

THE QUALITATIVE AND QUANTITATIVE COMPOSITION OF LIPIDS IN BIOMASS OF STREPTOMYCETES ISOLATED FROM SOILS OF CENTRAL PART OF REPUBLIC OF MOLDOVA

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Abstract. The qualitative and quantitative composition of lipids from biomass of soil streptomycetes was studied. Streptomyces strains were isolated from soil samples with the different content of humus (2.4-6.8%) from central part of Republic of Moldova. Storage of Streptomyces strains were performed on agar media Czapek with glucose and oatmeal agar. The biomass was obtained after cultivation on liquid complex medium M-I (basic source of carbon was corn flour) for 5 days at 27°C on the agitator. Were established that the amount of biomass of studied strains varies from 6.64 to 19.6 g/l, the percentage of lipids in biomass from - 4.94 to 13.5%. Amount of physiologically important fractions - phospholipids are 4.76-14.66%, sterols - 3,84 - 19.76% and triglycerides 15.11 - 44.23%. These strains can be considered as a basis for biological products recommended for animal husbandry.

Keywords: streptomycetes, biomass, lipids, lipids fractions.

INTRODUCTION

Search for new antibiotics effective against multi-drug resistant pathogenic bacteria is presently an important area of antibiotic research. Natural products having novel structures have been observed to possess useful biological activities. Filamentous soil bacteria belonging to the genus *Streptomyces* are widely recognized as industrially important microorganisms because of their ability to produce many kinds of novel secondary metabolites including antibiotics. Indeed, different *Streptomyces* species produce about 70-80% of commercially and medically useful antibiotics. In the course of screening for new antibiotics, several studies are oriented towards isolation of new *Streptomyces* species from different habitats [1, 2, 18, 32].

Among biologically active substances of microbial origin, lipids occupy a special place. Lipids constitute a group of naturally occurring molecules that include fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E and K), mono-glycerides, diglycerides, triglycerides, phospholipids, and others. For a long time it was considered that lipids play a modest part in the vital activity of cells. Later was established that lipids ensure a complicated metabolic processes, are the part of the structural elements of the cell are an advantageous energy substrate [16, 29]. The antimicrobial properties of lipids were identified after numerous observations of biochemists and microbiologists [31].

The actinomycetes synthesize and accumulate significant amount of lipids. The content of lipids in actinomycetes mycelium vary from 5 to 40% and more, depending on nutrient media composition and individual properties of organism [8, 17, 30].

The lipids have different biological activity: antibacterial (against a number of gram-positive and gram-negative bacteria and yeast-like fungi of the genus *Candida*), immunological, growth stimulating, antitumor, etc [8, 12, 25]. Furthermore, lipid fractions

of actinomycetes have antioxidant activity and intramuscular injection increase natural resistance and growth rate of pigs, reducing the costs of feed [9, 13].

For example, new antibiotics, pseudomycins or antimycotics, that affects agents of human fungus diseases, were isolated. Chemically they are lipopeptides [10].

Bulgarian scientists studied the fatty acid composition from mycelia of *Streptomyces hygroscopicus* strains. They shown that antibiotic spectrum correlates with some shifts of the fatty acid content. The differences in fatty acid formation in *S. hygroscopicus* strains had shown their variability. It reflects the adaptation capability of the species *S. hygroscopicus* by changes of the composition of the cell membrane to favor particular antibiotic biosynthesis [14].

Ukrainian scientists have shown a close connection between the avermectine-synthesizing ability of the strain *Streptomyces avermitilis* UCM Ac-2179 and lipids content in its biomass. On its basis an antiparasitic preparation Avercom with content of 1400-1800 mcg/ml avermectine were elaborated. In the composition of lipids of biomass *S. avermitilis* UCM Ac-2179 and of Averkom preparation such bioactive fractions prevailed: phospholipids (22.1-28%), sterols (24-34%), free fatty acids (10-8%), also mono-, di-, and triglycerides, sterols esters, wax and unidentified fractions. Significant quantity of linoleic (26%), oleic (21%) and palmitic (17%) acids, in addition to the low molecular weight fatty acids C4 - C10, the precursors of avermectine, were found in a fatty acid composition of lipids extracted from the mycelium of *S. avermitilis* UCM Ac-2179 [3].

We have also carried studies to determine the antimicrobial activity of lipids of microorganisms (actinomycetes, micromycetes) and plants. On the example of phospholipids of *Streptomyces griseus* 420 strain has been shown that even such a minimal dose of 0.001 mg/ml exhibit antimicrobial effect, while higher doses - a complete growth inhibition of a number of

test strains [8]. In other words, the literature date and our results suggested that further study of the possibility to use microbial lipids for creating new biological products are promising.

Thus, the aim of our study was to investigate the qualitative and quantitative composition of lipids from biomass of soil streptomycetes and to reveal promising strains for biotechnological purposes.

MATERIALS AND METHODS

Streptomycetes strains isolated from soil samples with the different content of humus (2.4-6.8%) from central part of Republic of Moldova were used in this study. These strains were isolated from the soils of following localities: *Streptomyces sp.* 11, 19 – Bacioi village (Botanica zone, Chisinau Municipality), black earth carbonate, (humus 2,4-2,5) under monoculture corn; *Streptomyces sp.* 42, 44, 49, 66, 120 – Truseni village (Chisinau Municipality), typical black earth, (humus 2, 6); *Streptomyces sp.* 145 – Ghidighici village (Chisinau Municipality), black earth carbonate (humus 3, 5), medium loamy; *Streptomyces sp.* 193 – Cornesti City (Ungheni district), forest black earth (humus 6, 8).

All test cultures of streptomycetes kept on agar media Czapek with glucose and oatmeal agar [4, 10]. Inoculum was cultivated on liquid mineral media Duloney, in Erlenmeyer flasks of 200 ml during 3 days at 27°C on the agitator [33].

To obtain a biomass, inoculum in an amount of 8% was added to the flasks with liquid complex medium M-I (basic source of carbon was corn flour) of 200 ml for 5 days at 27°C on the agitator.

To determine the productivity, 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 [5, 20, 21].

The intracellular lipids were extracted from biomass by Folch method, modified in the laboratory [6].

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 [6, 8].

RESULTS

The value of microbial biomass is determined by the presence of vitamins, microelements and other unique nutrients, including the lipids [19, 28].

The total amount of lipids in microorganisms varies widely from 0.2% to 40% of the cell substance, but in the conditions favorable to the accumulation of fats it may reach higher levels. Actinomycetes synthesize and accumulate in their cells, a significant amount of fat. The lipid content in the mycelium of actinomycetes depends on the composition of nutrient medium and the individual characteristics of the organism [4, 5, 8, 11, 21, 27, 33].

When actinomycetes are cultivated on rich organic media, they are able to synthesize from 18% up to 39% of lipids from dry weight of cells, whereas on the synthetic media the content of lipids vary between 1.9 to 5.6% [5, 8, 33].

On the first stage of our research we have tested the ability of new isolated actinomycetes strains of genus *Streptomyces* from soil of R. Moldova, to accumulate biomass at cultivation on liquid complex nutrient medium M-I, with corn flour as a main source of carbon (Tab. 1).

The obtained results revealed that the biomass productivity of *Streptomyces* strains ranged from 6.64 g / l (*Streptomyces sp.* 49) to 17.03 g / l (*Streptomyces sp.* 120) of ADB. Maximal quantity of biomass - 19.6 g / l, was accumulated by the strain *Streptomyces sp.* 66.

The next stage of our research was to study the accumulation of lipids in the streptomycetes biomass (Tab.1). The figure shows that amount of lipids in ADB of streptomycetes varies. The highest lipid content in biomass was observed at the strain *Streptomyces sp.* 49 - 13.5%. A good result has shown the strain *Streptomyces sp.* 44: the lipid content in ADB was 13.17%. The lowest percent of lipids content was observed in strain *Streptomyces sp.* 145 - it was 4.94%. The lipids content in the ADB of remaining strains ranged from 5.24% to 12.10%.

Determination of the fractional composition of lipids revealed two unidentified fractions that are not found in all the studied strains of streptomycetes. One fraction was detected at the strains *Streptomyces sp.* 44, *Streptomyces sp.* 49 and *Streptomyces sp.* 66; other fraction – in lipids of *Streptomyces sp.* 145. Both unidentified fractions have been identified only at strain *Streptomyces sp.* 120.

Table 1. The productivity of biomass and lipids by *Streptomyces sp.* strains on complex medium M-I

| <i>Streptomyces</i> strains | ADB, g/l | Lipids in ADB, g/l | Lipids, % in ADB |
|-----------------------------|------------|--------------------|------------------|
| 11 | 7.92±0.49 | 0.64±0.01 | 8.18 |
| 19 | 14.15±0.35 | 1.71±0.22 | 12.10 |
| 42 | 13.99±0.38 | 0.73±0.13 | 5.24 |
| 44 | 14.13±0.19 | 1.86±0.18 | 13.17 |
| 49 | 6.64±0.19 | 0.89±0.11 | 13.50 |
| 66 | 19.6±0.58 | 1.32±0.21 | 6.74 |
| 120 | 17.03±0.51 | 1.06±0.18 | 6.26 |
| 145 | 10.03±0.94 | 0.49±0.19 | 4.94 |
| 193 | 10.56±0.59 | 0.65±0.17 | 6.23 |

As could be seen from fig. 1 the content of the major lipid fractions (phospholipids, sterols and triglycerides) in the biomass is not the same and varies as follows: phospholipids - from 4.76 to 14.66%, sterols - from 3.84 to 19.76 % and triglycerides from 15.11 to 44.23%. The maximal amount of phospholipids was determined in biomass of *Streptomyces sp. 11* (14.66%), sterols in *Streptomyces sp. 120* (19.76%), and triglycerides - *Streptomyces sp. 193* biomass (44.23%).

As could be seen from fig. 2 the amount of mono- and diglycerides in the biomass of streptomycetes strains also vary from strain to strain. Maximal quantity of monoglycerides were found in the lipids of *Streptomyces sp. 11*, *Streptomyces sp. 145* and *Streptomyces sp. 193* - from 9.3 to 9.61%. Minimal amount of monoglycerides were detected in the lipids of *Streptomyces sp.44* - 0.96%.

Quantity of diglycerides in lipids of all investigated strains of streptomycetes is 1.33-8.57% from sum of lipids. Only the strain *Streptomyces sp. 19* has the same amount of mono- and diglycerides equal with 4.16%.

The amount of free fatty acids fraction in the lipids of studied streptomycetes strains ranged from 6.6% (*Streptomyces sp.11*) to 24.3% (*Streptomyces sp.66*). Fraction of sterols esters and waxes is 14.78-26.92% of the total lipids of streptomycetes.

It should be noted the strains that differ in their lipid composition. For example, the strain *Streptomyces sp.11*, has the greatest amount of phospholipids (14.66%), a sufficiently large amount of triglycerides (41.33%) and waxes and sterol esters (20%), but a small amount of sterols (6.6%).

Strain *Streptomyces sp.19* has the same number of phospholipids and sterols (each 12.5%), mono- and diglycerides (by 4.16%), small amount of free fatty acids (8.33%) and up to 39% of triglycerides.

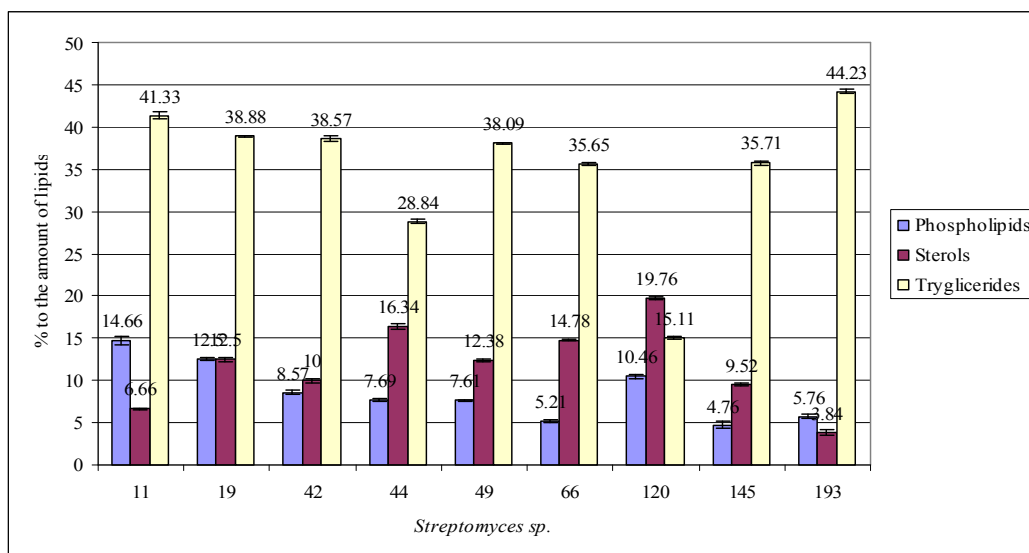


Figure 1. Quantity of the major lipid fractions in lipids of *Streptomyces sp.* strains

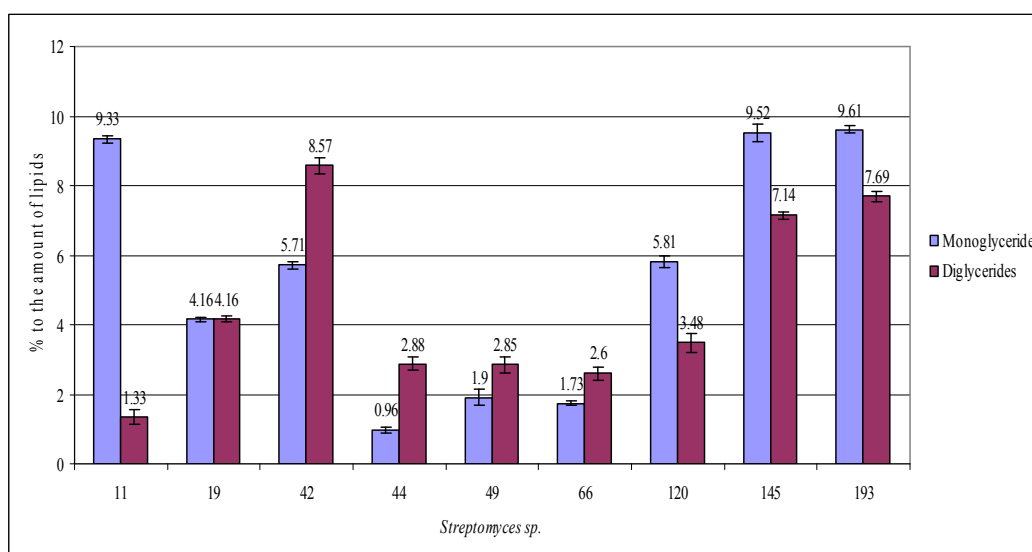


Figure 2. Quantity of mono- and diglycerides in lipids of *Streptomyces sp.* strains

DISCUSSIONS

Literature data concerning content of lipids in biomass of actinomycetes are few. The most complete data are available on the composition of fatty acids of the lipids of streptomycetes – producers of antibiotics – *S. levoris*, *S. olivaceus*, *S. streptomycini*, actinomycetes from Kazakhstan soils which are producers of antibiotics, and producer of antiparasitic antibiotic avermectin – *S. avermitilis* UKM Ac-2179, actinomycetes of yellow group of Ukrainian soils, actinomycetes of different ecological niches of Mongolia [3, 15, 22, 23, 24, 26].

By the example of three strains of actinomycetes (*S. olivaceus* 3, *S. olivaceus* 1073, *S. globisporus* 383) was revealed dependence of the content of individual lipid fractions in dependence of phosphate amount in the media. When the amount of phosphorus is less than 5 times (0,0356 g/100 ml in media), actinomycetes accumulate less biomass, but the lipid content increased from 3.92 % to 9.56 %. According to the Konova I. (1983), dynamics of microbial lipids content reflects a response to the conditions of existence. Thus, the level of phospholipids in the mycelium of actinomycetes (*Streptomyces*) and fungi (*Blakeslea* and *Entomophthora*) depends on the content of phosphate in the media. The concentration of exogenous phosphate, determining the degree of utilization of carbohydrates from media, is an essential factor in the regulation of growth of culture and the accumulation of lipids [20].

Comparing the obtained results with data published earlier by us [8], it should be noted that the collection strains cultivated on complex media M-I accumulated maximum 12.82% of phospholipids and 15.67% of sterols (*Streptomyces canosus* 71); *Streptomyces massasporeus* 36, had 11.31% of phospholipids and 8.84% of sterols, while the new isolated strains from soil of R.Moldova accumulate the maximum amount of phospholipids 14.66% (*Streptomyces sp.*11) and sterols 19.76% (*Streptomyces sp.*120) .

It should be noted that the amount of biomass at collection strains ranged from 8.21 g / l to 24.79 g/l, while at new strains - from 6.4 to 19.6 g/l. Lipids in biomass of collection strains was 4.78-37.93% (0,39 g / l - 9,4 g / l), while in biomass of new strains - from 4.94% to 13.5% (0,31 g / l – 2,64 g / l).

In conclusion our studies suggest that the process of biomass and lipids accumulation of *Streptomyces sp.* strains, isolated from soils of R. Moldova, depends on the species and culturing conditions, in particular from the composition of the nutrient media. Thus the amount of biomass varies from 6.64 to 19.6 g/l, the percentage of lipids from - 4.94 to 13.5% (0,49 g / l – 0,89 g / l). Amount of physiologically important fraction for both micro and for macro organisms - phospholipids are 4.76-14.66%, sterols - 3.84 - 19.76% and triglycerides 15.11 - 44.23%. These strains can be considered as a basis for biological products recommended for animal husbandry.

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