International Journal of Research Studies in Science, Engineering and Technology
Volume 2, Issue 8, August 2015, PP 62-70
ISSN 2349-4751 (Print) & ISSN 2349-476X (Online)

Carboxylic Acids of Saccaromyces Cerevisiae Grown in Different Culture Media

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Abstract: *The work studies the composition and content of carboxylic acids in the biomass of the yeast Saccharomyces cerevisiae and growth media using the methods of capillary electrophoresis and gas-liquid chromatography. The yeast biomass is predominated with palmitic $C_{16:0}$, linoleic $C_{18:2\omega-6,}$ dihomo-γ-linolenic $C_{20:3\omega-6,}$ and arachidonic $C_{20:4\omega-6}$ acids (that is up to 33.4, 34.3, 11.8 and 20.2% of the fatty acid amount). Geothermal water in the composition of the nutrient medium contributes to the increase of dihomo-γ-linolenic and arachidonic acids in the yeast biomass more than 12 times and to the increase of linoeladic and linoleic acids in aqueous-alcoholic solutions (distillate of the growth medium) almost threefold. The results obtained enable to consider S. cerevisiae Y-503 strain as a potential source of palmitic and polyene acids: linoleic, dihomo-γ-linolenic and arachidonic.*

Keywords: *Saccharomyces cerevisiae, nutrient media, geothermal water, carboxylic acids.*

1. INTRODUCTION

Being unicellular eukaryotes, the yeast *Saccharomyces cerevisiae* are found to be a suitable model for the study of cellular metabolism including the processes of accumulation or excretion of metabolites in various growth conditions [1-4]. Molasses has long been used in the composition of nutrient media for yeast cultivation as a universal source of carbohydrates, organic acids and mineral compounds [5, 6]. A number of works are devoted to the construction of new nutrient media containing the water of geothermal spring. [7-9].The study of the fatty acid composition of the yeast cultivated on different nutrient media is of great interest in terms of possibility to use these microorganisms as producers of bioactive lipids [10, 11].

Thermal waters as a part of nutrient growth media causes an interest due to their enrichment with mineral components and organic compounds: oil, hydrocarbon gases, humic substances and bitumen. Humus is known to contain individual (including specific) organic compounds, reaction products and organic compounds in the form of organic and mineral formations. Bitumen is chemical and analytical analogs of fats and fat-like substances which contain hydrocarbons: methanic, aromatic, n-alkanes, isoalkanes, cyclopentanes, fatty acids etc. [12]. Moreover, the use of geothermal water for the cultivation of yeast on a commercial scale will contribute to the development of high-performance high-performance economy sectors of the regions rich in groundwater resources.

Currently there are few publications on the excretion of fatty acids by *S. cerevisiae FMC-16* [13], *S. cerevisiae M-5* and *S. uvarum I-7* [14], *S. cesevisiae UCMY-524* [15] strains and other cultures of genus *Saccharomyces* [10, 16] but lipogenesis is not sufficiently studied when grown on nutrient media of different composition. This work studies the content and composition of carboxylic acids including fatty acids, some characteristics of growth and lipogenesis of yeast *S. cerevisiae* depending on the growth conditions.

2. METHODS

We used *Saccharomyces cerevisiae Y-503* strain (from the collection of the Scientific Center of Russian Federation Research Institute for Genetics and Selection of Industrial Microorganisms) engineered in the Caspian Institute of Biological Resources, Dagestan Scientific Center of the Russian Academy of Sciences [17].

The strain was shown to belong to to the *S. cerevisiae* taxon by UP-PCR method in the group of S. Bulat (Laboratory of Eucaryote Genetics, Department of Molecular and Radiation Biophysics,

Petersburg Nuclear Physics Institute, Russian Academy of Sciences). The *Y-503* strain is heterozygous tetraploid, as was shown [18, 19].

To cultivate *S. cerevisiaeY-503*, culture media with the following composition were used (g / l):

Control medium: molasses - 488.74, $(NH_4)_2HPO_4$ – 1.53, $(NH_4)_2SO_4$ – 4.6, H_2O – water, pH 4.5;

Development medium №1: molasses - 488.74, $(NH_4)_2HPO_4$- 2.58, H_2O-geothermal water from well №36 of Makhachkala field (chloride - sulphate – hydrocarbonate sodium), pH 4.5;

Development medium №2: the composition is identical to №1 but H_2O-geothermal water from well №7-Tof Kizlyar field (hydrocarbonate -chloride - sulphate - sodium).

In the development media (№1 and №2) molasses was diluted with geothermal water of different composition and in the control medium molasses was diluted with water till hydrocarbon content amounted to 20.0 g/100 cm^3 (in each variant).

To optimize *S. cerevisiae* cultivation conditions the selection of media composition was made beforehand [20].

The yeast was cultivated in 3000 ml flasks (working volume 1500 ml) for 120 h at 30^oC, pH 4.5 in the laboratory using the depth method in a batch mode under anaerobic conditions. Cell cultures grown in nutrient media that were identical to control or development media (№1 and №2) were used as inoculum. To obtain inoculum (with hydrocarbon content 20 g/100 cm^3) the cells were sequentially adapted to the media containing hydrocarbon: 7.9 – 10.8 – 12.4 – 20.0 g/100 cm^3. The inoculum was cultivated for 5 days at temperature 30± 1^oC in a batch mode under anaerobic conditions. The content of inoculum was 10% of culture medium volume. 80 % struktol – oily substance – (0.1 ml/1.5 l of medium) was used as defoamer. After 120 hours of cultivation and separation of the yeast by centrifugation (5000 g, 15 min) in a stationary laboratory centrifuge CLS - 344.2, the nutrient medium was named «fermented substrate». Aqueous – alcoholic solutions («alcohol distillate») were obtained using distillation of the fermented substrate. Ethanol concentration in the medium was determined using techniques [21].

The content study of free low-molecular organic aids in nutrient media, fermented substrates and aqueous-alcoholic solutions was carried out by the capillary electrophoresis device "Capel-105" (Russia).

The method of direct interesterification with methanol solution of sodium methoxide was used to obtain methyl esters of fatty acids from triglycerides [22]. The fractionation of methyl esters of fatty acids (in nutrient media, fermented substrates, biomass and aqueous-alcoholic solutions) and determination of impurities [23] in ethanol were carried out with the gas chromatography using chromatograph «TRACE – 2000» (Italy) with a flame ionization detector (PID) on a capillary gas chromatographic column HP-FFAP (USA) (50 m x 0.32 mm x 0.52 mm) with 10% diethylene glycol – succinate (USA). The column temperature was 185 °C; the evaporator temperature - 230^oC. Nitrogen was used as the carrier gas (1.8-2.7 dm^3/h).

Acids were identified according to the period of retention of individual fractions using standard solutions of certain fatty acids. The content of methyl esters of fatty acids was obtained by the methods of statistical analysis and presented as mean values with standard deviation. The results of 2-3 independent measurements were presented**.**

The morphology of the cells was examined using the light microscope Leica DMLP (Germany) and electron microscope JEM - 100 C (Japan) at an operating voltage of 3.5 MW. To study the structure of the cells, yeast cell suspension was washed by distilled water three times precipitating the cells by centrifugation. The cell pellets were sequentially fixed at 4^oC by 1.5% aqueous $KMnO_4$ solutions for 6 - 18 hours. Further fixation was carried out by 1% OsO_4 solution in 0.1 m phosphate buffer (pH 6.8) solution for 2 hours. To increase the contrast of objects, chromic anhydride was added in OsO_4 solution and then the material was put into Epon - 812. Ultrathin sections were obtained with the device Ultratom LKB - 4800 (Sweden). 46 cells of *S. cerevisiaeY-503* were studied in the development variants and 52 cells in the control type.

3. Results And Conclusion

3.1. The Study of Carboxylic Acids Content in Nutrient Media

We preliminarily studied the dependence of *S. cerevisiae Y-503* cell amount on the carbohydrate content in different media for inoculum cultivation. The closest values of the cell amount in the control and development variants were observed at carbohydrate content 20g/100cm^3. To standardize the conditions of cultivation, exactly this carbohydrate content was taken as the basis for preparation of inoculum growth media (see Materials and methods). The content of organic acids in the composition of different media: nutrient medium prior to the cultivation of *S. cerevisiae Y-503* cells, the fermented substrate and aqueous-alcoholic solution is presented in Table 1. In all nutrient media the quantitative content of acetic, lactic, oxalic, malic, succinic, butyric, tartaric and citric acids ranges from 0.03 to 80.7% of all acids amount. Succinic and citric acids were not detected in control and development (№2) variants. Tartaric acid was not detected in development variant №1. High content of acetic acid was observed in all three variants of the media (development medium №1: development medium № 2: control medium, 80.7:79.9:74.9%, respectively).

Table1. *Organic acids produced by S. cerevisiae Y-503 strain in different nutrient media.*

Organic acids	Content of organic acids, g/l								
	Nutrient medium			Fermented substrate			Aqueous-alcoholic solution		
	DV №1	DV №2	CV	DV №1	DV №2	CV	DV №1 (Ethanol 11.4%)	DV №2 (Ethanol 10.3%)	CV (Ethanol 9.3%)
Acetic $C_{2:0}$	26.24	25.40	28.48	0.09	0.10	1.50	11.94	12.07	12.94
Lactic $C_{3:0}$	1.92	2.03	1.28	16.65	17.38	4.27	12.01	12.42	13.76
Oxalic $C_{4:0}$	0.58	0.07	2.82	-	3.02	7.25	4.27	1.83	5.44
Malic $C_{4:0}$	0.11	0.86	0.01	-	4.71	-	1.38	1.40	1.92
Succine $C_{4:0}$	1.76	-	-	5.01	5.16	-	0.15	0.49	0.46
Butyric $C_{4:0}$	1.01	1.64	0.83	14.72	19.72	10.0	0.02	0.09	1.05
Tartaric $C_{4:0}$	-	1.80	1.95	-	0.54	2.94	3.07	3.34	2.98
Citric $C_{6:0}$	0.89	-	-	-	1.02	1.74	0.20	0.80	0.73
Total:	32.51	31.80	35.37	36.47	51.65	27.70	33.04	32.44	39.28

Note to Table 1: *Development variant (DV), Control variant (CV).*

The content of lactic acid increased in the fermented substrate from 3 to 8 times relative to the initial nutrient medium. The concentration of the acid in the fermented substrate was 45.7 and 33.7% of all acids amount in development variants №1 and №2. The content of butyric acid was more than 10 times higher than in the initial nutrient medium prior to cultivation and it was 40.4% (development variant №1), 38.2% (development variant №2) and 36.1% (control variant) of all acids amount. Malic and succinic acids were not detected in the fermented substrates of the control variant of the media. As for oxalic, malic, tartaric and citric acids they were not observed in development variant №1. Thus, after 120 hours of *S. cerevisiae Y-503* cultivation significant changes were observed in the composition and quantitative content of organic acids in the fermented substrate relative to the initial nutrient media: acetic acid content decreased with a simultaneous increase of lactic and butyric acids; in some variants the content of malic and succinic acids increased. It was observed that there was a tendency of increasing the content of acetic acid when compared to the fermented substrate in the aqueous-alcoholic solution obtained after the distillation of the fermented substrate.

3.2. The Study of Fatty Acid Composition of Nutrient Media

The content of fatty acids in the composition of different media (nutrient medium prior to the cultivation of *S. cerevisiae Y-503* cells, the fermented substrate and aqueous-alcoholic solution) is presented in Table 2.

The applied method of fractionation of methyl esters of fatty acids in nutrient media allowed to identify 12 acids. All nutrient media were predominated with palmitic $C_{16:0,}$ linoleic $C_{18:2\omega\text{-}6}$, lauric $C_{12:0}$ and linoeladic $C_{18:2}$acids in quantitative content. There were no significant differences observed in the amount of saturated, unsaturated acids (including polyene acids) in the development media and control variant, respectively (Table. 2). However, in all variants of nutrient media the content of saturated fatty acids predominated and averaged 60.5%, unsaturated acids - 39.5% of total acid amount, respectively, of which the polyene was 28.7%.

Table2. *Content and composition of fatty acids when grown S. cerevisiae Y-503 in different nutrient media*

Fatty acids	Content of fatty acids, % of amount								
	Nutrient medium			Fermented substrate			Aqueous-alcoholic solution		
	DV №1	DV №2	CV	DV №1	DV №2	CV	DV №1 (Ethanol 11.4%)	DV №2 (Ethanol 10.3%)	CV (Ethanol 9.3%)
Pentyl formic $C_{6:0}$	3.05	2.95	3.48	4.93	2.01	7.23	0.04	0.09	0.66
Hexyl acetic $C_{8:0}$	-	-	1.57	3.06	0.75	3.17	0.12	0.22	1.45
Capric $C_{10:0}$	4.21	4.79	-	1.99	0.93	2.04	-	-	3.98
Formic $C_{10:0}$	2.55	6.37	4.15	0.11	0.65	0.11	2.10	1.06	1.44
Lauric $C_{12:0}$	11.14	9.77	11.18	3.73	5.88	2.93	3.20	3.24	9.26
Tridecyl $C_{13:0}$	-	-	-	-	-	13.33	-	-	-
Tetradecanoic $C_{14:0}$	0.91	0.39	1.08	-	-	-	0.25	-	-
Myristoleic $C_{14:1}$	1.18	0.97	-	-	-	-	1.54	1.07	-
Palmitic $C_{16:0}$	30.09	29.24	34.14	32.86	34.46	24.18	4.42	-	35.26
Heptadecylic $C_{17:0}$	1.91	1.88	1.04	2.85	3.20	-	0.10	1.19	0.66
Stearic $C_{18:0}$	-	-	-	2.52	1.43	3.84	2.03	1.55	-
Elaidic $C_{18:1\omega-9}$	-	-	-	-	-	-	0.04	0.63	-
Oleinic $C_{18:1\omega-9}$	-	-	-	-	-	6.32	0.02	0.18	-
Linoeladic $C_{18:2}$	10.64	9.80	9.83	8.96	8.99	9.94	27.57	27.76	8.05
Linoleic $C_{18:2\ \omega-6}$	26.43	23.83	26.32	18.82	22.46	11.08	51.51	59.09	27.45
α-Linolenic $C_{18:3\omega-3}$	-	-	-	-	-	-	-	0.06	-
Arachic $C_{20:0}$	5.04	6.45	4.10	6.20	6.63	6.56	1.17	0.53	3.73
Eicosadienoic $C_{20:2\omega-6}$	-	-	-	-	-	-	-	0.13	-
Eicosatrienoic $C_{20:3\omega-3}$	-	-	-	-	-	-	0.97	0.88	-
Dihomo-γ-linolenic $C_{20:3\omega-6}$	-	-	-	-	-	-	-	0.36	-
Arachidonic $C_{20:4\omega-6}$	2.85	3.56	3.11	8.68	11.60	3.84	1.02	-	1.25
Docosadienoic $C_{22:2\omega-6}$	-	-	-	-	-	-	0.36	0.28	-
Behenic $C_{22:0}$	-	-	-	5.29	1.01	5.43	0.06	0.22	0.90
Docosahexaenoic $C_{22:6\omega-3}$	-	-	-	-	-	-	0.86	-	-
Tricocyl $C_{23:0}$	-	-	-	-	- -	-	0.79	0.84	-
Nervonic $C_{24:1\omega-9}$	-	-	-	-	-	-	1.83	0.62	5.91
Total :	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Including unsaturated	41.10	38.16	39.26	36.47	43.03	31.18	85.71	91.06	42.67
polyene	29.28	27.39	29.43	27.50	34.04	21.24	56.60	62.22	34.61
saturated	58.90	61.84	60.74	63.53	56.97	68.82	14.29	8.94	57.33
Unsaturated fatty acids /saturated fatty acids	0.70	0.62	0.65	0.57	0.76	0.45	6.00	10.19	0.74

Note to Table 2: *Development variant (DV), Control variant (CV)*

3.3. The Study of the Fermented Substrate

Changes occurred in the qualitative content and quantitative ratio of fatty acids with the increase of ethanol concentration in the medium while cultivating cell culture. Palmitic $C_{16:0,}$ linoleic $C_{18:2\omega-6}$ and linoeladic $C_{18:2}$acids were also predominant as in the nutrient media. Unlike the nutrient media, stearic $C_{18:0}$ and behenic $C_{22:0}$ acids were detected in all variants and in the development variants the content of arachidonic $C_{20:4\omega-6}$ acids increased threefold. After 120 h of cultivation in development (№1, №2) and control variants it was observed that palmitic $C_{16:0}$ and linoleic $C_{18:2\omega-6}$ acids became predominant. Ethanol is known to have inhibitory effect on *S. cerevisiae* cell suspension, which is less pronounced with linoleic $C_{18:2\omega-6}$ acid that increases fluidity of membranes [24]. We identified this essential acid in all variants of the media. According to the quantitative content in the fermented substrate it came second (after palmitic acid). When compared to the nutrient media it was observed that the amount of lauric and linoleic acids considerably decreased. According to Table 2 the content of capric $C_{10:0}$, formic $C_{11:0}$, lauric $C_{12:0}$ and linoleic $C_{18:2\omega-6}$ acids (in the acid amount) reduced in the process of cell cultivation relative to the nutrient medium. Myristic $C_{14:0}$ and myristoleic $C_{14:1}$acids, which were present in little amount in the nutrient media prior to the cultivation process, were not detected in all variants of the digested media. In general, the total index of value of fatty acid pools in all variants of the experiment and the ratio of unsaturated and saturated acids practically remained unchanged during the yeast cultivation (Table. 2). Moreover, in development variants №1 and №2 we observed less accumulation of biomass: 7.8: 8.0 g / l, respectively, versus 17.7 g / l in the control type.

3.4. The Study of Alcoholic Fermentation Products

Table 3 shows the data on the quantitative content of impurity compounds in fermented substrates. The comparison of volatile impurities chromatograms revealed the identical qualitative composition except for phenylethanol detected in development variant №1. As a rule, during the long-term cultivation in nutrient media the oxidation of unsaturated fatty acids by atmospheric oxygen was observed which resulted in production of alcoholic acids, aldehydes, ketones and esters. The content of ethanol in the development media was slightly higher than in the control variant (Table. 3). As for the content of by-products (impurity compounds) it was lower according to most indicators (Table. 3). Alcoholic fermentation with the release of more carbon dioxide amount led to the increased accumulation of acetaldehyde (by 15.0-17%) in the control variant when compared to the development types. Higher alcohols were represented by propyl alcohol-2, isobutanol, butanol-1, izoamylol and hexanol-1 where izoamylol was about 50% of their total content.

Table3. *Effect of nutrient media content on the formation of impurity compounds in fermented substrates, mg/dm^3*

Product of fermentation	Variants		
	Development №1	Development №2	Control
Acetic aldehyde	34.61	35.30	41.53
Acetone	6.78	4.23	3.5
Ethyl acetate	0.94	2.90	3.00
Propyl alcohol-2	2.78	3.33	2.94
Isobutanol	17.73	16.44	23.18
Butanol-1	0.43	2.5	2.56
Isoamyl	165.64	178.57	198.77
Hexanol-1	12.60	14.08	13.11
Phenylethanol	0.53	-	-
Benzaldehyde	3.77	13.95	18.86
Ethyl butyrate	86.43	91.63	98.68
Total:	332.24	362.93	406.13

Note to Table 3: *the content of methanol is identical in all variants– 0.002 vol. %.*

3.5. The Study of Yeast Biomass

Table 4 shows that regardless of culture medium polyene acids accumulated from 41.09 % (in the control type) up to 58.10 : 51.44% (in development variants №1 : №2) of the acid amount in the biomass of yeast cells. The content of dihomo-γ-linolenic $C_{20:3\omega-6}$ and arachidonic $C_{20:4\omega-6}$ acids significantly exceeded control indices (more than 10 times) in the yeast biomass in the development variants.

Table4. *Content and composition of fatty acids in biomass of S. cerevisiae Y-503 grown in different nutrient media*

Fatty acids	Fatty acids, % of acid amount		
	Development variant №1	Development variant №2	Control variant
Pentyl formic $C_{6:0}$	1.95	1.69	1.47
Capric $C_{10:0}$	5.22	5.97	2.88
Lauric $C_{12:0}$	4.77	4.48	4.34
Palmitic $C_{16:0}$	27.76	25.48	33.36
Heptadecylic $C_{17:0}$	-	-	2.31
Margaric-oleinic $C_{17:1}$	2.20	2.09	1.31
Stearic $C_{18:0}$	-	-	0.49
Oleinic $C_{18:1\omega-9}$	-	-	3.17
Linoeladic $C_{18:2}$	9.03	8.85	12.74
Linoleic $C_{18:2\ \omega-6}$	26.14	22.09	34.34
Dihomo-γ-linolenic $C_{20:3\ \omega-6}$	11.75	9.95	0.83
Arachidonic $C_{20:4\omega-6}$	20.21	19.40	1.56
Nervonic $C_{24:1\omega-9}$	-	-	1.20
Total:	100.00	100.00	100.00
Including unsaturated	58.10	60.29	53.83
polyene	58.10	51.44	41.09
saturated	41.90	39.71	46.17
Unsaturated fatty acids /saturated fatty acids	1.39	1.52	1.17

It is known that $C_{20:4\omega-6}$ plays an important role in the metabolism of microorganisms as an intracellular messenger and hormone-like compounds precursor [25]. High content of essential linoleic $C_{18:2\omega-6}$ and palmitic $C_{16:0}$ acids was observed in all variants of the experiment. The composition of polyene acids in the yeast biomass was identical to their composition in the nutrient media except for arachidonic acid $C_{20:3\omega-6}$ that was absent in the media.

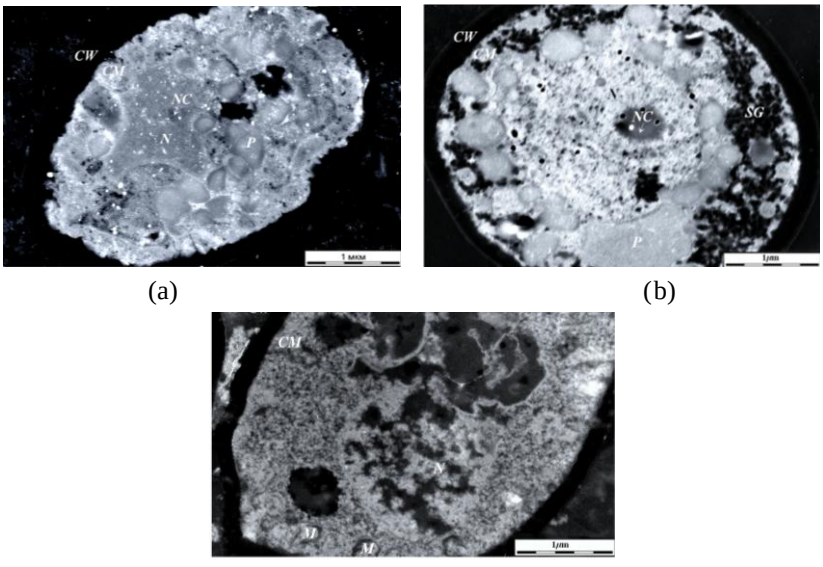
3.6. The Study of Aqueous-Alcoholic Solution

The study of fatty acid pools revealed specific characteristics in the fatty acid composition depending on the nutrient media. In all variants of the media the amount of saturated fatty acids considerably reduced when compared to the nutrient media and fermented substrates (Table. 2). High content of unsaturated fatty acids was detected in development variants №1:№2 (85.71: 91.06%) when compared to the control variant (42.67%). Among unsaturated fatty acids linoleic $C_{18:2\omega-6}$ and linoeladic $C_{18:2}$ acids were predominant in the development variants and linoleic acid $C_{18:2\omega-6}$ predominated in the control variant. The content of linoleic acid $C_{18:2\omega-6}$ in development variants №1 and №2 was more than 50% and the content of linoeladic acid $C_{18:2}$ was 27-28% of all acids amount.

It was found out that the development aqueous- alcoholic solutions contained a little amount of polyene acids: elaidic $C_{18:1\omega-9}$, oleinic $C_{18:1\omega-9}$, α-linolenic $C_{18:3\omega-6}$, eicosadienoic $C_{20:2\omega-6}$, eicosatrienoic $C_{20:3\omega-3,}$ dihomo-γ-linolenic $C_{20:3\omega-6}$, docosadienoic $C_{22:2\omega-6}$ and docosahexaenoic $C_{22:6\omega-3}$ which were not detected in the control variant. However, the content of nervonic acid $C_{24:1\omega-9}$ in the control type was higher than in the development variants. As is known unsaturated fatty acids are a part of cellular membranes as well as have a high biological activity and play a role of essential nutritive factors. For example, $C_{22:6\omega-3}$ is one of the important trophic factors necessary for microorganisms [26]. Besides, polyunsaturated fatty acids with linear chain of 20 carbon atoms are precursors of prostaglandins - mediators with pronounced physiological effect.

The study of fatty acid composition of *S. cerevisiaeY-503* shows that the strain synthesizes a great variety of acids and palmitic acid $C_{16:0}$ is predominant in the nutrient medium, fermented substrate, aqueous-alcoholic solution (except for development types) and biomass in all variants of the experiment that is specific for genus *Saccharomyces* [13-16]. It is shown that the development alcoholic solutions obtained from the geothermal water contain elaidic $C_{18:1\omega-9}$, α-linolenic $C_{18:3\omega-3}$, eicosadienoic $C_{20:2\omega-6}$, eicosatrienoic $C_{20:3\omega-3,}$ dihomo-γ-linolenic $C_{20:3\omega-6}$, docosadienoic $C_{22:2\omega-6,}$ docosahexaenoic $C_{22:6\omega-3}$ and nervonic $C_{24:1\omega-9}$ acids as well as low content of unwanted products of metabolism that were not detected in the media and fermented substrates.

3.7. Electron-Microscopy Study



(a) (b)

(c)

Fig. *Ultrathin structure of the cells S. cesevisiae Y-503 strain grown in development media №1 and №2 with geothermal waters (a, b) and in the control variant (c).*

Note to Fig: *cell wall (CW), cytoplasmic membrane (CM), nucleus (N), nucleolus (NC), mitochondrion (M), peroxisomes (P), secretory granules (SG). Samples were taken after 120 hour fermentation.*

Fig. shows ultrathin section of *S. cerevisiae Y-503* cells grown in different nutrient media. It was observed that there was nucleus with nucleolus in the cells in all sections of the development variants. Besides, there were large dull-luminous peroxisomes with small-grained electronic and dense matrix closely packed and gathered in clusters as well as secretory granules in close proximity to the nucleus and nucleolus (Fig. a, b). Electron-microscopic section of the control culture cells showed the presence of a large nucleus and rare small mitochondria on the periphery (Fig. c).Vacuoles were rarely observed in sections of the development and control variants; Golgi apparatus and the endoplasmic reticulum were not detected.

It is known that hyper-synthesis of proteins is observed in the microorganism cells in nutrient media in the presence of non-specific energy sources: alkanes - polycyclic hydrocarbons (C_8 to C_{23}) fatty acids, various alcohols (from methanol to high molecular weight alcohols) [27]. In our experiment in the development media such sources may be the organic components of geothermal water represented by humic substances and bitumen. As is known, mass formation of peroxisomes is not required to metabolize carbohydrates (particularly glucose), so higher fatty acids, hydrocarbons and methanol without peroxisomes cannot be metabolized. Peroxisomes can perform various metabolic functions [28, 29] including β-oxidation of long-chain ($C_{24\text{-}26}$ carbon atoms and more) and dicarboxylic fatty acids (C_{26} or more) as well as α-oxidation of fatty acids, synthesis of lipid esters, plasmogenes etc. The differences detected in the structural elements of *S. cerevisiae Y-503* cells in the development and control variants (Table 5) may be caused by changes in carbohydrate and oxidative metabolism which is indicated by occurrence of secretory granules and peroxisomes in the development types.

Table5. *Cytological properties of S. cerevisiae Y-503 cells grown in different nutrient media*

Cell structure	Nutrient media	
	Development variants №1, №2	Control variant
1. Cell wall	Cell wall is not affected	Many cells have canals in the cell wall through which the content runs out
2. Cytoplasmic membrane	Invagination of cytoplasmic membrane specific to fermentative cells	Invagination of cytoplasmic membrane specific to fermentative cells
3. Nucleus	Nucleus is of irregular form with dispersed chromatin that indicates to activity of a cell	Nucleus is big. Chromatin is not detected that may indicate reduce in metabolic activity
4. Nucleolus	Nucleolus is observed in nucleus in all sections. The formation of nucleolus is specific to the cells capable of active growth and synthesis of large amount of protein.	Nucleolus is not detected
5. Mitochondrion	A little amount	A little amount
6. Peroxisomes	Particularly all cells have peroxisomes gathered in clusters of 2-9 granules	Not detected
7. Secretory granules	There is clump of secretory granules in cytoplasm	Not detected

The results showed that the nutrient media containing geothermal water could be used for cultivation of the yeast *S. cerevisiae*. The predominance of linoleic $C_{18:2\omega\text{-}6,}$ arachidonic $C_{20:3\omega\text{-}6}$ and dihomo-γ-linolenic $C_{20:4\omega\text{-}6}$ acids in the biomass enables to consider yeast *S. cerevisiae* as a potential source for obtaining these acids at the optimization of growth conditions and use of geothermal waters in the composition of nutrient media. Using geothermal water for the yeast cultivation will reduce the cost of nutrient media on an industrial scale and this, in turn, will contribute to the development of high-performance economy sectors of the regions rich in groundwater water resources.

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