CHANGES IN THE FRACTIONAL COMPOSITION OF LIPIDS OF STREPTOMYCETES BIOMASS CULTIVATED ON COMPLEX MEDIA WITH DIFFERENT NUTRIENTS

Svetlana BOORTSEVA*, Maxim BYRSA*, Yulia BEREZIUK*, Oleg CHISELITSA*, Natalia CHISELITSA*, Vladimir SHEPTITSKII**, Anastasia VASILCHUK*, Alexandru MANCIU***, Nicolai STARCHUK***

*Institute of Microbiology and Biotechnology, Chisinau, Republic of Moldova
**Institute of Physiology and Sanocreatology, Chisinau, Republic of Moldova
***State Agrarian University of Moldova, Chisinau, Republic of Moldova
Corresponding author: Svetlana Boortseva, Institute of Microbiology and Biotechnology, 1 Academiei Str., MD2028, Chişinau, Republica Moldova, phone: 0037322725055, e-mail: burtseva.svetlana@gmail.com

Abstract. Importance of lipids in nature can not be underestimated. It is not only a group of substances which participate in process of metabolism; these substances can be wide using in fields of science and industry. Due to increase cost of animal and vegetal lipids, an alternative can serve microbial lipids. Actinobacteria is the group of microorganisms which is an excellent source of lipids, especially strains of genus *Streptomyces*. In next research changes of fractional composition of lipids of *Streptomyces* spp. cultivated on complex media with different nutrients were studied. *Streptomyces* spp. were isolated from soil samples of Republic of Moldova, cultivation was realized on complex media M-I and R. Maximal quantity of lipids in biomass were obtained after cultivation of strains on medium M-I (12.1-14.3%) and on medium R (12.27-18.69%). The most important fraction as sterols achieved following results: after cultivation on medium M-I it varied between 8.3-14.6% and on medium R – 8.7-10.98%. For fraction of phospholipids were fixed next data: after cultivation on medium M-I, the quantity was 9.1-14.3% and on medium R – 8.7-17.16%. Obtained results show perspective of application of bioproducts based on streptomycetes lipids in agriculture and husbandry due to sufficient quantity of total lipids and some fractions apart.

Keywords: streptomycetes; lipids; fractional composition; medium; nutrients; phospholipids; sterols.

INTRODUCTION

Industrial bioprocesses require the use of complex media containing carbohydrates and oils as sources of carbon. Oils added to the medium can increase the number of antibiotics synthesized by micromycetes, in particular by streptomycetes, and can become a cheap alternative to carbohydrates as sources of carbon [29]. In addition, it is believed that for creation of technology for obtaining new drugs, an important stage is not only the selection of the original producer, but also the elaboration of cultivation conditions that ensure its high biosynthetic activity. For synthesis of biologically active substances, the composition of the cultivation medium and the balance of nutrients in it are important. Therefore, researchers are optimizing the media, dividing its nutrients in 2 groups:

1 – by basic source of macronutrients (carbon, nitrogen, phosphorus) can be used: flour (soybean, maize, wheat, etc.), salts of nitric and phosphoric acids, sugars, starch, glycerin, peptone, amino acids;

2 – by mineral composition (vary of micro and macro elements) [51].

Many industrial bioprocesses require the use of complex media [1, 15, 31, 32, 34].

Sources of nitrogen affect the production of antibiotics by actinobacteria. So, for example, Li Liangzhi et al. (2007) [32] studied the effect of peptone, glutamic acid and asparamide on the synthesis of streptolidine. It result, after application of them, 2 antibiotic analogues were detected, and during using soy flour, 3 analogues of this antibiotic were detected. Experimentally, the authors proved that the best sources of nitrogen for the production of this antibiotic are glutamic acid and peptone. Typically, in media for the cultivation of microorganisms, nitrogen is in oxidized or reduced forms. Sources of nitrogen are salts of nitric acid, seldom nitrous acid, ammonium salts, or amino acids, products of pyrolysis of proteins (peptones, hydrolysates). In organic media with undetermined composition, which include soybean flour, maize flour and maize extract, nitrogen is a main part of proteins. Nutritional value of these media depends on the capacity of microorganisms to assimilate these proteins [14, 24].

According to the literature, it can be seen that the concept of the role of lipids has greatly expanded in recent decades. It is suppose that lipids are not only reserve substances, but they perform a barrier function, participate in membrane transport, in reactions catalyzed by membrane enzymes, excitation and stimulation processes, thermo sensor reactions, etc. [37, 46, 52]. In the literature of the last years of the 20th century and the beginning of the 21st century, it is increasingly possible to find reports on the development of new therapeutic and prophylactic products, which necessarily include phospholipids that possess hypolipidemic and hypocholesteroleic properties of antioxidants. Such products can be used in the treatment of patients with diabetes mellitus, strengthen intestinal motility, and also are able to inactivate heavy metals [10, 11, 20, 25, 33]. There is a generalized significant factual material on the production and use of microbial sterols; ways to increase the efficiency of ergosterol production are considered. In addition, data are given indicating the expediency of using sterols of microorganisms for chemical or biological transformation into vitamins of group D and androstane hormones [23]. The technology of obtaining active preparations using entomophthorales fungi – mycoafidine and entomophtorin has been developed. It is suggested that in the processes of pathogenesis both the spores of entomophthorales fungi and their metabolites, presumably of a lipid nature, play an active role [38]. Taking into account the physiological role of eicosanoids, sterols and other lipid compounds, which constitute up to one third of the cellular content, their active role in the entomopathogenic effect is assumed.

Of definite practical and theoretical interest are reports of the role of triglycerides of microbial origin as precursors of phospholipids and their comparison with vegetable oils [30, 52].

The most significant is the development of biotechnology for the preparation of pharmacologically active lipids containing arachidonic and eicosapentaenoic acids, which have a wide range of biological activity, in particular, antimicrobial properties, by developing medical, veterinary and cosmetic preparations [12, 21, 28, 35, 39, 45, 47].

Interest in lipids is associated with a special role in a number of vital cellular processes – the transfer of information, the secretion of metabolites, enzymes and hormones, proliferation, resistance to stress, and also in connection with practical interest as to medicinal preparations [18]. Lipids are important structural elements of biological membranes and determine many of their functional features. In the structure of lipids, the possibility of obtaining bioregulators is laid. Thus, the polyene fatty acids (γ-linolenic, arachidonic, eicosapentaenoic) present in the form of acyl residues in the molecule of phospholipids and may under certain conditions be transformed into prostaglandin which was involved in the regulation of more than 40 biological processes and exhibit therapeutic effects in many diseases [17]. Preparations based on microbial lipids (lower fungi, streptomycetes, bacteria) can be used to reduce cholesterol as anticarcinogens, act as chaperones to stress conditions, for the diagnosis of tuberculosis, diphtheria and others [2, 36, 38, 40, 48, 50].

To synthesize hormonal drugs, actinobacteria are used as effective biocatalysts for many processes of bioconversion of steroids [13, 19, 26, 38, 44].

MATERIAL AND METHODS

In studies were involved strains of genus *Streptomyces* isolated from soil samples with the different content of humus (2.4-6.8 %) from central part of Republic of Moldova. These strains were isolated from the soils of following localities: *Streptomyces* spp. 19 – Bacioi village (Botanica zone, Chisinau Municipality), black earth carbonate, (humus 2.4-2.5 %) under monoculture maize; *Streptomyces* spp. 33, 36, 47, 49, 66 – Truseni village (Chisinau Municipality), typical black earth, (humus 2.6 %); *Streptomyces* sp. 205 – Singera (Botanica zone, Chisinau Municipality), black earth polluted with pesticides.

In laboratory conditions, strains were kept on agar media Czapek (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, $FeSO_4$ – 0.01 g/l, agar – 20.0 g/l, pH=7.0-7.3), Gause (soluble starch – 20.0 g/l, KNO_3 – 1.0 g/l, K_2HPO_4 – 0.5 g/l, $MgSO_4$ – 0.5 g/l, NaCl – 0.5 g/l, $FeSO_4$ – 0.01 g/l, agar – 20.0 g/l, pH=7.2-7.4) and oatmeal agar (oatmeal flour – 40.0 g/l, agar – 20.0 g/l, pH=7.2) [12, 14].

Inoculum was cultivated on liquid mineral media Dulaney (glucose – 20.0 g/l, $(NH_4)_2HPO_4$ – 7.5 g/l, NaCl – 5 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), in Erlenmeyer flasks of 200 ml during 3 days at 27ºC on the stirrer [49].

To obtain the biomass, inoculum in an amount of 8 % was added to the flasks with liquid complex media M-I (maize meal – 20.0 g/l, baker's yeast – 5.0 g/l, $CaCO_3$ – 1.5 g/l, pH=7.0) and R (maize meal – 20.0 g/l, soluble starch – 15.0 g/l, NH_4NO_3 – 7.0 g/l, $CaCO_3$ – 5.0 g/l, NaCl – 3.0 g/l, KH_2PO_4 – 0.2 g/l, pH=6.8-7.0) of 200 ml for 5 days at 27°C on the agitator [3].

For determination the quantity of biomass, it was been separated of cultural liquid on a centrifuge (5000 r/min. during 20 min.). Quantity of absolute dry biomass was determined by weight method [7, 27].

The intracellular lipids were extracted from biomass by Folch method, modified in the laboratory (main modification is substitution of methanol with ethanol):

- wet biomass was diluted with system of solvents chloroform + ethanol (ratio 1:5) on stirrer for 20 min.;
- addition of chloroform to the system, for obtaining chloroform + ethanol in ratio 2:1 and agitation for 20 min.;
- separation of dissolved lipids in system chloroform + ethanol from biomass via filtration;
- separation of lipid fractions dissolved in chloroform from ethanol fractions with separating funnel by using distillate water (fivefold) and filtration through anhydrous Na_2SO_4;
- removal of lipid fractions on rotary evaporator from chloroform;
- quantity of total lipids was determined by weight method, expressed as %/quantity of absolute dry biomass [8].

Fractional composition of the lipids was determined by thin layer chromatography with „Sorbfil" plates (100x150 mm), in the solvent mixture hexane - diethyl ether - glacial acetic acid system (73:25:5), the quantity of each lipid fraction was determined by the method of densitometry [8, 9].

RESULTS

It is known, when identifying the potential of microorganisms to produce biologically active substances, the selection of nutrient media is given the most serious attention. The main reason for the attention of researchers to use maize flour and starch as a nutrient base and source of carbon is their availability, unique chemical composition, biological value and low cost [6]. Earlier, was found that during cultivation, streptomycetes isolated from soil of Republic of Moldova on complex media produce a larger amount of biomass than on synthetic media [5]. Were no significant differences observed in the amount of biomass obtained after cultivation on M-I and R media, except for strains *S.* sp. 33 in which the biomass was lower on M-I medium than on R medium (by 30.91 %) and *S.* sp. 47 in which the biomass was lower on R medium than on M-I medium (by 62.25 %).

Table 1. Quantity of absolute dry biomass after cultivation on complex media, g/l

Medium / *Streptomyces* sp.	M-I	R
19	14.15±0.21	13.92±0.16
33	7.24±0.56	10.48±0.13
36	12.1±0.12	12.27±0.24
47	9.35±0.49	3.53±0.04
49	6.64±0.42	7.93±0.53
66	19.6±0.07	18.72±0.14
205	4.2±0.11	3.85±0.09

The amount of lipids in the biomass of the studied strains (table 2) was also almost the same for *S.* spp. 19 and 66. For *S.* sp. 47 the amount of lipids in biomass obtained on medium R was more by 30.69 % than on medium M-I, and for *S.* sp. 205 the largest quantity of lipids in biomass was obtained after cultivation on medium M-I than on medium R (by 32 %).

Table 2. Quantity of total lipids after cultivation on complex media, %/absolute dry biomass

Medium / *Streptomyces* sp.	M-I	R
19	12.1±0.27	12.57±0.63
33	6.32±0.38	8.73±0.25
36	5.9±0.44	12.81±0.32
47	14.3±0.3	18.69±0.87
49	13.5±0.17	11.4±0.5
66	6.74±0.48	7.11±0.43
205	2.2±0.15	1.5±0.26

The study of the qualitative composition of the lipid fractions by thin layer chromatography was made. The lipids of *S.* spp. 19 and 47 had a fraction of free fatty acids. Opposite for strain *S.* sp. 205, isolated from soil polluted with pesticides, where was not found a fraction of free fatty acids and an unidentified fraction, which, as a rule, are part of the lipids of soil streptomycetes [9]. For strains of *S.* sp. 19, 36 and 47 fractions of mono and diglycerides on chromatograms were visible as a single spot, but in the case of strains *S.* spp. 66 and 205 were on chromatograms as separate spots of larger sizes.

As can be seen in figure 1 and 2, the amount of physiologically important fraction of phospholipids in the studied strains varied on both media from 8.7 to 17.16 %. The greatest amount of phospholipids was found in strains *S.* spp. 19, 47 and 205 after cultivation on medium M-I (13.78-14.3 %), and for strains *S.* spp. 36, 47, 66 and 205 on medium R (12.2-17.16 %). Exception are strains *S.* spp. 47 and 205, in which the maximal amount of phospholipids was practically the same in biomass lipids obtained after growing on both media M-I (14.0 and 14.3 %, respectively) and on medium R (17.16 and 16.5 %, respectively).

For other physiologically important lipid fraction – sterols (figure 1), it is necessary to note the following: the largest amount of this lipid fraction – 11.27-14.6 % was found in the lipids of strains *S.* spp. 19 and 66, the maximal being seen in lipids of strain *S.* sp. 66 after growth on medium M-I (14.6 %).

Amount of triglyceride fraction in strains of *S.* spp. 19, 47, and 66 was low (15.02-22.0 %) and slightly larger in the lipids of strain *S.* sp. 205 (23.8 %), in the case of using medium M-I. Maximal quantity of this fraction was observed in the lipids of strain *S.* sp. 36 after cultivation of this on both media M-I and R – 53.8-55.1 % (figure 1 and 2).

By comparison the quantity of fractions of mono and diglycerides in the studied strains, the following was observed:

- according to figure 3, strains *S.* spp. 19 and 36 have low amounts of mono- and diglycerides after cultivation on medium M-I (5.76 and 6.3 %, respectively);
- according to figure 4, strains *S.* spp. 19, 36 and 47 have low amounts of mono- and diglycerides after cultivation on medium R (6.84, 9.7 and 6.43 %, respectively);
- maximal quantity of mono- and diglycerides was noted in lipids of strain *S.* sp. 33 cultivated on both nutritive media M-I and R (26.7 and 21.8 %, respectively).

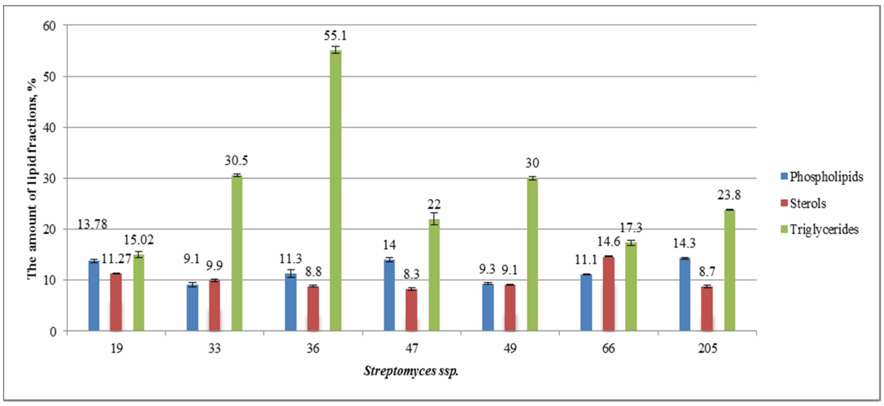

Figure 1. The amount of main lipid fractions of *Streptomyces* spp. after cultivation on medium M-I, %/total lipids

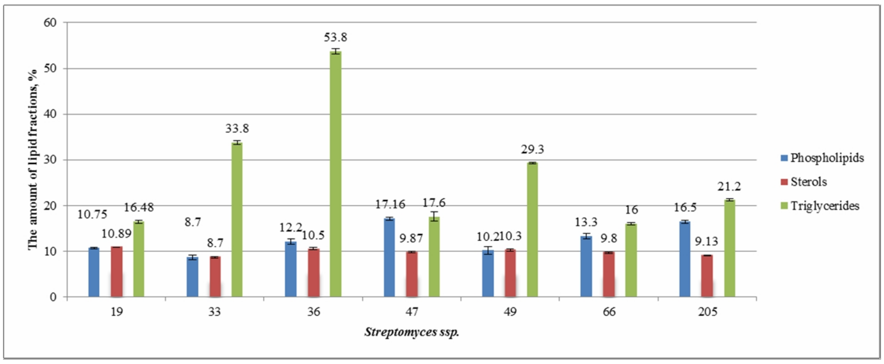

Figure 2. The amount of main lipid fractions of *Streptomyces* spp. after cultivation on medium R, %/total lipids

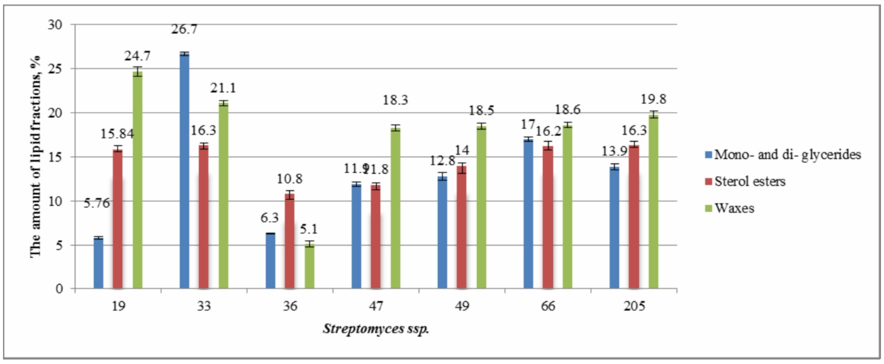

Figure 3. The amount of secondary lipid fractions of *Streptomyces* ssp. after cultivation on medium M-I, %/total lipids

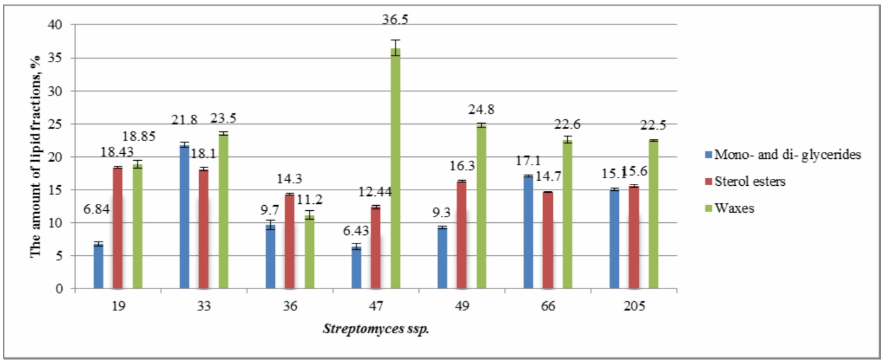

Figure 4. The amount of secondary lipid fractions of *Streptomyces* ssp. after cultivation on medium R, %/total lipids

By the amount of the fraction of sterol esters, it should be noted that in strain *S.* sp. 19 characterized by high degree of sterols synthesis, the number of sterol esters was 18.43 % (figure 4).

For the waxes fraction, it can be concluded that the streptomycetes studied contain more of this fraction in lipids of the biomass grown on organic media. Thus, in the strain *S.* sp. 19, waxes are 18.85-24.7 % in lipids of biomass obtained on M-I and R media, and only 10.9-11.5 % on the Czapek and Dulaney media. For *S.* sp. 47 the quantity of waxes varied between 18.3-36.5 %, but for *S.* sp. 66 – 18.6-22.6 % on complex media. The least amount of waxes was found in the lipids of *S.* sp. 36 – 5.1 and 11.2 % after cultivation on both media M-I and R, respectively (figure 3 and 4).

As it was previously mentioned, in lipids of all studied strains, an unidentified fraction was located on chromatograms between the spot corresponding to phospholipids and a spot corresponding to monoglycerides, with the exception of the strain *S.* sp. 205, in which this fraction was absent. This strain also did not reveal a fraction of free fatty acids, which was recorded on chromatograms of *S.* spp. 19 and 47 in the amount of 8.11-12.43 % after cultivation of these strains on both media M-I and R.

Studies which deals with change in the composition of lipids of streptomycetes isolated from the soil of Republic of Moldova, cultivated on complex media (containing flour, starch, mineral salts, etc.) have shown that the qualitative composition of the lipids of the strains studied is not the same:

- a fraction of free fatty acids was not found in strains *S.* spp. 36, 66 and 205, also for strain *S.* sp. 205 lacks an unidentified fraction;
- the amount of phospholipids is more after cultivation on medium R for 4 strains of streptomycetes out of 7 studied (with the exception of strain *S.* sp. 19);

- the amount of sterols is more during cultivation of 3 streptomycetes out of 7 strains on medium R (except for strains *S.* spp. 19 and 66);
- the amount of fractions of mono- and diglycerides are more during cultivation of 4 strains of streptomycetes out of 7 studied on medium R (except for *S.* sp. 47 – 6.43 %);
- the amount of triglycerides fraction is more in 4 strain of streptomycetes out of 7 studied after cultivation on M-I medium (except for *S.* sp. 19 – 15.02 %).

Because the amount of biomass produced by streptomycetes is larger after cultivation on medium M-I, the total lipids are slightly larger in the biomass of some strains after use of medium R: for obtain biomass containing lipids with the largest number of physiologically important fractions such as phospholipids and sterols, strain *S.* sp. 19 should be cultivated on medium M-I, and strains *S.* spp. 36, 47, 66 and 205 on the complex medium R.

All studied strains were found except for strain *S.* sp. 205 unidentified fraction, which was located in chromatogram between phospholipids and monoglycerides fraction, the amount varied from 4.7 % of strain *S.* sp. 19 (on medium M-I) to 12.3 % of strain *S.* sp. 47 (on medium R).

It should be noted that lipids of strains studied have an appreciable amount such as a fraction of the sterol esters and waxes. For all strains except *S.* sp. 36, waxes fraction in % was higher than the sterol esters and more of them were found in the lipids after cultivation on complex media, except of strain *S.* sp. 205, in which this fraction was not much more after cultivation on synthetic media Czapek and Dulaney. This strain had amount of sterol esters greater in total lipids of biomass obtained on synthetic media.

DISCUSSION

It is known that the medium for the cultivation of microorganisms should include a certain qualitative and quantitative composition of the components or individual elements necessary for the constructive and energy metabolism of the organism (sources of nitrogen, carbon, phosphorus, a number of microelements, vitamins, and growth substances). In this case, the qualitative characteristics of the individual components of the medium are important. This is due to the fact that the presence in the medium of some forms of nitrogen and carbon sources, their ratios, provokes the organism to react differently to them depending on the presence of certain enzyme systems and their activity in the microorganism, to release the silent genes responsible for biosynthesis of substances, definitely directs the metabolic reaction. This helps to identify the potential properties of microorganisms, or, conversely, inhibits the formation of a particular substance [6].

The results of the studies showed that when using 5 different nutrient media, the biosynthetic activity (biomass, protein and lipid synthesis) in the *S. canosus* CNMN-Ac-04 strain, which had a greater antimicrobial activity against bacteria and fungi than the original culture *S. canosus* CNMN-Ac-02, was not the same. To increase the production of biomass and lipids, the author recommended conducting cultivation on organic medium containing soybean flour [43]. The use of medium containing maize and soybean flour (medium SR-I) promoted the stimulation of biomass accumulation and the process of lipid synthesis in *S. massasporeus* CNMN-Ac-06 by 1.7 and 2.6 times, as well as an increase in the amount of the fraction of phospholipids by 21.3-56.8 % and sterols by 10.5-28.4 %, while the addition in the medium contained maize flour of soybean flour did not caused changes in the amount of triglycerides in lipids [43].

In their experiments, Ukrainian researchers showed a direct relationship between the accumulation in the biomass of antibiotics avermectins and lipid fractions such as phospholipids, sterols and triglycerides [41]. For strain *S. nastri* YG62, a positive correlation between triglycerides, phospholipids and the amount of antibiotic synthesized was also established [16]. Results of the present article coincide with the results obtained by the Ukrainian scientists in studying the qualitative composition of streptomycetes lipids. Phospholipids, mono-, di-, triglycerides, sterols, free fatty acids, sterol esters, waxes were found in the lipids of the strains which they studied [4]. Earlier, we also showed that the amount of biomass from the soil of Republic of Moldova grown on a complex maize medium varied from 12.1 to 17.7 g/l, the lipids in the biomass of these strains were from 7.0 to 27.0 %. However, the amount of phospholipids was 4.4-11.3 %, and sterols from 5.2 to 15.7 %, whereas the study of the biosynthetic activity of new strains isolated from samples of black earth with different humus content in the central part of Republic of Moldova, strains of streptomycetes are capable to accumulate biomass up to 18.72 and 19.6 g/l (strain *S.* sp. 66) during cultivation on organic complex media M-I and R. Was found strain *S.* sp. 47, in which the amount of lipids is 18.69 % in biomass grown on a complex organic medium R. New strains also differ in the amount of phospholipids. So, for *S.* sp. 19 their share in the total lipids was 13.78 %, for *S.* sp. 47 – 14.0 %, and for *S.* sp. 205 – 14.3 %, after cultivation on medium M-I. Cultivation on another complex medium R, showed next results in amount of phospholipids: *S.* sp. 47 – 17.16 % and *S.* sp. 205 – 16.5 %.

According to obtained results by us on the study of the role of lipids in the protection of spermatozoa from the negative effect of low temperatures, which showed that the total lipids of actinobacteria are most effective for increasing the cryoresistance of the rams spermatozoa, which served as the basis for developing the LOLA medium for freezing sperm of rams. It has been established by experiments that this medium increases the productivity of artificial insemination of sheep by 24.95 % [22]. In another series of our experiments it was found that adding to the ration feed of chickens the biomass of *S. canosus* CNMN-Ac-03 with high content of total lipids and phospholipids caused an increase in the weight of chickens: on 15 day by 18-25 % and on 45 day by 32.55-38.8 % [42]. In addition, the biomass of *S. canosus* CNMN-Ac-04 can also be recommended for the prophylaxis of infectious diseases of bees (American foulbrood and European foulbrood) during the spring-summer period. Thus, the conducted studies have shown the prospects of using strains of streptomycetes isolated from the soil of Republic of Moldova in husbandry and veterinary medicine, due to their ability to accumulate in sufficient quantities biomass during cultivation on complex media with an increased content of lipids in it, and, most importantly, such physiologically active lipid fractions as phospholipids and sterols.

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