydration media (RhM):

Czapek (Cz): Glucose – 20,0 g/l; $NaNO_3$ – 2,0 g/l; K_2HPO_4 – 1,0 g/l; $MgSO_4*7H_2O$ – 0,5 g/l; KCl – 0,5 g/l; $FeSO_4*7H_2O$ – 0,01 g/l; pH = 7,0-7,3.

Dulaney (Dl): Glucose – 20,0 g/l; $(NH_4)_2HPO_4$ – 7,5 g/l; NaCl – 5,0 g/l; K_2HPO_4 – 2,0 g/l; $MgSO_4*7H_2O$ – 1,0 g/l; $CaCl_2$ – 0,4 g/l; $ZnSO_4*7H_2O$ – 0,01 g/l; $FeSO_4*7H_2O$ – 0,01 g/l; pH = 7,0.

Distillate water (DW) [46,49].

Viability determination. The content of a vial after lyophilization was suspended in 1,0 ml of rehydration medium. The viability of the strains before and after lyophilization was determined using the method of successive dilutions, suspensions were inoculated on the agarized medium, the units forming colony (UFC) count after 7-14 days of incubation at 27°C [29, 49]. Number of viable cells was expressed in log_{10} of UFC in 1,0 ml of suspension.

The bacterial survival ratio (BSR) is reported as the ratio of the *log* of the number of bacterial cells present in the suspension after lyophilization (AL) to the *log* number of viable cells before lyophilization (BL) multiplied by 100, i.e., BSR = (*log*AL/*log*BL) × 100 [50]. Averages and standard deviations were calculated from at least three independent lyophilization analyses.

The antimicrobial activity of strains before and after lyophilization on protective medium Gel1%+S10% [37] was determined by the Egorov's method, applying to the agar blocks [7]. As the test cultures were used three strains of bacteria: *Corynebacteruim michiganense* 10_2, *Agrobacterium tumefaciens* 8628, *Erwinia carotovora* 8982 and 7 strains of phytopathogenes fungi: *Aspergillus niger, Aspergillus flavus, Penicillium funiculosum, Thielaviopsis basicola, Fusarium oxysporum, Alternaria alternata* and *Botrytis cinerea*, causing various plant diseases.

Experimental data were subjected to statistical processing by program Office Excel 2010.

Results and discussions

In National Collection of Nonpathogenic Microorganisms (NCNM) more than 500 strains of microorganisms with biotechnological importance, including the actinomycetes of genus *Streptomyces* are persevered. The strain *Streptomyces canosus* CNMN-Ac-02 and its variants, obtained by γ-ray and UV, *Streptomyces canosus* CNMN-Ac-03 and *Streptomyces canosus* CNMN-Ac-04 and the species *massasporeus* – *Streptomyces massasporeus* CNMN-Ac-06, *Streptomyces massasporeus* CNMN-Ac-07 and *Streptomyces massasporeus* CNMN-Ac-08 are producers of bioactive substances: phytohormones (auxin, gibberellins), briefs and immunoactive amino acids, lipids containing highly unsaturated fatty acids. Abovementioned strains and strain *Streptomyces levoris* CNMN-Ac-01, isolated from soils of Moldova and its natural variants *S. levoris* var. k-1 and *S. levoris* var. 4 possess high antimicrobial activity against a broad spectrum of phytopathogenic fungi and bacteria, which can cause various diseases in crop plants and *Apis mellifera*.

Actually, the main activities in the field of preservation of cultures of microorganisms are directed on the task of preserving the maximum number of viable and intact cells with the original genome and phenotypic characteristics that are important for their identification and their use in scientific research and biotechnological processes. Different species display different degree of freeze-drying survival, Gram-negative bacteria often showing lower survival than Gram-positive bacteria [45]. In addition to species, freeze-drying tolerance also depends on freeze-drying medium and rehydration conditions [42].

Thus, strains of actinomycetes were lyophilized by using different protection media and regenerated with different media for rehydration, 2 of 3 – Cz and Dl, with different minerals in its composition (Table 1, Fig.1). In the result of our study was established, that all species of actinomycetes survive after the lyophilization process, but their viability is different and depends largely on used media of protection and rehydration.

If we analyze the viability of the species *S. canosus* average viability depending on used protection and rehydration media, we highlight two media: Gel 2,5% + S 7,5% + Cz and Gel 2,5% + G 7,5% + DW, where strains of this species exhibit maximum viability after lyophilization - 82,1 and respectively, 86,9% (Fig.1).

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Table 1

Viability of strains *Streptomyces canosus* CNMN-Ac-02, Ac-03 and Ac-04, after lyophilization on different protective and regeneration media with different rehydration media

Strain					*S. canosus* CNMN-Ac-02		*S. canosus* CNMN-Ac-03		*S. canosus* CNMN-Ac-04	
					Titer *log*UFC ml^{-1}	BSR %	Titer *log*UFC ml^{-1}	BSR %	Titer *log*UFC ml^{-1}	BSR %
BL					7,4±0,03	100	10,1±0,1	100	7,4±0,03	100
AL	CM	SM	RM	DW	5,6±0,3	76,0±4,3	8,0±0,1	79,4±1,9	8,3±0,04	76,4±0,3
				Cz	6,1±0,2	82,1±2,7	8,1±0,8	79,8±7,5	8,7±0,1	79,4±1,2
				Dl	5,8±0,03	78,3±0,5	8,2±0,2	81,1±2,2	7,7±0,7	70,4±6,5
		SM+ G.7%		DW	6,1±0,1	82,2±2,1	8,1±0,06	79,8±1,5	7,3±0,06	67,0±0,4
				Cz	6,4±0,04	85,7±0,9	8,5±0,1	84,0±1,6	7,7±0,1	70,2±1,2
				Dl	6,4±0,02	86,3±0,6	8,3±0,1	82,1±1,6	8,3±0,8	75,7±7,4
		Gel.1% + S.10%		DW	6,6±0,1	88,4±0,9	7,5±0,1	73,7±1,8	8,9±0,02	81,5±0,4
				Cz	6,8±0,03	91,0±0,7	7,3±0,1	72,0±1,3	8,9±0,6	81,3±4,6
				Dl	6,5±0,06	86,9±1,1	7,3±0,2	71,9±1,7	8,9±0,03	81,7±0,9
		Gel.2,5% + S.7,5%		DW	6,0±0,08	80,8±1,1	7,6±0,6	75,0±6,6	8,2±0,7	75,0±6,5
				Cz	6,1±0,1	82,4±1,4	8,2±0,1	80,6±2,2	9,1±0,1	83,3±0,7
				Dl	6,2±0,1	83,1±2,3	8,0±0,2	78,8±2,2	8,9±0,02	81,2±0,4
BL					8,7±0,5	100	11,1±0,6	100	11,1±0,8	100
AL	CM	Gel.2,5% + G.7,5%	RM	DW	7,8±0,6	90,3±2,0	9,0±0,8	81,3±2,3	9,9±0,7	89,2±0,2

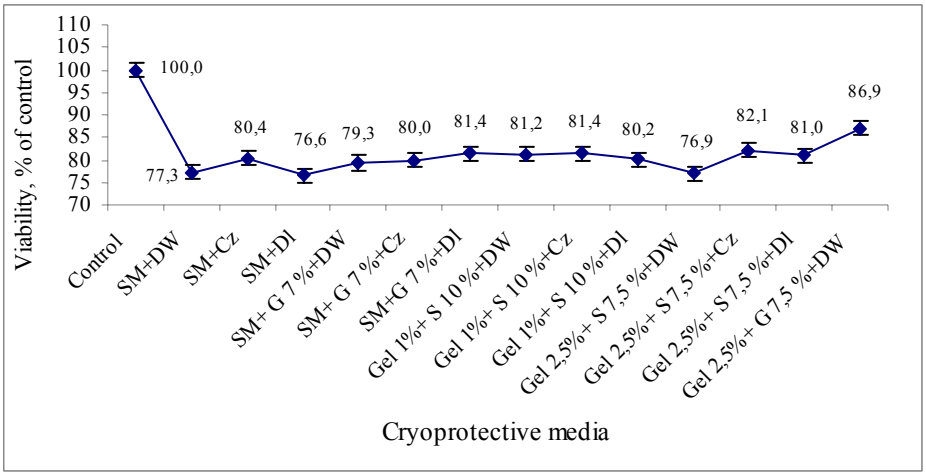

Fig.1. Average viability of species *Streptomyces canosus* (3 strains) before and after lyophilization, in dependence of used protective and rehydration media.

Maximum viability after lyophilization the strains of *Streptomyces canosus* manifest on protective media containing sucrose or glucose and gelatin in various proportions. Significant influence on the viability of these strains had also the used rehydration medium, DW and Cz were the optimal.

The initial strain of *Streptomyces levoris*, as well as its natural variants, showed obvious morbidity on protective media with SM and gelatin-sucrose in comparison with the gelatin-glucose, indifferent of the used rehydration medium. Thus, protective media SM, SM + G 7%, Gel 1% + S 10% and Gel 2,5% + S 7,5%, titer strains after lyophilization significantly decreased on average by 4,5 *log* UFC ml^{-1}, compared with 1-1,1 *log* UFC ml^{-1} on medium Gel 2,5% + G 7,5%. This has conditioned significantly lower viability of strains

of this species on the respective protective media, which reached values of only 65-71%, compared with 89,8 to 90,7% registered on medium Gel 2,5% + G 7,5%. Significant morbidity probably is due to initially very high titer of 13,5-13,8 *log* UFC ml^{-1} [44] and not to the composition of a protective medium (Table 2).

As in case of *S.canosus* species, the species *Streptomyces levoris*, the same two protective media ensure maximum viability of the species, but the effectiveness of medium Gel 2,5% + G 7,5% + DW is superior. This protective medium provides a viability of the species average about 90%, which is with 20,5 to 24,7% more compared with the others (Fig. 2). Thus, the major influence on the viability of strains of *Streptomyces levoris* after lyophilization were established: initially cell titer - at 10-11 *log* UFC ml^{-1} and the medium Gel 2,5% + G 7,5%, rehydration with DW. The highest species viability was of about 90%.

Table 2

Viability of strains *Streptomyces levoris* CNMN-Ac-01, k-1 and 4, after lyophilization on different protective and regeneration media with different rehydration media

Strain					*S. levoris* CNMN-Ac-01		*S. levoris* k-1		*S. levoris* var. 4	
					Titer *log*UFC ml^{-1}	BSR %	Titer *log*UFC ml^{-1}	BSR %	Titer *log*UFC ml^{-1}	BSR %
BL					13,5±0,1	100	13,8±0,1	100	13,8±0,1	100
AL	CM	SM	RM	DW	8,8±0,8	65,3±6,1	9,0±0,2	65,4±1,6	9,1±0,2	65,9±1,0
				Cz	9,3±0,05	69,1±0,6	9,3±0,05	67,1±0,2	9,3±0,06	67,3±0,3
				Dl	9,3±0,7	68,7±4,9	9,4±0,06	68,3±0,6	9,0±0,7	64,9±4,9
		SM+ G. 7 %		DW	8,8±0,8	65,0±5,9	9,1±0,2	65,8±1,0	9,2±0,1	66,6±0,6
				Cz	9,2±0,2	68,4±1,3	9,3±0,05	67,6±0,3	9,4±0,1	67,7±1,0
				Dl	9,2±0,6	68,4±4,7	9,4±0,09	68,0±0,6	9,4±0,1	68,2±1,0
		Gel. 1 % + S. 10 %		DW	8,7±0,8	64,7±5,9	9,3±0,09	67,6±0,5	8,7±0,7	62,6±4,8
				Cz	9,4±0,1	69,5±0,8	9,2±0,2	66,8±1,2	9,3±0,06	67,2±0,6
				Dl	9,5±0,05	70,7±0,2	9,4±0,1	67,8±0,6	9,5±0,05	68,9±0,4
		Gel. 2,5% + S. 7,5 %		DW	9,0±0,1	66,6±1,0	9,3±0,09	67,7±0,4	8,9±0,9	64,1±0,6
				Cz	9,2±0,2	68,0±1,5	9,2±0,2	66,8±1,2	9,3±0,1	67,5±0,6
				Dl	9,6±0,04	71,1±0,1	9,5±0,02	68,6±0,2	9,6±0,01	69,4±0,1
BL					11,0±0,6	100	11,0±0,7	100	11,0±0,7	100
AL	CM	Gel. 2,5% + G. 7,5 %	RM	DW	9,9±0,7	89,8±1,2	10,0±0,7	90,7±0,8	9,9±0,7	90,1±1,6

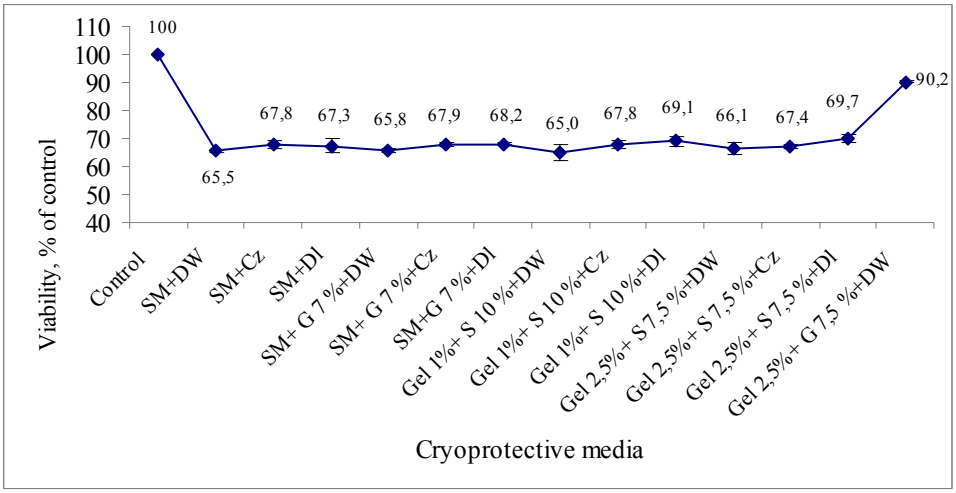

Fig.2. Average viability of species *Streptomyces levoris* (3 strains) before and after lyophilization, in dependence of protective and rehydration media used.

From the studied strains of *S. massasporeus*, the lowest viability had *S. massasporeus* CNMN-Ac-06: 69,3 to 75,7%, corresponding to titer of 7,5 and 6,8 *log* UFC ml^{-1}, respectively. Despite of higher titer registered after lyophilization on all used protective and rehydration media, a maximal viability of this strain, 75,7%, was recorded on the medium Gel 2,5% + G 7,5% rehydrated with DW with lowest titer – 6,8 *log* UFC ml^{-1} (Table 3).

Table 3

Viability of strains *Streptomyces massasporeus* CNMN-Ac-06, Ac-07 and Ac-08, after lyophilization on different protective and regeneration media with different rehydration media

Strain					*S. massasporeus* CNMN-Ac-06		*S. massasporeus* CNMN-Ac-07		*S. massasporeus* CNMN-Ac-08	
					Titer *log*UFC ml^{-1}	BSR %	Titer *log*UFC ml^{-1}	BSR %	Titer *log*UFC ml^{-1}	BSR %
BL					10,8±0,1	100	10,8±0,1	100	10,8±0,1	100
AL	CM	SM	RM	DW	7,5±0,05	70,1±0,7	8,4±0,07	77,8±0,6	8,5±0,04	78,6±0,4
				Cz	8,0±0,04	73,9±0,7	7,9±0,4	73,4±3,1	8,6±0,07	79,8±0,6
				Dl	8,0±0,1	74,4±1,4	8,7±0,03	80,7±0,5	8,4±0,05	77,9±0,4
		SM+ G. 7 %		DW	7,8±0,04	72,9±0,2	8,4±0,07	77,9±0,6	8,5±0,07	78,8±0,8
				Cz	7,9±0,04	73,2±0,2	8,7±0,02	80,2±0,1	8,6±0,05	79,9±0,4
				Dl	8,0±0,1	74,3±1,2	8,6±0,5	79,9±0,7	8,8±0,02	81,1±0,3
		Gel. 1 % + S. 10 %		DW	7,9±0,03	73,4±0,1	8,7±0,05	80,4±0,4	8,4±0,1	77,5±0,9
				Cz	7,9±0,04	73,2±0,4	8,7±0,02	81,0±0,1	8,9±0,01	82,2±0,3
				Dl	8,1±0,02	75,2±0,3	8,8±0,01	81,2±0,2	8,8±0,03	81,1±0,4
		Gel. 2,5% + S. 7,5 %		DW	7,5±0,2	69,3±1,2	8,6±0,06	79,9±0,7	8,6±0,1	79,3±1,5
				Cz	8,1±0,04	75,1±0,7	8,7±0,04	80,9±0,3	8,7±0,06	80,3±0,7
				Dl	7,9±0,04	73,4±0,6	8,8±0,01	81,1±0,2	8,8±0,02	81,4±0,2
BL					8,9±0,7	100	10,4±0,7	100	8,2±0,7	100
AL	CM	Gel. 2,5% + G. 7,5 %	RM	DW	6,8±0,6	75,7±1,4	9,4±0,6	90,8±0,4	6,8±0,7	83,1±2,0

Analysis of the results showed that natural variants of *S. massasporeus* CNMN-Ac-06, *S. massasporeus* CNMN-Ac-07 and *S. massasporeus* CNMN-Ac-08, unlike of the initial strain, had a higher degree of viability: about 73,4 to 90,8% and from 77,5 to 83,1%, detected maximum values for protection medium Gel 2,5% + G 7,5 % and DW, as rehydration medium.

Maximum values of viability of strains *S. massasporeus* CNMN-Ac-07 reactivated on Dulaney and *S. massasporeus* CNMN-Ac-08 reactivated on medium Czapek were obtained for the same protective medium – Gel 1% + S 10%: 81,2% and 82,2% respectively (Table 3).

Analysis of the results presented in Table 3 allowed evaluation of several protective and rehydration media for lyophilization of species *S. massasporeus*, which are composed of gelatin and sucrose or glucose in different proportions, which average viability of the species ranges between 78,6 and 83,2%. The media Gel 1% + S 10% + Dl and Gel 2,5% + G 7,5% + DW, recorded maximum results 79,2 and 83,2%, respectively (Fig. 3).

Thus, the protective media containing gelatin and sucrose or glucose in various concentrations are effective for lyophilization of actinomycetes, and the results confirm the data of literature on the effectiveness undeniable of media protection on maintaining the viability of strains, a major role awarded to the composition of medium and the initial cell density.

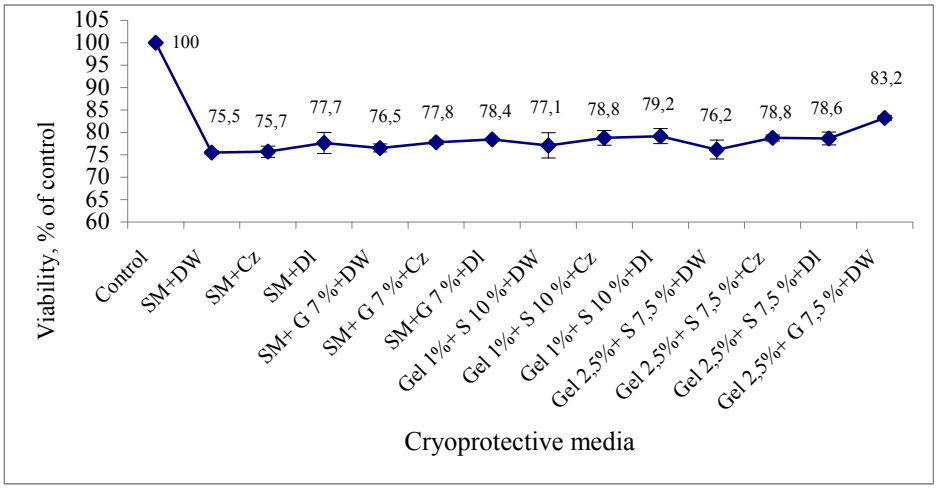

Fig.3. Average viability of species *Streptomyces massasporeus* (3 strains) before and after lyophilization, in dependence of the used protective and rehydration media.

The results of the evaluation of antimicrobial activity of streptomycetes before and after lyophilization (Tables 4 and 5), highlight that the strain *S. massasporeus* CNMN-Ac-06 and its variants attest the maximum antimicrobial activity compared to the other studied cultures. These strains all showed antagonism against test-strains phytopathogenic bacteria and against to 5 of 7 phytopathogenic fungi. The highest retention areas have been registered against pathogens of fusariose – *Fusarium oxysporum* (24,0 mm after lyophilization), tomato bacterial wilt – *Corynebacteriul michiganense* 10_2 (24,0 mm after lyophilization) and rottenness rot and soft of vegetables – *Erwinia carotovora* 8982 (20,0 mm after lyophilization). It has also been reported antimicrobial activity against *Aspergillus niger*, *Penicillium funiculosum* and *Botrytis cinerea*, the growth of which has not been retained by other strains of streptomycetes (Table 4 and 5).

The strain *S. levoris* CNMN-Ac-01 and their variants were less active against *Agrobacterium tumefaciens* 8628, demonstrating no antagonist relations. This strain has pronounced antifungal activity against *Fusarium oxysporum*, which causes fusaria wilting of the plant (retention zone diameter increasing from 19,0 to 30,0 mm) and to *Thieloviopsis basicola*, which causes black rot of plants (ø retaining area growth – 20,0- 27,0 mm).

Analyzing of the antimicrobial activity of *S. canosus* CNMN-Ac-04, allows to conclude that this strain is more active, compared with *S. canosus* CNMN-Ac-02 and *S. canosus* CNMN-Ac-03 (ø 28,0-30,0 mm), retaining grown of *Corynebacteriul michiganense* and showing also a pronounced antibiotic activity against *Aspergillus niger*, *Aspergillus flavus* and *Fusarium oxysporum*.

An increasing of antimicrobial activity against *Agrobacterium tumefaciens* 8628 (9,37 and 3,57%, respectively) was fixed after lyophilization (AL) of *S. canosus* CNMN-Ac-04 and *S. massasporeus* CNMN-Ac-07, while to *Corynebacterium michiganense* 10_2 - 6,25%. Meanwhile, antimicrobial activity against *Erwinia carotovora* 8982 at *S. massasporeus* CNMN-Ac-06 and at *S. levoris* var. 4 decreased after lyophilization by 23,1% and 5,0%, respectively.

Table 4

The antibacterial properties of streptomycetes before and after lyophilization

Strains	Diameter of the growth inhibition zone, mm					
	Corynebacteruim michiganense 10_2		*Agrobacterium tumefaciens* 8628		*Erwinia carotovora* 8982	
	BL	AL	BL	AL	BL	AL
S. canosus CNMN-Ac-02	–	–	–	–	10,0±1,3	10,0±0
S. canosus CNMN-Ac-03	–	–	–	–	13,0±0,7	12,5±0
S. canosus CNMN-Ac-04	28,0±1,1	28,5±0,7	16,0±0,7	**17,5±1,7**	18,0±0,9	17,0±0
S. levoris CNMN-Ac-01	20,0±0,7	20,0±0	–	–	18,0±1,3	18,0±1,3
S. levoris var. K-1	20,5±0,9	20,0±1,1	–	–	20,5±1,3	20,0±1,1
S. levoris var. 4	20,5±1,3	20,0±1,1	–	–	20,0±1,1	19,0±1,1

S. massasporeus CNMN-Ac-06	24,0±0	24,0±1,1	15,0±0,7	15,0±0,9	26,0±0	20,0±0
S. massasporeus CNMN-Ac-07	16,0±0	**17,0±0**	14,0±0	**14,5±0**	24,0±0	23,5±0
S. massasporeus CNMN-Ac-08	17,0±0	16,5±0,7	11,0±1,1	11,0±0	16,0±0	16,0±1,1

An increasing of antifungal activity against *Fusarium oxysporum* was established only after lyophilization (AF) of *S. massasporeus* CNMN-Ac-06 – 14.6%, while the activity of *S. massasporeus* CNMN-Ac-08 against *Alternaria alternata* and *Penicillium funiculosum* was decreased by 12,5 and 14,3%, respectively, at *S. canosus* CNMN-Ac-04 –*Fusarium oxysporum* - with 11,25%, at *S. levoris* var. K-1 against *Fusarium oxysporum* and *Alternaria alternata* – with 5.0 and 9.1%, respectively. Thus, experimental results confirm that streptomycetes possess antimicrobial activity against various bacteria and fungi [38] and emphasizes the possibility of keeping of streptomycetes antimicrobial activity after lyophilization, in proportions of 80-90% from baseline [51].

The results of this research will contribute significantly to streamlining regulation of technological lyophilization of strains of microorganisms of the genus *Streptomyces* kept in NCNM.

Table 5

Antifungal properties of streptomycetes before and after lyophilization

Test-strain	Diameter of the growth inhibition zone, mm					
	S. canosus CNMN-Ac-02		***S. canosus*** CNMN-Ac-03		***S. canosus*** CNMN-Ac-04	
	BL	AL	BL	AL	BL	AL
A. niger	9,0±0	9,0±0	14,0±1,1	14,0±1,1	16,0±0,9	16,0±0,9
A. flavus	–	–	–	–	–	–
P. funiculosum	–	–	–	–	–	–
T. basicola	–	–	–	–	12,0±1,1	12,0±0
F. oxisporum	–	–	12,0±0	12,0±0	**18,0±**1,3	16,0±0,7
A. alternata	–	–	–	–	–	–
B. cinerea	–	–	–	–	–	–
	S. levoris CNMN-Ac-01		***S. levoris* k-1**		*S. levoris* var. 4	
	BL	AL	BL	AL	BL	AL
A. niger	13,0±0,7	13,0±0,9	14,0±0	14,0±1,3	14,0±0,7	14,0±0
A. flavus	20,0±1,1	20,5±1,3	18,0±0	18,0±1,3	14,0±0,7	14,0±0
P. funiculosum	–	–	–	–	–	–
T. basicola	27,0±1,7	27,5±0,9	20,0±0	20,0±0	11,0±1,1	11,0±0
F. oxisporum	30,0±0	30,0±0	**20,0±0**	19,0±0	20,0±0	19,5±0,7
A. alternata	11,0±0	10,5±0,9	**11,0±0**	10,0±0	12,0±1,1	11,5±0,9
B. cinerea	–	–	–	–	–	–
	S. massasporeus CNMN-Ac-06		*S. massasporeus* CNMN-Ac-07		***S. massasporeus*** CNMN-Ac-08	
	BL	AL	BL	AL	BL	AL
A. niger	14,0±0	14,0±1,7	15,0±0,9	14,5±0	–	–
A. flavus	18,0±0	18,0±0	–	–	–	–
P. funiculosum	12,0±0,9	12,0±0	10,0±0,7	9,5±0,9	**12,0±0**	10,5±0,9
T. basicola	24,0±0,9	20,5±0	10,0±1,1	10,0±0	9,5±0,9	9,0±0
F. oxisporum	20,0±0	**24,0±1,1**	17,0±1,1	17,0±0	10,0±0	9,5±0
A. alternata	10,0±0	–	12,0±1,3	12,0±0	**14,0±0**	12,0±0
B. cinerea	14,0±1,1	14,0±1,7	10,0±0	9,0±0	10,0±0	10,0±0

Conclusions

So research results allow concluding the following:

1. Given in attention the sensitivity of different strains of the same species of streptomycetes at lyophilization, significant impact of protective media and initially titer on their viability were established, selecting the optimum parameters for lyophilization, requiring individual approach at the level of species or strain.

2. Lyophilization of streptomycetes strains on effective protective media containing gelatin and sucrose or glucose in different concentrations ensures a maximum viability after lyophilization registered for most strains. The optimal factors were: protective medium gelatin 2,5% + glucose 7,5%, rehydration with distilled water and the initial cell density of 9-11 *log* UFC ml^{-1}.

3. Lyophilization change insignificantly antimicrobial properties of studied streptomycetes strains, coupled with high activity.

Repferences:

1. DOLOTKELDIEVA, T., TOTUBAEVA, N. Biodiversity of *Streptomyces* of high-mountainous ecosystems of Kyrgyzstan and its biotechnological potential. In: *Antonie Van Leeuwenhoek*, 2006, vol.89(3-4), p.325-328.
2. GRIGOREAN, L.N., BATAEVA, Yu.V. Issledovania streptomitsetov v pochvennyh aecosistemah aridnoy zony. V: *Materialy vserossiyskogo simpoziuma s mezhdunarodnym uchastiem «Sovremennye problemy fiziologiy, aecologiy i biotechnologiy microorganizmov»*. Moskva, 2014, s.68. (Ru).
3. JANSO, E.J, CARTER, T.G. Biosynthetic potential of phylogenetically unique endophytic actinomycetes from tropical plants. In: *Applied and environmental microbiology*, 2010, July, p.4377-4386.
4. KRASIL'NIKOV, N.A. *Luchistye griby*. M., 1970. 535 p. (Ru).
5. MERZAEVA, O.V. *Aktinomitsety prikornevoy zony zlakov i klevera*: Avtoreferat na soiskanie uchionoy stepeni kanddata biologicheskih nauk. Moskva, 2007. 24 s. (Ru).
6. BERDY, J. Bioactive microbial metabolites. In: *J. Antibiotics*, 2005, vol.58, p.1-26.
7. EGOROV, N.S. *Osnovy uchenia ob antibiotikach. Opredelenie antibioticheskoy aktivnosti microorganizmov*. Moskva: Nauka, 2004. 155 s. (Ru).
8. HOPWOOD, D. *Streptomyces in nature and medicine*. The Antibiotic Makers. New York: Oxford University Press, 2007. 250 p.
9. MERZAEVA, O.V. Kolonizatsiya aktinomitsetami razlichnych rodov prikornevoy zony rasteniy. V: O.V. Merzaeva, I.G. Shirokich. *Microbiologiya*, 2006, vol.75, №2, s.271-276. (Ru).
10. PALMER, T., HUTCHINGS, M. Protein Secretion in Streptomyces. In: *Streptomyces: Molecular Biology and Biotechnology*, 2011, vol.4, p.87-104.
11. RUDIC, V., POPOVA, N., CRIVOVA, A., BOORTSEVA, S., RASTIMESHINA I. Biosynthesis of lipoxygenase, lipids and fatty acid composition of Actinomycetes and Yeast. In: *Romanian Biotechnological Letters*, 2002, vol.7, p.711-716.
12. MOSIN, V.A., KRUGLEAK E.B., BEREZKINA N.E., NOVIK, T.S., DRINEAEV, V.A. Shtammy actinomitseta *Strepmyces avermitilys* CCM 4697 – produtsent avermictinov. Patent 2156301, Rossiya, MPK[7], C12P 17/18, C12P 17/08, № 98110940/13, Zaeavleno 09.06.98. Opublikovanno. 20.09.00, Biuliten №26. (Ru).
13. SERGEEVA, A.G. Actinomitsety kak produtsenty biologicheski aktivnych veshestv. Doklad Regional'noy Nauchnoy Konferentsii «Diagnostika, prognozirovanie techenia i lechenia kardio-respiratornyh narusheniy v pul'monologii». V: A.G. Sergeeva, N.G. Kimova. *Biuliten' fiziologii i patologii dyhania. Blagoveshensk*, 2006, №22, p.88-90. (Ru).
14. FILIPPOVA, S.N. Mnogoletnee hranenie collectsionnych kul'tur actinobacterii. V: S.N. Filippova, N.A. Surguchiova, V.F. Gal'chenko. *Microbiologiya*, 2012, vol.81, №5, p.682-690. (Ru).
15. ORLOVA, R.S. Prodolzhitel'nost' zhizni i fiziologo-biohimicheskaya activnost' actinomicetov. V: *Trudy Instituta Microbiologii i Virusologii AN Kazahskoy SSR «Technologiya poluchenia productov microsinteza»*, 1988, vol.34, p.147-154. (Ru).
16. POHILENKO, V.D., A.M. Baranov, K.V. Detushev. Metody dlitel'nogo hranenia collectsionnyh cul'tur microorganizmov i tendentsii razvitiya. Izvestiya vyshich uchebnych zavedeniy. V: V.D. Pohilenko, A.M. Baranov, K.V. Detushev. *Povolzhskiy region. Meditsinskie nauki. Obzor literatury*, 2009, vol.12, №4, p.99-121. (Ru).
17. SIDEAKINA, T.M. *Konservatsia microorganismov*. Puschino: ONTI NCBI, 1985. 63 p. (Ru).
18. GOLOVACH, T.N. Vlieanie kriokonservatsii i liofilizatsii na sintez ekzopolisaharida i jiznesposobnost' *Xantomonas campestris pv. campestris* IMB B-7001. V: T.N. Golovach, L.I. Groma. *Microbiologichnyi zhurnal*. 2013, vol.75, №1, p.14-20. (Ru).

19. GURINA, T.M. Standartizatsiya protsessa crioconservirovaniya v collectsiyach i bankach promyshlennych shtammov microorganizmov. V: T.M. Gurina, I.P. Vysekantsev, O.M. Babinets. *Microbiologichnyi zhurnal*, 2013, vol.75, №5, p.33-39. (Ru).
20. KAMENSKIH, T.N. Osobennosti crioconservatsii alkanotrofnyh actinobacterii roda *Rhodococcus*. V: T.N. Kamenskih, E.A. Kalashnikova, I.B. Iwshina. *Vestnik Permskogo Universiteta. Biologiya. Microbiologiya*, 2010, vol.1, №1, p.15-20. (Ru).
21. KUPLETSKAYA, M.B. Zhiznesposobnost' liofilizirovannych microorganizmov posle 50 let chranenia. In: M.B. Kupletskaea, A.I. Netrusov. *Microbiologiya*, 2011, vol.80, №6, p.842-846. (Ru).
22. MORGAN, C.A., HERMAN, N., WHITE, P.A., VESEY, G. Preservation of microorganisms by drying: A review. In: *Journal of Microbiological Methods*, 2006, vol.66, №2, p.183-193.
23. ROTHROCK, M.J., VANOTTI, M.B., SZOGI, A.A., GONZALES, M.C.G., FUJI, T. Long-term preservation of anammox bacteria. In: *Applied Microbiology and Biotechnology*, 2011, vol.92, p.147-157.
24. TSUTSAEVA, A.A. Opyt dolgosrochnogo chranenia promyshlennych shtammov microorganizmov. V: A.A. Tsutsaeva, A.E. Anan'ina, L.M. Balyberdina, L.V. Stepaniuk, N.V. Pavlenko. *Microbiologiya*, 2008, vol.77, №5, p.696-700. (Ru).
25. PORTER, J. N. Cultural conditions for antibiotic-producing microorganisms. In: *Methods in enzymology*. N.Y.: Acad. Press, 1975, vol.43: Antibiotics, p.3-23.
26. SMITH, D. ONIONS, A.H.S. *The preservation and maintenance of living fungi*. Kew (Richmond); Surrey (England): Commonwealth Mycol. Inst. Publ., 1983. 51 p.
27. DONEV, T. *Methods for Conservation of Industrial Microorganisms*. Sofia, 2001. 93 p.
28. UZUNOVA-DONEVA, T., DONEV, T. Anabiosis and conservation of microorganisms. In: *Journal of culture collections*. 2004; 2005, vol.4, p.17-28.
29. FILIPPOVA, S.N. Optimizatsia zashitnyh sred dlea chranenia actinomicetov v zhidkom azote. V: S.N. Filippova, N.A. Surguchiova, V.D. Kuznetsov, G.I. Ael'-Registan, V.F. Gal'chenko. V: *Microbiologiya*, 2007, vol.76, №4, p.573-576. (Ru).
30. TADDEI, A., TREMARIAS, M.M., CAPRILES de, C.H. Viability studies on actinomycetes. In: *Mycopatologia*. 1999, vol.143, p.161-164.
31. BLANKOV, B.I., KLEBANOV, V.L. *Primenenie liofilizatsii v microbiologii*. Moskva: Medgiz, 1961. 282 p. (Ru).
32. POSTOLACHI, O., BURTEVA, S., POSTOLATI, G. Modificarea spectrului aminoacid în biomasa streptomicetelor păstrate timp îndelungat. In: *Transaction on metrology and analytical methods of research*. ASM, Institute of Chemistry. Chișinău, 2010, p.231-237.
33. RIFAAT, H. Viability study of some locally isolated streptomycetes. In: *Journal of culture collections*, 2008-2009, vol.6, p.38-41.
34. IVANITSA, V.O. Rozrobka metodiv zbaeraezhenniya colectsii praktichno korysnich cul'tur microorganizmiv Odaes'kogo Universitetu. V: V.O. Ivanitsa, O.L. Rachimova, G.A. Kozhanova, T.V. Gudzenko i dr. *Tezisy Dokladov Nauchnoy Konferencii Microbni Biotechnologii*, Odesa. 2006, p.8. (Ua).
35. GHERNA, R. *Hranenie microorganizmov* / Pod red. F. Gerhardta I dr. Moskva: Mir, 1983, p.512-534. (Ru).
36. LAPAGE, S.P., SHELTON, J.E., MITCHELL, T.G., MCKENZIE, A.R. *Culture collections and the preservation of bacteria. Methods in Microbiology*. L.: Academic Press, 1970, vol.3A, p.135-228.
37. RUGAL', A.A. Vlieanie saharov na degidratatsiu biopolimernych molekul v protsesse crioconservatsii. A.A. Rugal', V.N. Barvinchenko, N.P. Galagan, I.V. Siora, V.V. Turov. V: *Problemy kriobiologii*, 2007, vol.17, №4, p.374-384. (Ru).
38. SEMENOV, S.M. Sravnitel'naya otsenka nekotorych sred suspendirovania pri liofilizatsii actinomicetov. V: *Antibiotiki*, 1973, vol.18, №11, p.1026-1028. (Ru).
39. TSUTSAEVA, A.A. Vlieanie usloviy hranenia na svoystva criokonservirovannoy i liofilizirovannoy kulitury *Streptomyces fradiae* 25Ab12. V: A.A. Tsutsaeva, A.E. Anan'ina. *Problemy criobiologii*, 2001, №1, p.52-59. (Ru).
40. MEYER, E.D., SINCLAIR, N.A., NAGY, B. Comparison of the survival and metabolic activity of psychrophilic and mesophilic yeasts subjected to freeze-thaw stress. In: *Applied Microbiology*, 1975, June, vol.29, №6, p.739-744.
41. ABADIAS, M., BENABARRE, A., TEIXIDÓ, N., USALL, J., VIÑAS, I. Effect of freeze drying and protectants on viability of the biocontrol yeast *Candida sake*. In: *International Journal Food Microbiology*, 2001, vol.65, №3, p.173-182.
42. COSTA, E., USALL, J., TEIXIDO, N., GARCIA, N., VINAS, I. Effect of protective agents, rehydration media and initial cell concentration on viability of *Pantoea agglomerans* strain CPA-2 subjected to freeze-drying. In: *Journal Applied Microbiology*, 2000, vol.89, p.793-800.
43. KORNIENKO, A.F. Vplyv liofilizatsii i stroku zbaeriganiya zhyttezdatnisti ta biochimichni vlastivosti gruntovych aktinomitsetiv. V: A.F. Kornienko, R.T. Safarova, O.G. Gudkova. *Mikrobiologichniy zhurnal*, 1977, vol.39, №5, p.642-643. (Ua).

44. KUPLETSKAYA, M.B. Metody dlitel'nogo hraneniya collectsii microorganizmov kafedry microbiologii Moskovskogo Gosudarstvennogo Universiteta. In: M.B. Kupletskaya, Z.A. Arcad'eva. *Microbiologiya*, 1997, vol.66, №2, p.283-288. (Ru).
45. MIYAMOTO-SHINOHARA, Y., IMAIZUMI, T., SUKENOBE, J., MURAKAMI, Y., KAWAMURA, S., KOMATSU, Y. Survival rate of microbes after freeze-drying and long-term storage. In: *Cryobiology*, 2000, vol.41, p.251-255.
46. MORGAN, C. Freeze-Drying of Microorganisms. In: *Enciclopedia of Microbiology (Third Edition)*. Academic Press, 2009, p.162-173.
47. GAUZE, G.F., PREOBRAZHENSKAYA, T.P., SVESHNIKOVA, M.A., TERECHOVA L.P., MAXIMOVA, T.S. *Opredelitel' aktinomitsetov*. Moskva: Nauka, 1983, p.24. (Ru).
48. ZENOVA, G.M. *Pochvennye aktinomitsety*. Moskva: izdatel'stvo MGU, 1992, p.87. (Ru).
49. NETRUSOVA, A.I. *Praktikum po microbiologii*. Moskva, 2005. 603 p. (Ru).
50. MUÑOZ-ROJAS, J., BERNAL, P., DUQUE, E., GODOY, P., SEGURA, A., RAMOS, J. Involvement of Cyclopropane Fatty Acids in the Response of *Pseudomonas putida* KT2440 to Freeze-Drying. In: *Applied Environmental Microbiology*, 2006, vol.72, №1, p.472-477.
51. YOCHEVA, L., NAJDENOVA, M., DONCHEVA, D., ANTONOVA-NICOLOVA, S. Influence of the long-term preservation on some biological features of three streptomycetes strains, producers of antibiotic substances. In: *Journal of culture collection*, 2002, vol.3, p.25-32.

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