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Influence of Culturing Conditions on Biomass Yield, Total Lipid, and Fatty Acid Composition of Some Filamentous Fungi

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Abstract**—**In this work the effect of culturing conditions of filamentous fungi *Penicillium raistrickii, Penicillium anatolicum, Fusarium* sp. on biomass yield, the content of total lipids and fatty acids was studied. It has been established that in time the process of lipids accumulation correlated with biomass growth of cultures, reaching maximum values in stationary growth phase.

Biomass yield and accumulation of general lipids was increased by adding zinc to the culture medium. The more intensive accumulation of biomass and general lipids was observed at temperature 18°C. Lowering the temperature of culturing has changed the ratio of saturated: Unsaturated fatty acids in the direction of increasing the latter.

Keywords**—**Biomass, culturing conditions, fungi, fatty acids (FA), growth dynamics, lipids.

I. Introduction

POLYUNSATURATED FATTY ACIDS (PUFAs) have attracted attention because of their high physiological, industrial and pharmaceutical importance. In living organisms, they affect transport process and stimulate cellular response, including lipid metabolism, immune response and adaptation to the cold. In addition, undergoing biotransformation these compounds lead to the formation of numerous low-molecular bioregulators of important processes occurring in cells, tissues and body as a whole [1], [2].

Promising method for commercial production of PUFAs is to use unique biosynthetic capabilities of microorganisms able to synthesize them [3].

Filamentous fungi are promising producers of lipids and their components due to the high rate of growth on simple media, the possibility of accumulation of lipids large amounts and individual PUFAs, and the ability of their metabolism manipulation [4], [5].

Production of PUFAs from microorganisms on an industrial scale is closely related to culturing conditions which affect both the overall content of lipids and fatty acids [6], [7].

The influence of culturing time, temperature, zinc ions addition to the culturing medium on the biomass yield, total lipid and fatty acid composition of three fungi cultures *Fusarium* sp., *P. raistrickii* and *P. anatolicum* were studied.

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II. Materials and Methods

The objects of research were cultures of filamentous fungi isolated from soil and water of Kazakhstan: *Penicillium raistrickii, Penicillium anatolicum, Fusarium* sp.

Biomass of fungi was grown in a Petri dish (Pd) on Sabouraud medium during 3-7 days. The initial biomass was introduced in an amount of 0.001g/Petri dish. Growth dynamics of fungal cultures were evaluated by gravimetric method (according to the growth of biomass). To investigate the effect of Zn^{2+} ions on yield biomass and lipids $ZnCl_2$ in concentration of 5mg/l was added to the culturing medium. Effect of Zn^{2+} on fatty acids content in the cultures of fungi was evaluated after 4 days of culturing. As a control the culture grown on Sabouraud medium was used. Effect of temperature on the yield of biomass and lipids was investigated by incubating the cultures at temperatures 18°C and 28°C during 4 days.

Lipid extraction was performed according to [8]. Total lipids were determined gravimetrically.

Preparation of fatty acid methyl esters (FAMEs) was carried out according to [9]. Fatty acid composition of total lipid fractions was examined by gas chromatography. Chromatographic separation of fatty acids esters was performed on a gas chromatograph Clarus 500 (PerkinElmer 8500, USA). Programming heating up to 170°C - 3 minutes, heated to 220°C at a speed 4°C/min, holding during 15min. FAMEs were identified by comparison of retention times of the peaks with those of the standard G411 FA (Nu-Chek Prep. Inc., Elysian, MN, USA), Chromatogram processing program - Perkin-Elmer Total Chrom Navigator.

III. Results and Discussion

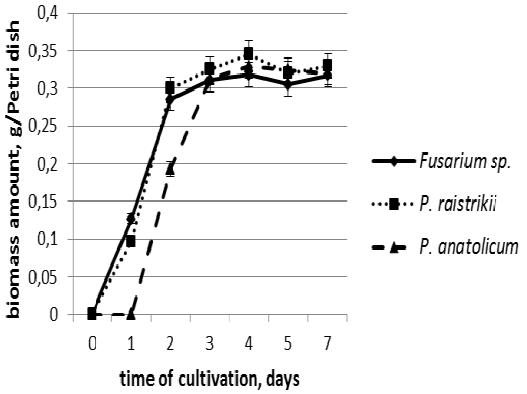
The content of total lipid, as well as the qualitative composition of fatty acids depends on the physiological state of culture associated with the phases of fungal development, growth intensity and lipogenesis, culturing conditions and the composition of the medium [10], [11].

Since PUFAs are endogenous compounds, to obtain their large scale amount their high biomass yield is necessary.

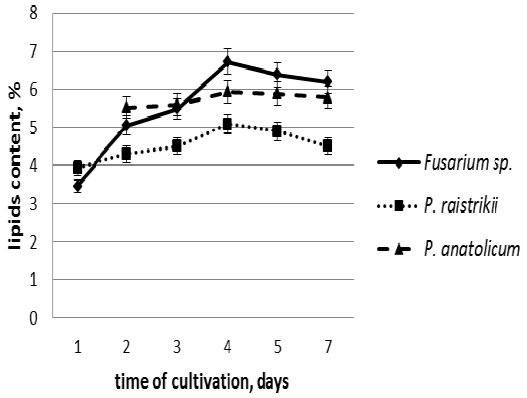
To study the effect of culturing time, control of biomass and total lipid fraction was performed on 1, 2, 3, 4, 5, and 7 days of fungi growth. On Fig. 1 are presented the results of the

growth dynamics and accumulation of total lipid fractions in studied fungal cultures. It has been shown that biomass reached maximal values on the fourth day (access to the stationary phase) of culturing and increased (compared to the initial) 318 times for *Fusarium* sp., 345 times for *P. raistrickii* and 329 times for *P. anatolicum* (0.318g /Pd, 0.345g /Pd and 0.329 g/Pd, respectively).

Maximum amount of total lipids was observed in all studied cultures in the stationary growth phase and reached 6.73% for *Fusarium* sp., 5.1% for *P. raistrickii* and 5.95% for the culture *P. anatolicum*



(a)



(b)

Fig. 1 Dynamics of biomass (a) and total lipids (b) accumulation by fungi cultures *Fusarium* sp., *P. raistrickii* and *P. anatolicum*

It was found that the amount of biomass of cultures correlated with the amount of total lipid fraction. Correlation coefficient was for *Fusarium* sp. 0.91, for *P. raistrikii* – 0.78, for *P. anatolicum* – 0.77.

By changing the composition of nutrient medium it's possible to increase the yield of biomass, total lipids, including their components - polyunsaturated fatty acids [12], [13].

Role of variable valency metals in the metabolism of fungi determined by their participation in the structure and functioning of several important enzymes, and in stabilization of cell membranes. One of them is zinc - microelement that plays an important role in metabolism of microorganism cells. Zinc is involved in synthesis and breakdown of nucleic acids and proteins metabolism of ethanol and is a part of many enzymes (transferases, oxidoreductases, hydrolases, etc.) [14], [15].

By adding zinc in concentration 5mg/l to *Fusarium* sp. biomass yield increased 3.2 times and reached to 1.017g / Pd, whereas in the control this value was 0.318g/ Pd. In culture *P. raistrikii* biomass growth on medium supplemented with zinc increased 5.07 times to 1.75g / Pd. In *P. anatolicum* biomass grown on a medium with zinc was 2.53g / Pd, that is 7.7 times higher in comparison with control (Fig. 2).

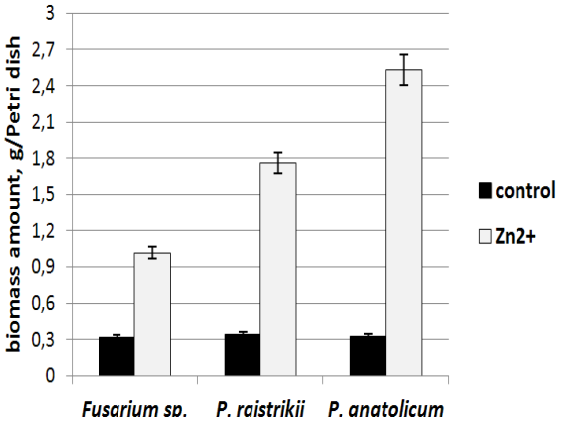


Fig. 2 Effect of zinc on the growth of fungal biomass

It has been shown that the presence of zinc in the medium of fungi culturing increased total lipid content in comparison with the control (Fig. 3). For culture *Fusarium* sp. addition of zinc increased total lipid yield the amount of which was 15.8%, that is 2.35 times larger as compared with the control. For culture *P. raistrikii* this value increased 2.2 times (11.2%) and for *P. anatolicum* 3.3 times and amounted to 19.6%.

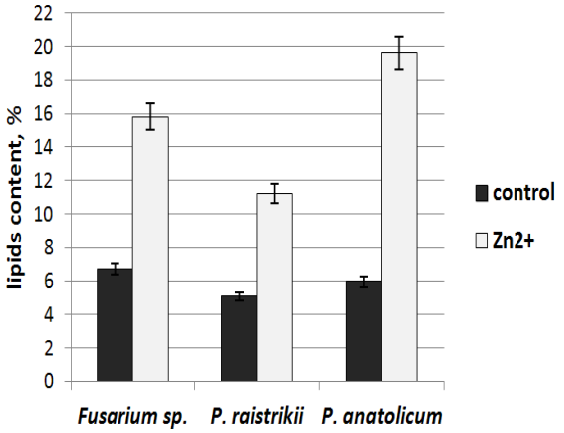


Fig. 3 Effect of zinc on the content of total lipid fraction of fungi

The addition of zinc to culture medium did not cause significant changes in the ratio of saturated and unsaturated

fatty acids in the total lipid fraction (Table I). In all three fungal cultures unsaturated fatty acids dominated.

TABLE I
INFLUENCE OF Zn^{2+} ON THE CONTENT OF FATTY ACIDS IN TOTAL LIPID FRACTION EXTRACTED FROM FUNGAL CULTURES

Culture	Fatty acids	Control	Zn^{2+}
P. raistrikii	saturated	27.62%±0.98	27.32%±0.94
	unsaturated	67.29%±2.02	72.69%±2.53
	monoenic	15.55%±0.78	50.17%±1.22
	polyenic	51.74%±1.03	22.52%±0.99
	unidentified	5.16%±0.009	0.20%±0.005
Fusarium sp.	saturated	31.68%±0.89	35.91%±1.12
	unsaturated	65.51%±2.28	63.04%±1.98
	monoenic	21.46%±1.03	58.96%±1.37
	polyenic	44.06%±1.35	4.08%±0.38
	unidentified	2.81%±0.11	1.23%±0.06
P. anatolicum	saturated	33.25%±0.98	29.20%±1.10
	unsaturated	65.96%±2.23	70.26%±2.98
	monoenic	21.41%±0.69	48.10%±1.46
	polyenic	44.55%±1.11	22.16%±1.57
	unidentified	0.84%±0.007	0.90%±0.008

In total lipid fractions isolated from cultures grown on medium without zinc, the amount of unsaturated fatty acids is approximately 2 times (1.98-2.4 times) higher than that of saturated acids. The percentage of unsaturated FA on media containing zinc exceeded that of saturated acids in culture *Fusarium* sp. 1.75 times, cultures *P. raistrikii* and *P. anatolicum* 2.7 and 2.4 times respectively as compared with the control.

When added zinc to the culture medium the ratio of monoenic: polyenic acids increased towards monoenic fatty acids. Thus, for *P. raistrikii* the content of monoenic FA on medium with zinc increased 3.2 times, for *Fusarium* sp. the level of monoenic acids increased 2.7 times and for *P. anatolicum* 2.2 times and amounted to 50.17%, 58.96% and 48.10%, respectively.

With the increase of monoenoic acids level in media containing zinc the content of polyenic acids reduced 2 times in fungi *P. raistrikii* and *P. anatolicum* and reached 22.52% and 22.6%, respectively (51.74% from initial and 44.55%). For *Fusarium* sp. amount of polyenic fatty acids in media containing zinc, decreased 10.7 times and reached 4.08%.

Study of temperature impact on fungal cultures growth showed that more intensive biomass accumulation occurred at a temperature of culturing 18°C. Thus, biomass of *P. raistrikii*, *Fusarium* sp., *P. anatolicum* increased on average 1.5 times in comparison with culturing at 28°C and was 0.364 g/ Pd, 0.39g / Pd and 0.592g/ Pd, respectively. The results are shown in Fig. 4.

Similar trends were observed in the study of temperature effect on accumulation of total lipid fraction (Fig. 5). Culturing at temperature 18°C resulted in the increase of total lipid fraction amount 1.4 times for *Fusarium* sp., 1.6 times for *P. raistrikii* and 2 times for *P. anatolicum* compared with lipid content at 28°C.

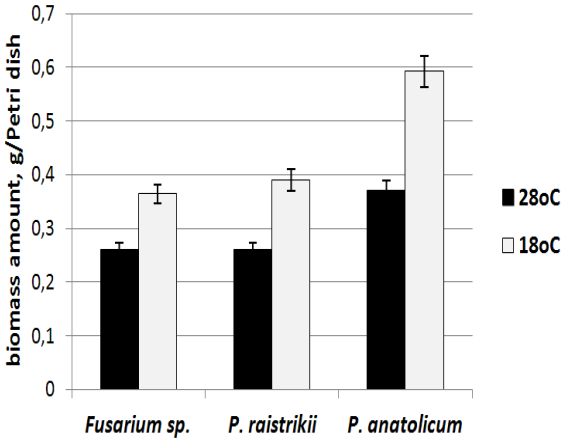


Fig. 4 Dependence of fungi biomass growth on temperature of culturing

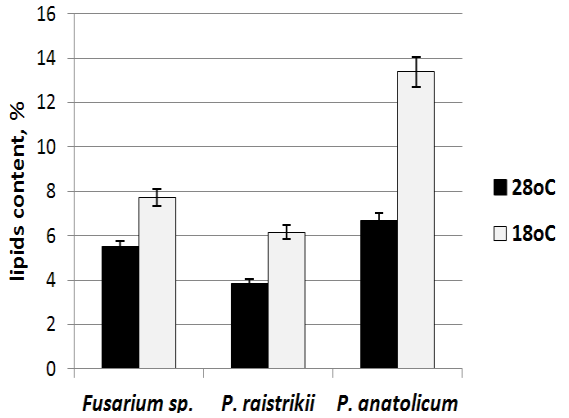


Fig. 5 Dependence of the total lipid fraction amount on temperature of fungi culturing

Investigation of the effect of different temperature culturing regimes on fatty acid composition of total lipid fraction showed that with temperature decrease the level of saturated fatty acids declined (Table II). Thus, culture *Fusarium* sp., in the total lipid fraction the content of saturated fatty acid with a decrease in culturing temperature by 10°C (from 28°C to 18°C with) fell by 9.26% and was 18.35%, for *P. raistrikii* and *P. anatolicum* this index decreased by 3.1% and 2.79%, respectively (and amounted 16.21% and 20.51%, respectively). With the reduction of saturated fatty acids at a decrease of temperature the increase in amount of unsaturated fatty acids was observed. Their level increased by 10.03% in *Fusarium* sp., whereas in *P. raistrikii* and *P. anatolicum* increase occurred on average of 3.8%. At decrease of culturing temperature the content of polyenic fatty acids increased. In cultures of fungus *Fusarium* sp. PUFAs level increased by 6.79% and reached 55.38%, in the cultures of *P. raistrikii* and *P. anatolicum* by 4.41% (55.49%) and 2.27% (52.14%), respectively.

TABLE II
FUNGI CULTURING TEMPERATURE EFFECT ON FATTY ACID CONTENT IN TOTAL LIPID FRACTION

Culture	Fatty acids	Temperature of culturing	
		28ºC	18ºC
P. raistrikii	saturated	19.31%±0.98	16.21%±0.85
	unsaturated	78.95%±2.03	82.84%±3.78
	monoenic	27.87%±1.78	27.36%±1.43
	polyenic	51.08%±1.99	55.49%±1.78
	unidentified	1.75%±0.083	1.12%±0.069
P. anatolicum	saturated	23.30%±1.04	20.51%±1.00
	unsaturated	74.79%±2.95	78.55%±3.12
	monoenic	24.91%±1.03	26.41%±1.52
	polyenic	49.87%±1.68	52.14%±1.99
	unidentified	1.91%±0.069	0.94%±0.007
Fusarium sp.	saturated	27.61%±0.97	18.35%±0.84
	unsaturated	71.51%±2.88	81.54%±3.52
	monoenic	22.92%±1.14	26.16%±1.08
	polyenic	48.59%±1.66	55.38%±2.31
	unidentified	0.27%±0.009	0.89%±0.051

Thus, the obtained results allowed concluding that over time the accumulation of total lipid fraction correlated with the growth of biomass cultures, reaching maximum values in the stationary growth phase of cultures. Yield of biomass and accumulation of total lipid fraction increased when zinc was added to culturing medium. When zinc was added to culture medium the ratio of saturated:unsaturated fatty acids significantly did not change, but the level of monoenic acids increased with respect to polyenic fatty acids.

The greatest increase in biomass for studied fungal cultures occurred at 18°C cultivation. Reduction of the culturing temperature on 10°C (from 18°C to 28°C) affected on total lipid fraction content that increased with decrease of temperature. Decrease of the temperature was reflected in quality ratio of saturated: unsaturated fatty acids towards the increase of the latter. This trend is more specific in fungi *Fusarium* sp.

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