THE AMINO ACID COMPOSITION OF THE BIOMASS OF THE STRAIN *Streptomyces fradiae* CNMN-Ac-11, CULTIVATED ON A COMPLEX MEDIUM WITH BIO PRODUCTS OF A CYANOBACTERIAL NATURE

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Abstract. The article deals with importance of proteins and amino acids in vital processes of all organisms and finding new ways for obtaining its in sufficient quantity to meet the growing demands. Because strains of genus *Streptomyces* are source of proteins and amino acids, the aim of research was to study changes of amino acid composition of the biomass of the strain *Streptomyces fradiae* CNMN-Ac-11, which was obtained during cultivation on medium R supplemented with bio products of cyanobacterial nature. Medium for cultivation R was improved by adding of cyanobacterial bio products BioR and Psh*ZnS. For analysis of amino acid composition were selected biomasses of experimental variants obtained after cultivation on medium R + 0.1 % BioR and medium R + 30.0 % Psh*ZnS, because of their benefic influence on lipogenesis. The addition of the BioR preparation in a concentration of 0.1 % had a significant effect on the productivity of the biomass of the strain and the synthesis of amino acids, increasing their yield by 24.2 %. The quantity of individual amino acids, such as asparagine, serine, valine, threonine and phenylalanine, has more than doubled. The addition of Psh*ZnS particularly affected the biosynthesis of methionine, increasing its yield by 83.8 %. The significant increase in the amount was mainly seen for essential amino acids.

Keywords: essential amino acids; proteins; *Streptomyces*; cyanobacterial bio products; biomass; lipids.

INTRODUCTION

The protein component of nutrition is a very important part of the daily diet of all organisms. The protein that comes with food in the gastrointestinal tract is split to amino acids that are absorbed into the internal environment of the organism and then used to synthesize the protein, supplying the nitrogen atom and the portion of the carbon chain to form nitrogen-containing compounds [14]. In turn, the body proteins synthesized from amino acids perform the following functions: structural, enzymatic, transport, contractile, protective, regulatory, storing [3, 20, 23, 34].

Depending on the structural formula and the functions performed in the metabolic processes, several functional groups of free amino acids are remarked.

According to the ability of the organism to synthesize one or another amino acid, the essential amino acids (which are synthesized by the organism) are distinguished. It is established that protein synthesis is carried out due to 20 proteinogenic amino acids.

In addition to this, on the first plan appear immunoactive amino acids: threonine, valine, tryptophan, aspartic and glutamic acid, serine, alanine, cystine, γ-aminobutyric acid. They form immunoactive proteins of the organism, participate in the formation of specific antibodies; accelerate the production of T-lymphocytes [1, 2].

According to the functional classification of amino acids, glycogenic and ketogenic amino acids are also remarked. Glycogenic amino acids as a result of gluconeogenesis are converted into glucose, thereby providing energy needs of the body. Ketogenic amino acids also serve as an energy source, but are converted to acetoacetyl-CoA or acetoacetate. There is currently no clear separation among amino acids for these groups. Some amino acids (isoleucine, phenylalanine and tyrosine) are referred to both groups.

An important role in the metabolism processes are played by sulfur-containing amino acids - cysteine, methionine, cysteic acid, cystathionine. Sulfur, which is an important part of these amino acids, is a part of biologically active substances (histamine, biotin, lipoic acid, etc.), participates in the cell during energy transfer and is a component of the sulfhydryl group of organic compounds, which ensures their enzymatic function [11].

Currently, more than half of the world's population is experiencing an acute protein deficiency. That is why amino acids have a big role in the problem of its elimination, since the widespread use of amino acid additives is one of the crucial conditions in the development of forage production and animal husbandry. At present, the microbiological industry serves as an important supplier of various amino acids, such as glutamic acid, lysine, methionine, threonine, valine, etc. [16]. Among microorganisms-producers one of the first places is the order of actinobacteria [19], which contains numerous active strains in the synthesis of amino acids. In particular, the genus *Streptomyces* attracts attention as one of the sources of obtaining these substances [19, 24]. As a result of microbial synthesis, L-forms of amino acids, whose production is relatively environmentally safe, are also obtained by directional microbial synthesis; enzymes can be obtained for the enzymatic method of producing amino acids.

The obtained amino acids are widely used in the food industry for the production of food additives (glutamic acid), in agriculture for feeding animals, for correcting the inferiority of vegetable proteins. In particular, the so-called fodder additives in the form of microbial biomass can be used in animal husbandry.

These supplements contain L-forms of amino acids, in particular, a set of essential amino acids [21]. Thus, the amount of essential amino acids in various strains of streptomycetes ranges from 40.5 to 53.6 % of the biomass protein [7, 13, 35].

The aim of research was to study the amino acid composition of the biomass of the strain *Streptomyces fradiae* CNMN-Ac-11, obtained during cultivation on medium supplemented with bio products of cyanobacterial nature.

MATERIAL AND METHODS

The object of the study was strain *Streptomyces fradiae* CNMN-Ac-11, isolated from the soil of the central part of Moldova. Strain was isolated on starch-ammonia agar medium (water-soluble starch – 10 g/l; K_2HPO_4 – 1 g/l; $MgSO_4$ – 1 g/l; NaCl – 1 g/l; $(NH_4)_2SO_4$ – 2 g/l; $CaCO_3$ – 2 g/l; agar – 20-25 g/l; pH=7.0-7.4). The strain was stored in two ways: by periodic transfer, using 3 agar media - Czapek, Gause and oatmeal agar, and also in a lyophilized form [5, 8, 12]. To conduct research on the amino acid composition of biomass, the inoculum of investigated strain was grown on orbital shaker (180-200 rpm) for 3 days at 27ºC in Erlenmeyer flasks (V=1,000 ml) with 200 ml of liquid mineral media Dulaney with glucose of 7.0 % (pH=7.0) [32]. For obtaining biomass, cultivation of inoculum continued in the same conditions on medium R (corn flour – 20 g/l; water-soluble starch – 15 g/l; NH_4NO_3 – 7 g/l; KH_2PO_4 – 0.2 g/l; $CaCO_3$ – 5 g/l; NaCl – 3 g/l; pH=6.8-7.0) on the stirrer for 5 days. To study the effect of the composition of the cultivation medium on the accumulation of biomass, total protein and the amino acid composition, cyanobacterial preparations were added to the basic nutrient medium R in different concentrations. The used bio products – amino acids and oligopeptides (BioR) and zinc sulfated polysaccharides (Psh*ZnS) from *Spirulina platensis* CNMN-CB-02 were kindly provided by staff of Laboratory of Phycobiotechnology [30].

The biomass was separated from broth culture by centrifugation (5.000 rpm for 20 min). Absolutely dry biomass (ADB) was determined by classical dry weight [5].

The intracellular lipids were extracted from biomass by the modified Folch method [10].

The amino acid composition of the obtained biomass was determined by ion exchange chromatography on an amino acid analyzer AAA-339 M „Microtehna".

In complex biological materials, both bound and free amino acids are determined. For the preparation of biomass samples, the method of hydrolysis with 6N-hydrochloric acid was used [18]. The sample is weighed and quantitatively transferred into test tubes from Pyrex or Siala, to which 6N-hydrochloric acid is added in a double excess. The 12N-hydrochloric acid is added to the liquid samples in the sample volume. When choosing samples, reference data are used, as 1 mg of protein contains 0.3-1 μmol of individual amino acids. The tubes are sealed, and then complex samples are held in an air thermostat at 110±10°C for 24 hours. After hydrolysis, the tubes are cooled; the contents of the tubes are quantitatively transferred and filtered. The acid in the resulting liquid is evaporated in a vacuum rotary evaporator at 400°C, to a pH of 2.2. The usual hydrochloric acid hydrolysis of the complex mixture on the chromatogram gives the actual amount of protein bound and free amino acids.

RESULTS

In experiments, was optimized the nutrient medium for growth of the studied strain with preparations from microalgae *Arthrospira platensis* CNMN-CB-02, since biomass contains up to 70.0 % of the protein represented by all essential amino acids, a complex of vitamins, including β-carotene, B vitamins (B_1, B_2, B_3, B_5, and especially B_{12}), a large number of macro- and microelements in bioavailable organic form [28]. Cultivation of the strain on the improved complex nutrient medium R showed an increase in productivity in the yield of biomass, total protein, and individual amino acid groups. The effect on the vital activity of the studied strain of the preparation BioR - an extract of amino acids and peptides from *Arthrospira platensis* CNMN-CB-02 is especially pronounced [29].

As can be seen from table 1, the largest concentration of lipids in biomass was observed in the samples with the addition of substances of the cyanobacterial nature of BioR at a concentration of 0.1 % and Psh*ZnS in a concentration of 30.0 % to the main nutrient medium. Therefore, was decided to investigate precisely these samples for the ability to accumulate protein and amino acids.

Table 1. The effect of cyanobacterial preparations on the productivity of biomass and lipids in strain *Streptomyces fradiae* CNMN-Ac-11

Variants	ADB, g/l	Lipids, %
Medium R	12.38±0.19	4.34±0.2
Medium R + 0.05 % BioR	13.98±0.19	3.22±0.44
Medium R + 0.1 % BioR	14.64±0.23	3.54±0.37
Medium R + 1.0 % BioR	14.27±0.21	2.1±0.25
Medium R + 2.0 % BioR	13.76±0.17	2.25±0.28
Medium R + 5.0 % BioR	13.13±0.28	1.9±0.25
Medium R + 5.0 % Psh*ZnS	13.43±0.23	3.16±0.25
Medium R + 10.0 % Psh*ZnS	13.88±0.27	3.14±0.38
Medium R + 20.0 % Psh*ZnS	12.63±0.41	3.16±0.22
Medium R + 30.0 % Psh*ZnS	11.36±0.22	4.27±0.49
Medium R + 50.0 % Psh*ZnS	10.92±0.48	3.12±0.19

As can be seen from the data presented in figure 1, the studied strain showed an increase in the productivity of biomass when BioR was added to the main nutrient medium. When the preparation was added at a concentration of 0.1 %, the biomass yield increased by 18.0 %. Introduction of Psh*ZnS in a concentration of 30.0 % caused a decrease in the amount of biomass by 8.0 %.

The results presented in figure 2 indicate a positive effect on the accumulation in the biomass of the protein, by adding of the BioR preparation to the main nutrient medium. Here, the increase in protein yield was 23.5 % compared to the control value. Addition of the Psh*ZnS caused a decrease of protein yield in biomass by 22.7 %.

Further, the task was to determine the qualitative composition of the protein obtained in the course of biomass experiments. Analysis of the total amino acid content in the biomass of *Streptomyces fradiae* CNMN-Ac-11 showed an increase in the yield of practically of all groups, except for non-essential amino acids, when BioR was added to the nutrient medium. As can be seen from the data presented in table 2, the increase in the number of essential amino acids was 78.2 %, immunoactive - 14.2 %, the amount of glycogenic amino acids increased by 40.6 %, ketogenic - by 71.25 %, proteinogenic - by 24.3 %, and sulfur-containing - by 55.5 %. A decrease in the yield by 3.3 % was observed only in non-essential amino acids.

When the Psh*ZnS was added to the main nutrient medium, the opposite tendency was observed. Practically in all amino acid groups was registered decrease. Thus, in comparison with the control sample, the decrease in the amount was 35.7 % for non-essential amino acids, 30.1 % for immunoactive, 17.2 % for glycogen, 6.65 % for ketogenic and 22.6 % for proteinogenic amino acids respectively. An increase in yield of 3.07 % and 11.7 % was observed for essential and sulfur-containing amino acids, respectively.

Next, we conducted a comparative analysis of the amino acid content in the biomass of strain *Streptomyces fradiae* CNMN-Ac-11. The results of this analysis are presented in Table 3.

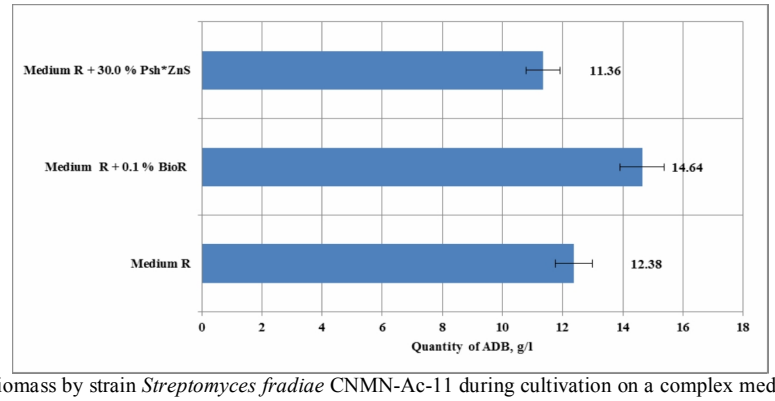

Figure 1. Accumulation of biomass by strain *Streptomyces fradiae* CNMN-Ac-11 during cultivation on a complex medium R and on medium R with cyanobacterial additives

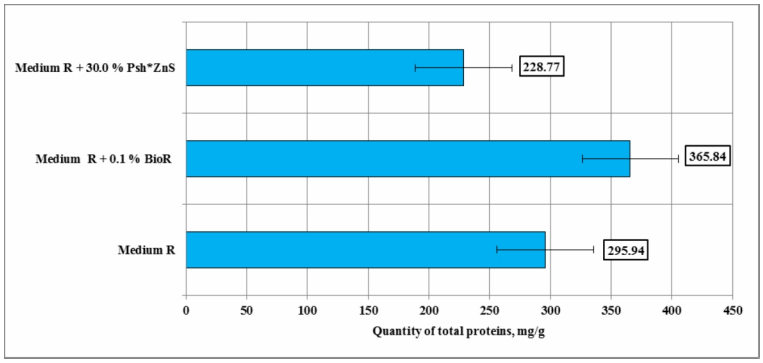

Figure 2. Protein content in biomass of strain *Streptomyces fradiae* CNMN-Ac-11 during cultivation on a complex medium R and on medium R with cyanobacterial additives

Table 2. Effect of additives to the main nutrient medium on the total amino acid content in the biomass of strain *Streptomyces fradiae* CNMN-Ac-11

Σ	Control (medium R)		Medium R + 0.1 % BioR		Medium R + 30.0 % Psh*ZnS	
	mg/g	%	mg/g	%	mg/g	%
Amino acids	291.11	100	361.56	124.20	225.71	77.53
Non-essential amino acids	189.26	100	183.04	96.71	121.63	64.27
Essential amino acids	96.82	100	172.54	178.21	99.79	103.07
Immunoactive amino acids	179.35	100	204.80	114.19	125.35	69.89
Glycogenous amino acids	99.81	100	140.35	140.61	82.66	82.82
Ketogenic amino acids	57.22	100	97.98	171.25	53.41	93.35
Proteinogenic amino acids	286.08	100	355.58	124.29	221.42	77.40
Sulfur-containing amino acids	14.53	100	22.60	155.54	16.23	111.70

Table 3. Amino acid content in biomass of strain *Streptomyces fradiae* CNMN-Ac-11 cultivated on medium R and medium R with cyanobacterial additives

Amino acid	Control (medium R)		Medium R + 0.1 % BioR		Medium R + 30.0 % Psh*ZnS	
	mg/g	%	mg/g	%	mg/g	%
Cysteic acid	3.32	100	3.94	118.85	2.41	72.59
Asparagine	15.49	100	33.65	217.21	14.87	95.99
Threonine	12.85	100	26.88	209.26	11.21	87.26
Serine	7.56	100	16.04	212.09	6.59	87.10
Glutamic acid	84.96	100	62.40	73.45	45.07	53.04
Proline	18.11	100	13.37	73.82	10.17	56.14
Glycine	15.10	100	14.24	94.34	12.12	80.25
Alanine	35.99	100	23.62	65.64	22.10	61.41
Valine	12.82	100	25.91	202.07	15.77	123.01
Cysteine	7.97	100	14.26	178.86	7.87	98.68
Methionine	3.24	100	4.40	135.74	5.95	183.77
Isoleucine	7.25	100	11.72	161.51	8.87	122.25
Leucine	27.26	100	43.51	159.60	23.90	87.68
Tyrosine	4.08	100	5.46	133.75	2.86	70.02
Phenylalanine	8.06	100	17.29	214.49	8.61	106.81
γ-aminobutyric acid	1.71	100	2.04	119.13	1.88	109.83
Lysine	10.56	100	20.02	189.46	9.18	86.89
Histidine	4.32	100	8.37	193.86	4.08	94.37
Arginine	10.46	100	14.46	138.20	12.23	116.87

The increase in the quantity of individual amino acids after use of optimized medium with extracts of cyanobacteria is very pronounced. For example, the weight of threonine increased by 109.26 %, serine by 112.1 %, valine by 102.1 %, phenylalanine by 114.5 %, compared to the control sample of biomass when BioR was added to the nutrient medium. The increase in the number of amino acids such as cysteine, leucine, isoleucine, lysine, histidine is less pronounced, so the yield of cysteine increased by 78.86 %, leucine by 59.6 %, isoleucine by 61.5 %, lysine by 89.5 %, histidine – almost 94.0 %.

Concerning the addition of the Psh*ZnS to the nutrient medium, there was a pronounced increase in such amino acid as methionine - by almost 84.0 %. All amino acids, the number of which has increased high, except for serine and cysteine, are essential. The amount of the last conditionally non-essential amino acid arginine increased only by 38.2 %.

DISCUSSION

The problem of the lack of protein raw materials is quite acute. More than half of the world's population is deficient in protein. Also, this problem is relevant for animal husbandry and poultry [27]. So, it is known that methionine in poultry diets is the first limiting amino acid, since methionine replenishes the body with sulfur, is a universal source of methyl groups, whose synthesis in a living organism is limited. Therefore, in the USA, rations for broilers count not only for raw protein, but also for methionine and lysine. In experiments with the addition of 0.12 and 0.25 % methionine to the diet, the live weight of chickens and turkey-broilers for 7 weeks increased by 10.3 and 11.0 % [26]. For pigs, the first limiting amino acid is lysine. Lysine is a part of all proteins, it affects oxidation-reduction reactions in the body, catalyzes the processes of reamination and deamination. Thus, in the experiment with the addition of the preparation "L-lysine monochlorohydrate fodder" to the main ration of young pigs, registered daily increase in the live weight of the experimental animals by 14.1 % in comparison with the control group [22]. Lysine also promotes the absorption of calcium and phosphorus [31].

Since there is an inadequacy of the feed material for the aforementioned amino acids, it is important to add to the feeds synthetic amino acids of industrial production. In particular, in 2005 the consumption of synthetic amino acids in Russia Federation increased by 29.9 %. Production of amino acids is about 400 thousand tons per year, half of production is accounted for glutamic acid, methionine is produced up to 150 thousand tons, and lysine 45-50 thousand tons [14].

It is known that many strains of streptomycetes have a pronounced ability to accumulate amino acids in biomass. Previously, it was shown that in the biomass of the strain *S. massasporeus* 36, isolated from the soils of Moldova, contains about 44.0 % protein, a sufficiently large number of essential amino acids [6, 32]. Studies have shown that in mycelium strains of streptomycetes contains 14-16, up to 18 of amino acids, the composition of which is practically the same for all, but there are changes in the amount of individual acids. In general, active strains of streptomycetes accumulate leucine, alanine, aspartic and glutamic amino acids [33]. Our experiments has demonstrated the ability of strain *Streptomyces fradiae* CNMN-Ac-11 accumulate in biomass 19 amino acids, including 9 of different quantities of essential acids. The importance of these amino acids could not be overestimated. Thus, threonine involved in the synthesis of immunoglobulins, valine support body serotonin level, phenylalanine participates in the synthesis of melanin and insulin, leucine and isoleucine are the building and energy material for muscle tissue, lysine promotes calcium accumulation in the body, histidine is the starting material for the synthesis of

histamine as well as in large amounts in hemoglobin, methionine acts as a donor of methyl groups for the synthesis of other biologically active substances. Conditionally non-essential amino acid arginine by its presence stabilizes the secondary and tertiary structure of the protein. Non-essential serine amino acid is part of the active site of some enzymes; complex lipids, sulfur-containing amino acid cysteine are involved in the synthesis of coenzyme A [14].

An increase in the quantitative yield of an amino acid in biomass can be obtained by changing the composition of the nutrient medium for the cultivation of microorganisms. Thus, in studies on the cultivation of streptomycetes on the Dulaney medium, an increased synthesis of glutamine, alanine, valine, leucine, and histidine was noted [17]. In an experiment to optimize the growth parameters of the keratinase producer *Streptomyces ornatus* s 1220, an increase in the yield of biomass and keratinase activity was established when calcium carbonate was added to the main nutrient medium and when the temperature was maintained during cultivation at 30°C [25]. Alanine and cysteine, supplemented, can increase the growth and accumulation of protein in the mycelial fungus of *Fusarium* by 1.5-3.0 times [36]. Also, increasing the productivity of producer strains can be affected by the use of physical agents. So, after applying electromagnetic radiation in the millimeter range of low intensity for 1 minute, an increase of 20.6 % in the biomass of *S. massasporeus* CNMN-Ac-06 of valine was observed, after 18 minutes of isoleucine - by 18.75 %, and after 5 and 15 minutes of γ-aminobutyric acid by 42.1 and 52.6 %, respectively [4, 9].

The experiments showed that the addition of the BioR preparation in a concentration of 0.1% had a significant effect on the productivity of the biomass of the strain and the synthesis of amino acids, increasing their yield by 24.2 %. The number of individual amino acids, such as asparagine, serine, valine, threonine and phenylalanine, has more than doubled. The addition of Psh*ZnS particularly affected the biosynthesis of methionine, increasing its yield by 83.8 %. The tendency of a significant increase in the amount was mainly seen for essential amino acids. This indicates the expansion of the possibility of obtaining these amino acids in much larger quantities using the streptomycetes of soils of Republic of Moldova.

Thus, the obtained data allow us to consider the use of cyanobacterial extracts BioR and Psh*ZnS to increase the biosynthetic activity of *Streptomyces fradiae* CNMN-Ac-11, in particular, to increase biomass accumulation with improved qualitative and quantitative composition of amino acids, treating it as the basis of new biological products for farm animals and poultry.

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Received: 29 September 2017
Accepted: 29 October 2017
Published Online: 2 November 2017
Analele Universităţii din Oradea, Fascicula Biologie
http://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433
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