ACCUMULATION OF BIOMASS AND SYNTHESIS OF LIPIDS BY *Streptomyces canosus* CNMN-Ac-02 CULTIVATED ON COMPLEX MEDIUM ADDED WITH CYANOBACTERIAL POLYSACCHARIDES

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Abstract. The effect of cyanobacterial zinc sulfated polysaccharides from *Arthrospira* (*Spirulina*) *platensis* CNMN-CB-02, as a supplement added in complex liquid medium M-I, on accumulation of biomass, synthesis of total lipids and fractional lipid composition of strain *S. canosus* CNMN-Ac-02 was studied in laboratory conditions. Maximal quantity of biomass compared to control was obtained using 30.0 %/V Psh*ZnS (8.83 g/l) concentration, with an increase of 63.91 %. In the experiment total lipids were lower than in the control. However, no significant difference was observed at 5.0 %/V Psh*ZnS concentration (95.82 %) in comparison to control. At all concentrations of Psh*ZnS applied, fractions of phospholipids and sterols were higher than those observed in the untreated control. Significant increase of triglycerides (20.63 %) was obtained adding in the growth medium 10.0 %/V Psh*ZnS. All tested concentrations of Psh*ZnS had no effect on mono- and diglycerides in comparison to control, whereas waxes were significantly lower. Quantity of sterol esters was higher in comparison to control only at concentrations of 1.0-20.0 %/V Psh*ZnS. Total lipids were increased by the increase of biomass, by 15.9 % in comparison to control, after the addition of Psh*ZnS at 30.0 %/V concentration.

Keywords: *Streptomyces*; cyanobacterial zinc sulfated polysaccharides; biomass; fractional lipid composition.

INTRODUCTION

According to the data reported in literature of last three decades of twentieth century and the beginning of twenty-first century there are many information deals with biosynthesis of lipids by microorganisms including actinobacteria. After the first important investigations worldwide some conclusions were reached on a smaller importance of lipids in cellular metabolism. New data, on the contrary show a high importance in metabolic processes of lipids, because they take part in composition of cell structural elements and serve as energetic substrate [21]. For example, total lipids of actinobacteria of „yellow" group mentioned by Kozyritskaia (1980), had a large percent content, variable from 9.2 to 57.1 %, in dried biomass. Synthesis of lipids and their percent content can vary according to the strain, i.e. *Streptomyces longisporus flavus* 3129 and *S. longisporus flavus* 2193 produce 40.6 % and 13.2 %, respectively, with 3 times less percentage for the second biological agent in comparison to the first agent. In the composition of total lipids was demonstrated the importance of presence of groups with free fatty acids and triglycerides. Phospholipids, sterols and fatty acids were also found in the composition of total lipids [15].

The identification of lipid content in the biomass of actinobacteria of different ecological niches of Mongolia, has been evaluated are constituted by phospholipids, mono- and diglycerides, sterols and their esters, free fatty acids, triglycerides, and waxes (wherein triglycerides and fatty acids free predominate) [5, 13, 32]. About prevalence of triglyceride fraction, investigation carried out by Muzabparov (1987) demonstrated that a new strain *S. antibioticus* UMB 25/779 is able to synthesize triglycerides much more 1.4 times than the original strain. Moreover, the optimization of SR-I by sucrose, increased by 3 times biosynthetic capacity [17]. During studies on the synthesis and biological activity of the complex avermictin, produced by *S. avermitilis,* Petruk (2005) demonstrated that in composition there are lipid fractions of phospholipids, mono- and diglycerides, sterols and their esters, free fatty acids and waxes. He discovered the correlation between accumulation of avermictins in biomass and important physiological lipid fractions consisting of triglycerides, phospholipids and sterols that represented 45%, 21% and 13% of the total amount of lipids, respectively [20].

The presence of new data in the literature on the study of the most important cellular connections - such as lipids that enter into the composition of the double-layered membranes, performing reserve function, demonstrates their important role in various cellular processes - transfer information, metabolites secretion, enzymes and hormones, proliferation, stability against stress, and of course with practical interest as the suministration of drugs [12].

To increase total lipids in biomass of *Streptomyces* spp., can be used two modalities: by using media which have in composition stimulators for synthesis of lipids or media enriched with nutrients to increase quantity of accumulated biomass and consequently total lipids which will be resulted higher than in the control. For second modality as source could be proposed extracellular polymeric substances as proteins, uronic acids, polysaccharides and other compounds not yet fully characterized [19].

Exopolysaccharides are substances with relatively complex structure [16], produced by many microorganisms as a strategy to growth successfully and surviving to adverse climatic conditions [18, 23, 24]. They can increase nutrition value for culture medium and they can be easily assimilated by

streptomycetes due to symbiosis with cyanobacteria [2].

The possible exploitation of cyanobacteria took place last decade by the growing industrial interest towards polysaccharides of microbial origin, that often show advantages over the polysaccharides extracted from plants or marine macro algae. As a result, a wide research for bacterial strains able to produce high yields of new polysaccharides with potentially useful properties has been undertaken also involving cyanobacteria because of the well-known capability of some strains to excrete mucilaginous material. On the other hand, polysaccharides produced by these cyanobacteria are essentially used in various industrial applications [27, 28]. The range of polysaccharides produced by microalgae is large and has own specific functions [22]. Microbial polysaccharides are attracting and increasing interest for their potential applications in food, cosmetic, pharmaceutical industries and remediation methods especially of water. That is why, they competing with other natural polysaccharides obtained from plants [14, 25, 26, 30].

The aim of present reserach was to found out favorable concentrations of zinc sulfated polysaccharides, obtained from biomass of *Arthrospira* (*Spirulina*) *platensis* CNMN-CB-02, to increase biomass yield and to analyze its total and fractional lipid composition derived from *Streptomyces canosus* CNMN-Ac-02 cultivated on complex liquid medium M-I.

MATERIALS AND METHODS

Streptomyces canosus CNMN-Ac-02 of the National Collection of Non-pathogenic Microorganisms of the Institute of Microbiology and Biotechnology of Academy Science of Moldova [7] was grown on agar media Czapek with glucose, Gause and oatmeal agar [3, 10]. The inoculum was cultivated on liquid mineral media Dulaney with glucose (pH=7.0), in Erlenmeyer flasks (V=1,000 ml) with 200 ml of medium for 3 days at 27ºC on a stirrer [32].

To obtain biomass, the inoculum in a percentage of 8 % was added to the flasks with liquid complex medium M-I containing maize flour as basic source of carbon (pH=7.0), and then a biological product of cyanobacterial zinc sulfated polysaccharides (Psh*ZnS), at different concentrations, was added. Psh*ZnS concentrations were: 1.0, 5.0, 10.0, 20.0, 30.0 and 40.0 %/V. They were obtained by *Arthrospira* (*Spirulina*) *platensis* strain CNMN-CB-02. The cultivation was carried out using Erlenmeyer flasks with 200 ml of medium, for 5 days at 27°C on a stirrer.

Biomass was separated from culture broth by centrifugation (5,000 r/min for 20 min). Quantity of absolutely dry biomass (ADB) was determined by the weight method [3].

The intracellular lipids were extracted from biomass by Folch's method, modified in the laboratory [5].

Fractional composition of the lipids was determined by thin layer chromatography using „Sorbfil" plates (100x150 mm), in the solvent mixture hexane-diethyl ether-glacial acetic acid system (73:25:5). Quantity of each lipid fraction was determined using the technique of densitometry [5, 7].

RESULTS

The productivity of biomass, by different groups of microorganisms, depends on composition of growth medium. In the last years many studies have been conducted to develope new balanced nutritive media, taking into account the needs of each strain. The aim was to adjust a qualitative and quantitative composition that can provide maximal biomass yield and a minimal rest of unused elements [4, 7, 9, 12, 20, 21, 29, 31, 32].

The experiments shown that adding in complex medium M-I (basic source of carbon and nitrogen – maize flour) a biological product Psh*ZnS from *Arthrospira platensis* in different concentrations has positive effect on studied *Streptomyces* strain.

According to the table 1, the quantity of biomass increased from 12.75 to 63.9 %, in comparison with the untreated control (0 %/V Psh*ZnS), by the addition of *Arthrospira* (*Spirulina*) *platensis* strain CNMN-CB-02, in different concentrations, in the nutritive medium. The highest ADB production (8.83 g/l) was obtained at 30.0 %/V Psh*ZnS concentration, with an increase of 63.91 % in comparison to that obtained in the untreated control (5.38 g/l). A positive linear correlation was noted between concentration of the biological agent *A. platensis* CNMN-CB-02 and ADB production, until the 30.0 %/V concentration (table 1). So, by the increase of concentration increased also ADB production. A further increase at 40.0 %/V concentration caused a biomass reduction (7.8 g/l) in comparison to 30.0 %/V concentration.

Table 1. Quantity of absolutely dry biomass (ADB) and amount of total lipids of *S. canosus* CNMN-Ac-02 during cultivation on medium M-I added with zinc sulfated polysaccharides (Psh*ZnS)

Concentration	ADB (g/l)	% ADB compared to control	% Total lipids	% Total lipids compared to control
Control (0 %/V Psh*ZnS)	5.38±0.23	100	15.14±0.43	100
1.0 %/V Psh*ZnS	6.07±0.35	112.75	10.52±0.53	69.5
5.0 %/V Psh*ZnS	7.00±0.14	129.93	14.51±0.89	95.82
10.0 %/V Psh*ZnS	7.59±0.04	141.01	9.78±0.49	64.6
20.0 %/V Psh*ZnS	7.89±0.15	146.59	10.83±0.71	71.54
30.0 %/V Psh*ZnS	8.83±0.15	163.91	11.59±1.62	76.52
40.0 %/V Psh*ZnS	7.8±0.73	144.81	10.62±0.8	70.13

Lipogenesis of *S. canosus* CNMN-Ac-02, growth on complex medium M-I, was negatively affected by the addition of different concentratons of the biological product obtained from *A. platensis* CNMN-CB-02. At all tested concentrations a significant reduction of % total lipids was observed in comparison to the untreated control with the exception of the use of 5.0 %/V of Psh*ZnS (14.51 % total lipids) which percentage was not so different from that in the control (15.14 %) (table 1). At this concentration total lipids were 95.82 % of those in the control.

At 30.0 %/V Psh*ZnS concentration percent total lipids was 11.59 corrisponding to 76.52 % of that verified in the control (15.14%).

At 1.0 and 40.0 %/V Psh*ZnS, percent total lipids, compared to control content, were 69.5 and 70.13 %, respectively.

According to data reported in the literature, streptomycetes of „gray" group isolated from European Part of Russia, Ukraine and Moldova, and also Kazakhstan and Mongolia, has an identical qualitative composition of lipids. Basic lipid fractions are phospholipids; mono-, di-, triglycerides; free fatty acids; sterol esters and waxes. In the composition of lipids of some strains were found fractions of hydrocarbons and 1-3 unidentified fractions situated on the plates for thin-layer chromatography immediately after phospholipids. Sometimes they were found also after diglycerides or before sterols and their esters and rarely after triglycerides [1, 7, 15]. In exceptional cases, during cultivation on organic complex media, unidentified fraction is absent as fraction of hydrocarbon [7].

The amount of a determined fraction depends by species and cultivation medium. *S. avermitilis* UCM Ac-2179 has next lipid composition: phospholipids 21.1-28.0 %; sterols 24.0-34.0 %; free fatty acids 8.0-10.0 % [1, 7].

Previously, it was observed the fact that stimulation of *S. canosus* CNMN-Ac-02 influenced by a biological product of *A. platensis* had an effect on the important fraction of phospholipids, with a yield that ranged between 111.9 and 115.8% [7].

The addition in the complex medium M-I of Psh*ZnS in concentration variable from 1.0 to 40.0 %/V increased phospholipids in the range 13.53-16.03 % compared to control (11.5 %) (figure 1). The smallest stimulation of phospholipids synthesis was observed at 10.0 %/V Psh*Zn concentration corrisponding to 117.64 % of that in the control, whereas the largest yield was obtained at 1.0 %/V concentration with 16.02 equivalent to 139.34 % compared to control (figure 1).

An increase of fraction of sterols was also observed although less than that observed for phospholipids. The percent increase of sterols in comparison to control varied from 1.34 to 16.9 %. In comparison to control, the largest amount of sterols was obtained by the addition of 1.0 %/V Psh*Zn concentration and the smallest by the addition of 20.0 % concentration. However the addition of 10.0 %/V Psh*Zn concentration resulted similar to that obtained at 20.0 %/V concentration with an increment of 2.45% (figure 1).

Analysis of fraction of triglycerides showed an increase of this fraction until the addition of 10.0 %/V Psh*Zn at the growth M-I medium. In comparison to the control at 1.0, 5.0 and 10.0 %/V Psh*Zn was recorded an increase of 2.0, 21.3 and 28.8 %, respectively. A further increase in Psh*Zn concentration percentually reduced the amount of triglycerides. At the last concentrations of 20.0, 30.0 and 40.0 %/V was observed a reduction of 25, 32.7 and 12.5%, respectively (figure 1).

According to Petruk's findings (2005), quantity of sterols in *S. avermitilis* was till 13.0 % of total lipids, similar to that we found in lipid composition of *S. canosus* CNMN-Ac-02. Some variants of selected *S. avermitilis* grown on liquid media, with soya flour and potato broth, dehydrated yeasts or yeast extract, glucose, maize extract and soluble starch, synthesized avermectin with dominance of triglycerides in lipid biomass (maximal amount – 45.0 %), phospholipids (till 21.0 %) and sterols (till 13.0 %) [20].

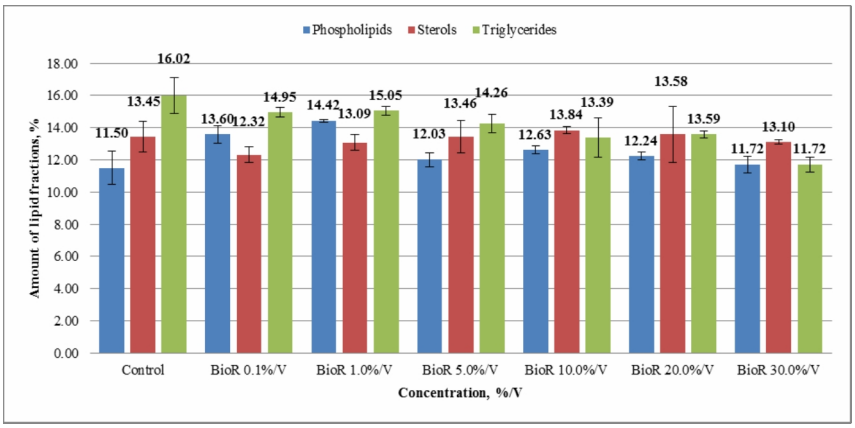

Figure 1. The amount of main lipid fractions of *S. canosus* CNMN-Ac-02 after cultivation on medium M-I added with zinc sulfated polysaccharides (Psh*ZnS) at different concentrations, %

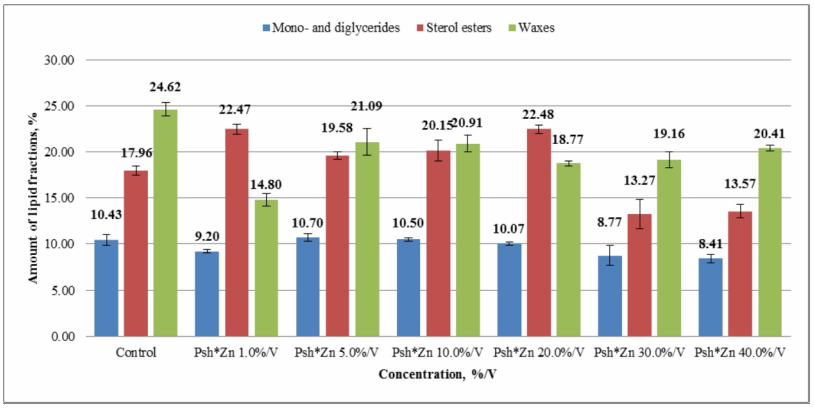

Figure 2. The amount of secondary lipid fractions of *S. canosus* CNMN-Ac-02 after cultivation on medium M-I with adding of Psh*ZnS

Composition of used medium in our research was more simple with low amount of triglycerides and phospholipids and sterols higher. Increase of sterols could be obtained adding Psh*ZnS in concentration variable from 1.0 to 5.0 %/V. Same concentrations of 1.0-5.0 %/V Psh*ZnS can increase also phospholipids amounts by 34.0-39.4 % in comparison to control.

Fractional lipid composition of *S. canosus* CNMN-Ac-02 grown on complex liquid medium M-I added with the biological product obtained from *A. platensis* CNMN-CB-02 – Psh*ZnS is reported in figure 2.

Analyzing the plates for thin layer chromatography, it was observed that mono- and diglycerides were in one spot. The biological product in small concentration (1.0 %/V) did not caused positive effect on synthesis of this fraction, and intermediate concentrations (5.0, 10.0 and 20.0 %/V) had neutral effect. Higher concentrations (30.0-40.0 %/V) reduced the fraction by 16.0-19.4 % in comparison to control (10.43 %) (figure 2).

Sterol esters increased by 9.02-25.16 % in comparison to control by the use of concentrations of the biological product variable from 1.0 to 20.0 %/V. Reductions of their quantity of 20.1 and 24.4 were observed in comparison to control by addition of 30.0 and 40.0 %/V, respectively.

The waxes fraction in all used concentrations was lower, from 16.1 to 39.89 %, than that observed in the control (24.62) (figure 2).

Additionally was found also an unidentified fraction, which resulted for all used concentrations higher by 7.14 to 40.03 % to those recorded in the control, with the exception of 1.0 %/V concentration in which it resulted lower.

DISCUSSION

As a rule, on complex organic media, actinobacteria produce essentially more biomass in comparison with synthetic media as Dulaney, Czapek and others. For example, microorganisms of the „white" group of *S. albodenitrificans* 13[a], cultivated on synthetic medium Dulaney, produce a quantity of biomass of 3.6 g/l, whereas on complex medium I after cultivation of *S. ravidus* 221 („gray" group) – 13.2 g/l, 5.3 g/l and 14.8 g / l; *S. aurigineus* 2377 („yellow" group) – 3.1 g/l and 22.2 g/l; *S. aureoverticillatus* 1306 – 4.4 and 16.3 g/l (in dependence of season conditions), respectively. Quantity of total lipids of studied strains was more in biomass cultivated on complex medium than on synthetic one (8.0-37.9 % on I and 5.1-20.8 % on Dulaney, respectively).

Some strains isolated from soils of Republic of Moldova produced biomass during the cultivation on medium M in an amount variable from 8.3 to 17.7 g/l and lipids was 7.0-27.0 % [6].

Cultivation of *S. canosus* CNMN-Ac-02 on different media showed that the greatest amount of produced biomass (12.76 g/l) was obtained by cultivation on medium R, which composition include maize flour, soluble starch and mineral salts. Cultivation on medium M-I, which contain only maize flour and mineral salts, showed a biomass of only 4.37 g/l. Results obtained after use of PM medium, containing maize flour, phosphates salts and other minor components, increased total lipids of 17.6 % in comparison to 6.22 % obtained on M-I medium [21].

Addition of microalgae in growth medium had different influence on accumulation of biomass and lipogenesis of *S. canosus* CNMN-Ac-02. Increase of biomass took place under the influence of biological products of *Porfiridium cruentum* by 4.78-17.52 % and *Spirulina platensis* by 8.06 %. Were identified doses of cyanobacterial biological products which caused the increase amount of lipids: by *P. cruentum* with 6.1-64.3 %; by *S. platensis* with 4.8-31.9 % and by *Nostoc linckia* with 11.8-19.2 % [7].

Previously investigations were carried out on „white", „gray", „yellow" and „orange" groups of actinobacteria on their capacity for lipid biosynthesis. The biosynthesis was different according to microorganism strain and growth medium composition [33]. Of course there are many physical chemical methods which can be used to increase quantity of total lipids. The most distinguished method is irradiation of biological material by different types of waves [21]. Quantity of lipids was 8-38 %, common yield of biomass per unit of the cultivation medium was highest at *S. canosus* 89 (24.8 g / l) and *S. aurigineus* 2377 (22.2 g / l) [7]. According to lipidic composition of biomass produced by actinobacteria, 8 classes of compounds and 3 unidentified fractions were found,

which presence dependeds on species of strain and cultivation medium, as above reported. Maximum quantity of phospholipids was 9 %, sterols – 9 %, free fatty acids – 13.3 % and triglycerides – 30.4 % [6].

The comparative investigation on amount of lipids of R-, S- and M-variants of *Rhodococcus rubroperstictus* strain 104 showed that main lipid fraction regardless temperature were triglycerides, phospholipids, saturated fatty acids and mycolic acids. Lipid amount of 22 % was obtained in variant R after cultivation at 30ºC, while in the variants S and M it was 14 and 16 %, respectively. By increasing the temperature of cultivation from 30 to 37ºC, quantity of free lipids decreased, whereas phospholipids increased. Waxes and spirits were not found [11].

Investigations on changes of lipid composition by other representative microorganisms as of genus *Rhodococcus – R. erythropolis* by long term storage on laboratory medium MPA (meat-peptone agar) showed that the variant S do not cause reconstruction of cell wall of the original strain and oncoming by micolats and possibility to growth on medium with carbohydrates to form variant R. Modification of hydrophobic degree of cell wall allows their quick adaption to different types of organic substrates. Authors found out substantial difference in synthesis of neutral lipids: cells of S variant did not accumulated waxes at all, but amount of triglycerides was by 1.4 times less in comparison with R variant [8].

Adding the biological product obtained from *A. platensis* as Psh*ZnS, can stimulate accumulation of biomass for *S. canosus* CNMN-Ac-02 from 12.8 to 63.9 %, increase fractions of phospholipids from 17.6 to 39.3 % and sterols from 10.9 to 16.9 % according to the used different concentrations.

Because of reduction of total lipids in experimental samples, their lower amount is compensated by obtained biomass. After recalculation the quantity of total lipids to the control per liter of medium, it was assessed that in samples with 30.0 %/V Psh*ZnS concentration increase yield by 15.9 %. By continue the idea, in result increasing and quantity of lipid fractions.

Results obtained show interesting perspectives for use of cyanobacterial biological products to stimulate biomass accumulation and increase of important physiological lipid fractions as phospholipids and sterols, during cultivation of different microorganisms, especially streptomycetes.

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