doi: https://doi.org/10.15407/microbiolj82.05.041

CHANGES IN LIPID COMPOSITION OF *STREPTOMYCES MASSASPOREUS* CNMN-AC-06 BIOMASS AFTER LONG-TERM STORAGE

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Aim. *The aim of the research was to determine changes in content and composition of Streptomyces massasporeus CNMN-Ac-06 strain biomass lipid complex during cultivation in various nutrient media after long-term storage.* ***Methods.*** *To obtain the inoculum, S. massasporeus CNMN-Ac-06 strain was cultivated in Dulaney medium. For biomass accumulation, inoculum was cultivated in M-I, SP-I and SP-III. The amount of biomass was determined on the 5th day of culture growth. Intracellular lipids were extracted from streptomycete biomass by Folch method modified in our laboratory. The qualitative and quantitative characteristic of lipid complex composition was determined by thin-layer chromatography. 10 % solution of phosphomolybdic acid in ethanol was used as developer. The quantity of individual lipid fractions was determined by densitometry.* ***Results.*** *The studied strain was cultivated in three nutrient liquid media. Cultivation of the strain in M-I medium increases the biomass yield up to 11.53 g/l. In case of SP-III, on the contrary, at the beginning of research, the biomass yield was higher than after storage. The best result of the synthesis of total lipids was noted after SP-I medium use. Analysis of the quantity of main lipid fractions in the biomass showed that the maximum percentage of phospholipids was 12.15 % after cultivation in SP-I medium. The amount of sterols in biomass was: in M-I medium – 8.96 %, in SP-I medium – 12.15 % and in SP-III medium – 14.17 %. The smallest amount of mono- and diglycerides in the total lipids of the biomass of this strain was observed after cultivation in SP-III medium, sterol esters in SP-I medium, and waxes in M-I medium. The studies shown that the highest amount of biomass of the strain S. massasporeus CNMN-Ac-06 was noted after cultivation in nutrient medium M-I (11.53 g/l), and the maximum percentage of total lipids after cultivation in medium SP-I (15.85 %).* ***Conclusions.*** *The experiments shown that in order to increase the biomass of S. massasporeus CNMN-Ac-06 strain, it is the best to cultivate this microorganism in complex SP-I medium. But, a significant amount of such physiologically important lipid fractions like phospholipids was obtained in SP-I medium and sterols in SP-I and SP-III media.*

Keywords: Streptomyces massasporeus, storage, biomass, lipids, phospholipids, sterols.

A scientific approach to elaboration of balanced nutrient media continues to develop by paying attention to the individual needs of each studied microorganism strain [1]. The balanced nutrient medium ensures accumulation the maximum amount of biomass at a certain speed with minimal residual concentrations of elements [2]. It is known that the conditions of the cultivation of microorganisms have a significant effect on lipogenesis [3]. The content of lipids in the biomass of producer strains depends on substances added to the nutrient medium as a carbon source [4].

Konova et al. (1986) studied the influence of exogenous lipids sources, such as soybean flour, corn flour, corn extract, vegetable oils and plant extracts on the growth and accumulation of biomass and lipids of actinobacteria and fungi, and concluded that the composition of total lipids was a reflection of two processes: *de novo* lipid synthesis and assimilation of exogenous lipids. Researchers also noted the importance of the nutrient medium composition for actinobacteria growing [5].

Actinobacteria during the growth process can be splitted into variants that differ in biochemical and morphological characteristics: resistance against external influences, the ability to synthesize various substances, the ability of xenobiotics and hydrocarbons destruction, and according to needs in nutrients [6].

Actinobacteria as sources of lipids demonstrate a number of advantages over plants and animals. They are able to produce and store a wide variety of lipids. Their productivity per unit volume and energy consumed can be up to 5-6 times more than that of plants, and even more than animals [7].

It is known that for a long time, lipids were given a rather modest role in the cell life as a form of metabolic fuel reserve. Lipids are a large group of substances that play an important role in the life of microorganisms. Lipid synthesis occurs at all stages of the growth of microorganisms.

It was also found that lipids have another important function: they are the main structural components of cell membranes. It has now been established that lipids are the most important biological effectors, regulators, and mediators involved in almost all the most important physiological processes in the body and in biochemical reactions in animal and human cells [8]. According to modern ideas of lipidology about the functional role of lipids, the following directions of their use for biomedical purposes can be noted: transport function; energy; regulation of cell activity; immunological; restoration; emulsifying [9].

The aim of the study was to determine the lipid composition of *Streptomyces massasporeus* CNMN-Ac-06 strain biomass during cultivation in various nutrient media after long-term storage.

Material and methods. An object of the study was *Streptomyces massasporeus* CNMN-Ac-06 strain, isolated from Moldavian soil samples and deposited in the National Collection of Non-Pathogenic Microorganisms of the Institute of Microbiology and Biotechnology, Republic of Moldova. The strain was maintained by subculturing on Czapek medium. To obtain the inoculum, a spore suspension was transferred into a 250-ml flask in Dulaney medium (%): NaCl – 0.5, K_2HPO_4 – 0.2, $CaCl_2$ – 0.04, $ZnSO_4x7H_2O$ – 0.001, $FeSO_4x7H_2O$ – 0.001, $MgSO_4$ – 0.1, $(NH_4)_2HPO_4$ – 0.7, glucose – 2.0, pH 7.0–7.2. The inoculum was grown for 72 hours at 27°C on a shaker (180-200 rpm). Obtained inoculum was transferred into Erlenmeyer flasks in media of the following composition (%):

- medium M-I: corn flour – 2.0, yeast – 0.5, $CaCO_3$ – 0.15, pH 7.0–7.2 [9];
- medium SP-I: corn flour – 2.0, soy flour – 1.0, NaCl – 0.5, $CaCO_3$ – 0.1, pH 7.0–7.2 [10];
- medium SP-III: corn flour – 2.0, soy flour – 1.0, NaCl – 0.5, $CaCO_3$ – 0.1, K_2HPO_4 – 3.0, pH 7.0–7.2.

Cultivation was carried out at 27° C on shaker (180–200 rpm). Biomass was separated from the culture fluid by centrifugation. The amount of biomass was determined on the 5th day of culture growth.

Intracellular lipids were extracted from streptomycete biomass by Folch method, modified in the laboratory [11].

The qualitative and quantitative composition of lipids was determined by thin-layer chromatography on Sorbfil plates in a solvent system: hexane – diethyl ether – glacial acetic acid (73:25:5). 10 % solution of phosphomolybdic acid in ethanol was used as developer. The quantity of individual lipid fractions was determined by densitometric method [12].

Results. Complex or organic media are widely used among the liquid nutrient media proposed for the cultivation of actinobacteria, with flour (soy, corn, wheat, etc.) as the main source of carbon, and various additives (corn extract, baker's yeast, yeast extract) and mineral salts [2]. Despite the large number of compositions of nutrient media for the cultivation of actinobacteria, the questions dealt with the increase of the amount of biomass and reduce its cost and increase the yield of certain metabolites remain relevant [13]. Previously, experiments were conducted examining the relationship among nutrient medium composition and biosynthesis of lipids and individual lipid fractions of the strain *Streptomyces canosus* CNM-89. The composition of the cultivation medium affected the biosynthesis of particular classes of lipids by this strain of streptomycetes. For example, the predominant fraction was the fraction of triglycerides. The amount of phospholipids varied, depending on substances added to the basic medium. To determine the optimal cultivation medium, which allows to obtain biomass with increased amount of lipids, a number of media were tested in which corn flour served as the main carbon source. In order to reduce the cost of complex M medium (40.0 g flour/l medium), in one variant (M-I) the amount of corn flour was halved (20.0 g/l). The best result was obtained with the addition of vegetable oils, especially olive or mustard. Thus, the addition of 0.1 % mustard oil to M-I medium contributed for the accumulation of biomass in an amount similar to medium with 40.0 g/l corn flour [13].

Table 1

The accumulation of biomass and total lipids by *S. massasporeus* CNMN-Ac-06 strain after long term storage in different media with periodic subculturing

Cultivation medium	Absolute dried biomass, g/l		Total lipids, % / absolute dried biomass	
	2006 year	*2019 year*	*2006 year*	*2019 year*
M-I	7.18±1.07	11.53±0.83	4.96±0.90	11.96±0.01
SP-I	12.27±1.19	10.56±1.29	12.81±1.64	15.85±0.01
SP-III	11.93±0.16	9.86±0.49	13.79±0.79	13.52±0.01

Legends: $p<0.05$.

Based on literature data, studies were conducted to determine the accumulation of biomass and total lipids of *S. massasporeus* CNMN-Ac-06 strain after prolonged storage by periodic subculturing (Table 1).

The studied strain was cultivated in three complex liquid media. The results of studies showed that cultivation of this strain on M-I medium after long-term storage by subculturing (13 years) contributes to significant increase the biomass yield (Table 1). In SP-I medium supplemented with 3.0 g of K_2HPO_4 (SP-III), on the contrary, at the beginning of research, the biomass yield was higher than after storage for 13 years (Table 1). The percentage of total lipids of *S. massasporeus* CNMN-Ac-06 strain biomass is bigger after 13 years of storage and after cultivation in SP-I medium then at the beginning of experiment [14].

Table 2

The accumulation of the main lipid fractions in the biomass of *S. massasporeus* CNMN-Ac-06 strain during cultivation in the medium M-I

Experimental period	Phospholipids	Sterols	Triglycerides
2006	14.95±0.10	7.45±0.08	32.62±0.09
2019	6.65±0.29	8.96±0.17	13.02±0.05

Legends: $p<0.05$.

By thin-layer chromatography, it was found that the lipid complex of the studied streptomycete strain includes: phospholipids, sterols, mono- and diglycerides, triglycerides, sterol esters, waxes and non-identified fractions.

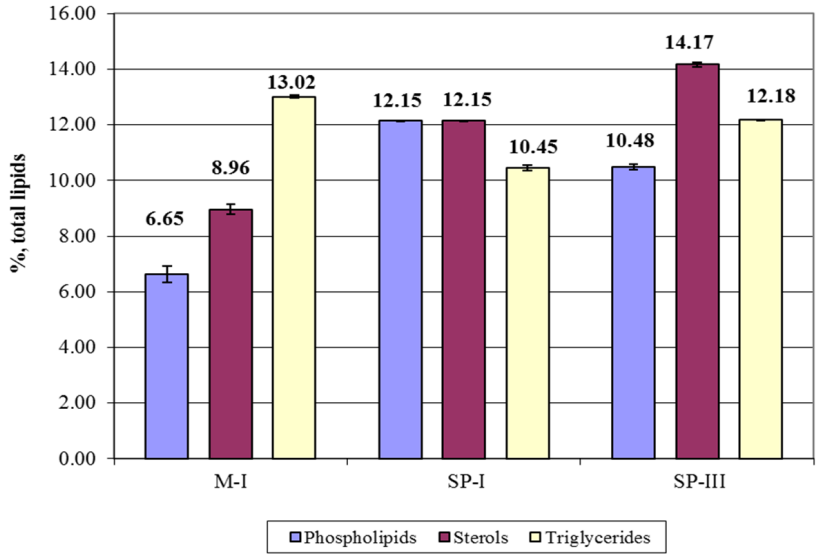
The experiments showed that with a constant qualitative composition of the lipids of streptomycetes cultivated in M-I, SP-I, SP-III media, quantitative changes in the main lipid fractions occurred.

Investigation the ability to synthesize phospholipids and sterols of the studied strain after long-term storage by subculturing and cultivation in M-I nutrient medium showed that the quantity of these physiologically important lipid fractions have changed significantly (Table 2). Analyzing the change in the content of phospholipids fraction in *S. massasporeus* CNMN-Ac-06 lipids, their more than 2 times decrease was found. Amount of triglycerides fraction decreased by 2.5 times after 13 years of storage, while the amount of sterol fraction slightly increased.

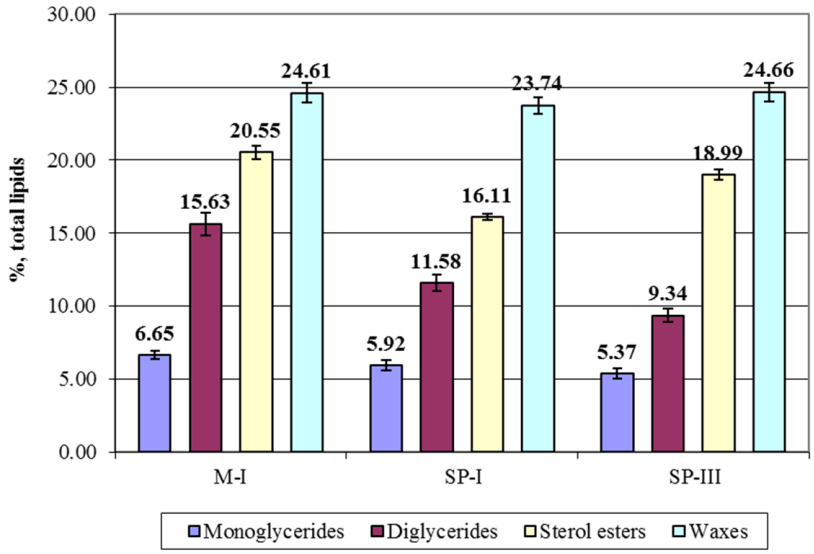
Analysis of the quantity of main lipid fractions in the biomass of *S. massasporeus* CNMN-Ac-06 strain after cultivation in three nutrient liquid media showed that the maximum percentage of phospholipids was registered after cultivation in SP-I medium. The quantity of this important lipid fraction can be increased by cultivating this strain in complex media that additionally contain corn flour and soy flour (Fig. 1). It is necessary to be noted that the amount of another physiological lipid fraction – sterols, can be increased by cultivating strain in SP-I and SP-III media, then the amount of triglycerides during cultivation of the same media decreased.

Analyzing the changes in quantity of secondary lipid fractions of *S. massasporeus* CNMN-Ac-06 strain, we can conclude that, depending on the composition of the cultivation medium, the percentage varies (Fig. 2). The smallest amount of mono- and diglycerides in the total lipids of the biomass of this strain is contained after cultivation in SP-III medium, sterol esters in SP-I medium, and waxes in M-I medium. Quantitative changes

in the biomass accumulation followed by changes in lipid fractions during long-term storage by periodic subculturing are explained by the high heterogeneity of streptomycetes. Long-term storage causes changes in the ratio of the natural variants of the strain resulting from exposure to various environmental factors. Earlier, by literature data were noticed changes in the antimicrobial activity of the studied strains of streptomycetes after prolonged storage by periodic subculturing [15].



F i g. 1. The quantity of main lipid fractions of *S. massasporeus* CNMN-Ac-06 strain after cultivation in different nutrient media (p<0.05)



F i g. 2. The quantity of secondary lipid fractions of *S. massasporeus* CNMN-Ac-06 strain after cultivation in different nutrient media (p<0.05)

Thus, analyzing the ability of streptomycetes to synthesize physiologically active lipid fractions after cultivation in various media, it should be noted that the composition of the nutrient media is important. The studies shown that the largest yield of biomass of *S. massasporeus* CNMN-Ac-06 strain was accumulated after cultivation in nutrient medium M-I (11.53 g/l), and the maximum percentage of total lipids in biomass was detected after cultivation in medium SP-I (15.85 %).

Thus, biosynthetic activity of *S. massasporeus* CNMN-Ac-06 strain is strictly individual and depends on the composition of the cultivation media.

Discussion. It is known that microorganisms, and especially actinobacteria, are easy to change even with conventional storage methods. Moreover, quite often complete or partial loss of antibiotic activity or other properties was observed. The loss of biosynthetic activity was often observed during their cultivation in rich media and with frequent subculturing. A number of methods are currently used to preserve strains – producers of biologically active substances, ensuring their long stay in an active state. These methods are based on the principle of delayed development of microorganisms and the principle of conservation. For each type of microorganism, the most suitable method of conservation, which allows cells to be kept in an active state for a relatively long time should be selected [16].

Considering that lipids are integral components of cell membranes, researchers believe that they, especially phospholipids and sterols, play a significant role in adaptation processes during long-term storage of microorganisms. Changes in the phase-structural organization of the phospholipid fractions of actinobacteria can serve as an indicator of the stability of their membrane structures [17]. So, Coronelli (1996) showed that prolonged storage on a complex organic medium and on a medium with hexadecane led to cell wall rearrangement, a change in the lipid composition of *Rhodococcus erythropolis* actinobacteria cells, which allows cells to adapt to various types of organic substrate [18].

Ability of strains isolated from the soil of Russia, Germany, Argentina, and Japan to synthesize basic lipid fractions as phospholipids, sterols, and triglycerides was studied. It was found that after cultivation in complex medium M-I of *Streptomyces griseus* 15 strain, the proportion of lipid fractions in complex of total lipids was next: phospholipids – 4.2 %, sterols – 4.1 % and triglycerides – 72.8 %; at *Streptomyces griseofavillus* 31: phospholipids – 3.8 %, sterols – 9.1 % and triglycerides – 43.0 %; whereas for *Streptomyces galbus* 1616-Z-3 – phospholipids – 4.4 %, sterols – 5.0 % and triglycerides – 40.7 % [19]. *S. massasporeus* CNMN-Ac-06 strain was isolated from the soil of the central part of the Republic of Moldova. Its lipid fractions were registered in next proportion: phospholipids – 6.65 %, sterols – 8.96 % and triglycerides – 13.02 %. Then we concluded that it biosynthetic activity regarding phospholipids and sterols was higher than that of other strains of streptomycetes. Long-term storage by periodic subculturing led to quantitative changes in biomass, accumulated during cultivation in complex media, as well as to changes in the amount of synthesized lipids and lipid fractions such as phospholipids, sterols, triglycerides. The experiments shown that to increase *S. massasporeus* CNMN-Ac-06 strain biomass, it is better to cultivate it in complex SP-I medium, that it also contributes to an increase in the content of lipids in the biomass, and, most importantly, an increase of physiologically important lipid fractions such as phospholipids and sterols.

The study of changes in the cultural and biochemical properties of streptomycetes after long-term storage revealed the possibility of controlling their productivity when grown in various complex media that increase the accumulation of biomass (by 2.5–3 times) and biologically active substances of different chemical nature and action (antibiotics, plant and animal growth regulators, lipids, including phospholipids and sterols) [20].

ЗМІНИ ЛІПІДНОГО СКЛАДУ БІОМАСИ *STREPTOMYCES MASSASPOREUS* CNMN-AC-06 ПІСЛЯ ТРИВАЛОГО ЗБЕРІГАННЯ

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Резюме

Метою досліджень було визначення змін у змісті і складі ліпідного комплексу міцелію штаму *S. massasporeus* CNMN-Ac-06 при культивуванні на різних поживних середовищах після тривалого зберігання. **Методи.** Для нарощування інокуляту штам *S. massasporeus* CNMN-Ac-06 культивували на середовищі Дюлоне. Для отримання біомаси інокулят додавали в комплексні середовища M-I, SP-I і SP-III. Кількість біомаси визначали на 5-й день росту штаму. Ліпіди екстрагували з біомаси стрептоміцета методом Фолча, модифікованим в лабораторії. Якісний і кількісний склад ліпідів визначали методом тонкошарової хроматографії. Як проявник використовували 10 % етанольний розчин фосфомолібденової кислоти. Кількість окремих ліпідних фракцій визначали денситометрично. **Результати.** Досліджуваний штам культивували на трьох комплексних рідких середовищах. Після тривалого зберігання штаму на середовищі M-I збільшується вихід біомаси до 11,53 г/л. На середовищі SP-III, навпаки, вихід біомаси після зберігання знизився. Найбільший вміст загальних

ліпідів в біомасі відзначено після культивування штаму на середовищі SP-I. Аналіз співвідношення основних ліпідних фракцій показав, що максимальний відсоток фосфоліпідів після культивування штаму на середовищі SP-I становив 12,15 %. При культивуванні на середовищі M-I в ліпідах біомаси штаму кількість стеринів становила 8,96 %, а на середовищах з додаванням соєвого борошна кількість їх збільшувалася до 12,15 % (SP-I) і 14,17 % (SP-III). При аналізі змін у кількісному вмісті вторинних ліпідних фракцій було встановлено, що найменша кількість моно- і дигліцеридів у комплексі загальних ліпідів біомаси цього штаму міститься після культивування на середовищі SP-III, складних ефірів стеринів – на середовищі SP-I, а восків – на середовищі M-I. Дослідження показали, що найбільша кількість біомаси була відзначена після культивування штаму *S. massasporeus* CNMN-Ac-06 на поживному середовищі M-I (11,53 г/л), а максимальна кількість загальних ліпідів – після культивування на середовищі SP-I (15,85 %). **Висновки.** Встановлено, що для збільшення накопичення біомаси штам *S. massasporeus* CNMN-Ac-06 краще культивувати на складному середовищі SP-I. Але значне збільшення вмісту фізіологічно важливих ліпідних фракцій у екстрагованому комплексі загальних ліпідів, таких як фосфоліпіди, було отримано при культивуванні на середовищі SP-I, а стеринів – на середовищах SP-I і SP-III.

Ключові слова: Streptomyces massasporeus, зберігання, біомаса, ліпіди, фосфоліпіди, стерини.

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Received 13.03. 2020