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Selecting the Optimal Growing Medium for Strain of Streptomycetes *S.canosus* CNMN-Ac-04

Olga POSTOLACHI, Svetlana BURŢEVA

Institute of Microbiology and Biotechnology of ASM,
1, Academiei str., Chişinău, Moldova, 2028
oleseap@yahoo.com

Abstract. The biosynthetic activity (accumulation of biomass, proteins and lipids) of strain *Streptomyces canosus* CNMN-Ac-04, which possesses antimicrobial activity against several bacteria and fungi, was studied using five nutrient media. The medium S-I, with soybean flour, was recommended for the increase the quantity of biomass, proteins and lipids. The biomass of this strain can be used for the prophylaxis and treatment of bacterial diseases of honey bees.

Key words. streptomycetes, antimicrobial activity, medium, biomass, protein, lipids.

INTRODUCTION

Contemporary tasks in the field of antibiotics are primarily related to the necessity to combat pathogen's resistance to existing preparations [1, 19]. To overcome this problem the scientists resort to screening of new groups of antibiotics with broad spectrum, with a mechanism of action, pharmacokinetic and pharmacological favorable properties, which means that the search for new active strains of actinomycetes is actual at present, because of their well-known ability to synthesize the substances with antibacterial, antifungal, antiviral and antitumor properties. Antibiotics synthesized by actinomycetes are characterized by a broad antimicrobial spectrum. [1, 8, 11].

Defining of composition of culture medium is an important step, which allows, in a measure, to directing enzymatic processes and accumulation of biomass with predicted biochemical content [5, 9, 12, 20]. Growing medium must provide energy, carbon and nitrogen sources, mineral salts, corresponding to the physiological necessity of strain and providing a maximum accumulation of biomass with a high content of bioactive principles. Should be mentioned that the media, which provide enhanced growth of microorganisms, not always stimulates the synthesis of the desired bioactive substances [6, 9, 17, 20]. Problem of antibiotic biosynthesis regulation, depending on the nature of nitrogen source, is studied today. For example, the cycle of experiments with S. *galbus (F) subs*. 695 *achromogenes* was shown that the addition to the cultivation medium of L-asparagines, L-tyrosine, DL-phenylalanine and DL-histidine lead to synthesis of new antibiotics fractions [15]. Frequently, the source of amino acids is a corn flour, soybean, ammonium salts or nitrates. On media with organic sources of nitrogen the biosynthesis of antibiotics is increased [7, 9, 13, 14].

Considering the foregoing, the aim of our investigation was to select the optimum growth medium for streptomycetes strain *S.canosus* CNMN-Ac-04, which possesses antimicrobial activity, which will favor maximum accumulation of biomass with an increased content of biologically active substances, such as lipids and essential and immunoactive amino acids.

MATERIALS AND METHODS

The object of research was strain *Streptomyces canosus* CNMN-Ac-04, from National Collection of Nonpathogenic Microorganisms. The strain was stored in laboratory conditions on solid Czapek medium at about 4^0C (refrigerator). In investigations were used five complex media, using in microbiological practice to cultivate actinomycetes strains:

1. M-I – on the basis of a corn flour;
2. SP-I - on the basis of a corn flour and a soy flour;
3. S-I - on the basis of a soy flour;
4. R - on the basis of a corn flour and starch;
5. AM - on the basis of a corn flour with minerals.

To prepare an inoculum strains were first grown in liquid medium Duloney in Erlenmeyer flasks on shaker for three days at 27^0C. After the inoculations of complex media to be investigate actinomycetes were cultivated for 5 days under continuous stirring at constant temperature (27^0C). Medium M-I, developed in the laboratory, served as controls [2].

To determine the productivity, biomass has been separated from cultural liquid on a centrifuge (5000 rev/min. during 20 min.). Quantity of biomass was determined by a weight method. The amount of total protein and amino acid composition was identified at the Center for Automation and Metrology. Analyses were performed by ion-exchange liquid chromatography analyzer AAA-339 "Microtechnology" (Czech). Intracellular lipids were extracted from biomass according to Folch method, modificated in the laboratory [3]. Fractional composition of lipids was determined by the chromatography in a thin layer plates "Sorbfil" (100x150 mm) in the system hexane - diethyl ether - glacial acetic acid (73:25:5) and densitometrically [2].

Antimicrobial characteristics were studied by the agar diffusion test, using agar blocks [16, 17]. The strain was incubated on Petri dishes with agarizated Czapek medium and over 5-7 days can be used to prepare blocks inserted into the Petri dishes with test-strains.

RESULTS AND DISCUSSION

Strain *S. canosus* CNMN-Ac-04 was obtained after the combine irradiation with γ-rays and ultraviolet of strain *S. canosus* CNMN-Ac-02 [10]. Strain is characterized by accumulation of relatively stable quantities of biomass (4.0 to 5.0 g / l), with an increased content of lipids and proteins (50% and 180% more than the initial strain). Also *S.canosus* CNMN-Ac-04 differs from the initial strain with its ability to retain growth of a number of test microorganisms, including pathogens of bees: *B. alvei*, *B. larvae*, S*tr. apis*, *A. flavus*, *A. apis* (Table 1).

The necessity to improve the biosynthetic properties of studied strain, required to seek for new culture media. To determine the optimal medium, which ensures maximum accumulation of biomass with high content of lipids and protein were investigated five complex media, in wich the base source of C, N, and P served corn flour and soy flour. As controls served M-I medium.

The obtained results showed that depending on the used medium, the productivity of biomass and content of lipid and protein in it has changed substantially. The greatest quantity of absolutely dry biomass (ADB) was obtained from cultivation on medium with soy flour - SP-I - 15.36 g / L (Table 2). The stimulation of the biomass accumulation combine with the increased of productivity of proteins on g/L was established, the maximum quantity being on

the medium containing soy flour – S-I 5.30 g/L to 2.16 g/L in control, which is almost 2.5 times more.

Tab. 1.

Antimicrobial activity of strain *S.canosus* CNMN-Ac-04

Test-microorganisms	Diameter of growth retention zone, mm	
	S.canosus CNMN-Ac-02	*S.canosus* CNMN-Ac-04
Bacillus alvei	0	24,0±2,3
Bacillus larvae	0	27,5±2,5
Streptococcus apis	0	21,5±2,0
Escherichia coli B-60	11,0±1,1	16,0±1,4
Bacillus subtilis B-117	0	14,0±1,2
Erwinia carotovora 8982	0	18,0±1,7
Corynebacterium michiganense 13A	0	20,0±1,7
Ascosphaera apis	0	10,0±0,8
Aspergillus flavus	0	12,0±1,1
Aspergillus niger	0	10,0±0,8
Fusarium solani	0	13,0±1,2

„0"- absence of growth retention zone

For the long time was considered that lipids play a fairly modest cellular activity. Especially lipids were seen as a source of energy reserve. Extensive research in the last decades has been established that lipids are important biological effectors, regulators and mediators involved in all physiological processes. It is known that cultivation of actinomycetes on organic media rich in nutrients, promote lipids synthesis on 18.0%-37.9% of dry cell mass, while cultivation on synthetic medium increased the quantity of lipids only on 1.9% -5 6%. Lipid genesis depends on the amount and nature of substances, which were added to the nutrient medium as a source of carbon and phosphorus [5]. Thus lipids productivity of *S. canosus* CNMN-Ac-04, cultivated on media with soybean flour, was increased on 90.4% on SP-I medium and on 2 times on medium S-I and.

Tab, 2.

Biosynthetic activity of strain *S.canosus* CNMN-Ac-04 in dependence of growth medium

Experimental media	ADB		Total Protein		Total Lipids	
	g/L, (M±m)	% on control	g/L, (M±m)	% on control	g/L, (M±m)	% on control
M-I (control)	4,39±0,12	100,0	2,16±0,22	100,0	0,40±0,06	100,0
SP-I	15,36±0,63	349,8	3,72±0,31	172,2	0,77±0,12	190,4
S-I	13,58±0,57	309,3	5,30±0,44	245,3	0,82±0,13	201,1
R	13,56±0,54	309,1	3,32±0,31	154,4	0,52±0,09	129,1
PM	12,95±0,53	294,9	2,75±0,25	127,3	0,64±0,08	157,9

Fractional analysis of lipids, obtained by cultivation on experimental media also confirmed the modification of content of the main lipids fractions depending on growing media. Thus, synthesis of phospholipids obvious increased on cultivation on the medium S-I (25.2% of the lipids), contents of sterols vary up-to-date of control and triglyceride synthesis was stimulated to the growing on media with corn flour - 13.8% (R) and 16.3% (PM) of total lipids and was inhibited on media with soya flour - 9.3% (SP-I) and 9.4% (SI).

Results of investigations of amino acid content showed that its spectrum depends on medium. It was established that media containing soy flour promotes protein synthesis with increased content of essential and immune active amino acids. This can be explained by the fact that in soybean flour nitrogen content is higher than in corn flour. Thus, protein obtained on S-I medium contains the increased quantity of essential amino acids, such as lysine, isoleucine, threonine, phenylalanine and immunoactivi amino acids: aspartic acid, serine and arginine compared to the control. On the medium SP-I was determined increasing of the amount of lysine, alanine and cysteine. On the medium R – was established increased quantity of lysine and proline. On the medium PM the increased level of leucine, proline, alanine and tyrosine is characterized the protein. Biomass obtained on the control medium M-I, unlike the experimental media, includes more valine, histidine, glutamine and glycine.

After the investigations we can say that increasing the amount of biomass is related to increased protein and lipids content in it. Qualitative analysis of proteins confirmed the fact that high nitrogen content in soybean flour media provides increased synthesis of essential and immune active amino acids. Analysis of fractional composition of lipids revealed increased amounts of phospholipids, which is due to the high content of phosphorus in soybean flour [20]. Taking into account the obtained data, we can recommend medium S-I for cultivation of strain *S.canosus* CNMN-Ac-04, which allows to get richer biomass in protein and lipids.

Considering the antimicrobial properties of strain *S.canosus* CNMN-Ac-04 we supposed that biomass produced on the medium S-I can be used for prophylaxis and treatment of bee's bacteria diseases especially of American and European foul brood. Testing biomass of *S. canosus* CNMN-Ac-04 in apiary was conducted by Prof. V. Derjanski from the Laboratory of Entomology of the Institute of Zoology of the ASM.

Were formed two groups of seven families of bees infected with American foulbrood (control and experimental group). The experiment was performed during the period of maximum development of infection. Hives were selected taking into account the degree of infection with American foulbrood and family power. Experimental group received *S.canosus* CNMN-Ac-04 biomass mixed with powdered sugar in ratio of 1.8 to 2.2 g per 100 g powder [4]. Dose per family was 100 g mixture. The mixture was administered in a single half. Biomass efficacy was assessed by the degree of infection of brood before and after the experiment.

Tab. 3.

Efficiency of *S. canosus* CNMN-Ac-04 biomasson American foul brood

Groups	% of the infected brood		Efficiency of treatment (%)
	Before the experiment	After the experiment	
Control group	20,0 ± 2,0	28,0 ± 4,0	-
Experimental group	24,0 ± 3,0	2,0 ± 0,5	91,6

Monitoring the health status of bee families (presence or absence of died larvae, quantity of cap brood, number of populated frame, family's productivity), the collection of pathological material showed that the percentage of damage of bee's larvae in the experimental variants made up only 2.0%. Also, in the spring experimental groups of bees were more active. Daily family grew with 0.5 frames, and quantity of honey was on average 10 kg / hive higher than in control groups.

In conclusion we can say that improving conditions for cultivation of strains of streptomycetes, is an effective way to stimulate the biosynthetic activity of this group of organisms. And biomass of strain *S. canosus* CNMN-Ac-04, obtained after cultivation on the medium S-I, can serve as a basis for developing of bioproducts for treatment and prophylaxis of a number of infectious diseases of bacterial nature that affect honey bee.

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