QUALITATIVE AND QUANTITATIVE COMPOSITION OF LIPIDS OF BIOMASS OF STREPTOMYCETES AFTER CULTIVATION ON MEDIA WITH DIFFERENT COMPOSITION

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Abstract. The growing of biomass, lipid synthesis and lipid fractions of streptomycetes isolated from soils of Republic of Moldova was studied. Strains of streptomycetes were cultivated on four media: complex media (M-I and R – main source of Carbon and Nitrogen – corn flour and starch) and synthetics classic media (Czapek and Dulaney). After cultivation for 5 days at 27°C on agitator, it was found that the amount of biomass after cultivation on a complex media, varies from 3.5 to 19.6 g/l, and less after cultivation on synthetic media – 2.26-6.32 g/l. The amount of lipids on the complex medium is from 1.5% to 18.69%, and on synthetic media – 3.12-12.6%.The qualitative composition of lipids in the studied strains of streptomycetes was almost identical: the main lipid fraction were submitted by phospholipids, sterols, mono-di and tri-glycerides, sterol esters and waxes. Strains *S. ssp.* 12, 19 and 36, the amount of phospholipids was more after cultivation on synthetic media (12.6-15.4%) and at *S. ssp.* 47, 66 and 205 - on complex media (11.1-17.16%), the amount sterols changed, depending on the strain and nutritive medium from 7.3 to 14.6%, triglycerides from 16.48 to 55.1%, sterols esters – 10.8-20.0%, waxes – 5.1-36.5%, free fatty acids from 8.11 to 18.8 %. Of greatest interest are strains *S. ssp.* 19, 36, 47 and 66 constituting the largest amount of biomass after cultivation on complex media (12.1-19.6%), wherein the amount of lipids is from 5.9 to 18.7% and the physiologically active lipid fraction (phospholipids, sterols) - 10.75-17.2% and 9.8-14.6%, respectively. Indicators allow us to consider the strains as the basis of new biological drug for animal husbandry.

Keywords: Streptomycetes; medium for cultivation; biomass; lipids; lipid fractions.

INTRODUCTION

Lipids - a large group of substances that are important in the life of microorganisms. The study of lipids started in the 19th century, when it was discovered that many yeasts form a large amount of lipids. Fungi are also intensively studied from the point of view of the commercial production of lipids, as found that they contain fatty acid molecules of C10 to C26. The formation of lipid occurs at all stages of growth of the microorganism, but most intensely - during the logarithmic growth phase. Most modern production of lipids is biphasic: 1 - accumulation of biomass producer, 2 - synthesis of lipids in the media with a high ratio of C (Carbon) number.

It is believed that the fungal oil can be an alternative of vegetable oils (olive, sunflower, etc.) [18, 29, 33]. Lyapkov et al. (1993) also studied the composition of the triglycerides of genus *Candida* yeast and comparing them with vegetable oils [23]. Lipids of microorganisms (*Streptomyces rimosus, Fusidium coccineum*) propose to use as anti-foam agent in the synthesis process and oxytetracycline, fucidin for replacement sunflower oil, lard and synthetic fat "adekolol" [17]. Regulations developed practical use of microbial lipids in the paint industry, technology of extracting gold from ore, use them in the oil industry, cleaning soil and water from oil pollution in geochemistry.

In recent years, began to develop a scientific approach to the preparation of balanced nutrient media, taking into account the needs of individual strains of microorganisms in the individual components. By "balanced" nutrient understand such its qualitative and quantitative composition that meets the needs of strain, provides the maximum amount of biomass at a certain rate with minimal residual concentrations of elements [9, 13, 25-28, 30, 36]. The amount of lipid in the biomass-producing strains depends upon what substance added to the medium as a Carbon source [1, 12, 15, 22, 24]. The concentration of Nitrogen in the nutrient medium has a significant effect on the growth and synthesis of lipids in microbial cell: considerably reducing the amount of Nitrogen increases lipid synthesis, but reduces the yield of biomass [8, 10, 16, 35]. Konova et al. (1986) studied the effect of exogenous sources of lipids, soybean and corn flour, corn extract, growth of vegetable oils and biomass accumulation of actinomycetes and fungi, came to the conclusion that the lipid composition is a reflection of two processes: enhancing growth and lipid synthesis *de novo*. The same authors also note the importance of mineral-based environment for the cultivation of actinomycetes. The best source of Nitrogen for actinomycetes and fungi, the authors consider $(NH_4)_2SO_4$, Potassium - KH_2PO_4, Calcium - $CaCO_3$, Sodium – NaCl [19].

The aim of our study was to investigate the ability of streptomycetes isolated from soils of Moldova to accumulate biomass after cultivation on synthetic and complex media, as well as to determine the qualitative and quantitative composition of lipids biomass of these strains.

MATERIALS AND METHODS

The objects of our studies were *Streptomyces ssp.* 12, 19, 33, 36, 47, 49, 66 isolated from soils of Central Part of Republic of Moldova and *S. sp.* 205 isolated from soils polluted with pesticides. The strains were isolated by using subculturing on starch-ammonia agar (SAA) from soil samples of the black soil of the Central Part of Republic of Moldova, differing by amount of humus (2.4-6.8%). After examination of colonies grown on SAA, were observed:

- colonies are leatherback, grows into the substrate, with well-developed to varying degrees of the substrate and aerial mycelium;
- size of the colonies are 1-10 mm;
- surface of colonies – powdery, most velvety, in rare cases fluffy .

In direct microscopy of isolated colonies on a Petri dish can be seen on the spore-bearing hyphae of the aerial mycelium the spores in fixed chain (monopodially or in bundles). Chains of spores of the majority isolates were straight, but it was possible to meet and wavy, spiral - in the form of hooks.

The above features have allowed to carry studied strains to the genus *Streptomyces* [14, 32, 34, 36].

All strains of streptomycetes were kept on agar medium Czapek with glucose with pH=7.0 [11]. Inoculum was cultivated on liquid mineral media Dulaney, in Erlenmeyer flasks of 200 ml during 3 days at 27ºC on the agitator [31].

To obtain a biomass, inoculum in an amount of 8% was added to the flasks with liquid complex medium M-I ($CaCO_3$, baker's yeast, basic source of Carbon was corn flour), medium R (NH_4NO_3, KH_2PO_4, $CaCO_3$, NaCl, basic source of Carbon were corn flour and soluble starch), medium Czapek ($NaNO_3$, K_2HPO_4, $MgSO_4*7H_2O$, KCl, $FeSO_4$, basic source of Carbon was glucose) and medium Dulaney ($(NH_4)_2HPO_4$, NaCl, K_2HPO_4, $MgSO_4*7H_2O$, $CaCl_2$, $ZnSO_4*7H_2O$, $FeSO_4*7H_2O$, basic source of Carbon was glucose) of 200 ml for 5 days at 27°C on the agitator.

To determine the productivity of strains after cultivation on different medium, biomass has been separated from cultural liquid on a centrifuge (5000 r/min during 20 min). Quantity of absolutely dry biomass (ADB) was determined by a weight method [3, 20, 21].

The intracellular lipids were extracted from biomass by Folch method, modified in the laboratory, by change methanol with ethanol [4].

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 using the method of densitometry on densitometer DO-1M [4, 7].

RESULTS

Among the nutrient media offered for the cultivation of actinomycetes, including the streptomycetes, wide application obtained mineral media - Dulaney, Pruss, Klein, Czapek with glucose or sucrose, as well as complex or organic medium in which the main source of Carbon - flour (soybean, corn, etc.), and various additives (molasses, corn steep liquor, yeast hydrolysate) and mineral salts [14, 28, 36]. We have previously carried out experiments to study relation between composition of medium and lipid biosynthesis, and individual lipid fractions from collection strain *Streptomyces canosus* 89. The maximum biomass was obtained on the medium Dulaney with 10% molasses (20.4 g/l) or Dulaney medium with 5% of corn steep liquor (20.2 g/l) [7].

The experiments of the cultivation of the studied strains in media of different composition have shown that in complex nutritive media (M-I and R) biomass was almost 2-3 times more than on "classic" synthetic media Dulaney and Czapek (Table. 1). The composition of the medium to a lesser extent affected the lipid content in the biomass of the studied strains. For example, *S. sp.* 33 cultivated on a complex medium M-I and R, contained 6.32 and 8.73% of lipids in biomass, and on synthetic media Czapek and Dulaney - 8.36 and 5.87% (Table 2).

Strain *S. sp.* 49 after cultivation on complex media contained an amount of lipids in the biomass more (13.5% and 11.4%) than after cultivation on synthetic media (9.3 and 8.4%). Strain *S. sp.* 66 greatest quantity of biomass in comparison with other strains obtained after cultivation on media M-I and R (19.6 and 18.72 g/l) as could be seen in table 1, but in the biomass of the strain of the lipid content is greater when strain was cultivated on the "classical" synthetic media Czapek and Dulaney (10.81% and 8.94%). Strain *S. sp.* 36 had sharp differences in the ratio of biomass/lipids after cultivation on complex and synthetic media. For example, after cultivation on the complex media M-I and R, at this strain is almost the same amount of biomass (12.1 and 12.27 g/l), but sharp differences in lipid content (5.9% and 12.81%), and on synthetic media the amount of biomass is negligible (4.0 and 5.1 g/l), but substantially larger lipid content than the other strains cultivated on these two synthetic media. Strain *S. sp.* 19 after cultivation on synthetic media, ratio in the amount of biomass and lipid content in it is seen not as dramatically as, for example, the strain *S. sp.* 36.

Strain *S. sp.* 12 at low biomass yield on complex medium M-I (5.6 g/l), the ability to produce lipids listed in more than on other media (12.1% compare with 5.0-7.7% other media). Of certain interest have strain *S. sp.* 47, which was seen the following features: after cultivation on complex medium M-I and R in the biomass and lipids were 14.3 and 18.69%, i.e., largest amount in comparison with other strains studied. Strain *S. sp.* 205 isolated from soil contaminated with pesticides, amount of biomass after cultivation in the media under study was small (3.05-4.2 g/l), and lipids content in it was smaller than other strains (1.5-4.35%).

That is, studies have shown that the strains of streptomycetes isolated from soil of Central part of Republic of Moldova have high potential: after cultivation on complex media, biomass can reach till 20 g/l, and its content of the total lipids may vary from 5.9 to 18.69%. On synthetic media studied strains accumulate significantly less biomass and lipids make it 1.5-12.6%.

Table 1. Quantity of biomass after cultivation on different media, g/l (ADB)

Medium / *Streptomyces sp.*	M-I	R	Czapek	Dulaney
12	5.6±0.43	4.6±0.2	4.3±0.37	3.8±0.16
19	14.15±0.21	13.92±0.16	4.8±0.28	2.26±0.34
33	7.24±0.56	10.48±0.13	3.72±0.12	4.1±0.42
36	12.1±0.12	12.27±0.24	4.0±0.19	5.1±0.23
47	9.35±0.49	3.53±0.04	5.1±0.25	5.9±0.32
49	6.64±0.42	7.93±0.53	5.8±0.27	3.32±0.38
66	19.6±0.07	18.72±0.14	6.32±0.15	5.83±0.19
205	4.2±0.11	3.85±0.09	3.05±0.18	4.1±0.16

Table 2. Quantity of total lipids after cultivation on different media, % (ADB)

Medium / *Streptomyces sp.*	M-I	R	Czapek	Dulaney
12	12.1±0.4	5.0±0.9	7.7±0.3	6.83±0.14
19	12.1±0.27	12.57±0.63	6.3±0.41	4.9±0.65
33	6.32±0.38	8.73±0.25	8.36±0.54	5.87±0.27
36	5.9±0.44	12.81±0.32	12.6±0.11	9.3±0.5
47	14.3±0.3	18.69±0.87	8.34±0.43	7.32±0.37
49	13.5±0.17	11.4±0.5	9.3±0.45	8.4±0.2
66	6.74±0.48	7.11±0.43	10.81±0.39	8.94±0.29
205	2.2±0.15	1.5±0.26	4.35±0.32	3.12±0.18

In strains isolated from soil taken in the territory of scientific and experimental base of the Academy of Sciences of Moldova (near Chisinau municipality), verification of the potential ability to synthesize lipids after cultivation on complex nutritive media, showed that their share in the biomass has 10.4-27.0% lipids. The maximum amount of biomass was 17.7 g/l and the minimum - 8.5 g/l [5, 6], studied strains of streptomycetes after cultivation on complex media M-I and R, lipids varied depending on the strain belonging to the composition of the medium, from 1.5% to 18.69%.

Composition of the nutritive medium affected the biosynthesis of individual lipid fractions of streptomycetes. Most of the lipid content are triglycerides fractions of studied streptomycetes, the quantity of which ranged from 28.7 to 56.3%, whereas 4.9-11.4% are phospholipids and sterol fraction – 5.2-15.7%, when the strains were grown on the complex media M and M-I (corn) [5].

The next step of research was to determine the qualitative and quantitative composition of lipids of biomass of studied strains obtained after cultivation on complex (M-I and R) and synthetic media (Czapek and Dulaney). As can be seen in the figures 1-4, amount of basic lipid fractions - phospholipids, sterols and triglycerides changed depending on the composition of the nutritive medium.

For example the amount of phospholipids in the lipid was most in biomass after cultivation on synthetic media of strains *S. ssp.* 12, 19, 36 (Figure 3 and 4), and strains *S. ssp.* 47, 66 and 205, the number of phospholipids was more in biomass growing on the media M-I and R (Figure 1 and 2).

Quantity of sterols were more in lipid of biomass obtained after cultivation on complex media M-I and R, only at strain *S. sp.* 19, but in lipid content of these strains as the *S. sp.* 66 and 205 the number of sterols was slightly larger as well on complex media.

Quantity of triglycerides was greater from lipid of biomass obtained after cultivation of strains *S. ssp.* 19, 47, 66 and 205 on synthetic media Czapek and Dulaney.

Such strains, like *S. sp.* 33 and 49, which were only cultivated on complex media, the quantity of such lipid fractions as phospholipids and sterols was less than other strains and triglycerides were 29.3-33.8%, which constitute the average position on scale proportion of this fraction to other lipids studied of streptomycetes strains in which the minimum amount of triglycerides was 14.66% (*S. sp.*12 cultured on medium R) – figure 2, and the maximum amount of 55.1% in strain *S. sp.* 36 after cultivation on medium M-I (Figure 1).

For other lipid fractions found in the lipid composition of biomass strains studied to be noted that the total amount of mono-, di- glycerides ranged from 5.7% at *S. sp.* 19 (medium M-I) to 26.7% in *S. sp.* 33 (medium M-I). As a rule, the remaining number of strains fractional mono- and di- glycerides prevailed after cultivation on a synthetic media (Czapek and Dulaney).

In the chromatograms were discovered spots on the appropriate level of R_f (coefficient of refraction) for FFA (free fatty acids) in 4 out of 8 strains of streptomycetes *S. ssp.* studied - 19, 33, 47 and 49. The amount of this fraction predominant in strain *S. sp.* 33, after cultivation on complex media (16.3 % and 18.8 % on the media M-I and R respectively).

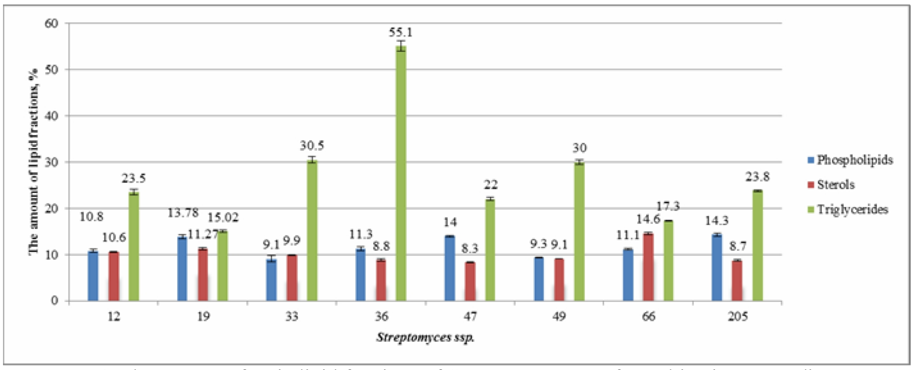

Figure 1. The amount of main lipid fractions of *Streptomyces ssp.* after cultivation on medium M-I

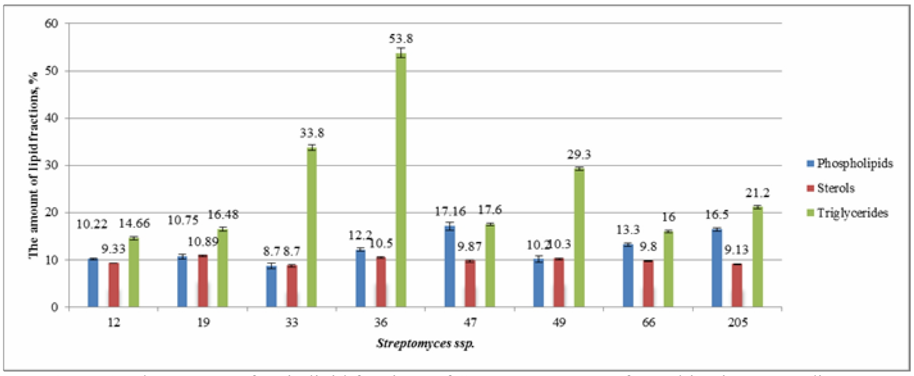

Figure 2. The amount of main lipid fractions of *Streptomyces ssp.* after cultivation on medium R

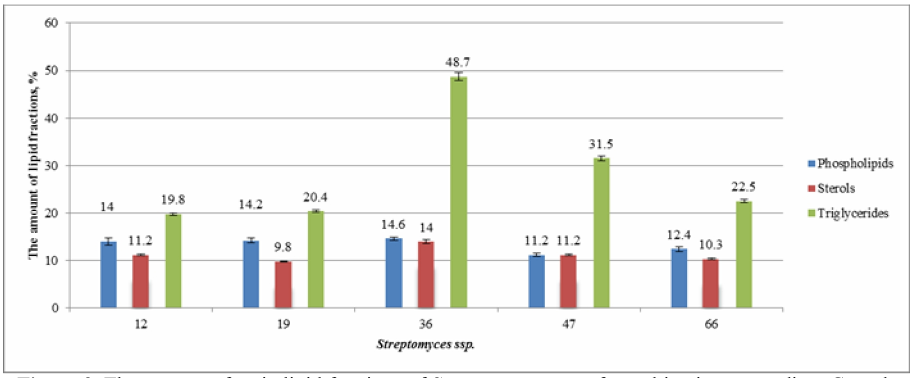

Figure 3. The amount of main lipid fractions of *Streptomyces ssp.* after cultivation on medium Czapek

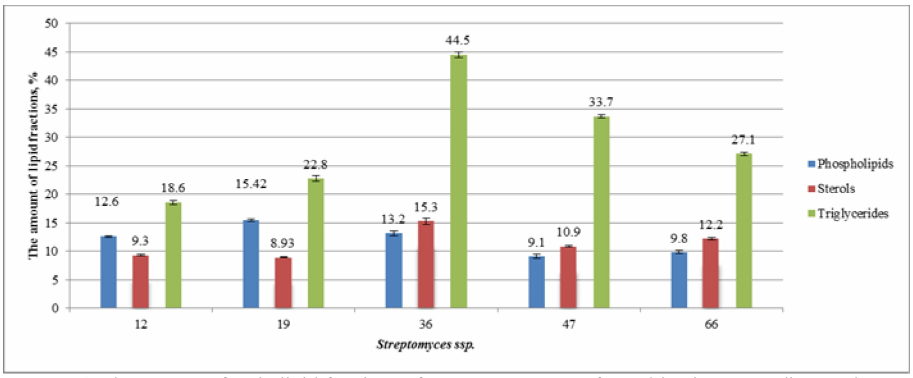

Figure 4. The amount of main lipid fractions of *Streptomyces ssp.* after cultivation on medium Dulaney

DISCUSSION

Analyzing the data on the ability of streptomycetes to synthesize physiologically active lipid fractions after cultivation on media of different composition, it should be noted that the greatest interest present strains *S. ssp.* 19, 36, 47 and 66, since these strains detecting the greatest number of fractional phospholipids (from 11.1% to 17.16%) and sterols (from 11.27% to 15.3%). However, given the amount of biomass which accumulates streptomycetes strains after cultivation on complex media, highlight strain *S. sp.* 19 and 66, as they have the greatest amount of biomass, after cultivation on complex media (13.92 - 19.6 g/l), in which lipids constitute 6.74-12.57%.

Of certain interest present and strain *S. sp.* 47, in which the amount of lipid in the biomass is 14.3 and 18.69% after cultivation on complex media, but the quantity of the biomass of the strain on media M-I and R is small - 9.35 g/l and 3.53 g/l respectively.

Comparing the biosynthetic activity of streptomycetes strains studied with our earlier results from other streptomycetes strains isolated from soils of Republic of Moldova, we can see that the strain *S. sp.* 11 had quantity of phospholipids also high - 14.6%, but this strain on complex medium accumulate only 7.9 g/l of biomass.

According to the content of sterols fraction, our previously observed strains *S. sp.* 44 and 120, in which the lipid fraction was 16.34 and 19.6% for the ability to accumulate biomass on medium M-I of 14.13 g/l and 17.03 g/l. With these parameters on the fraction of the sterols it can only be compared with *S. sp.* 66, which, as we have already mentioned can accumulate a significant amount of biomass on complex media, M-I and R (18.72-19.6 g/l), respectively, and in which there are more phospholipids and sterols in 1 gram of biomass produced per liter of medium. Can be also on this productivity of the physiologically active fraction (phospholipids and sterols) mentioned strain *S. sp.* 19 [2].

Our previous studies on the effect of the composition of the medium of cultivation on the formation of biomass and lipid synthesis of collection strains of streptomycetes have shown that these strains also tend to accumulate more quantity of biomass on complex medium M (the main source of N and C - corn flour, 40 g/l) than on synthetic medium Dulaney. For example at *S. griseus* 15 on synthetic medium accumulate biomass - 4.7 g/l and on the complex medium M - 14.7 g/l, total lipid content 4.5% and 12.7%, respectively; strain *S. griseofavillus* 31 accumulate biomass - 3.1 g/l and 13.6 g/l and total lipid content 13.6% and 26.9%; *S. galbus* 1616-Z-3 accumulate biomass - 5.5 g/l and 15.2 g/l and total lipid content 12.0% and 8.0% [7]. That is among the collection strains cannot be clearly traced a pattern between the amount of biomass and lipid content in it and depending on the nutritive medium. Our results support the view of researchers that biosynthetic activity of each strain is strictly individual and depends on the physiological characteristics of each strain.

When comparing collection strains on the ability to synthesize these lipid fractions as phospholipids, sterols and triglycerides, previously it has been found that after the cultivation on complex medium M, strain *S. griseus* 15 proportion of phospholipids in lipid - 4.2%, sterols 4.1% and triglycerides - 72.8%, in *S. griseufavillus* 31 - phospholipids (3.8%), sterols (9.1%) and triglycerides (43.0%), and *S. galbus* 1616-Z-3 - phospholipids (4.4%), sterols (5.0%) and triglycerides (40.7%).

That is clearly evident that the isolated from the soil of the Central Part of the Republic of Moldova, streptomycetes actively synthesize physiologically active lipid fractions than that stored for a long time in collection strains of streptomycetes isolated from soil of Russia, Germany, Argentina and Japan. It should also be noted that, regardless of the nutritive medium of strains of soil of the Republic of Moldova, content of triglycerides contained about 1.5 - 3 times less than other collection strains isolated from other regions.

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