Influence of carbon-bearing raw material on microfungus *Blakeslea Trispora* biomass producing

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Abstract

Introduction. This paper investigates influence of hydrated fullerenes on degree of accumulation bioactive substances of microfungus Blakeslea trispora.

Materials and methods. In this research effort detection of fatty-acid composition in amino acids, carotenoids and sterols biomass by means of using methods of high-performance liquid chromatography, adsorption and disjunctive chromatography in thin-layer sorbent and spectrophotometric; gravimetric method; method of direct spectrophotometration in benzene took place.

Results and discussion. It has been induced that application of hydrated fullerenes in microfungus Blakeslea trispora nutrient medium promotes increasing accumulation in biomass quantity of carotene on 32,3 %; asparaginic, glutamic acids and leucine.

Reproportion carbon to nitrogen by means of adding to microfungus Blakeslea trispora nutrient culture medium hydrated fullerenes did not influence on the biomass amino acid structure any.

Obtained data of fatty-acid composition in microfungus Blakeslea trispora lipoid fraction indicate about significant predominance unsaturated fatty acids and, as a result of this, we have advance of use microfungus Blakeslea trispora biomass as a source of biologically active substances for establishing a new kind of prophylactic action goods.

Introduction

Microfungus *Blakeslea trispora*, owing to its high carotinsynthesizing ability, is the most promising producer of β-carotin and is advanced industrial basic material among other microorganisms and plants [1]. Hydrated fullerenes — high-persistent fine-dispersed aqueous solution of native fullerenes, which, unlike other fullerenes, have properties of lyophobic molecular-colloidal systems and are growth-stimulating agents and antioxidants

[2, 3, 4]. Special properties of fullerenes are their water repellency and their ability to oxidation due to high electronegativity, notably their ability to attach any more than six free electrons to oneself [5].

Materials and methods

In this research effort detection of fatty-acid composition in amino acids, carotenoids and sterols biomass took place. A quantitative composition of biologically active substances in the microfungus *Blakeslea trispora* biomass samples, obtained in different culture conditions: in traditional nutrient medium and with adding to it hydrated fullerenes; was studied.

Definition of fatty-acid composition in biomass pilot samples was carried out with use of method of high-performance liquid chromatography by the instrumentality of chromatograph Shimadzu Gl-14B with electronic data processing [7].

For detection sterols in microfungus *Blakeslea trispora* biomass, we obtained unsaponifiable fractions of biomass samples. For complete total (qualitative and quantitative) definition 3β-hydroxysterols we applied methods of adsorption chromatography and thin-layer chromatography and spectrophotometric. Saponification of samples was carried out with the use of petroleum-ether (40…60 °C) as extractant, elimination of solvent — in vacuum under temperature 30…35 °C, while detection of unsaponifiable fraction — with gravimetric method.

Content of carotenoids we estimated by means of method of direct spectrum-photometric measurements in benzol under wave length 456 nm. Content of carotenoids calculated in conformity with 100 g band-and-hook hinge of virgin sample [9].

For detection of amino acids quantity in microfungus *Blakeslea trispora* biomass samples, we previously applied normalized amino acids mixture with well-known concentration of every amino acid on the column of autoanalyser [10]. In accordance with chromatogram, we calculate peak area (or peak height) of every amino acid. Formula evaluation of number of micromoles in every amino acid (X_1) in observable solution stated below:

$$X_1 = S_1 / S_o, \quad (1)$$

where S_1 - peak area (or peak height) of amino acid in observable sample;

S_0 - peak area (or peak height) of the same amino acid in solution of normalized amino acids mixture, which corresponds to 1 micromole quantity of every amino acid.

In the samples of microfungus *Blakeslea trispora* dried biomass we carried out content test for carotenoids and vitamin E, as well as content test for ubiquinone (coenzyme Q_{10}) [16]. For detecting this substances, we saponified band-and-hook hinge of biomass samples via potassium hydroxide alcoholic solution and extracted via diethyl ether. Extracts were washed off with water up to neutral pH magnitude, were dried by means of anhydrous sulfuric sodium, were filtrated and evaporated by the use of rotary evaporator. Unsaponifiable fraction (solid residual) was dissolved in benzol.

Extracts of unsaponifiable matter had been simultaneously separated and determined content of carotenoids, ubiquinone and vitamin E in them [15].

Content of vitamin E and ubiquinone in aliquots of summarized unsaponifiable matter in observable samples was determined after two sequential chromatographies: adsorption (preparative) thin-layer chromatography with silicagel mark LS 5/40 μm in system hexane-ether (correlation respectively 70:30), and analytic chromatography on impregnated plates

(5 % hexadecane in light petroleum ether, fraction 40…60 °) Silufol uv 254 in system acetone-water (correlation accordingly 90:10). In the capacity of control samples we used standards Q_9, Q_{10} and α-tocoferol ("Serva").

Content of ubiquinone we determined after chromatographic separation components of unsaponifiable fractions of samples in re-computation on dry fraction at wave length 275 nm in ethanol according to difference between extinctions of oxidated and reduced forms in 15 minutes after adding to probe 0,02 ml 2,5 % borane sodium aqueous solution.

Content of vitamin E we determined after chromatographic separation components of unsaponifiable fractions of samples by the use of Emery-Engel reaction with photometric method at wave length 520 nm.

For determining sterols in microfungus *Blakeslea trispora* biomass, we obtained unsaponifiable fractions of biomass samples. For complete general (qualitative and quantitative) detection 3β-hydroxysterols we used methods of adsorption and disjunctive chromatography in thin-layer sorbent and spectrophotometric. Saponification of samples was carried out with the use of petroleum-ether (40…60 °C) as extractant. Elimination of solvent was carried out in vacuum under temperature 30…35 °C. Detection of unsaponifiable fraction was carried out with gravimetric method.

For qualitative and quantitative detection 3β-hydroxysterols we used method of thin-layer chromatography. Localization of investigated materials evaluated per standards: ergosterine, cholesterol, fucosterol, β-sitosterol. Supplementary standard was precursor of sterols — squalene.

Quantitative determinations were carried out using method of color chemical reactions with the use of spectrophotometric measurements.

Components of unsaponifiable fraction we separated on carotenoid fraction (contain only squalene) and noncarotenoid fraction (contain sterols and steroids).

Results and discussion

Composition of amino acids in microfungus *Blakeslea trispora* samples, that were obtained in various culture conditions, denotes on increasing number of certain amino acids in the samples of biomass (table 1) and content of carotenoids (table 2).

From this findings of investigation (table 1), it can be concluded that total content of amino acids in microfungus *Blakeslea trispora* biomass presented with all principal amino acids. Reproportion carbon to nitrogen by means of adding to nutrient medium hydrated fullerenes did not influence on the biomass amino acid structure any.

As a result of findings, which is stated below in the Table 4, content of unsaturated fatty acids in check and test biomass samples, that were obtained under different culture conditions, practically is quite indistinctive.

Table 1

Amino acid composition of microfungus *Blakeslea trispora* biomass test specimens

Amino acids	Content in biomass on conventional nutrient solution		Content in biomass with adding to nutrient medium hydrated fullerenes	
	mg	*%, in mg*	*mg*	*%, in mg*
Lysine	0,3555 ± 0,02	6,79 ± 0,41	0,8340 ± 0,07	8,25 ± 0,68
Histidine	0,1634 ± 0,02	3,02 ± 0,22	0,2554 ± 0,02	2,52 ± 0,12
Arginine	0,2111 ± 0,01	3,84 ± 0,24	0,3882 ± 0,04	3,84 ± 0,21
Asparaginic acid	0,5168 ± 0,17	9,63 ± 0,81	1,6141 ± 0,17	15,94 ± 1,34
Threonine	0,3598 ± 0,03	6,74 ± 0,53	0,4956 ± 0,05	4,90 ± 0,39
Serine	0,3395 ± 0,03	6,43 ± 0,42	0,4957 ± 0,05	4,91 ± 0,42
Glutamic acid	1,8597 ± 0,02	16,05 ± 1,02	1,9887 ± 0,21	19,65 ± 1,71
Proline	0,1100 ± 0,01	1,91 ± 0,13	0,0978 ± 0,008	0,91 ± 0,06
Glycine	0,5289 ± 0,04	9,93 ± 0,64	0,6127 ± 0,08	6,05 ± 0,53
Alanine	0,4109 ± 0,03	7,65 ± 0,52	0,8006 ± 0,09	7,91 ± 0,65
Cystine	0,0109 ± 0,00	0,18 ± 0,01	0,0190 ± 0,002	0,19 ± 0,02
Valine	0,2319 ± 0,01	4,47 ± 0,36	0,3968 ± 0,04	3,92 ± 0,35
Methionine	0,0206 ± 0,00	0,38 ± 0,25	0,0573 ± 0,006	0,57 ± 0,05
Isoleucine	0,5231 ± 0,04	4,44 ± 0,18	0,4762 ± 0,05	4,70 ± 0,34
Leucine	0,5299 ± 0,04	9,87 ± 0,37	1,0238 ± 0,07	10,11 ± 0,97
Tyrosine	0,2321 ± 0,02	4,12 ± 0,22	0,3249 ± 0,02	3,21 ± 0,21
Phenylalanine	0,2339 ± 0,02	4,54 ± 0,24	0,2422 ± 0,03	2,39 ± 0,06

Table 2

Content of carotenoids in microfungus *Blakeslea trispora* biomass test specimens

№	Denomination of a sample	Content of carotenoids, g/100 g
1	Biomass on conventional nutrient solution	0,720 ± 0,064
2	Biomass with adding to nutrient medium hydrated fullerenes	0,800 ± 0,082

Content of sterols in microfungus *Blakeslea trispora* biomass samples is adduced in the Table 3.

Table 3

Content of sterols in microfungus *Blakeslea trispora* biomass samples

№	**Denomination of a sample**	**Unsaponifiable fraction,** % from biomass sample	**Squalen,** % from unsaponifiable fraction	**Noncarotenoid fraction**, %	**β-sitosterol,** % from unsaponifiable fraction
1	Biomass on conventional nutrient solution	**10,89 ± 1,12**	**7,50 ± 0,78**	**1,71 ± 0,19**	**0,15 ± 0,02**
2	Biomass with adding to nutrient medium hydrated fullerenes	**14,41 ± 1,51**	**9,06 ± 1,01**	**1,95 ± 2,02**	**0,26 ± 0,04**

Table 4

Fatty-acid composition of microfungus *Blakeslea trispora* biomass

№	**Name of acid**	**Content in biomass on conventional nutrient solution**, mg %	**Content in biomass with adding to nutrient medium hydrated fullerenes, mg %**
1	$C_{14:0}$ myristic	-	0,8329 ± 0,09
2	$C_{16:0}$ palmitic	8,6913 ± 0,9	8,957 ± 0,9
3	$C_{16:1}$ palmitic-oleic	0,8381 ± 0,8	-
4	$C_{18:0}$ stearic	4,6892 ± 0,5	4,6211 ± 0,5
5	$C_{18:1}$ oleic	21,9756 ± 2,1	20,9858 ± 2,3
6	$C_{18:2}$ linoleic	61,0342 ± 6,2	59,0434 ± 4,8
7	$C_{18:3}$ linolenic	1,2153 ± 0,1	3,2598 ± 0,3
8	$C_{20:1}$ gadoleic	0,4410 ± 0,03	-
9	$C_{22:0}$ behenic	1,2102 ± 0,1	1,0332 ± 0,1

Obtained data of fatty-acid composition of lipid fraction in microfungus *Blakeslea trispora* biomass indicate about significant predominance unsaturated fatty acids, that stipulates high indices of acidity and peracidity numbers. Withal presence of huge variety of fatty acids indicates about availability of employment of microfungus *Blakeslea trispora* biomass as a source of biologically active substances for the purpose of creation of new types of production with prophylactic action [17, 18].

Synthetic data, concerned in the question of content of biologically active substances in microfungus *Blakeslea trispora* biomass, are presented in the table 5.

Table 5

Content of biologically active substances (BAS) in microfungus *Blakeslea trispora* biomass samples

№	Biologically active substances	Content in biomass on conventional nutrient solution	Content in biomass with adding to nutrient medium hydrated fullerenes
1	Carotenoids, mg %	635 ± 49	840 ± 79
2	General lipids, %	19,5±2,2	18,6±1,6
3	Ubiquinones, mg %	0,10 ±0,01	0,08±0,006
4	β-sitosterol, % from unsaponifiable fraction	0,15±0,02	0,36±0,04
5	Squalen, % from unsaponifiable fraction	7,50±0,78	9,06±1,01
6	Protein, %	2,29±0,20	1,82±0,15
7	Phospholipids in re-calculation on lecithin, mg %	246,9 ± 20,12	252,0 ± 22,1

On the ground of presented data (in table 5), we can predicate about existence in microfungus *Blakeslea trispora* biomass not only carotenoids, but also another different biologically active constituents.

Conclusion

It has been induced that application of hydrated fullerenes in microfungus *Blakeslea trispora* nutrient medium promotes increasing accumulation in biomass quantity of carotene on 32,3 %, asparaginic, glutamic acids and leucine, and variation of some other amino acid composition characteristics.

Reproportion carbon to nitrogen by means of adding to microfungus *Blakeslea trispora* nutrient culture medium hydrated fullerenes did not influence on the biomass amino acid structure any.

Obtained data of fatty-acid composition in microfungus *Blakeslea trispora* lipoid fraction indicate about significant predominance unsaturated fatty acids and, as a result of this, we have advance of use microfungus *Blakeslea trispora* biomass as a source of biologically active substances for establishing a new kind of prophylactic action goods.

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