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The changes in lipid composition of *Pythium irregulare LX* oomycetes at a stressful situation created with crude oil

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A B S T R A C T

Pythium irregulare oomycetes adapts with environmental changes including crude oil concentration by changing the composition of lipids in the cytoplasmic membrane and providing the required characteristics for adaptation in improper and stressful environmental situations. It was found that cultivation of *Pythium irregulare LX* oomycetes in the nutrient media with different concentrations of crude oil with 1.0, 2.0, 3.0, 5.0 and 10.0 (%), incubated for 5 days at 26-28°C on a rotary shaker (200 rpm) in aerobic conditions and deep culturing caused an increase in the lipid content and the unsaturation degree of fatty acids, confirming the correspondence between the increase of polar lipid/free sterol in the composition of membrane lipids' ratio and that of polar lipids in general lipid fractions. Represented data shows that the process of adaptation of oomycetes to a stressful situation created with crude oil motivated the increase of the rate of membrane phospholipids with a high quantity of unsaturated fatty acids.

Key words: stress, oil, *Pythium irregulare*, lipid, phospholipids.

INTRODUCTION

Microorganisms become adapted against undesirable environmental changes. In this process, the cytoplasmic membrane gets involved, increasing the likelyhood of existence in the early stages. Changes of lipid compositions in the membrane provide the required characteristics for adaptation in improper and stressful environmental situations [1]. Factors such as the length of

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the fatty acids' chain, the unsaturated degree and its related chains, cholesterol rate and lipid polarity in the membrane's composition highly affect biomembrane permability [2]. For instance, tolerance to ethanol and high temperatures in Saccharomyces cerevisiae is due to the changes of unsaturated degree [3]. Similar results in *Escherichia coli*, *Schizosaccharomyces pombe* and *Lactobacillus* species have been observed [4]. Previous studies have shown that the incubation of microorganisms in the presence of stressful conditions results in changes of the membrane's lipid composition [5-8]. In this study, changes in *Pythium irregular* lipid composition resulting from adding crude oil to the medium as a stressful agent have been investigated.

MATERIALS AND METHODS

Medium and culturing condition: Lipogene micromycetes *Pythium irregulare LX* used for the present investigation was isolated in the Institute of Microbiology, National Academy of Science of Azerbaijan, Baku. The medium for the cultivation of oomycetes was composed of 2% glucose, 0.5% yeast extract (Fisher scientific) and 0.1% KH_2PO_4 (pH= 6.2). The microorganism was incubated for 5 days at 26-28 °C on a rotary shaker (200 rpm) in aerobic conditions and deep culturing. Crude oil with 1.00, 2.00, 3.00, 5.00 and 10.00 (%) concentrations was added to the main medium.

Separation of lipid compositions: For lipid extraction Floch and Bligh- Dyer methods were used [9, 10]. Polar lipids were separated from neutral lipids by a precipitation method with cold acetone. Polar lipids were segregated into different fractions with a chloroform- methanol- water base (65: 25: 4), and neutral lipids into different fractions with a hexane –diethylether acetic acid base (85: 25:4) all performed by thin-layer chromatography. The lipids' fatty acid composition was determined by highly effective liquid chromatography (HPLC). Metylation lipids, as well as a lipid methanolysis mix were separated by liquid chromatography with an ultraviolet detector ((λ=250 nm) KOBO marked). The structure of the fatty acids was determined by mass-spectrum and Iodine number, and TOCT 5475-69 [11]. The calculated data was meaningful at the $P<0.05$ level.

RESULTS AND DISCUSSION

The results extracted from the growth and lipogenesis in *Pythium irregulare LX* in a medium containing crude oil concentrations are presented in Table 1. From these observations, it is clear that the increase of crude oil concentration in the medium significantly delays the growth of the oomycetes species under study. Besides, general lipid rate in the biomass and the fatty acid unsaturated degree (with iodine number) increased in the mycelial biomass. The growth delay and the increase of the lipids' unsaturated degree by crude oil are significant.

Table 1: The effect of crude oil concentrations on growth and lipogenesis in *Pythium irregulare* LX

Crude oil concentration %	Biomass dry weight 100 ml/g in medium	Lipids %	Unsaturated degree (Iodin number)		
			General lipids	Neutral lipids	Polar lipids
Control	0.82	29.3	108.1	102.5	145.1
1.0	0.75	30.1	109.9	103.0	145.8
2.0	0.60	33.5	115.6	106.4	140.5
3.0	0.39	35.0	116.4	106.3	135.2
5.0	0.26	46.2	120.0	113.7	128.1
10.0	0.10	50.4	125.3	123.7	126.5

A biosynthesis of unsaturated fatty acids by desaturase in the cytoplasmic membrane was performed and found to be dependent on the situation of the membrane. To further clarify this issue, general lipids, as well as polar and neutral fractions were analyzed. The results are shown in Table 2.

Table 2: The effect of crude oil concentrations on lipids composition in *Pythium irregulare* LX

Lipids	Crude oil concentrations %					
	Control	1.0	2.0	3.0	5.0	10.0
PL	13.1	16.1	27.0	35.0	43.7	58.2
DAG	3.7	3.4	3.2	3.0	3.0	3.0
FS	6.8	6.2	6.0	6.0	5.3	5.5
FFA	6.6	6.0	5.3	5.1	4.9	5.6
TAG	55.4	54.2	51.1	43.0	33.0	20.2
ES	11.4	11.2	10.7	10.2	7.3	5.0
PL/FS	1.9	2.6	4.5	5.8	8.2	10.6
ES/FS	1.7	1.8	1.5	1.4	1.4	0.9

Note: Polar lipid (PL); Diacylglycerol (DAG); Free sterol (FS); Free fatty acids (FFA); Triacylglyserol (TAG); Ether sterol (ES)

The results show the effects of crude oil on different classes of lipids separately as compared with the control samples. Triglyceride rate decreased with increasing oil concentration in the medium while the rate of polar lipids increased. The composition of free sterol/ether sterol rate did not significantly change. These results show the membrane structure collision as well as its decrease of rigidity. In this situation, by increasing fluidity, the preservation of the membrane's structural- functional characters can be performed further by regulating fatty acids with an unsaturated acyl chain. In this research, the increase of the rate of polar lipids increased the lipids' unsaturated degree. To demonstrate this issue, the composition of neutral and polar lipid fatty acids was determined. The results are given in the Table 3.

Table 3: The effect of crude oil concentration on fatty acids compositions in neutral and polar lipids in *Pythium irregulare LX*

Fatty Acids	Crude oil concentrations %											
	Control		1.0		2.0		3.0		5.0		10.0	
	NL	PL	NL	PL	NL	PL	NL	PL	NL	PL	NL	PL
C<14	Trace	Trace	Trace	Trace	1.0	1.5	3.5	1.5	4.0	3.1	4.0	8.4
C16:0	26.5	20.1	26.5	16.0	31.6	16.3	30.3	10.0	30.3	7.1	30.3	6.0
C16:1	12.	14.8	12.0	17.3	16.0	18.1	15.5	18.4	15.	19.3	15.5	10.5
C18:0	4.3	4.0	3.8	3.4	2.6	3.0	3.1	2.0	3.3	7.6	2.8	11.6
C18:1	15.3	8.9	18.1	11.2	18.6	8.4	22.8	9.1	21.8	13.6	20.0	8.0
C18:2	17.1	20.3	16.0	20.2	10.1	17.4	7.2	16.8	8.4	15.6	8.9	9.5
C18:3	3.6	2.4	3.2	3.2	3.0	5.6	2.4	6.9	2.4	7.8	2.7	10.9
C20:1	2.2	2.9	2.8	3.2	3.0	3.2	3.0	6.0	3.4	3.0	3.2	2.0
C20:3	2.0	1.3	1.1	2.3	1.0	3.5	1.4	5.8	1.3	7.4	1.4	9.0
C20:4	9.2	12.5	9.0	10.5	6.1	11.5	6.1	12.2	6.4	12.5	6.4	13.4
C20:5	7.8	12.8	7.5	12.9	7.0	11.5	7.0	11.3	6.0	7.0	5.1	10.7

Note: Polar lipid (PL); Neutral lipids (NL)

Olein and linol acid rates in the neutral lipid's fraction composition were changed by crude oil impression. Adding oil to the medium up to 3%, gradually increased the rate of olein acid, but decreased at 5% and 10%. The rate of linol acid reduced from 1701 to 7.2 and increased a little at 5% and 10%. One of the significant changes was the increase in the rate of fatty acid including fewer than 14 carbon atoms. In the tested growth condition in polar lipids, the fatty acids' composition and unsaturated degree changed more than the neutral lipids. Saturated and monounsaturated fatty acid rate in the fraction was increased by increasing oil concentrations in the medium. Short chain fatty acids also increased 5-10%. In *Pythium irregulare LX*, the polar lipid unsaturated degree was more than that of the neutral lipid, but it decreased with increasing oil in the medium. Despite this, increasing the rate of polar lipid in general lipids composition inhibited the decreasing of iodine number. Hence, the unsaturated degree increased. The decrease of polar lipids' unsaturated degree can decrease cytoplasmic membrane fluidity. However, in this research, short chain fatty acid rate in the lipids' composition increased. This action can be attributed to membrane fluidity preservation in specific levels as cell defense reactions because the physical-chemical characteristics of short chain saturated fatty acids and long chain fatty acids are similar [12].

Certain strains and species of both yeast and filamentous fungi are known to produce high amounts of oils and fats. Fungal lipids and their fatty acids have been well recognized from the late nineteenth century and considerable studies have been done to determine the potential application of lipids and PUFA from microbial sources has been investigated and reported by a number of researchers [13]. The oils can be employed as a carbon source for microbial production [14]. Polyunsaturated fatty acids have been shown to play an important role in sexual development and spore germination of several filamentous fungi. In *Neurospora sp.*, for instance, alpha-linolenic acid stimulates the formation of fruiting bodies. In *Mucor sp.*, gamma- linolenic acid steadily increases during the germination process of spores where the Δ6 desaturase gene responsible for the biosynthesis of fatty acid is highly expressed. However, the exact mechanism underlying the process still needs to be defined [15]. The decrease of linol acid rate, fatty acid Δ12 desaturase activity and stearin acid and increase of palmitin acid demonstrate the decrease of the elongase enzyme activity by crude oil concentration. Thus, the fatty acid mechanism, their desaturase and more specifically elongase enzymes, have important roles in the membrane

lipids' fluidity regulation. Delta (Δ) 5 desaturase is a key enzyme for the biosynthesis of health-beneficial, very long chain polyunsaturated fatty acids (VLCPUFA) such as arachidonic acid (ARA, C20:4n-6), eicosapentaenoic acid (C20:5n-3) and docosahexaenoic acid (C22:6n-3) via the "desaturation and elongation" pathways [16]. VLCPUFA are essential components of cell membranes, and are precursors for a group of hormone-like bioactive compounds (eicosanoids and docosanoids) involved in the regulation of various physiological activities [17]. The role of the stearin mechanism is more conspicuous. Sterols cannot prepare the required conditions for membrane structural-functional performance in stress conditions. The results show correspondence between the increase of polar lipid/free sterol in the composition rate of membrane lipids and increase of polar lipids in general lipid fractions.

It can be concluded that the process of adaptation of *Pythium irregulare* micromycetes to an environmental stressful situation created with crude oil, induced an increment in the rate of membrane phospholipids with high quantity of unsaturated fatty acids.

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