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Analysis of Microalgae Lipids Isolated from Basin of Kazakhstan, to Assess the Prospects of Practical Use

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***Abstract*—**It was analyzed of fatty acid composition of 16 strains of microalgae lipid fractions isolated from different basins of Kazakhstan and characterized by stable active growth in the laboratory. Three species of green microalgae (*Oocystis rhomboideus, Chlorococcum infusionum, Dictyochlorella globosa*) and three species of diatoms (*Synedra* sp., *Nitzshia* sp., *Pleurosigma attenuatum*) are characterized by a high content of lipids and are promising for further study as a source of polyunsaturated fatty acids.

***Keywords*—**Fatty acids, lipids, microalgae.

I. Introduction

SPECIFICITY of microalgae metabolism associated with the production of valuable human metabolites has made them one of the important objects of biotechnology [1].

Lipids are important constituents of microalgae for their energy potential and structural-functional features [2], [3]. Under certain conditions of cultivation, several species of microalgae may accumulate up to 80% of the lipids by dry weight that exceeds the content of lipids in most oilseed crops. Lipids have a group of compounds that is polyunsaturated fatty acids and their derivatives, many of them have physiological activity are essential to mammals and are synthesized by microalgae only [4]. In this con-text, and because of the possibility of large-scale commercial production of microalgae biomass, the study of their lipid composition attracts the attention of many researchers. An urgent task is search high-yielding strains of microalgae, well adapted to local climatic conditions, capable of producing a large number of compounds having biotechnological interest, grow steadily and rapidly microalgae metabolism associated with the production of valuable human metabolites, has made them one of the important objects of biotechnology [1].

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II. Materials and Methods

The object of study was the strains of microalgae isolated from different basins of Kazakhstan and stored in the collection the Institute of Ecology problems Al-Faraby Kazakh National University. A list of types used in the work is presented below.

- Green microalgae:

Oocystis rhomboideus, Selenastrum gracilis, Nautococcopsis constricta, Scenodesmus acutiformis, Chlorococcum infusionum, Dictyochlorella globosa, Chlorella sp., *Cladophora globulina, Chlorococcum* sp.

- Diatoms microalgae:

Pinnularia sp., *Pleurosigma attenuatum, Nitzshia* sp., *Navicula* sp., *Achanthes hauckiana, Synedra* sp., *Fragillaria* sp.

To clean of the microalgae from related bacteria have used antibiotics cephasoline, streptomycin, penicillin, gentamicin, linkomicin and merkacin [5]-[7]. Microalgae were grown in cumulative mode, 16-hour photoperiod. The intensity of light at the surface of the solution was 8 kLx. Media temperature ranged +20-25°C. Fitzgerald medium is used as culture medium.

At the end of experience was obtained the dry mass of culture, as well as optical density of culture at 750nm (green microalgae) and 420nm (diatomaceous microalgae). For the transition from the optical density units to absolutely dry weight (ADW) used the factor k [8]:

$$k = 0.78 \text{ g} \cdot \text{l-1} \cdot \text{Un. opt. density -1},$$
$$\text{i.e. ADW} = k \cdot D750 \quad (1)$$

Extractions of lipids from cells of microalgae were on modified method Chi et al. [9]. Algae cells subjected to drying, then preliminary deactivate enzymes with isopropyl alcohol (5ml) at a temperature of +80°C for 15 minutes. Then samples were homogenized with 5 ml of chloroform-methanol mixture (1: 1).

Total lipids have divided on the neutral, glyco- and phospholipids by Silica column chromatography [10].

For gas chromatography used the ethyl esters of fatty acids [11]. The lipids dissolved in hexane (0.1 ± 0.02g: 2.0ml hexane), than added 2M sodium ethylate. The upper layer containing ethyl esters used for chromatography. Gas chromatograph is GC-2010 Plus, Shimadzu Corporation, Japan. The temperature of the evaporator is 250^0C, with the detector 250^0C, column 90^0C; Programming – 50^0C/min to 240^0C, 40min isotherm. Program processing chromatograms by GC Solution

III. Results and Discussion

From the collection were picked up 9 algological pure strains of green and 7 strains of diatom microalgae, characterized by stable growth in the laboratory. They were isolated from samples of water collected in basins of Kazakhstan. The axenic cultures was obtained after using some kinds of antibiotics (cephasoline, streptomycin, penicillin, gentamicin, linkomicin and merkacin), effective concentration of them was chosen experimentally for each strain. For accumulation of biomass algae were grown in identical conditions in luminostate within 14 days, it was adequate to the top of stationary phase of growth. After this the determination of the total lipid composition in green and diatoms microalgae was followed (Table I). Analysis of the results showed that the highest lipid content was detected in cultures of microalgae: diatomaceous - *Nitzshia* sp., *Synedra* sp., *Pleurosigma attenuatum*, green - *O. rhomboideus, C. infusionum, D. globosa*.

The lowest lipid content was found in cultures of the diatom *Navicula* sp., *Pinnularia* sp. Content of lipids in the species of microalgae ranged from 16.31 ± 1.1 (*A. hauckiana*) to 5.50 ± 1.1 g/100g of dry weight (*C. globulina*).

Thus, it has been found that three species of Diatoms microalgae (*Synedra* sp., *Nitzshia* sp., *P. attenuatum*) and three species of green microalgae (*O. rhomboideus, C. infusionum, D. globosa*) characterized by relatively high content of lipids (over 20% in terms of dry biomass).

Among the major lipid fractions of microalgae, which take for further analysis are phospholipids and glycolipids, which are major components of membranes and neutral reserve lipids that accumulate fatty acids for energy processes of cell division, a membranes status and other metabolic transformations.

TABLE I
Contents of the Various Classes of Lipids in Cell Cultures of the Diatom and Green Microalgae after 14 Days of Cultivation

Microalgae	Lipid weight in 100g of dry biomass, g			
	Total lipids	neutral lipids	glycolipids	phospholipids
Diatoms microalgae:				
A. hauckiana	16.31±1.1	5.23±1.9	4.31±0.7	0.31±0.05
Navicula sp.	0.50±0.01	0.20±0.01	0.10±0.01	0.10±0.01
Pinnularia sp.	0.42±0.02	0.20±0.04	0.10±0.05	0.09±0.01
Nitzshia sp.	21.62±2.3	9.45±0.2	1.98±0.03	0.24±0.06
Synedra sp.	22.45±1.3	11.74±2.1	2.04±0.2	0.51±0.01
P. attenuatum	20.58±2.4	4.53±0.7	4.94±0.5	0.41±0.01
Fragilaria sp.	6.34±0.9	2.84±0.5	1.87±0.01	0.97±0.04
Green microalgae				
S. acutiformis	16.09±0.5	9.56±0.9	2.38±0.2	1.67±0.06
O. rhomboideus	26.72±3.1	17.68±2.1	2.13±0.01	1.11±0.01
C. globulina	5.50±1.1	2.25±0.8	1.13±0.03	0.13±0.07
Chlorella sp.	5.60±0.8	1.90±0.01	0.84±0.05	0.21±0.09
C. infusionum	22.83±2.1	12.61±0.5	5.04±0.07	1.13±0.04
D. globosa	23.4±2.7	11.74±0.7	0.50±0.06	0.8±0.02
Chlorococcum sp.	8.85±0.9	4.47±0.01	1.87±0.09	1.11±0.01
N. constricta	13.9±1.4	8.21±2.3	3.21±1.1	1.1±0.07
S. gracilis	12.5±1.1	7.65±2.7	2.13±0.8	1.1±0.08

The most functionally important lipid fractions of microalgae are polyunsaturated fatty acids that contain two or more double bonds. Therefore, we have determined the content of the individual lipid classes. Fractions of neutral lipids, phosphor - and glycolipids from cell cultures of *D. globosa, N. constricta, S. gracilis* and other species of microalgae were extracted by the methods of Silica column chromatography of lipids (Table I). It was found that from the microalgae lipids the neutral lipid fraction is dominant, second is the fraction of glycolipids and the smallest percentage is a fraction of phospholipids. In cells of diatoms, characterized by a high content of total lipid fractions the ratio of neutral lipids: phospholipids: glycolipid was 39: 8: 1 for *Nitzshia* sp., 23: 4: 1 for *Synedra* sp., 10:10: 1 – for *P. attenuatum*. For three species of green algae with a high con-tent of total lipid fraction, this ratio was 2: 1: 17 (*O. rhomboideus)*: 14.6 : 0.6 : 1 (*D. globosa*) and 12: 5: 1 (*C. infusionum*).

For 3 of 6 species of microalgae, characterized by a high content of total lipid fraction, as well as for the culture of *S. acutiformis,* the content of total lipids in biomass of which amounted to 16g/100g of dry weight, was carried out gas chromatography analysis. Data of total fatty acids, as well as the distribution of fatty acids in various classes of lipids extracted from microalgae, is presented in Table II.

The data indicate that the most of the fatty acids in the total lipid fraction make up unsaturated acids, which account for 64% (*O. rhomboideus, Synedra* sp.), 59% (*S. acutiformis*) and 51% (*C. infusionum*) of the total fatty acids. Species vary in the ratio of mono- and polyene conjugated fatty acids presented in the fraction of total lipids.

TABLE II
RELATIVE CONTENT OF FATTY ACIDS OF SUMMARY AND INDIVIDUAL LIPID FRACTIONS OF MICROALGAE (%)

Lipids fraction		Fatty acids			
		saturated	unsaturated	monoene	polyene
Total lipids	*O.romboideus*	35.78±1.3	64.22±1.9	22.39±1.4	41.84±1.9
	S.acutiformis	40.09±2.3	59.06±1.9	34.90±1.5	24.15±1.7
	C.infusionum	49.07±1.9	50.93±2.1	38.80±1.7	12.12±1.5
	Synedra sp.	36.05±2.1	63.95±2.9	50.45±3.1	13.50±1.9
Neutral lipids	*O.romboideus*	25.02±0.9	74.98±1.5	36.16±1.1	38.82±0.8
	S.acutiformis	11.65±0.5	88.35±1.9	12.46±0.9	75.88±1.5
	C.infusionum	10.10±1.5	89.90±2.1	19.74±1.4	70.16±1.9
	Synedra sp.	6.29±0.1	93.71±3.1	18.33±1.2	75.38±2.4
Phospholipids	*O.romboideus*	not determined	not determined	not determined	not determined
	S.acutiformis	28.83±0.9	71.18±1.9	50.97±2.1	20.21±1.9
	C.infusionum	24.07±2.0	75.93±3.1	59.76±2.4	16.17±1.1
	Synedra sp.	17.91±1.9	82.09±2.3	64.88±2.9	17.21±1.4
Glycolipids	*O.romboideus*	not determined	not determined	not determined	not determined
	S.acutiformis	36.12±2.0	63.88±2.9	41.54±1.9	22.34±1.3
	C.infusionum	37.43±1.9	62.57±2.9	34.57±1.5	28.01±1.1
	Synedra sp.	35.56±2.3	64.44±2.9	47.25±1.5	17.19±1.6

Polyene acids are predominant in lipids extracted from *O. romboideus* cells, whereas in other species monoene fatty acids dominate. In the neutral lipid fraction of species *S. acutiformis, C. infusionum, Synedra* sp. polyene fatty acids are predominate (70-75% of unsaturated fatty acid fraction), in other classes of lipids the content of monoene fatty acids is in several times greater than the remaining fraction of lipids poliene.

Obtained data testify that the selected cultures are promising for further study as a source of unsaturated fatty acids.

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