CHANGES IN THE ANTIMICROBIAL PROPERTIES OF *Streptomyces canosus* CNMN-AC-02 AND ITS VARIANTS DURING LONG-TERM STORAGE BY SUBCULTURING

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Abstract. The paper deals with the research results on changes in antimicrobial activity of strain *Streptomyces canosus* CNMN-Ac-02 and its 3 variants (obtained using γ irradiation, combined (γ and UV) mutagenesis, low-frequency and low intensity magnetic field) after long-term storage (20 years) by subculturing. Antimicrobial activity was determined by the disk diffusion method, as test cultures were chosen opportunistic pathogenic bacteria and fungi which cause crop diseases. Analysis of the results obtained allow us to recommend oat agar medium for long-term storage of the original *S. canosus* CNMN-Ac-02 strain with antibacterial activity against phytopathogenic bacteria, and Czapek medium with glucose to preserve antifungal activity. For *S. canosus* CNMN-Ac-03, the antifungal activity is preserved on Gause medium, and for *S. canosus* CNMN-Ac-04 - on Czapek medium. For variant treated with magnetic field, higher antibacterial activity was registered against *Clavibacter michiganensis* 13[a] on Gause medium, by cca. 11.0 % more than original strain. Also it was noted, that strains synthesize several antibiotics, because of ability to inhibit develop both of phytopathogenic fungi and bacteria.

Key words: *Streptomyces canosus*; antimicrobial activity; subculturing; long-term storage; synthetic media; organic media.

INTRODUCTION

Actinobacteria (*Actinomycetes*) or filamentous bacteria represent an exceptional group of microorganisms, which combine the molecular, chemical and physiological peculiarities of prokaryotes with the morphological characteristics of eukaryotic fungi. Decomposers of organic substances, nitrogen fixers, which are found mainly in the soil, actinobacteria produce a wide range of bioactive substances with antibiotic, antifungal, antitumor, insecticidal and antiparasitic action. The most important characteristic of streptomycetes is their ability to produce secondary metabolites: enzymes, herbicides, anti-cancer remedies, vitamins, immunomodulators, plant growth substances [2, 3, 17, 27].

According to a study of about 20,100 scientific publications on *Streptomyces,* dated 2000–2017, these microorganisms are the richest known sources of bioactive substances. Along with this, due to their versatile metabolism, these bacteria are today the most important suppliers of biocatalytic tools (enzymes) for advanced applications in biotechnology, presenting a major interest in the context of the UN policy agenda "Bioeconomy to 2030" [34].

Among the various forms of relationships of microorganisms in natural habitats, we are interested in antagonistic relationships, which are characterized by the fact that one kind of microorganism in one way or another inhibits the development or inhibition the growth of other microorganisms [13].

According to many researchers, the synthesis of antibiotics by microorganisms during their cultivation in laboratory conditions is not manifested in all organisms. However, under appropriate cultivation conditions, all so-called inactive strains of streptomycetes are able to synthesize antibiotic substances under laboratory conditions [5]. The antibiotics produced by actinobacteria, according to the chemical structure, belong to various groups of compounds: from relatively simple (sarcomycin) to such complex structures as chromopeptides (actinomycin), glycopeptides (bleomycin) [12].

The studies on the use of antibiotics in agriculture, in the fight against phytopathogenic organisms, recommend the biological plant protection products that are more environmentally friendly and harmless than chemically synthesized pesticides [1].

Researches in recent years, in response to the need to provide the biotechnology industry with viable strains of microorganisms with stable biotechnological parameters, is aimed at developing and implementing efficient methods of conservation and preservation of active strains. The problem of preserving microorganisms includes a number of objectives, the most important of which is to detect the optimal conditions for conservation and preservation of microbial cells to avoid their damage [32].

Sustainable and efficient storage of microorganisms depends on the preservation method, parameters and protection media. Various conservation methods are currently known: subculturing, storage under mineral oil; storage in soil; drying; cryopreservation; lyophilization, etc. [10].

Currently, the widely used methods of storage of microorganisms both in laboratory practice and in production are subculturing and lyophilization.

The aim of the research was to study changes in the antimicrobial properties of *S. canosus* CNMN-Ac-02 and its variants after long-term storage by subculturing.

MATERIAL AND METHODS

The strain *Streptomyces canosus* CNMN-Ac-02, stored in the National Collection of Non-Pathogenic

Microorganisms (NCNM) of Institute of Microbiology and Biotechnology, Republic of Moldova, was used in the study. Strain improvement using γ irradiation, combined (γ and UV) mutagenesis, low-frequency and low intensity magnetic field, developed 3 variants.

The mutant *S. canosus* CNMN-Ac-03, was obtained using γ irradiation, performed at Institute of Genetics, Physiology and Plant Protection, Republic of Moldova (installation PXM-γ-20, activity 12750 Ci, capacity 0.67 Gy/s, radioactive ^{60}Co as source of γ-rays, doses of irradiation - 1000, 2000, 3000 and 4000 Gy).

The mutant *S. canosus* CNMN-Ac-04 was obtained by combined γ and UV of 260 nm irradiation, performed at the Faculty of Physics of Moldova State University [31].

The microbial variant *S. canosus* CNMN-Ac-02 (magnetic field), was obtained using low-frequency and low intensity magnetic field (1 - 10Hz / 40-50Ts) treatment of initial strain (cultivated in Petri dish), with an exposure time of 15 minutes. The device, elaborated at "Ghitu Institute of Electronic Engineering and Nanotechnologies", used for this purpose, makes it possible to obtain a low-frequency magnetic field, ecologically safe with parameters close to magnetic fields of the Earth [9, 14, 26].

According to NCNM passports data, strains of *Streptomyces canosus* synthesize lipids, essential and immunoactive amino acids, and principles with antimicrobial activity.

The studied streptomyces strains were subcultured during 20 years, every 3 months using Czapek agar medium ($NaNO_3$, K_2HPO_4, $MgSO_4 \times 7H_2O$, KCl, $FeSO_4$, agar, source of carbon – glucose, pH – 7.0-7.3), Gause agar medium (K_2HPO_4, $MgSO_4$, NaCl, KNO_3, $FeSO_4$, agar, source of carbon – soluble starch, pH – 7.2-7.4) and oat agar (OA) – oatmeal serving as source of carbon, nitrogen, micro and macro elements, agar, pH – 7.2.

Antimicrobial activity was determined by disk diffusion agar method. The strains were subcultured for 2 weeks on mentioned media in thermostat at temperature of 28ºC in Petri dishes for obtaining the lawn culture. During growth the strains synthesizes substances with antimicrobial properties which are diffused in agar medium. Agar blocks of 8 mm were cut out with a sterile cork drill from medium where streptomycete strains grew. Then agar blocks were transferred in prepared holes of nutrient agar medium with recently subcultured test cultures. Petri dishes were maintained in a cold place for 1 hour before incubation to allow the diffusion of antimicrobial substances. The diameter of growth inhibition zone was measured after incubation at 28°C for opportunistic pathogenic fungi and at 37°C for bacteria, respectively. The diameter of the growth inhibition zones was measured on 3rd, 14th and 21st day to assess the persistence of the effect. There were three replications for each test [12].

The opportunistic pathogenic fungi – *Alternaria alternata*, *Aspergillus niger*, *Fusarium solani*; and opportunistic pathogenic bacteria – *Clavibacter michiganensis* 13[a], *Erwinia carotovora* 8982 (renamed *Pectobacterium carotovorum* 8982), *Xanthomonas campestris* 8003[b], were used as test culture for the assessment of antimicrobial activity. Mentioned test cultures preserved in NCNM cause various crop diseases (*A. alternata* causes black spot on numerous crops; *A. niger*, agent of black mold; *F. solani* causes root rot disease; *C. michiganensis*, agent of soft rot disease on tomato; *E. carotovora*, agent of soft rot disease of several crops; *X. campestris*, agent of black rot disease) and are widely spread in Republic of Moldova.

For obtain lawn culture, phytopathogenic fungi grew up on a wort agar of 5.0ºBlg (pH – 5.8-6.0), phytopathogenic bacteria - on a potato agar (pH – 7.0-7.5) [6, 12, 22].

RESULTS

Among the most significant factors affecting the manifestation of the antibiotic properties of microorganisms isolated from natural sources, the composition of the medium is highlighted.

When identifying the potential of microorganisms to synthesize antibiotic substances, the selection of media must be given the most serious attention [18, 23, 24, 30, 33].

During laboratory storage of antagonist streptomyces strains isolated from natural substrates, a significant decrease in antibiotic activity or its complete loss is often observed. Therefore, the determination of changes in antimicrobial activity in the NCNM of streptomycetes was of particular interest in our studies.

Waksman et al. believed that antibiotics are synthesized only when there are nutrients in the medium that are favorable for this process [36]. This position in itself is completely correct and is confirmed by many facts. It is known that for the growth and biosynthesis of an antibiotic, the producer of novobiocin uses glucose, starch, maltose, and organic acids. A good yield of the antibiotic is observed on media containing glucose or starch as carbon sources [19].

Therefore, in our studies, for the subculturing of the studied streptomyces strains, we also selected nutrient media, which included glucose or starch and a number of mineral salts - Czapek medium with glucose and Gause medium with starch, as well as oat agar as a complex organic medium.

Many microorganisms, in the process of their vital activity, are able to simultaneously produce several antibiotic substances. For example, *Streptomyces alblreticuli* produces three antibiotics: iromycin, enteromycin and carbomycin [25]. In the culture of *Streptomyces netropsis*, antifungal polyene antibiotics (a mixture of tetraene, pentaene and heptaene) and an

antibacterial substance are simultaneously synthesized [12].

Determination of antimicrobial activity *S. canosus* CNMN-Ac-02 and its variants were carried out by pre-cultivation them on 3 media – synthetic (Czapek with glucose, Gause with starch) and organic medium OA.

It is also important to consider which carbon source contributes to the synthesis of antibiotics: glucose, maltose starch, or organic acids. So, for example, depending on the carbon source in the medium, *Streptomyces spheroides* produce not only an unequal amount of antibiotics, but also their different variants [12]. This fact was noticed in our study: when *S. canosus* CNMN-Ac-02 was cultivated on Gause medium, the antimicrobial activity was higher in relation to phytopathogenic bacteria than on Czapek medium and lower in relation to phytopathogenic fungi. The same phenomenon was noted in *S. canosus* CNMN-Ac-02 (magnetic field) variant (Tables 1 and 2).

That is, the biosynthetic activity of microorganisms can be controlled by changing the cultivation conditions and, first of all, by the composition of the nutrient medium [12].

Based on the obtained results, it can be seen that the studied streptomyces strains are able to inhibit the growth of not only phytopathogenic bacteria, but also of fungi, so it is most likely that these strains form at least two or more antibiotics, depending on the culture medium.

The growth of phytopathogenic bacteria and fungi was monitored every 2-3 days. At the first examination of Petri dishes with growing test cultures, the development of surface growth was noticed. It was especially active in bacterial strains, while in fungi aerial mycelium was already beginning to form, and one could even notice emerging growth inhibition zones. So, for example, under the influence of *S. canosus* CNMN-Ac-02 metabolites were clearly seen the differences between Czapek and Gause medium (inhibition zones from 7.3 to 19.0 mm). Clear inhibition zones against *X. campestris* 8003[b] test culture (up to 14.0 mm) were also seen in the variant with Gause medium (Tables 1 and 2).

Subsequent observations revealed that *S. canosus* CNMN-Ac-02, grown on organic medium OA produces substances with antibiotic properties to a greater extent than when cultivated on synthetic media. For example, the diameter of *C. michiganensis* 13[a] growth inhibition zones varied in the range of 15.0-18.3 mm under the influence of *S. canosus* CNMN-Ac-02 metabolites, obtained on Czapek and Gause media, and were much larger – 25.0 mm, after the growth on OA medium. The same pattern was observed against *E. carotovora* 8982 and *X. campestris* 8003[b], with inhibition zones of 23.5 mm and 18.0 mm, respectively. It was noticed, that in relation to phytopathogenic fungi, this strain was more active in the case of streptomyces cultivation on Czapek medium, than on Gause or OA medium (fungi growth inhibition zone of 19.7 mm compared to 8.0-13.3 mm in the case of streptomyces cultivation on Gause or OA media) (Tables 1-3).

Table 1. Antimicrobial activity of *S. canosus* CMNN-Ac-02 and its variants after long-term storage by subculturing on Czapek medium

Strain	Measure of zones	The diameter of the growth inhibition zones by strains *Streptomyces* spp., mm					
		A. alternata	*A. niger*	*F. solani*	*C. michiganensis* 13[a]	*E. carotovora* 8982	*X. campestris* 8003[b]
S. canosus CNMN-Ac-02	21 day, March 1998	0	0	0	0	0	C.i.
	3 day, March 2018	19.7±0.7	14.7±0.7	11.7±0.7	8.3±0.7	9.3±0.7	8.0±0
	14 day, March 2018	19.0±0	16.7±0.7	12.3±0.7	15.0±0	18.0±0	8.0±0
	21 day, March 2018	19.0±0	17.0±0	12.0±0	15.0±1.1	17.0±1.1	7.3±0.7
S. canosus CNMN-Ac-03	21 day, March 1998	0	13.7±0.7	12.0±0	0	0	C.i.
	3 day, March 2018	8.3±0.7	7.7±0.7	8.0±1.1	8.0±0	8.0±1.1	8.0±0
	14 day, March 2018	8.0±0	8.0±0	8.0±0	8.0±0	8.0±0	8.0±0
	21 day, March 2018	8.0±0	8.0±0	8.0±0	8.0±0	8.0±0	8.0±0
S. canosus CNMN-Ac-04	21 day, March 1998	0	10.0±0	0	23.0±1.1	14.0±0.7	0
	3 day, March 2018	17.3±0.7	17.0±1.1	9.3±0.7	8.0±0	8.0±0	8.0±0
	14 day, March 2018	20.0±0	20.0±1.1	9.0±0	8.0±0	8.0±0	8.0±0
	21 day, March 2018	18.0±0.7	18.0±1.1	9.0±0	8.0±0	8.0±0	8.0±0
S. canosus CNMN-Ac-02 (magnetic field)	21 day, March 1998	0	0	0	0	0	C.i.
	3 day, March 2018	9.0±0	14.0±0	9.0±0	9.0±0.7	8.0±0	8.0±0
	14 day, March 2018	9.0±0	14.0±0	9.0±0	9.0±0	15.0±1.1	8.0±0
	21 day, March 2018	8.0±0	11.0±0	8.0±0	9.0±0	15.3±0.7	8.0±0

C.i. – complet inhibition

Table 2. Antimicrobial activity of *S. canosus* CMNN-Ac-02 and its variants after long-term storage by subculturing on Gause medium

Strain	Measure of zones	The diameter of the growth inhibition zones by strains *Streptomyces* spp., mm					
		A. alternata	*A. niger*	*F. solani*	*C. michiganensis* 13[a]	*E. carotovora* 8982	*X. campestris* 8003[b]
S. canosus CNMN-Ac-02	21 day, March 1998	0	0	0	0	0	C.i.
	3 day, March 2018	13.0±0	10.0±0	8.0±0	9.3±0.7	10.0±0	14.0±1.1
	14 day, March 2018	13.3±0.7	10.0±0	8.0±0	18.0±1.1	16.0±0	14.3±0.7
	21 day, March 2018	11.0±0	9.0±0	8.0±0	18.3±0.7	16.0±0	14.0±0.7
S. canosus CNMN-Ac-03	21 day, March 1998	0	14.0±0	12.0±0	0	0	C.i.
	3 day, March 2018	10.0±0	10.0±0	8.0±0	8.0±0	8.0±0	9.0±0
	14 day, March 2018	14.0±0	12.0±0	8.0±0	8.0±0	8.0±0	9.0±0
	21 day, March 2018	11.0±0	10.0±0	8.0±0	8.0±0	8.0±0	9.0±0
S. canosus CNMN-Ac-04	21 day, March 1998	0	10.0±0	0	23.0±1.1	14.3±0.7	0
	3 day, March 2018	9.3±0.7	9.3±0.7	8.0±0	8.0±0	8.0±0	8.0±0
	14 day, March 2018	9.0±0	9.3±0.7	8.0±0	8.0±0	8.0±1.1	8.0±0
	21 day, March 2018	8.0±0	8.0±0	8.0±0	8.0±0	8.0±0	8.0±0
S. canosus CNMN-Ac-02 (magnetic field)	21 day, March 1998	0	0	0	0	0	C.i.
	3 day, March 2018	10.0±0	9.0±0	8.0±0	11.0±0	12.0±1.1	12.0±0
	14 day, March 2018	10.0±0	9.0±0	8.0±0	20.0±1.1	18.0±1.1	12.3±0.7
	21 day, March 2018	8.0±0	8.0±0	8.0±0	20.3±0.7	18.0±0	12.3±0.7

C.i. – complet inhibition

Table 3. Antimicrobial activity of *S. canosus* CMNN-Ac-02 and its variants after long-term storage by subculturing on OA medium

Strain	Measure of zones	The diameter of the growth inhibition zones by strains *Streptomyces* spp., mm					
		A. alternata	*A. niger*	*F. solani*	*C. michiganensis* 13[a]	*E. carotovora* 8982	*X. campestris* 8003[b]
S. canosus CNMN-Ac-02	21 day, March 1998	0	0	0	0	0	C.i.
	3 day, March 2018	11.0±0	8.0±0	8.0±0	8.0±0	8.0±0	18.0±0
	14 day, March 2018	11.0±1.1	8.3±0.7	8.0±0	25.0±1.1	23.5±1.1	18.0±0
	21 day, March 2018	8.0±0	8.0±0	8.0±0	25.0±0	23.5±1.1	18.0±0
S. canosus CNMN-Ac-03	21 day, March 1998	0	14.3±0.7	12.3±0.7	0	0	C.i.
	3 day, March 2018	15.0±1.1	9.0±1.1	8.0±1.1	8.0±0	8.0±0	8.0±0
	14 day, March 2018	15.0±0.7	9.0±0	8.0±0	8.0±0	8.0±0	8.0±0
	21 day, March 2018	10.0±0	8.0±0	8.0±0	8.0±0	8.0±0	8.0±0
S. canosus CNMN-Ac-04	21 day, March 1998	0	10.3±0.7	0	23.0±1.1	14.3±0.7	0
	3 day, March 2018	8.0±0	8.0±0	8.0±0	8.0±0	8.0±0	8.0±0
	14 day, March 2018	8.0±0	8.0±0	8.0±0	8.0±0	8.3±0.7	8.0±0
	21 day, March 2018	8.0±1.1	8.0±0	8.0±0	8.0±0	8.0±0	8.0±0
S. canosus CNMN-Ac-02 (magnetic field)	21 day, March 1998	0	0	0	0	0	C.i.
	3 day, March 2018	8.0±0	8.3±0.7	8.0±0	8.0±1.1	8.3±0.7	8.0±1.1
	14 day, March 2018	8.3±0.7	8.0±0	8.0±0	22.0±1.1	23.5±1.1	8.0±0
	21 day, March 2018	8.0±0	8.0±0	8.0±0	22.0±1.1	23.0±1.1	8.0±1.1

C.i. – complet inhibition

Analysis of the ability of *S. canosus* CNMN-Ac-03 and *S. canosus* CNMN-Ac-04 metabolites to inhibit the growth of test cultures, showed the appearance of clear inhibition zones only against phytopathogenic fungi. The growth inhibition of test bacteria by two mentioned streptomyces were practically not noticed (Tables 1-3).

The experiments showed that *S. canosus* CNMN-Ac-03 (the strain obtained after exposure of *S. canosus* CNMN-Ac-02 to γ rays) has practically no ability to inhibit the growth of phytopathogenic bacteria used in our experiments. The metabolites of this strain have the ability to inhibit the growth of *A. niger* (zones up to 12.0 mm in diameter) and *A. alternata* (zones up to 14.0 mm), when cultivated on Gause medium. Inhibition zones up to 15.0 mm against *A. alternata* were registered for *S. canosus* CNMN-Ac-03 metabolites, in the case of Gause and OA media (Tables 1-3).

The metabolites of the *S. canosus* CNMN-Ac-04 strain (obtained under the influence of combined irradiation of γ and UV rays) showed the ability to inhibit the growth of test fungi only (zones up to 20.0 mm against *A. niger* and *A. alternata*), provided in the case of streptomyces cultivation on Czapek medium (Table 1). The metabolites of *S. canosus* CNMN-Ac-04 strain, after its cultivation on Czapek medium, have inhibited the growth of *A. alternata* and *A. niger* (zones up to 20.0 mm).

The microbial variant *S. canosus* CNMN-Ac-02 (magnetic field) showed low antimicrobial activity against the most of phytopathogenes used in our study. A significant antibacterial action was registered only against *C. michiganensis* 13^a, after streptomyces variant cultivation on Gause medium, with inhibition zones higher by cca. 11.0 %, compared to original strain (Tables 1-3).

The monitoring of phytopathogenic cultures after 8, 10, 14 and 21 days, showed clear delimitation of inhibition zones boundaries in the test bacteria. In some variants of fungi, after 10-14 days, the appearance of a young aerial mycelium reaching the agar blocks with streptomycete was observed, but the growth inhibition zone was clearly visible on the background of the dense fungal mycelium grown during the observation period.

After long-term storage of the studied streptomyces strains, by subculturing on the Czapek agar medium, changes in their antimicrobial activity against the selected test cultures could be noted. Verification of the antimicrobial activity of the original strain *S. canosus* CNMN-Ac-02 after 20 years of subculturing showed that, as a result of cultivation on Czapek agar medium, this strain acquired the ability to inhibit the growth of phytopathogenic bacteria (inhibition zones 7.3-17.0 mm) and phytopathogenic fungi (inhibition zones 11.7-19.7 mm), with the exception of the ability to inhibit the growth of *X. campestris* 8003^b, that significantly decreased (minimal zones – 7.3 mm instead the initial complete inhibition). The loss of the ability of complete suppression of *X. campestris* 8003^b was observed as well after the storage of streptomyces strain on the Gause or OA medium, growth inhibition zones from 14.0 to 18.0 mm being noted, instead of completely suppressing this bacterium.

The variant *S. canosus* CNMN-Ac-03 showed the following changes in antimicrobial activity: the ability of the strain to completely suppress the growth of *X. campestris* 8003^b was not noted, with minimal zones of inhibition (8.0 mm), as in the case of another two strains of phytopathogenic bacteria. In relation to *A. niger,* the antifungal activity decreased (from 14.3 to 8.0 mm of the diameter of inhibition zones), while the ability to inhibit the growth of *A. alternata* increased (depending on the culture medium from 8.0 to 15.0 mm).

The antimicrobial activity of *S. canosus* CNMN-Ac-04 has changed, as well, during the storage. Thus, the decrease of ability to inhibit the growth of *C. michiganensis* 13^a (from 23.0 to 8.0 mm) and of *E. carotovora* 8982 (from 14.3 to 8.0 mm) was observed. An increase in activity against *A. niger* (zones from 10.0 to 20.0 mm) and the emergence of the ability to inhibit the growth of *A. alternata* (zones up to 20.0 mm) were noticed only when cultivated on Czapek medium.

DISCUSSION

It is known that bacterial, fungal and viral diseases cause significant damage to agriculture production. Despite the annual expenses on plant disease control, during the epiphytotics, up to 80% yield losses and the deterioration of agricultural products are recorded. Actinobacteria, as the most well-known producers of numerous extracellular enzymes and antibiotics, are also considered as producers of biological control substances against plant diseases around the world [8, 16, 29, 35]. Our study of the ability of streptomycetes isolated from the soils of the Republic of Moldova to inhibit the growth of phytopathogens showed that long-term (20 years) storage of streptomycetes by subculturing mainly reduces the ability to inhibit the growth of phytopathogens. In some cases, the antagonism to phytopathogens persists or changes insignificantly. It is quite likely that natural variants could appear in which antimicrobial activity manifests itself to a greater extent than in the initial strain. Such variants with increased antimicrobial activity it is advisable to isolate and store in a lyophilized form [7].

Analyzing the obtained results, it is necessary to take into account such an important fact that one of the characteristic features of actinobacteria is their great variability. They are more variable than other bacteria or fungi. The slightest deviations in the composition of the medium or in other external conditions cause changes in the growth, development, or in the biochemical activity of the culture of actinobacteria [11]. Krasilnikov also noted that in most cases antibiotic substances are produced only on media with

a certain composition: some species - on complex protein media, others - on simple synthetic media with mineral sources of nitrogen nutrition, and others - can produce antibiotics as on synthetic ones and on complex protein media. That is, in the selection of active producers of substances with antimicrobial properties, the most important is the selection of nutrient media. It is possible to have a very active strain, but not get antibiotics from it if the proper medium is not identified [20].

Comparing the ability of the studied streptomyces strains to maintain activity during subculturing on media of different compositions, it can be noted that long-term storage by mentioned method, significantly changed the level of activity, but however there is a tendency to maintain activity to a greater extent when cultivated the strains on Gause medium and even more evident - on organic medium - oatmeal agar.

Analyzing the results of the study of changes in the antimicrobial activity of streptomycetes stored for a long-term by subculturing on media of different compositions, it can be noted that frequent passages of the initial culture, *S. canosus* CNMN-Ac-02, led to noticeable changes in its antimicrobial properties in relation with phytopathogenic bacteria, especially this manifested itself in the variant of cultivating the strain on OA medium. Strain *S. canosus* CNMN-Ac-04 underwent changes in antifungal activity, which is especially noticeable in the variant of cultivation of this strain on Czapek synthetic medium. The effect of the magnetic field also contributed to some changes in the antimicrobial activity of the strain, which were manifested both on the Gause medium and on OA to a greater extent than on the synthetic Czapek medium. Therefore, our conclusions coincide with the opinion of number of researchers and practitioners working in the field that, considering their high variability, the actinobacteria need monitoring of the state of the culture, systematic check of its activity, periodic separation of the culture not only from extraneous microflora, but also from different variants that are formed in the process of its variability. A systematic work is required for obtaining new more active variants, which can be carried out in two ways: by the method of selection of active forms and a directed change in the initial culture. Great attention should be paid to the search for active strains in nature [4, 21, 28].

Thus, the conducted studies have shown that the composition of the culture medium of antagonist streptomycetes significantly affects their antimicrobial activity. The metabolites produced by them have a strict selectivity in relation with one or another representative of phytopathogens and are capable of influencing the growth of bacteria or fungi to varying degrees.

The analysis of the obtained results allows us to recommend OA medium for long-term storage of the original *S. canosu*s CNMN-Ac-02 strain with antibacterial activity against phytopathogenic bacteria, and Czapek medium with glucose to preserve antifungal activity. Gause medium contributes to the preservation antifungal activity of *S. canosus* CNMN-Ac-03, and Czapek medium is suitable for the storage of *S. canosus* CNMN-Ac-04.

Considering the need to preserve the viability and useful properties of actinobacteria (including their antimicrobial activity), and due to the high level of their genetic instability, in order to avoid the appearance of new natural variants during long-term storage of strains by subculturing, which can cause a change in their antimicrobial activity, the lyophilized form is recommended for the streptomyces strains storage [11, 15].

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