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Change in Phospholipid Composition and Phospholipase Activity of the Fungus *Lentinus tigrinus* VKM F-3616D during Growth in the Presence of Phenol and Lignocellulosic Substrates

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Abstract—Changes in phospholipid composition, phospholipase activity, and accumulation of lipid peroxidation products in mycelium of the lignin-degrading fungus *Lentinus* (*Panus*) *tigrinus* VKM F-3616D in the presence of phenol and lignocellulosic substrates in the cultivation medium are reported. It is shown that in fungal mycelium in the presence of both substrates the share of lysophosphatidylcholine sharply increases. The parity between separate groups of phosphatidylinositols also changes. The lysophosphatidylcholine content increase during cultivation is connected with activation of phospholipase A_2 (EC 3.1.1.4), and phosphatidylinositol parity change is associated with distinctions in affinity of phosphoinositide-specific phospholipase C (EC 3.1.4.11) to them.

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Key words: *Lentinus tigrinus*, phospholipids, phospholipase, xenobiotics, lignin, biodegradation

Basidiomycetes (*Basidiomycetes*), by producing a unique complex of extracellular oxidative and hydrolytic enzymes, are the most promising organisms for degrading numerous aromatic compounds that are resistant to natural decomposition. White-rot fungi, which are basidiomycetes, are the only organisms able to perform complete biodegradation of lignocellulosic substrates, products of their processing (including toxic chlorolignins) and a wide variety of xenobiotics (i.e. polycyclic aromatic hydrocarbons, pentachlorophenol, phenanthrene, etc.) [1-4]. These organisms and their enzymes can be used in paper and textile bleaching, production of biosensors, composite materials manufacture, and for other purposes [5-7]. Therefore, the number of publications devoted to the extracellular ligninolytic enzyme complex (ELEC) of these fungi grows every year.

Considering the fact that these complexes are extracellular, some authors believe that membrane structures, including phospholipids, play an important role in their secretion into the environment [8]. In addition, lipid peroxide radicals (products of lipid peroxidation (LPO)), which are derived from fatty acids, also participate in biodegradation of lignin and phenolic xenobiotics along with the ELEC according to several authors [9, 10]. As well as in higher eukaryotes, there is a correlation between physiological activity and composition of lipids, the intensity of LPO processes, and antioxidant activity in filamentous fungi [11-13].

Phospholipase plays an important role in the regulation of these processes in animal and plant cells. Phospholipase A_2 (PLA_2) (EC 3.1.1.4) together with acylCoA:lysophosphatidylcholine transferase participates in the renewal of membrane phospholipids, while lysophosphatidylcholine (lysoPtdChol) and fatty acids – the products of phospholipase reaction – are potent effectors of membrane processes [14].

Among phospholipases C, special attention is given to phosphoinositide-specific phospholipase C (PI-PLC)

Abbreviations: DAG, diacylglycerol; DC, diene conjugates; ELEC, extracellular ligninolytic enzyme complex; Ins(1,4,5)P_3, inositol-1,4,5-trisphosphate; LPO, lipid peroxidation; lysoPtdChol, lysophosphatidylcholine; MDA, malonic dialdehyde; PA, phosphatidic acid; PI-PLC, phosphoinositide-specific phospholipase C; PLA_2, phospholipase A_2; PtdChol, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdGly, phosphatidylglycerol; PtdIns, phosphatidylinositol; PtdIns(4)P, phosphatidylinositol-4-phosphate; PtdIns(4,5)P_2, phosphatidylinositol-4,5-diphosphate; Ptd-L-Ser, phosphatidylserine; SPH, sphingomyelin; TC, triene conjugates.

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(EC 3.1.4.11) that catalyzes the hydrolysis of phosphatidylinositol-4,5-diphosphate (PtdIns(4,5)P_2). The reaction products are physiologically active secondary messengers: diacylglycerol (DAG) and inositol-1,4,5-triphosphate (Ins(1,4,5)P_3). They play an important role in cell division, information transfer, regulation of membrane permeability, etc. [15].

However, the mechanisms of regulation of the qualitative and quantitative ratios of phospholipids/fatty acids as well as possible participation of lipolytic enzymes in these processes are not fully understood for microscopic fungi. The study of this problem may allow better understanding of the biodegradation mechanisms of phenolic xenobiotics and lignocellulosic substrates as well as ELEC secretion, thus promoting new ways of enhancing its synthesis and activity.

The objective of this work is to study changes in the phospholipid composition and phospholipase activity of the fungus *Lentinus tigrinus* VKM F-3616D grown in the presence of phenol and lignocellulosic substrates.

MATERIALS AND METHODS

The fungus *Lentinus* (*Panus*) *tigrinus* was isolated at the Department of Biotechnology of Ogaryov Mordovian State University from dry basidiocarp of the fungus growing on birch brushwood near Saransk and deposited in the All-Russian Collection of Microorganisms as strain VKM F-3616D [16].

The inoculum of *L. tigrinus* was grown on Czapek–Dox medium containing 15 g/liter of lignosulfonate. Fungi from a wort-agar slant were inoculated in liquid medium. Overgrown pieces of agar were added to 500-ml Erlenmeyer flasks with 100 ml of culture medium and grown for 4 days on an environmental shaker-incubator (235 rpm) at 26°C. The producer was grown further on modified medium [17] with birch sawdust (20 g/liter) to which 5% (v/v) inoculum was added. The fungal culture was grown submerged with stirring (235 rpm) at 26°C in 500-ml Erlenmeyer flask with 100 ml of culture medium for 6, 9, and 12 days with the addition of phenol at the concentrations of 1 and 5%. Phenol was added on the third and sixth day of culture growth. Culture medium [17] with birch sawdust without the addition of phenol was used as a control sample.

Lentinus tigrinus was solid-phase cultivated on machine-processed lignocellulosic substrate of 85% humidity with inoculation at the rate of 2 ml inoculum per gram of the substrate. The fungal cultures were grown under static conditions at 26°C for 3, 6, 9, and 12 days.

Lipids were extracted from the mycelium by the Bligh–Dyer method [18]. Phospholipids were separated from neutral lipids by precipitation with cold acetone [19]. The composition of phospholipids was analyzed by two-dimensional thin-layer chromatography on silica gel in the Broekhuyse systems: chloroform–methanol–28% ammonia–water (90 : 54 : 5 : 8) and chloroform–methanol–glacial acetic acid–water (90 : 40 : 10 : 4) [20]. Phosphatidylinositides were extracted from the mycelium by the Bligh–Dyer method as modified by Prokhorova [21]. Phosphatidylinositide composition was analyzed by one-dimensional thin-layer chromatography on silica gel in the solvent system: chloroform–methanol–28% ammonia (9 : 7 : 2). Chromatograms were sprayed with 5% sulfuric acid in methanol followed by heating at 180°C to reveal spots. Individual fractions of phospholipids and phosphoinositides were identified using pure substances as references, R_f values from the literature, and specific reagents [19]. The phospholipids and phosphoinositides were quantitatively analyzed following Vaskovsky et al. [22].

The content of diene and triene conjugates (DC and TC) was determined spectrophotometrically by the absorption in the UV region. Oxidation indexes were calculated as $OI_{DC} = A_{235}/A_{215}$, $OI_{TC} = A_{275}/A_{215}$ [23]. Malondialdehyde (MDA) was registered in the reaction with thiobarbituric acid [24].

PLA_2 activity (EC 3.1.4.11) was determined by the accumulation of free fatty acids during phosphatidylcholine (PtdChol) cleavage by gas–liquid chromatography on a Kristall 5000.1 chromatograph (Kristall, Russia) with HP-FFAR capillary column 50 m long with an internal diameter of 0.32 mm (HP-FFAR, USA). Column temperature was programmed to change by 4°C per min from 145 to 220°C. Free fatty acid content was assessed by the peak areas corresponding to methyl esters of fatty acids in comparison with the internal standard margarinic acid (1 mg/ml). The enzyme activity was expressed in micrograms of fatty acids formed in 1 h per mg protein [19]. The activity of PI-PLC (EC 3.1.1.4) was determined by a modified Palmer method. The enzyme activity was expressed in nanomoles of inorganic phosphorus formed in 1 min per mg protein [25]. The protein content was determined by the Lowry method.

Standard phospholipids and fatty acids were from Sigma (USA). Other reagents were of Russian origin with no lower than chemical purity. Freshly distilled solvents were used.

All results, obtained in no less then five parallel experiments, were statistically analyzed with Microsoft Excel 2000.

RESULTS

The addition of 5% phenol to the cultivation medium after 3 days of growth did not inhibit the growth of the ligninolytic fungus *L. tigrinus* culture. The culture growth was observed in the form of rapidly settling pellets with light pink color, which is probably due to the adsorption of phenol by the *L. tigrinus* mycelium [26].

Cultivation in the presence of phenol in the medium was accompanied by a change in proportions of individual phospholipid fractions without any changes in their qualitative composition. Upon addition of 1% phenol on the third and the sixth day, a decrease in the content of the main phospholipids (phosphatidylcholine (PtdChol) and phosphatidylethanolamine (PtdEtn)) and simultaneous increase in the total fraction of lysophosphatidylcholine and sphingomyelin (lysoPtdChol + SPH) was registered by the sixth day. Upon increasing the phenol concentration to 5%, the type of PtdChol and (lysoPtdChol + SPH) content changes remained the same but became more prominent. At the same time, there was a slight increase in the PtdEtn content. Among the other fractions of phospholipids, phosphatidic acid (PA) and phosphatidylglycerol (PtdGly) was noted, the amount of which increased upon addition of phenol. The total fraction of phosphatidylserine (Ptd-L-Ser) and phos-

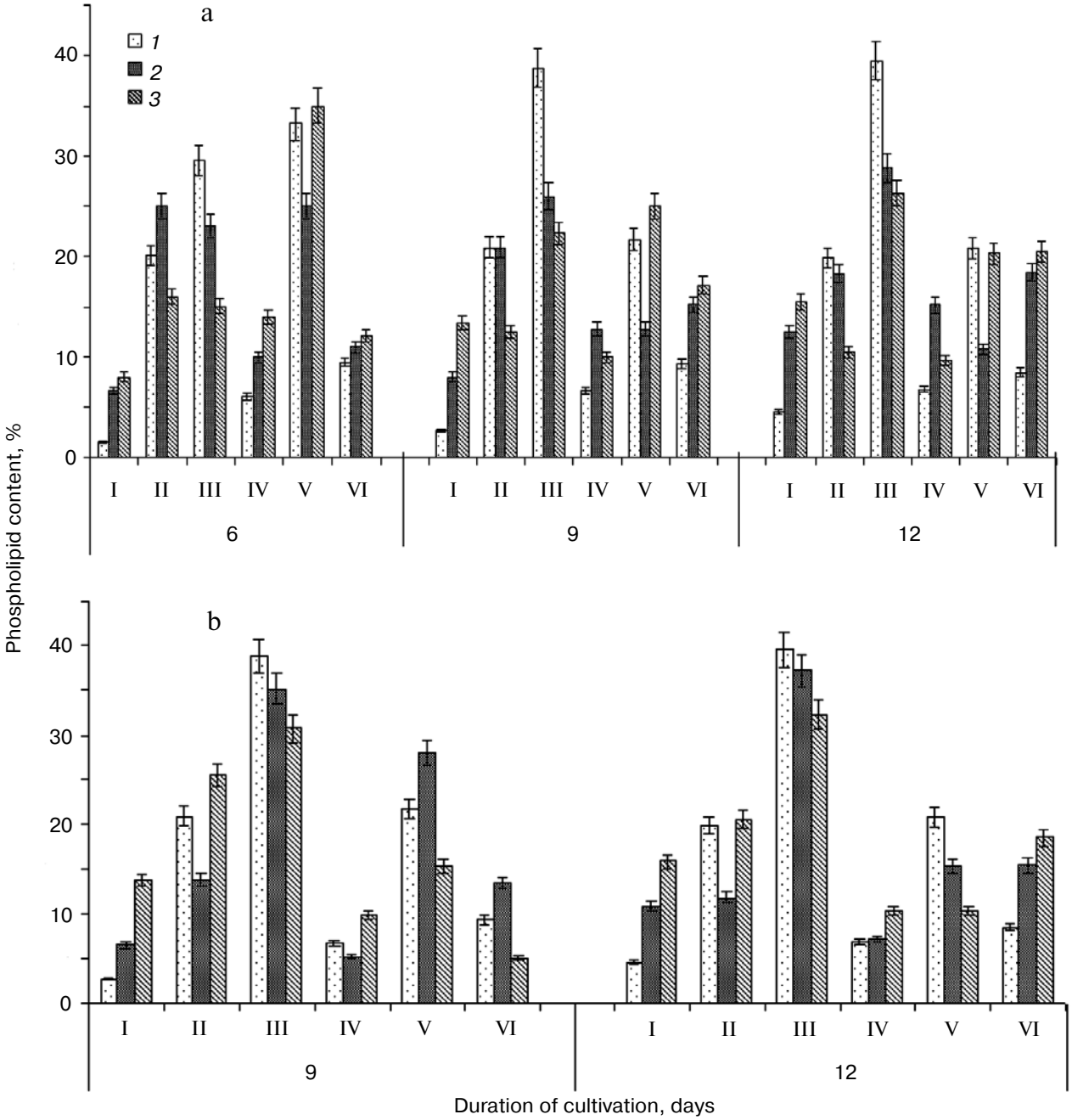

Fig. 1. Ratio changes in individual phospholipid fractions (% of total phospholipids) of *L. tigrinus* mycelium upon phenol addition on third (a) and sixth (b) day. *1*) Control; *2*) 1% phenol; *3*) 5% phenol. I, lysoPtdChol + SPH; II, Ptd-L-Ser + phosphatidylinositides; III, PtdChol; IV, PA; V, PtdEtn; VI, PtdGly.

phatidylinositides increased after 1% phenol addition, but decreased on raising the phenol concentration to 5%. During further growth, PtdEtn and (Ptd-L-Ser + phosphatidylinositides) fraction contents decreased, while (lysoPtdChol + SPH) and PtdChol shares increased, although they remained lower than in the control sample (Fig. 1a).

Upon addition of 1% phenol on the third day, the content of PtdEtn and (Ptd-L-Ser + phosphatidylinositides) decreased and the share of the combined (lysoPtdChol + SPH) fraction increased by the end of cultivation. Increasing the phenol concentration to 5% did not significantly affect the nature of the changes except for the (Ptd-L-Ser + phosphatidylinositides) fraction, the relative content of which increased slightly (Fig. 1b).

Similar phospholipid composition changes of *L. tigrinus* mycelium were also observed during lignocellulosic substrate decomposition. The major phospholipids of *L. tigrinus* mycelium, which was cultivated on a solid phase, i.e. mechanically processed lignocellulosic substrate, were PtdEtn and PtdChol, which quantitatively prevailed over the other phospholipids throughout the entire cultivation period. By the third day of growth, the mycelium of *L. tigrinus* was characterized by a high content of PtdEtn, but later its share decreased, with the content of PtdChol and its lysoforms increasing during the same time. Among other phospholipid fractions of *L. tigrinus* mycelium, one can note PA and PtdGly, the amount of which decreased during growth, as well as the total fraction of (Ptd-L-Ser + phosphatidylinositides), the content of which was almost unchanged during growth (Fig. 2).

Individual phosphatidylinositides were separately isolated and identified to assess their contributions to the changes in the combined fraction of (Ptd-L-Ser + phosphatidylinositides). Chromatography of phosphatidylinositides, isolated from total lipids of *L. tigrinus* mycelium, showed the presence of three fractions, which, according to the literature and the use of standards, were identified as phosphatidylinositol (PtdIns), phosphatidylinositol-4-phosphate (PtdIns(4)P), and phosphatidylinositol-4,5-diphosphate (PtdIns(4,5)P_2). On the third day of *L. tigrinus* growth in submerged culture in the control medium the proportion of individual phosphatidylinositides was as follows (% of total): PtdIns, 51.9; PtdIns(4)P, 38.9; PtdIns(4,5)P_2, 9.2. The qualitative composition of phosphatidylinositides did not change in the control sample and after the phenol addition during growth, but significant changes in their quantitative ratio were observed. In a control sample, the fungal growth was accompanied by a decrease in the PtdIns and PtdIns(4)P content and an increase in the PtdIns(4,5)P_2 content during the whole period of cultivation. In both experimental variants an increase in PtdIns occurred upon addition of 1 or 5% phenol, and its content was much higher than in the control sample by the 12th day of growth (Fig. 3a). The content of PtdIns(4)P during the *L. tigrinus* culture growth in these conditions was reduced upon addition of phenol in the trophic phase and was slightly increasing when it

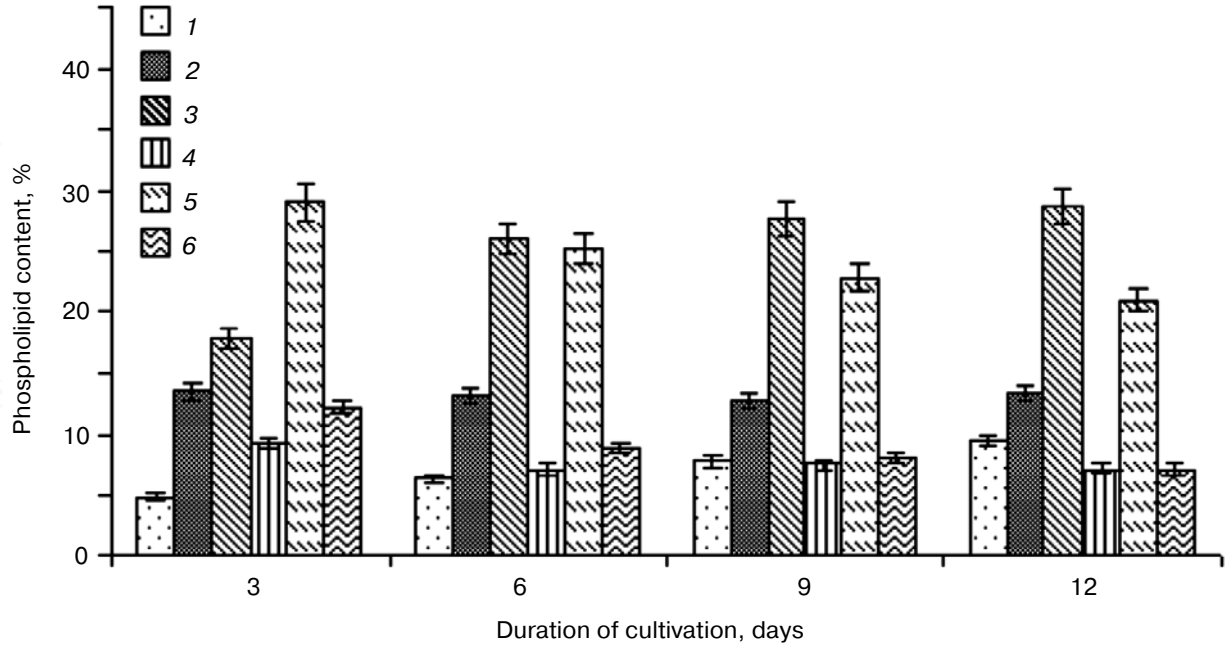

Fig. 2. Change in ratios of individual phospholipid fractions (% of total phospholipids) of *L. tigrinus* mycelium during biodegradation of mechanically processed lignocellulose substrate. *1*) lysoPtdChol + SPH; *2*) Ptd-L-Ser + phosphatidylinositides; *3*) PtdChol; *4*) PA; *5*) PtdEtn; *6*) PtdGly.

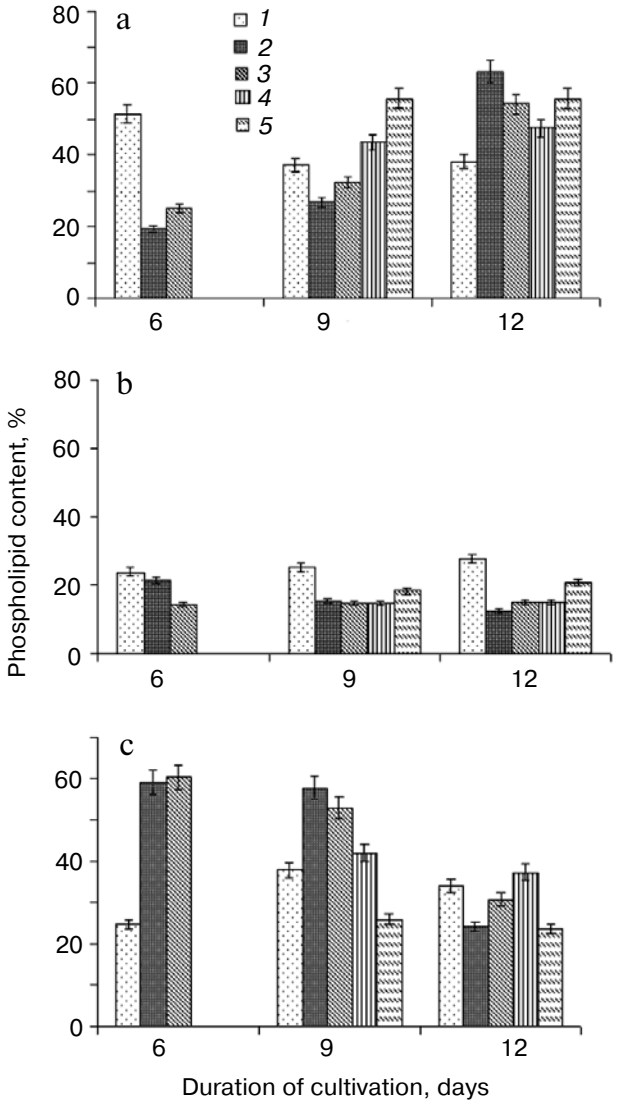

Fig. 3. Effect of phenol on ratio change (% of total phosphatidylinositides) of individual phospholipid fractions PtdIns (a), PtdIns(4)P (b), and PtdIns(4,5)P_2 (c) of *L. tigrinus* mycelium. *1*) Control; *2*) 1% phenol addition on third day; *3*) 5% phenol addition on third day; *4*) 1% phenol addition on sixth day; *5*) 5% phenol addition on sixth day.

was added during idio-phase, but it was lower than control values throughout the whole period of cultivation (Fig. 3b). However, an increase in PtdIns(4,5)P_2 level was observed regardless of the phenol concentration upon its entry into the trophic phase, the share of which at the time of the maximum ligninolytic activity (6-9 days) was much higher than in the control sample. However, its decline was observed by the 12th day of growth. When adding phenol during idio-phase, the content of this fraction also increased, but by the end of cultivation a decrease in the PtdIns(4,5)P_2 content was noted (Fig. 3c).

The combined amount of phosphatidylinositide in the control sample and after phenol addition in trophic phase decreased until the 9th day and then increased to the 12th day. When adding phenol in idio-phase, there was a slight increase in the overall content of phosphatidylinositide up to the end of cultivation.

Similar changes in the composition of *L. tigrinus* mycelium phosphatidylinositides were observed during the degradation of lignocellulosic substrates. Phosphatidylinositide qualitative composition did not change during the *L. tigrinus* growth in the solid-phase cultivation medium (mechanically processed birch sawdust). However, on this lignocellulosic substrate significant changes in their quantitative ratios were observed. The fungal growth was accompanied by a decrease in the content of PtdIns and PtdIns(4)P and an increase in the content of PtdIns(4,5)P_2 during the whole period of cultivation. The period of maximum ligninolytic activity of *L. tigrinus* (9-12th day) was characterized by higher levels of PtdIns(4,5)P_2 and lower content of PtdIns and PtdIns(4)P (Fig. 4).

Thus, during biodegradation of phenol and lignin by *L. tigrinus* changes occur in the ratio of mycelial phospholipids and the products of their degradation. The relative content of PtdEtn, phosphatidylinositides, and Ptd-L-Ser decreases, while the fraction of lysoPtdChol + SPH increases, probably because of greater stability of SPH more than compensating for lysoPtdChol [27]. Also, the biodegradation of xenobiotic and lignocellulosic substrates is accompanied by intensification of phosphoinositide metabolism. PtdIns and PtdIns(4)P dominated among the phosphatidylinositides at the beginning of cultivation, but PtdIns(4,5)P_2 was leading after 6 days. Intensive growth of mycelium corresponds to a higher content of the most labile and metabolically active fraction – PtdIns(4,5)P_2. Its share was already over 60% of the total phosphatidylinositides by the sixth day of growth.

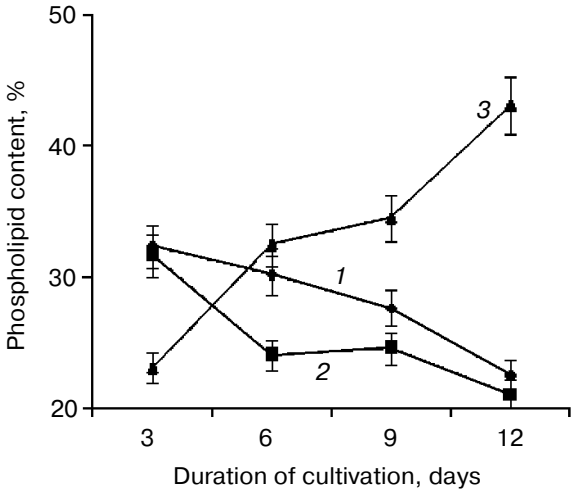

Fig. 4. Ratio change (% of total phosphatidylinositides) of individual phospholipid fractions PtdIns (*1*), PtdIns(4)P (*2*), and PtdIns(4,5)P_2 (*3*) of *L. tigrinus* mycelium during biodegradation of mechanically processed lignocellulose substrate.

Effect of phenol on the content of LPO products in *L. tigrinus* mycelium

LPO products	Cultivation time, days	Phenol concentration, %				
		Control	1		5	
			Time of addition, days			
			3rd	6th	3rd	6th
DC, arbitrary units/mg lipids	3	26.9 ± 1.3	26.9 ± 1.3	26.9 ± 1.3	26.9 ± 1.3	26.9 ± 1.3
	6	20.5 ± 1.0	10.4 ± 0.5	20.5 ± 1.0	13.5 ± 0.6	20.5 ± 1.0
	9	42.3 ± 2.1	29.8 ± 1.4	25.0 ± 1.2	15.9 ± 0.7	22.5 ± 1.1
	12	48.1 ± 2.4	22.2 ± 1.1	20.1 ± 1.0	12.3 ± 0.6	24.1 ± 1.2
TC, arbitrary units/mg lipids	3	24.2 ± 1.2	24.2 ± 1.2	24.2 ± 1.2	24.2 ± 1.2	24.2 ± 1.2
	6	15.7 ± 0.8	10.7 ± 0.5	15.7 ± 0.8	5.5 ± 0.2	15.7 ± 0.8
	9	30.8 ± 1.5	18.4 ± 0.9	25.7 ± 1.2	13.3 ± 0.6	22.0 ± 1.1
	12	36.2 ± 1.8	16.3 ± 0.8	23.1 ± 1.1	12.5 ± 0.6	23.1 ± 1.1
MDA, nmol/mg biomass	3	6.2 ± 0.3	6.2 ± 0.3	6.2 ± 0.3	6.2 ± 0.3	6.2 ± 0.3
	6	11.9 ± 0.6	24.6 ± 1.2	11.9 ± 0.6	16.2 ± 0.8	11.9 ± 0.6
	9	33.8 ± 1.6	30.2 ± 1.5	31.5 ± 1.5	27.7 ± 1.3	27.0 ± 1.3
	12	52.8 ± 2.6	28.1 ± 1.4	29.6 ± 1.5	25.1 ± 1.2	25.1 ± 1.2

Phenol addition to the nutrient medium, in addition to changes in phospholipid composition, caused changes in the accumulation of LPO products. Regardless of the concentration and time of its introduction, there was a reduction of the primary peroxidation products of lipids, DC and TC (table), and 5% phenol provided greater antioxidative effect.

However, an increase in MDA content was observed. This is probably due to the fact that MDA is a secondary product of LPO and is formed as a result of further conversion of DC and TC that accumulated during the first phase of growth. However, regardless of the concentration and the time of phenol addition, there is some reduction of MDA by the 12th day of growth. This is apparently the result of phenol decreasing the amount of primary LPO products (DC and TC), and since they are precursors of secondary LPO products including MDA, then, accordingly, a decrease in its content is observed.

Since according to several authors radical processes play a major role in lignolysis and biodegradation of xenobiotics including those involving LPO [28, 29], special attention should be devoted to the changes in the content of lysoPtdChol and LPO products. It is known that wood-destroying fungi, including *L. tigrinus*, have a relatively high content of lysoPtdChol compared to higher animals [30]. This can be explained by the constant need for availability of the sources of radicals – free fatty acids formed by the hydrolysis of phospholipids. Therefore, an increase in lysoPtdChol content should lead to increase in LPO product. Indeed, there is a dependence close to this observed in the control samples. However, the content of LPO products decreases in the presence of phenol, while at the same time lysoPtdChol portion increases. This is probably caused by the ability of phenol to inhibit chain oxidation by the interaction with peroxide radicals of oxygenating unsaturated fatty acids.

One of the most likely regulation mechanisms is PLA_2 activation. Our research shows that PLA_2 activity in fungal mycelium extracts was already observed by the third day of growth, and its level depends on the duration of cultivation and the presence of phenol. PLA_2 activity increased during *L. tigrinus* culture growth in control medium from 113 to 467 μg fatty acids per hour per mg protein by the 12th day (Fig. 5a).

Enzyme activity abruptly increased upon addition of 1% phenol in the trophic phase, exceeding the control value by the 6th day almost 2-fold. Its value continued to increase for 12 days of cultivation, but its difference from the control was insignificant. PLA_2 activity increased even more upon increasing phenol concentration 5% and was 2 times greater by the 6th day than in the 1% phenol variant and more than 3 times greater than in the control (6th day). It stayed fairly high even by the 12th day, greatly exceeding the control (Fig. 5a). The same trend was observed after adding phenol on the 6th day during idiophase. However, PLA_2 activity increase compared to the 1% phenol variant was less significant (Fig. 5c).

The same high PLA_2 activity in *L. tigrinus* mycelium was observed during the degradation of lignocellulose substrates. PLA_2 activity during solid phase cultivation on machine-processed lignocellulose substrate was detected from the 3rd day of growth and increased during *L. tigrinus* cultivation from 267 to 645.6 μg fatty acids per h per mg protein by the 12th day of growth (Fig. 6a).

It was shown earlier that fungal growth and development are accompanied by changes in the ratio between dif-

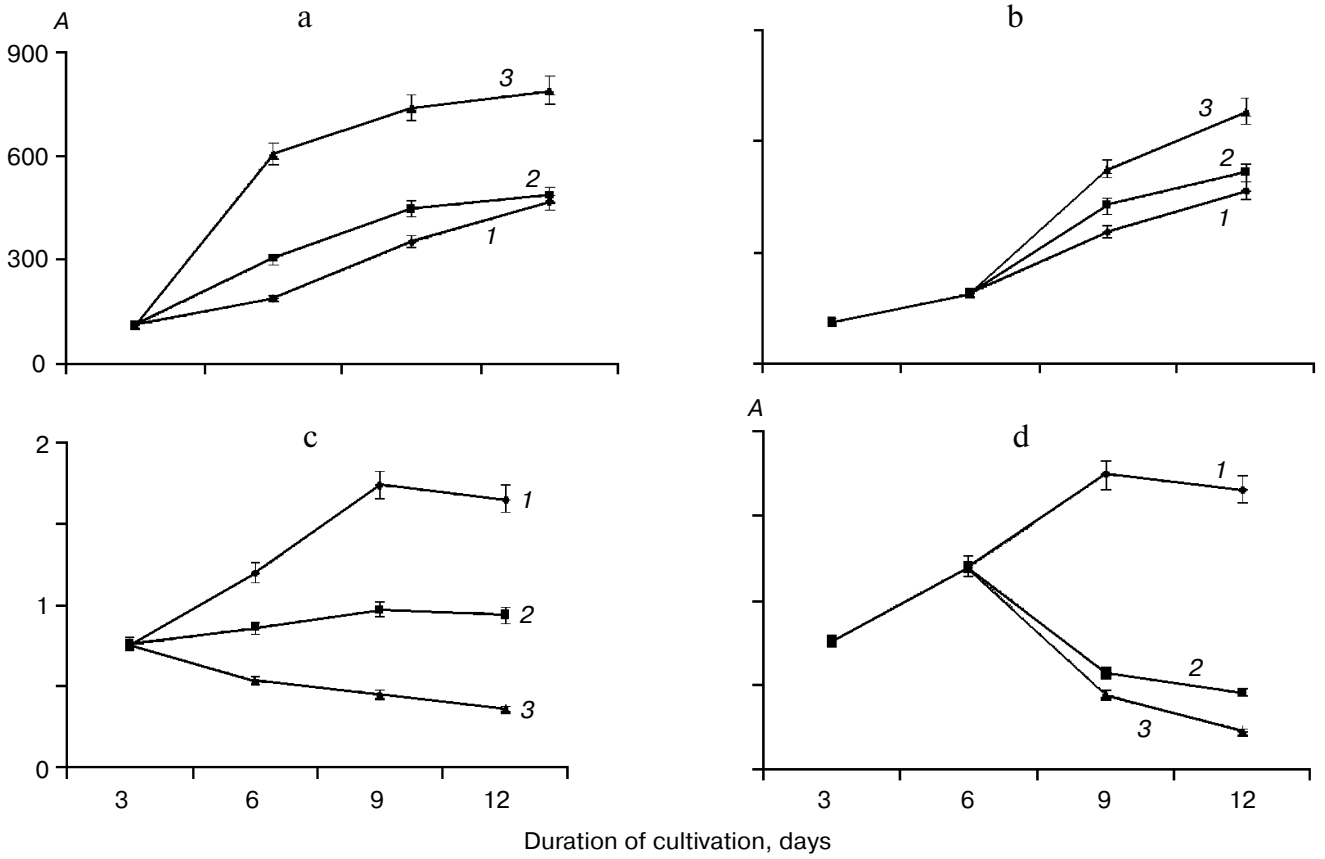

Fig. 5. Change in PLA_2 activity (μg fatty acids per h per mg protein) (a, b) and in PI-PLC activity (nmol phosphorus per min per mg protein) (c, d) in *L. tigrinus* mycelium upon addition of phenol on third (a, c) or sixth (b, d) day. *1*) Control; *2*) 1% phenol; *3*) 5% phenol.

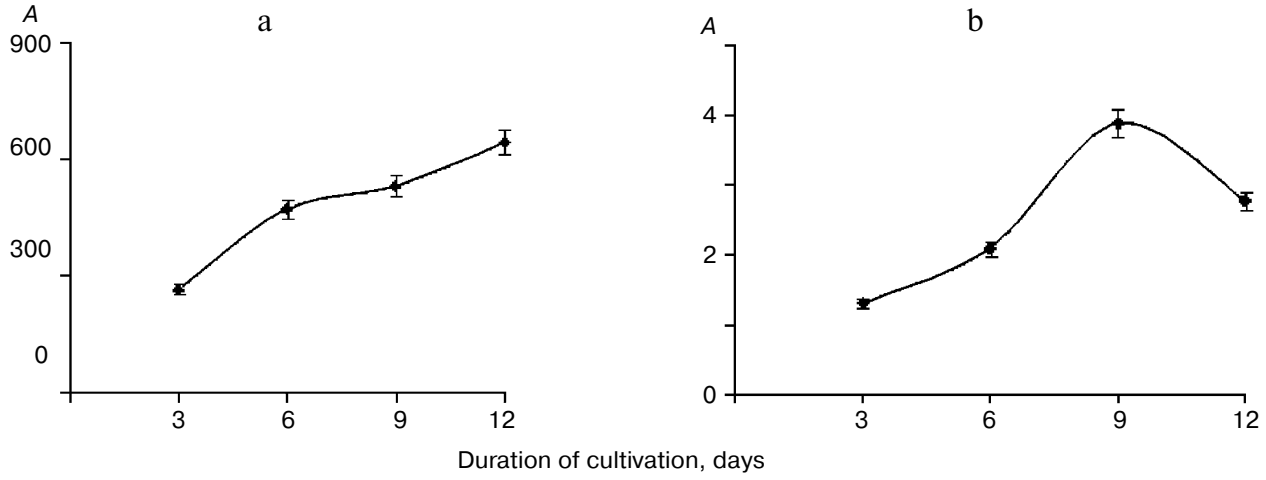

Fig. 6. Change in activity of PLA_2 (μg fatty acids per h per mg protein) (a) and PI-PLC (nmol phosphorus per min per mg protein) (b) in *L. tigrinus* mycelium during biodegradation of mechanically processed lignocellulose substrate.

ferent phosphatidylinositide fractions (PtdIns, PtdIns(4)P, and PtdIns(4,5)P_2) and that the pattern of these changes depends on the presence of phenol in the nutrient medium. According to the literature, ratios of individual phosphoinositide fractions play an important part in the activity of cells and the whole organism. First, this ratio affects the functional (physical) characteristics of the membrane – viscosity and permeability; second, the speed of hydrolysis

of these and calcium affinity varies greatly, thus their ratio considerably affects the rate of production of very important secondary messengers such as DAG and myo-inositols [31]. The transformation of PtdIns into PtdIns(4)P and further into PtdIns(4,5)P_2 are catalyzed by polyphosphoinositide phosphomonoesterase enzymes, and their phosphodiester cleavage is catalyzed by PI-PLC [32].

Our studies have shown that PI-PLC activity during *L. tigrinus* culture growth in the control medium is already observed by the third day, that it reached its maximum of 1.74 nmol of phosphorus per min per mg protein by the 9th day, and it remains virtually unchanged from thereon to the end of cultivation (Fig. 5b). Addition of phenol to the nutrient medium considerably lowered PI-PLC activity. PI-PLC activity during the whole cultivation period stayed virtually the same as in the control on the third day on addition of 1% phenol in the trophic phase. Increasing phenol concentration to 5% had an inhibitory effect on the activity of PI-PLC; its activity throughout the period of cultivation was reduced and was much lower than with 1% phenol and in the control sample (Fig. 5b). PI-PLC activity was also decreased when adding phenol during idio-phase throughout the whole period of cultivation, and its value was much lower than in control, with the addition of 5% phenol causing the greatest inhibitory effect (Fig. 5d). Comparison between content modification of individual phosphatidylinositide fractions and the PI-PLC activity indicates that the content of PtdIns(4,5)P_2 is dependent on the activity of PI-PLC. High activity of the enzyme corresponds to relatively low levels of PtdIns(4,5)P_2 in the control sample. Upon addition of either 1 or 5% phenol, inhibition of the enzyme activity occurs resulting in a higher content of PtdIns(4,5)P_2.

The same trend in the PI-PLC activity change of *L. tigrinus* mycelium was observed during lignocellulosic substrate degradation. PI-PLC activity was detected during solid-phase cultivation on lignocellulosic mechanically processed substrate by the third day of growth, and it reached a maximum value of 3.9 nmol/min per mg protein by the ninth day. However, following this, its value began diminishing, and it reached 2.78 nmol/min per mg protein by the end of cultivation (Fig. 6b).

Thus, the relationship was revealed between the studied activity of phospholipase, phospholipid composition, and content of LPO products in mycelium during the growth of the fungus. A relatively low activity of both PLA_2 and PI-PLC is observed in the absence of phenol in the medium. Addition of phenol leads to the activation of PLA_2, whereas the activity of PI-PLC is reduced.

DISCUSSION

Data obtained in the course of the study is further proof of the fact that radical processes play a significant role in lignolysis and biodegradation of phenolic xenobiotics by fungi. The dependence of ligninolytic properties of white-rot fungi on hydrogen peroxide for the enzymatic processes is well known [33]. During growth these organisms generate various reactive oxygen species that attack phenolic substrates [34]. Fatty acids with catalysis by acyl-CoA oxidase enzyme may serve as a source of peroxide radicals along with the special peroxide-generating enzymes in wood-destroying fungi [35]. Under these conditions, we think the role of PLA_2 is the formation of fatty acids (sources of peroxide radicals) and their constant influx to peroxide-generating enzymes (i.e. lipoxygenase). It is known that the phospholipids of wood-destroying fungi contain a large amount of unsaturated fatty acids in comparison with other organisms, especially linoleic acid, the content of which may reach 60% of the total fatty acids [36]. Thus, lysophospholipids and free fatty acids (mostly unsaturated) accumulate in the lipid bilayer of the mycelium with the activity of PLA_2. This, in turn, leads to a change in its structural and functional properties – permeability increase, activity changes of membrane-bound enzymes, and the initiation of LPO [37].

Nonenzymatic and enzymatic oxidation of unsaturated fatty acids is enhanced under the influence of these factors. The main contribution is probably from the oxidation of linoleic acid, which far exceeds all unsaturated fatty acids by the rate of oxidation except for arachidonic acid [38]. As a result, hydroperoxides capable of oxidizing phenols and other aromatics are formed. The negative charge in the phenolic aromatic ring shifts to oxygen due to the presence of the generalized system of π-electrons, which leads to a fairly easy separation of the OH group hydrogen atom with the formation of a reactive and unstable phenoxy radical. Phenoxy radical in turn acts as an interceptor of fatty acid radicals generated during LPO with the participation of reactive oxygen species and lipoxygenases, converting them into a molecular product, but a phenoxy radical is no longer able to participate in the continuation of a chain reaction since it undergoes dimerization with the formation of a carbon–carbon or carbon–oxygen bond [39]. This results in the polymerization of phenols (raising their molecular weight) and the formation of less toxic products. The more phenol in the medium, the greater is the need of unsaturated fatty acids (sources of peroxyl radicals), and vice versa.

Our study has also shown that the presence of phenol in the medium in the soluble form or adsorbed on the surface of the mycelium causes an increase in PLA_2 activity and, consequently, an increase in lysoPtdChol content. The sharp increase in the enzyme activity in the presence of phenol, probably due to the fact that fungal cells need more unsaturated fatty acids as sources of peroxide radicals for the removal of "phenolic barrier" (neutralization of xenobiotics) and seek to compensate for losses due to antioxidant properties of the xenobiotic. It can also be assumed that the reorganizations in the phenol molecule

caused by peroxide radicals of fatty acids reduce its toxicity and make it more accessible for ligninolytic enzymes. This is confirmed by the literature data that the affinity of some enzymes of the ELEC to substrates increases with increasing molecular weight of the substrates [40].

It is not yet established whether PLA_2 cleaves already oxidized unsaturated fatty acids. For example, it is established for plants that phospholipases recognize and selectively remove oxidized fatty acids from membrane lipids or oxidation occurs after cleavage. In any case, taking into account the availability of free and bound fatty acids to the action of PLA_2, it can be assumed that the second mechanism is preferred [41, 42].

The published data on the relationship between the activities of different types of phospholipases is very contradictory. According to some authors, decrease in PI-PLC activity in higher organisms leads to the lack of DAG in cells. As a result, the rate of synthesis of major phospholipids (PtdEtn, PtdChol) is also lower, which affects the activity of PLA_2 [43]. Meanwhile, this relationship may have different properties in lower organisms. For example, inositol, one of the products of PtdIns(4,5)P_2 hydrolysis by the PI-PLC enzyme, was shown to be an inhibitor of PLA_2 in yeast cells [44]. Apparently, the studied fungus exhibits a similar interconnection. In the presence of phenol, the cell needs a large amount of LPO products formed from unsaturated fatty acids, the amount of which depends on the activity of PLA_2. The activity of PLA_2 is regulated by the presence of inositol, a product of phosphatidylinositide hydrolysis by the PI-PLC enzyme. Thus, high activity of PLA_2 corresponds to low activity of PI-PLC.

There is evidence in the literature that endogenous and exogenous phospholipases play an important role in the interaction between pathogenic fungi and host; they cause formation of pores in host lipid bilayer membranes through which the hyphae of the mycelium penetrate [45, 46]. We suppose that in case of wood-destroying fungi the mechanisms of destruction of lignin and other phenolic structures can be supplemented with the following propositions. PLA_2 forms are activated on the surface of the substrate (wood) or in the presence of phenolic xenobiotics, resulting in a large amount of fatty acids being secreted into the near-mycelial layer. Under the action of lipoxygenase and reactive oxygen species, lipid peroxide radicals are formed which then neutralize (oxidize) the aromatic compounds present in the wood, which are inhibitors of fungi growth and include lignin. Simultaneously, the mycelium of the fungus secretes enzymes of ligninolytic complex into the extracellular medium. This leads to the removal of the barrier created by aromatic compounds (resins) and/or to increase in the availability of the resins to the enzymes of ligninolytic complex. Thus, a more favorable environment is formed around the mycelium of the ligninolytic fungus for the hyphae to attack and deeply penetrate the wood substrate.

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