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EXPERIMENTAL ARTICLES

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# Dynamics of the Cytosol Soluble Carbohydrates and Membrane Lipids in Response to Ambient pH in Alkaliphilic and Alkalitolerant Fungi

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**Abstract**—Comparative composition of lipids and cytosol soluble carbohydrates at different ambient pH values was studied for two obligately alkaliphilic fungi (*Sodiomyces magadii* and *S. alkalinus*) and for two alkalitolerant ones (*Acrostalagmus luteoalbus* and *Chordomyces antarcticus*). The differences and common patterns were revealed in responses to pH stress for the fungi with different types of adaptation to ambient pH. While trehalose was one of the major cytosol carbohydrates in alkaliphilic fungi under optimal growth conditions (pH 10.2), pH decrease to 7.0 resulted in doubling its content. In alkalitolerant fungi trehalose was a minor component and its level did not change significantly at different pH. In alkalitolerant fungi, arabitol and mannitol were the major carbohydrate components, with their highest ratio observed under alkaline conditions and the lowest one, under neutral and acidic conditions. In alkaliphiles, significant levels of arabitol were revealed only under alkaline conditions, which indicated importance of trehalose and arabitol for alkaliphily. Decreased pH resulted in the doubling of the proportion of phosphatidic acids among the membrane lipids, which was accompanied by a decrease in the fractions of phosphatidylcholines and sterols. Alkalitolerant fungi also exhibited a decrease in sterol level at decreased pH, but against the background of increased proportion of one of phospholipids. Decreased unsaturation degree in the fatty acids of the major phospholipids was a common response to decreased ambient pH.

**Keywords:** alkaliphily, alkalitolerance, *Sodiomyces magadii*, *Sodiomyces alkalinus*, *Acrostalagmus luteoalbus*, *Chordomyces antarcticus*, pH stress, membrane lipids, osmolytes, mannitol, arabitol, trehalose

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Metabolism of eukaryotes, including fungi, is greatly affected by proton and hydroxyl ion concentration in the environment. Biochemical processes in the cells require specific ranges of their concentrations, and pH changes alter availability of inorganic ions and compounds to the organism. Extremely high or low ambient proton concentrations are factors limiting life. Most fungi have growth optimum at acidic and neutral pH values. Obligate alkaliphiles are extremely rare among fungi. They were revealed among ascomycetes of the genera *Sodiomyces* (*Plectosphaerellaceae*, *Sordariomycetes*, *Ascomycota*) and *Thielavia* (*Chaetomiaceae*, *Sordariomycetes*, *Ascomycota*) inhabiting the coasts of soda lakes, i.e., environments with stable alkaline conditions (Bilanenko et al., 2005; Grum-Grzhimaylo et al., 2013, 2016).

Alkaliphilic fungi have growth optima at alkaline pH above 8 (Grum-Grzhimaylo et al., 2016). Alkali-

philes are divided into two groups: obligate and facultative alkaliphiles. Obligate alkaliphiles have growth optima at high pH (of 8 to 10) and do not grow at low pH (less than 5). Facultative alkaliphiles grow in a wider range of alkaline pH values and even at an acidic pH.

In contrast to the rare alkaliphilic fungi, alkalitolerant fungi are more widespread and their habitats are not limited to soda lakes and soils. Alkalitolerant species grow in a wide pH range but have growth optimum at neutral or even acidic pH.

Both alkalitolerant and facultatively alkaliphilic fungi are polyphyletic groups, which include representatives of different orders of *Ascomycota* inhabiting various environments. Plant and invertebrate pathogens, plant rhizosphere inhabitants, species associated with algae and lichens, and inhabitants of the surfaces of old limestone constructions have been found among

them (Grum-Grzhimaylo et al., 2016). Alkalitolerant fungi may be isolated from slightly acidic or neutral soils (Bondarenko et al., 2016). Temporary alkaline conditions occur ubiquitously both in natural (due to photosynthesis, ammonification, and sulfate reduction) and in anthropogenic habitats (e.g., in cultivated soils due to the use of fertilizers and in oil-contaminated soils). Apparently, alkalitolerant fungi do not lose the ability to grow under acidic conditions due to temporary local alkalization but gain a competitive advantages over the species unable to survive at high pH values.

Organic osmolytes, low-molecular compounds belonging to different classes including sugars, amino acids and their derivatives, amines and their derivatives, etc., play an essential role in the adaptation of the organisms not only to high osmolality, but also to other stresses (Yancey, 2005; Yancey and Siebenaller, 2015; Patel and Williamson, 2016). Cytoprotective compounds in fungi are represented by sugar alcohols and the disaccharide trehalose. The study of the mechanisms of adaptation of fungi to high pH values is currently at the initial stage.

It was previously shown that two carbohydrates, trehalose and glucose, were predominant in the cytosol of the obligately alkaliphilic fungus *Sodiomyces tronii* (optimum pH for growth 9.2), and trehalose content increased during the growth (Bondarenko et al., 2017). Changes in the ambient pH did not lead to the changes in trehalose content, whereas glucose was replaced with mannitol and arabitol under alkaline conditions (pH of 10.2) and practically disappeared under acidic conditions. The data obtained demonstrated the potential role of trehalose in alkaliphilic fungi as well as involvement of mannitol, arabitol, and trehalose in the response of the alkaliphilic fungus *Sodiomyces tronii* to unfavorable changes in the ambient pH. Moreover, the proportion of sterols and sphingolipids in membrane lipids increased significantly under acidic conditions, which may be associated with the formation of lipid rafts in the membranes. In this regard, it should be considered whether osmolytic adaptation is a universal stress response in alkaliphilic fungi and whether it is used for the adaptation to the external pH in alkalitolerant fungi. Moreover, changes in membrane lipid and fatty acids compositions caused by alterations in the external pH are also of high interest.

The goal of the present work was to study changes in the composition of cytosol soluble carbohydrates and membrane lipids in two obligately alkaliphilic fungi (*Sodiomyces magadii* and *S. alkalinus*) and in two alkalitolerant ones (*Acrostalagmus luteoalbus* and *Chordomyces antarcticus*) caused by the changes in the ambient pH.

## MATERIALS AND METHODS

Two obligately alkaliphilic fungi, *Sodiomyces magadii* (S.A. Bondarenko, A.A. Grum-Grzhim., A.J.M. Debets & Bilanenko CBS 137619) and *S. alkalinus* (Bilanenko & M. Ivanova, A.A. Grum-Grzhim., A.J.M. Debets & Bilanenko CBS 110278), as well as two strongly alkalitolerant fungi *Acrostalagmus luteoalbus* (Link) Zare, W. Gams & Schroers CBS ZBS5) and *Chordomyces antarcticus* (Bilanenko, Georgieva & A.A. Grum-Grzhim. CBS 120045) were the subjects of the study. These species belong to the family *Plectosphaerellaceae* (*Sordariomycetes*, *Ascomycota*). The fungi were isolated from different habitats: *S. magadii* was isolated from soda soil of the coast of Lake Magadi (Kenia); *S. alkalinus* was isolated from soda soil around a soda lake in Northeastern Mongolia; *C. antarcticus* was isolated from the soil of the coast of a soda lake of the Kulunda steppe (Altai, Russia) (Grum-Grzhimaylo et al., 2016); *A. luteoalbus* was isolated from sod-podzol soil from the vicinity of the of the Moscow State University Zvenigorod Biological Station (Moscow oblast, Russia) (Bondarenko et al., 2016).

Alkaliphilic and alkalitolerant fungi were grown on alkaline agar medium (pH of 10.2) (Grum-Grzhimaylo et al., 2013) and on malt extract agar, respectively. To achieve required pH, citrate (pH of 4.0, 4.5, 5.5, and 5.9), phosphate (pH of 7.0), and carbonate (pH of 10.2) buffer solutions were used (Bondarenko et al., 2017). Optimum temperature and sodium chloride concentration were determined by assessment of the growth rate in petri dishes at optimum pH. For biochemical studies, all fungi were grown in petri dishes with cellophane membranes in the dark at the optimal temperature. *S. magadii* was grown for 7 days at 25°C on the media with pH of 10.2, 7.0, and 5.5. *S. alkalinus* was grown for 7 days at 25°C on the media with pH of 10.2, 7.0, and 5.9. *C. antarcticus* was grown for 10 days at 25°C on the media with pH of 10.2, 7.0, and 4.0. *A. luteoalbus* was grown for 7 days at 25°C on the media with pH of 10.2, 7.0, and 4.5.

Grown mycelium was collected with a scalpel and the lipids were extracted. To analyze carbohydrates, mycelium samples were frozen and stored at -21°C.

Dry biomass was determined gravimetrically. Lipids and carbohydrates were analyzed according to the method described previously (Ianutsevich et al., 2016a).

The experiments were performed in triplicates. The results of typical experiments are presented on the figures. The dispersion of the results did not exceed 10%. The main trends were coinciding.

## RESULTS

**Optimal growth conditions for the fungi.** To study the effect of pH on the composition of cytosol carbohydrates and membrane lipids of alkaliphilic and alka-

litolerant fungi, optimal growth conditions should be evaluated.

In our previous works, we studied dependences of the growth rates of the studied fungi on the ambient pH. *Sodiomyces magadii* and *S. alkalinus* were shown to be obligate alkaliphiles having distinct pH optima exceeding 10 and unable to growth at pH values less than 5. *C. antarcticus* and *A. luteoalbus* were shown to be strongly alkalitolerant, able to grow in a wide range of pH values and to retain high growth rate under alkaline conditions. Both fungi had indistinct growth optima at circumneutral pH values and their growth rates insufficiently decreased under acidic conditions (Grum-Grzhimaylo et al., 2016; Bondarenko et al., 2016).

Evaluation of the growth rate of the fungi at different temperatures demonstrated that they had different temperature optima: 32°C for *S. magadii*, 28°C for *S. alkalinus* and *A. luteoalbus*, and 25°C for *C. antarcticus*.

Evaluation of the effect of sodium chloride on the growth of the fungi demonstrated that *S. magadii* did not require NaCl addition for the growth, whereas for other three fungi optimal NaCl concentration was 0.1 M.

**Dynamics of growth and spore formation.** To perform experiments accurately, fungal biomasses were obtained during comparable growth phases. Both alkalitolerant fungi, *C. antarcticus* and *A. luteoalbus*, were shown to form only conidia from the first days of the growth at all studied pH. Conidia (phialoconidia) were not colored, did not have ornamentation, and were similar to the vegetative cells. Conidia were formed by extrusion of the cytoplasm through the necks of the phialide into the formed conidium surrounded by a thin cell wall that is the continuation of the inner layer of the phialide cell wall. Sexual sporulation did not occur in these fungi. In contrast, alkaliphiles *S. magadii* and *S. alkalinus* formed fruit bodies after 5–7 days of the growth under optimal conditions (pH 10.2). At the early stage of the development, fruit bodies were not numerous and were not colored. Asexual sporulation was observed in *S. alkalinus* during the first days of growth but was not observed in *S. magadii*. In alkaliphiles, sexual and asexual (if occurs) reproduction decelerated at neutral pH and did not occur under acidic conditions. Based on these observations, the following duration of incubation was selected: 7 days for *S. magadii*, *S. alkalinus*, and *A. luteoalbus*; 10 days for *C. antarcticus*.

**Cytosol soluble carbohydrates.** Soluble carbohydrates comprised 16–17% of the dry weight of alkaliphile *S. magadii* under optimal conditions (pH of 10.2) and were represented mainly by mannitol (55%), trehalose (20%), and arabitol (20%) (Fig. 1a). Glucose, erythritol, inositol, and glycerol were minor sugars. At pH 7.0, content of carbohydrates decreased 1.5 times due to the decrease in the levels of mannitol and ara-

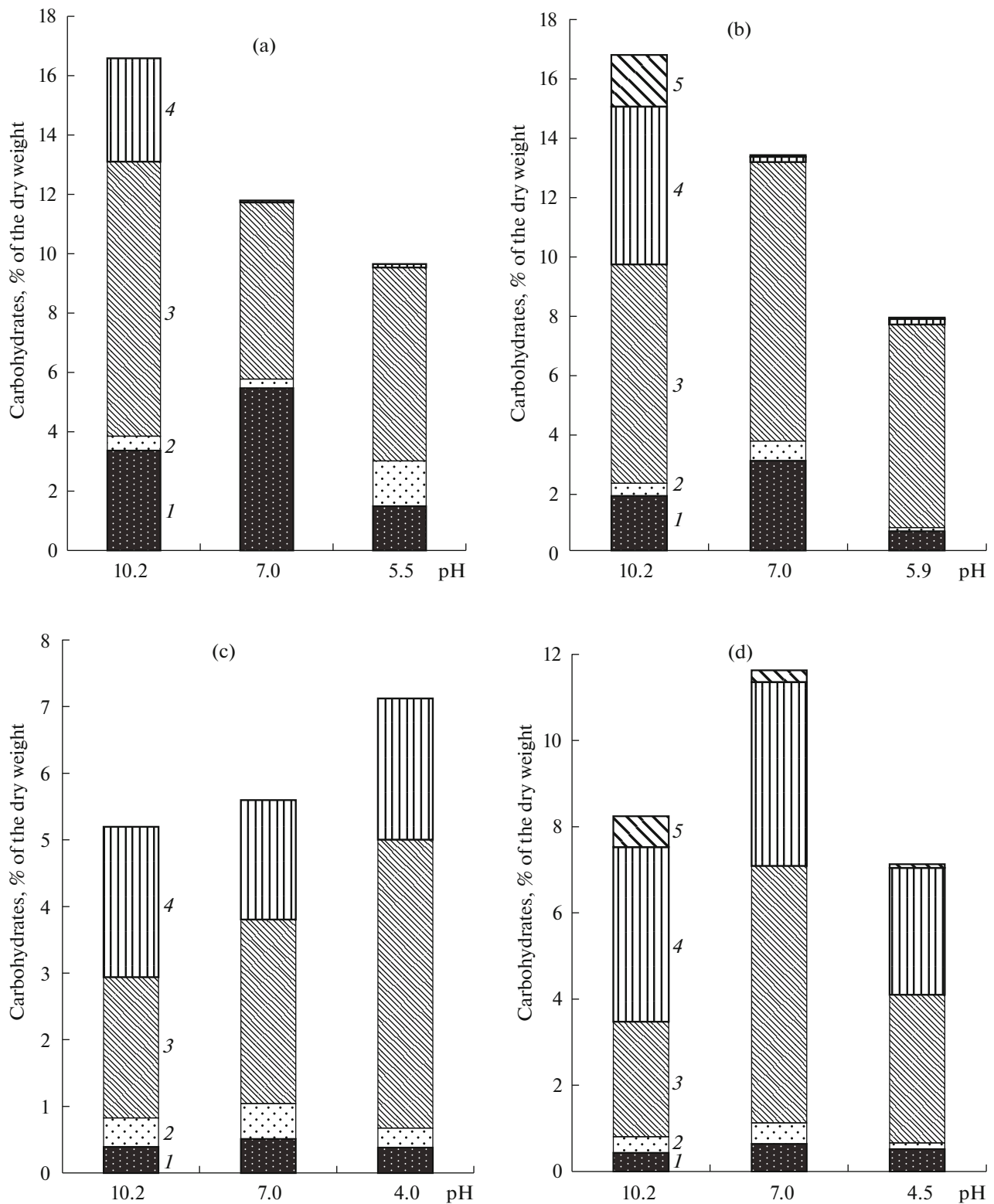
bitol (down to trace amounts). Trehalose content increased 1.5 times, which resulted in the increase in trehalose proportion (up to 45% of total sugar content). Under acidic conditions, carbohydrate content decreased 2 times, mannitol content did not change compared with the neutral conditions, and trehalose content decreased 2 times, which resulted in predominance of mannitol (up to 67% of total sugar content) and equalization of trehalose and glucose proportions (up to 15%).

Soluble carbohydrates comprised 17% of the dry weight of the other alkaliphile, *S. alkalinus* (Fig. 1b). Under optimal conditions (pH 10.2), mannitol (45% of the total sugar content) and arabitol (31%) were predominant carbohydrates, whereas proportions of trehalose and erythritol were 10–11%. Cultivation of the fungus under neutral conditions caused a 20% decrease in carbohydrate content and abrupt changes in carbohydrate composition. Amounts of arabitol and erythritol decreased down to traces, whereas trehalose content increased two times. As a result, mannitol (72%) and trehalose (22%) became predominating carbohydrates. Under acidic conditions, carbohydrates content decreased two times in comparison to the optimal conditions. Mannitol was predominant (85%), whereas trehalose content did not exceed 8%.

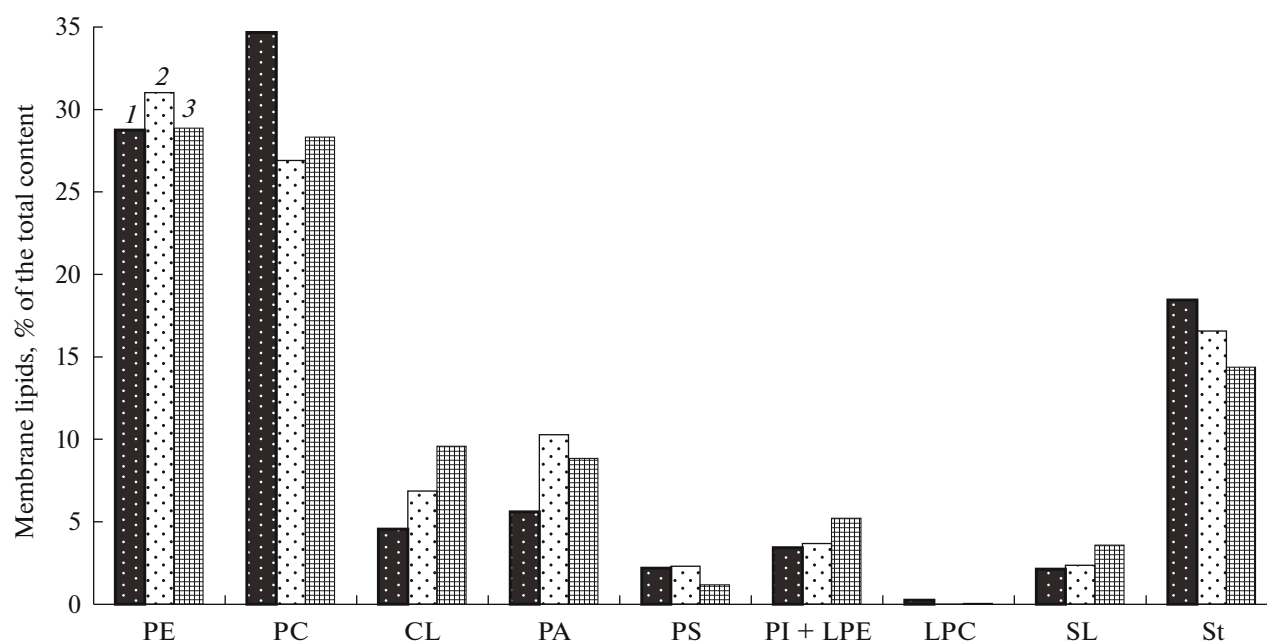
In the alkalitolerant fungus *C. antarcticus*, sugar contents were equal at pH values of 10.2 and 7.0 (5% of the dry weight), whereas it increased up to 35% at pH 4.0 (Fig. 1c). Mannitol (40–60% of the total sugar content) and arabitol (29–42%) were predominant. Trehalose content did not exceed 9% and did not change at decreased pH. The highest arabitol/mannitol ratio (1.1) was detected at pH 10.2, whereas a decrease in pH resulted in the decrease in arabitol content and arabitol/mannitol ratio (to 0.65 and 0.49 at pH values of 7.0 and 4.0, respectively).

In the alkalitolerant fungus *A. luteoalbus*, content of the cytosol carbohydrates was 6–12% of the dry weight (Fig. 1d). Cytosol carbohydrates were mainly represented by mannitol (32–51%) and arabitol (40–50%). Trehalose proportion did not exceed 7% and did not change at different pH. Polyols (glycerol, erythritol, and inositol) were the minor compounds. External pH values affected the total content of cytosol carbohydrates. The maximal content of soluble sugars was detected at pH 7.0 (11–12% of the dry weight), whereas pH increase up to 10.2 and decrease down to 4.5 decreased carbohydrate content 1.5 times. Quantitative composition of carbohydrates (mainly arabitol/mannitol ratio) was affected by the ambient pH. Arabitol/mannitol ratio reached the maximal value (1.53) at pH 10.2 and the minimum (0.73) at pH 7.0, whereas it was 0.80 at pH 4.5.

**Membrane and storage lipids.** In alkaliphile *S. magadii*, total lipid content was about 4% of the dry weight under optimal conditions and increased up to 40% under acidic conditions. In *S. magadii* grown



**Fig. 1.** Changes in the quantitative composition (%) of cytosol soluble carbohydrates at different pH values in alkaliphilic fungus *S. magadii* (a), alkaliphilic fungus *S. alkalinus* (b), alkalitolerant *C. antarcticus* (c), and alkalitolerant *A. luteoalbus* (d). Designations: trehalose (1), glucose (2), mannitol (3), arabitol (4), and erythritol (5).



**Fig. 2.** Changes in membrane lipid composition (% of the total content) in alkaliphilic fungus *S. magadii* at different pH values: 1—pH 5.5, 2—pH 7.0, 3—pH 10.2. Designations: PC, phosphatidylcholines; PE, phosphatidylethanolamines; CL, cardiolipins; PA, phosphatidic acids; PS, phosphatidylserines; LPE, lysophosphatidylethanolamines; PI, phosphatidylinositols; LPC, lysophosphatidyl cholines; SL, sphingolipids; St, sterols.

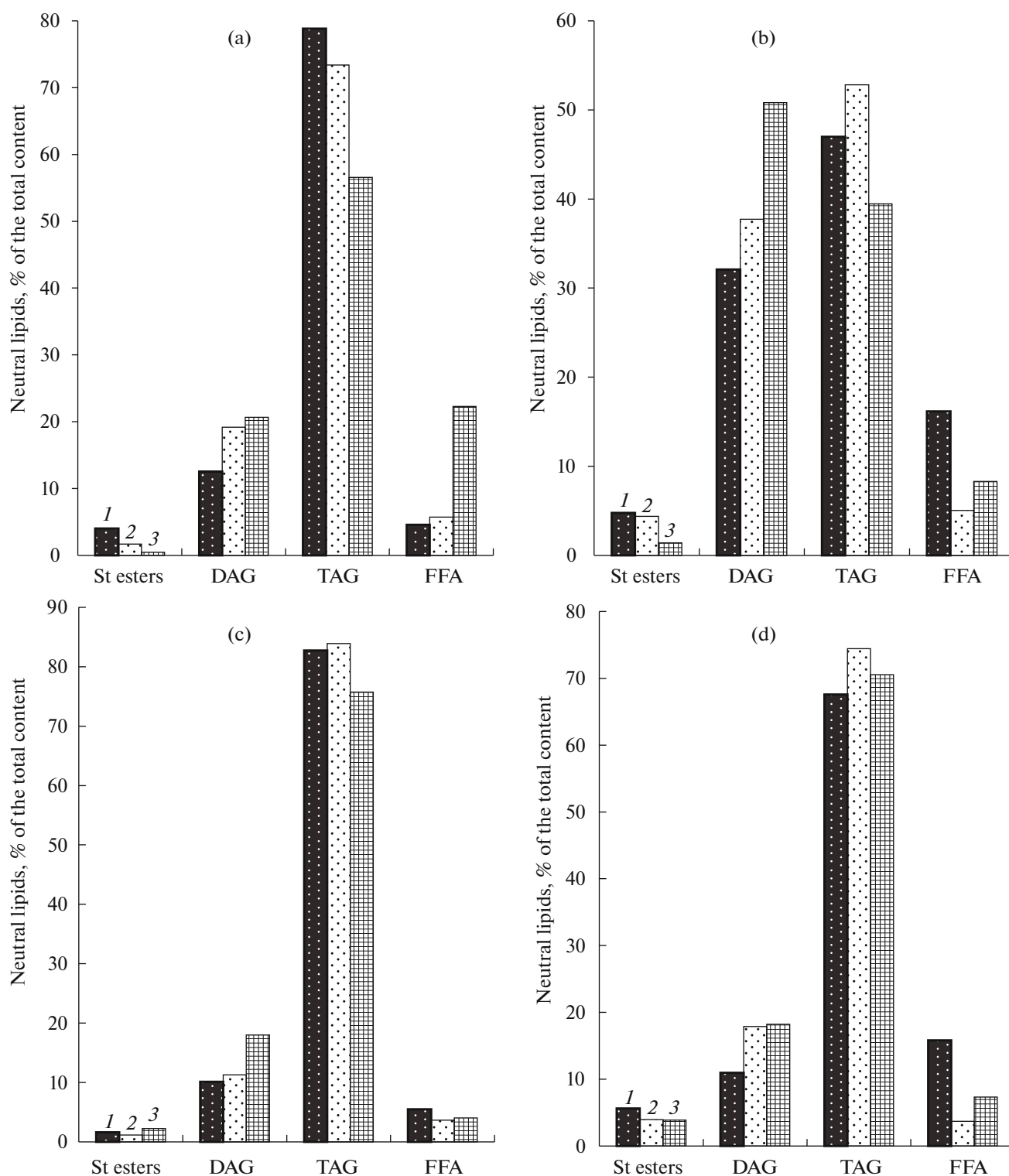
under optimal conditions, phosphatidylethanolamines (PE), phosphatidylcholines (PC), and sterols (St) were predominant membrane lipids, whereas the share of phosphatidic acids (PA) did not exceed 5% (Fig. 2). The decrease in pH down to neutral (7.0) and to 5.5 caused significant changes in lipid composition: PA increased two times, PC decreased, and the relative content of sterols did not change significantly. The most unsaturated phospholipids were typical under alkaline conditions. Decrease in pH resulted in the increase in the unsaturation degree of the fatty acids of the main phospholipids (PE and PC) due to the increase in the proportion of linoleic acid (Table 1).

Triacylglycerols (TAG) were predominant storage lipids under all conditions (56–78% of total lipid content) (Fig. 3a). Changes in neutral lipid content were observed only at pH of 5.5. Proportions of free fatty acids (FFA) and diacylglycerides (DAG) increased, whereas TAG proportion decreased.

In the alkaliphilic fungus *S. alkalinus*, lipid content comprised 4% of the dry weight under optimal conditions and increased 1.5 times under neutral and acidic conditions. Membrane lipids in all experiments were mainly represented by PE, PC, PA, and St (Fig. 4). The decrease in pH caused increase in PE and PA pro-

**Table 1.** Fatty acid composition of the main phospholipids of alkaliphilic fungus *S. magadii* grown at different pH

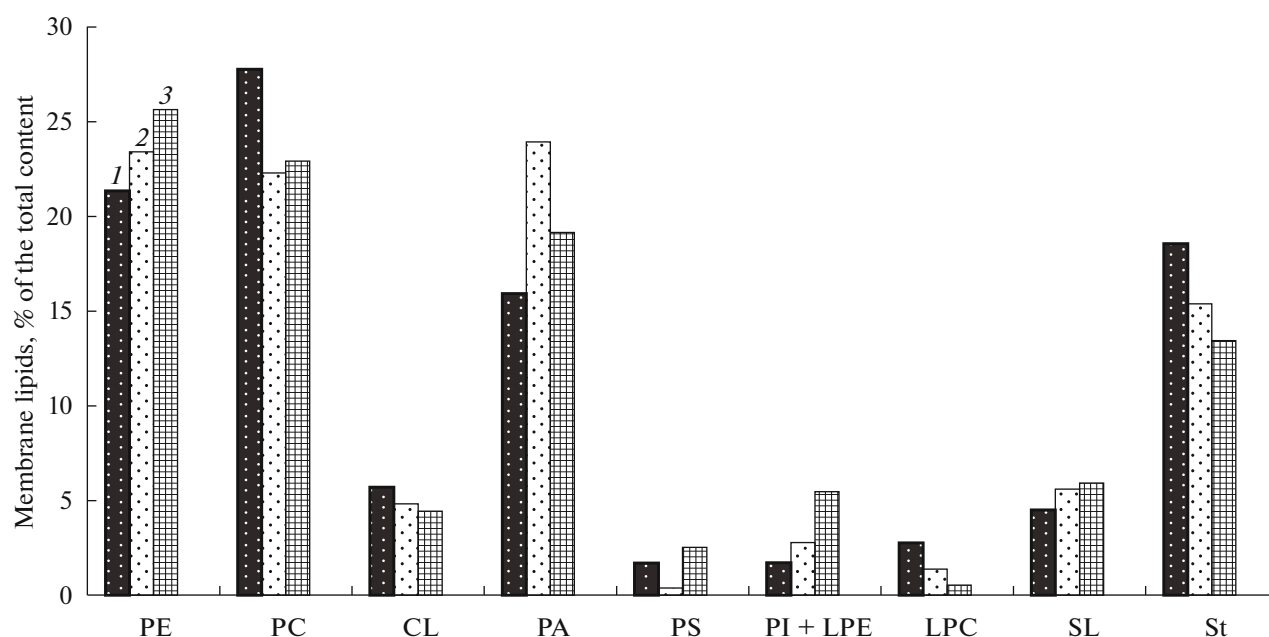
Fatty acid	pH 10.2		pH 7.0		pH 5.5	
	PE	PC	PE	PC	PE	PC
C <sub>14:0</sub>	0.8	0.7	0.0	0.6	0.5	0.5
C <sub>14:1</sub>	0.0	0.1	0.0	0.0	0.1	0.1
C <sub>16:0</sub>	30.2	21.2	26.6	13.4	26.4	10.9
C <sub>16:1</sub>	1.6	1.9	1.6	2.8	2.1	3.2
C <sub>18:0</sub>	5.6	4.9	3.0	2.4	1.9	1.7
C <sub>18:1n9c</sub>	27.6	22.4	27.7	20.7	30.4	24.8
C <sub>18:2n6c</sub>	32.7	46.1	39.2	55.1	36.1	53.0
C <sub>18:3n3</sub>	1.5	2.8	1.9	5.0	2.5	5.8
Unsaturation degree	0.99	1.25	1.13	1.49	1.12	1.51



**Fig. 3.** Changes in membrane lipid composition (% of the total content) in alkaliphilic fungus *S. magadii* (a), alkaliphilic fungus *S. alkalinus* (b), alkalitolerant *C. antarcticus* (c), and alkalitolerant *A. luteoalbus* (d) at different pH values: pH 5.5 (1), pH 7.0 (2), and pH 10.2 (3).

portions, whereas St proportion decreased and PC proportion did not change. Unsaturation degree of the fatty acids of the main lipids increased when pH

decreased due to the increase in linoleic acid content and decrease in oleic acid content (Table 2). TAG and DAG fractions in the neutral lipids were 40% each,



**Fig. 4.** Changes in membrane lipid composition (% of the total content) in alkaliphilic fungus *S. alkalinus* at different pH values: pH 5.9 (1), pH 7.0 (2), and pH 10.2 (3).

whereas FFA content did not exceed 13% (Fig. 3b). The decrease in pH caused an increase in DAG fraction and a decrease in TAG and FFA content.

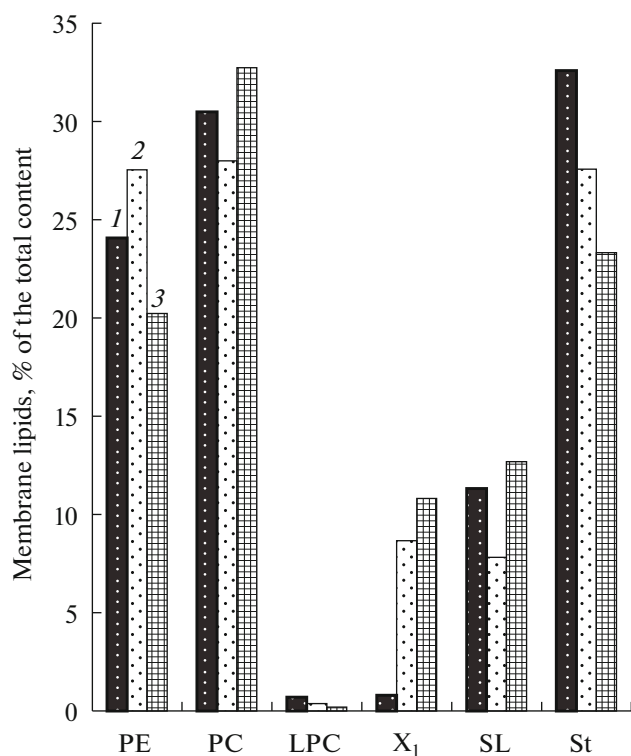
Total lipid amounts in the alkalitolerant fungus *C. antarcticus* at pH 4.0, 7.0, and 10.2 were 5.9, 5.8, and 8.3% of the dry weight, respectively. Membrane lipids were represented by the predominant phosphatidylcholines (28–33%), phosphatidylethanolamines (20–27%), and sterols (23–33%). The decrease in pH caused a decrease in sterols and PE fractions and increase in the fraction of an unidentified phospholipid X<sub>1</sub> (Fig. 5). Trace amounts of phosphatidic acids were presented at all pH values. Unsaturation degree of the fatty acids of phosphatidylcholines increased significantly during growth on the neutral and acidic

media in comparison to the alkaline due to the increase in linoleic acid content and decrease in palmitic and oleic contents (Table 3). Unsaturation degree in PE increased only on the acidic medium. TAG (70–80%) were predominant storage lipids. Under acidic conditions, DAG proportion increased and TAG fraction decreased, whereas the FFA fraction did not change (Fig. 3c).

Total lipid amounts in the alkalitolerant fungus *A. luteoalbus* at pH values 4.0, 7.0, and 10.2 were 3.8, 5.3, and 6.5% of the dry weight, respectively. Membrane lipids were mainly represented by phosphatidylcholines (25–35%), phosphatidylethanolamines (19–26%), and sterols (16–25%) (Fig. 6). The share of phosphatidic acids did not exceed 6%. The decrease in the ambient pH (10.2, 7.0, and 4.5) caused an

**Table 2.** Fatty acid composition of the main phospholipids of alkaliphilic fungus *S. alkalinus* grown at different pH

Fatty acid	pH 10.2		pH 7.0		pH 5.9	
	PE	PC	PE	PC	PE	PC
C <sub>14:0</sub>	0.6	0.4	0.7	0.4	0.5	0.4
C <sub>16:0</sub>	27.1	11.5	25.9	8.9	17.7	8.2
C <sub>16:1</sub>	0.8	1.1	0.5	0.6	1.0	1.2
C <sub>18:0</sub>	6.5	5.2	4.1	2.8	3.7	3.1
C <sub>18:1n9c</sub>	28.5	25.7	15.3	11.9	21.8	16.7
C <sub>18:2n6c</sub>	36.0	54.7	53.0	74.5	51.3	63.0
C <sub>18:3n3</sub>	0.5	1.4	0.5	0.8	4.0	7.3
Unsaturation degree	1.03	1.40	1.23	1.64	1.37	1.66



**Fig. 5.** Changes in membrane lipid composition (% of the total content) in alkalitolerant fungus *C. antarcticus* at different pH values: pH: pH 4.0 (1), pH 7.0 (2), and pH 10.2 (3).

increase in PC and PE fraction by 35% and a decrease in sterols content by 40%. Unsaturation degree of the fatty acid of two main phospholipids (PE and PC) was the lowest at the alkaline pH and increased under neutral and acidic conditions (Table 4). TAG were predominant storage lipids (67–75%). Increase in external pH caused an increase in FFA proportion and increase in DAG content (Fig. 3d).

## DISCUSSION

The results obtained in the present study make it possible to reveal the following general patterns of the dynamics of cytoplasmic carbohydrates and membrane lipids in the obligately alkaliphilic fungi:

(1) Trehalose as well as mannitol and arabitol were the main cytoplasmic carbohydrates at the optimal pH values;

(2) Trehalose content increased 2 times due to pH decrease to 7.0 and sharply decreased under acidic conditions;

(3) Arabitol was present only under alkaline conditions;

(4) Proportion of phosphatidic acids increased 2 times and those of phosphatidylcholines and sterols decreased at decreased pH;

(5) Unsaturation degree of the fatty acids of the main phospholipids increased at decreased pH;

(6) TAG proportion decreased under acidic conditions.

The following general patterns were revealed in alkalitolerant fungi:

(1) Mannitol and arabitol were the main cytosol soluble carbohydrates;

(2) Arabitol proportion increased under alkaline conditions, whereas mannitol fraction increased under neutral and acidic conditions;

(3) Proportion of phospholipids increased and proportion of sterols decreased under neutral and acidic conditions;

(4) Unsaturation degree of the fatty acids of the main phospholipids increased at decreased pH;

(5) Proportion of FFA decreased and proportion of DAG increased under acidic conditions.

Comparative analysis of the data obtained for alkaliphilic and alkalitolerant fungi revealed the following general patterns:

**Table 3.** Fatty acid composition of the main phospholipids of alkalitolerant fungus *C. antarcticus* grown at different pH

Fatty acid	pH 10.2		pH 7.0		pH 4.0	
	PE	PC	PE	PC	PE	PC
C <sub>14:0</sub>	0.7	0.9	0.5	0.8	0.4	0.4
C <sub>16:0</sub>	21.7	13.3	19.4	12.3	16.3	7.2
C <sub>16:1</sub>	1.2	1.6	2.7	3.7	1.2	1.9
C <sub>18:0</sub>	4.9	4.7	2.7	4.1	2.6	0.0
C <sub>18:1n9c</sub>	26.7	16.9	26.0	17.0	27.1	14.6
C <sub>18:2n6c</sub>	40.0	52.1	44.2	52.7	44.1	54.8
C <sub>18:3n3</sub>	4.8	10.6	4.5	9.5	8.4	21.1
Unsaturation degree	1.22	1.54	1.31	1.54	1.42	1.89



**Table 4.** Fatty acid composition of the main phospholipids of alkalitolerant fungus *A. luteoalbus* grown at different pH

Fatty acid	pH 10.2		pH 7.0		pH 4.5	
	PE	PC	PE	PC	PE	PC
C <sub>14:0</sub>	1.5	0.9	0.3	0.4	0.4	0.5
C <sub>16:0</sub>	25.9	13.6	16.3	8.7	16.1	7.8
C <sub>16:1</sub>	1.0	0.6	0.5	0.5	0.4	0.5
C <sub>18:0</sub>	8.5	4.8	3.8	2.8	3.7	3.0
C <sub>18:1n9c</sub>	36.5	20.1	29.9	18.8	30.2	18.5
C <sub>18:2n6c</sub>	25.9	54.0	46.8	61.3	46.9	61.9
C <sub>18:3n3</sub>	0.7	6.0	2.4	7.4	2.4	7.8
Unsaturation degree	0.91	1.47	1.31	1.64	1.32	1.66

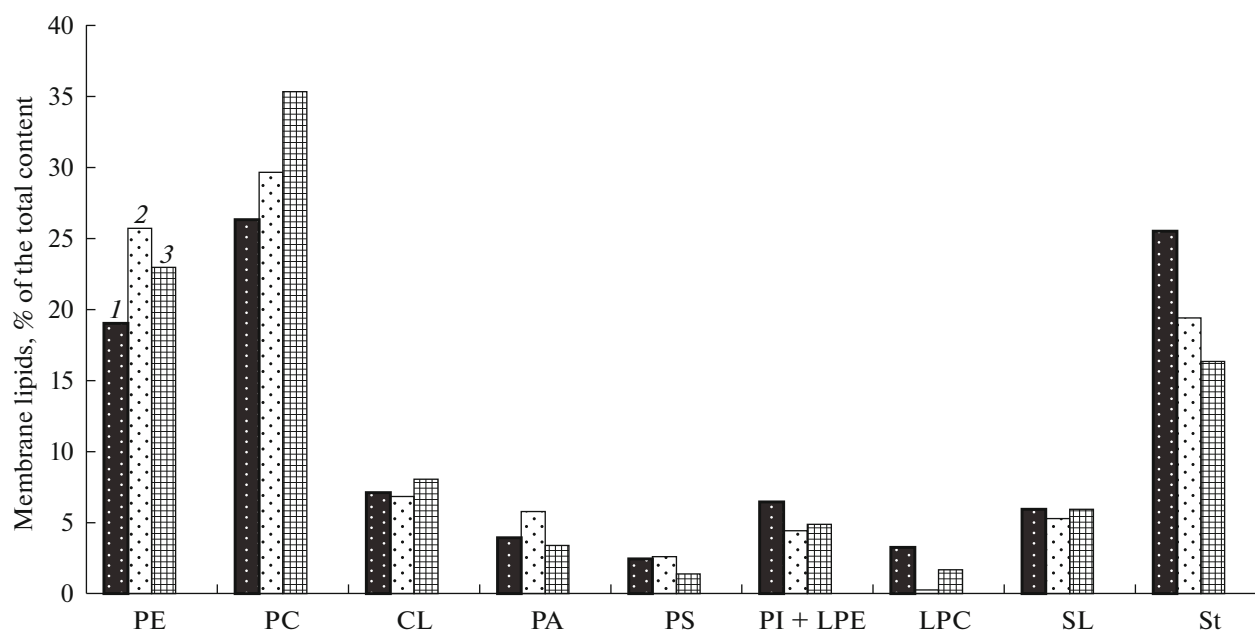
(1) Unsaturation degree of the main phospholipids increased at decreased pH;

(2) Arabitol proportion increased under alkaline conditions;

(3) Proportion of the sterols in membrane lipids decreased at decreased pH.

At the same time, several differences between alkaliphilic and alkalitolerant fungi were shown. First, in obligately alkaliphilic fungi, trehalose was the main carbohydrate and its amount increased under neutral conditions, whereas in alkalitolerant fungi, it was a minor component and its level did not change at different pH. Second, alkaliphilic and alkalitolerant fungi differed in arabitol content. In the studied alkaliphiles with pH optimum of 10.2 this polyol was

observed only under alkaline conditions, whereas in alkalitolerant fungi arabitol and mannitol were the main cytosol carbohydrates at all pH values. It should be noted that in these fungi arabitol predominated over mannitol only under alkaline conditions, whereas mannitol was predominant under neutral and acidic conditions. In the previously studied alkaliphilic fungus *S. tronii*, arabitol was found in trace amounts under optimal conditions (pH of 9.2) but its content sharply increased at increased pH (10.2) (Bondarenko et al., 2017). This confirmed our hypothesis that this osmolyte is important for growth at pH 10.2. Significant amounts of another low-molecular polyol, erythritol, were present both in alkaliphilic *S. alkalinus* and in alkalitolerant *A. luteoalbus* only under alkaline conditions. According to previous studies, arabitol and

**Fig. 6.** Changes in membrane lipid composition (% of the total content) in alkalitolerant fungus *A. luteoalbus* at different pH values: pH 4.5 (1), pH 7.0 (2), and pH 10.2 (3).

mannitol contents in the obligate alkaliphile *S. tronii*, carbohydrates of which were mainly represented by glucose and trehalose under the optimal conditions, essentially increased due to the increase in pH up to 10.2 (Bondarenko et al., 2017). In the present study, mannitol was the main carbohydrate in the studied alkaliphilic fungi, whereas glucose was a minor component. Arabitol content significantly increased under alkaline conditions, whereas increase in mannitol content was observed only in *S. magadii*.

We obtained the results confirming the difference in the role of trehalose in alkaliphilic and alkalitolerant fungi. High trehalose content was previously detected in sterile mycelium of the obligate alkaliphile *S. tronii* at all stages of growth both under optimal conditions and during changes of the ambient pH (Bondarenko et al., 2017). It can be assumed that trehalose is extremely important for alkaliphily. In contrast to *S. tronii*, trehalose content sharply decreased under acidic conditions in both alkaliphiles studied in the present work. This may be explained by the differences in pH optima (9.2 for *S. tronii* and 10.2 for *S. magadii* and *S. alkalinus*), i.e., acidic conditions were more unfavorable for *S. magadii* and *S. alkalinus*. Trehalose content in *S. alkalinus* was lower than those in *S. magadii* and *S. tronii*. It is probably associated with the habitats of the fungi and differences in their optimum temperatures (30–32°C for *S. magadii* and *S. tronii* and 28°C for *S. alkalinus*). However, high trehalose content cannot be explained solely by the thermotolerance. For example, alkaliphilic *S. alkalinus* and alkalitolerant *A. luteoalbus* with the same optimum temperature significantly differ in the composition of cytosol soluble carbohydrates.

According to the modern data, osmolytes represented in fungi by trehalose and polyols are metabolic and neutralizing cytoprotectors (Yancey, 2005; Yancey and Siebenaller, 2015). This view is fundamentally different from the earlier hypothesis about “compatible compounds,” which do not affect metabolism even at high concentrations that implies their interchangeability (Brown and Simpson, 1972). Recent studies demonstrated that fungal cells usually contain several osmolytes, many of them possess cytoprotective activity, and certain compounds are typical under specific growth conditions. The results obtained confirmed our previous assumption that trehalose is important for alkaliphily, one of the types of extremophily (Bondarenko et al., 2017). Trehalose content in the cells of alkaliphiles is comparable to that in thermophilic fungi (Ianutsevich et al., 2016b) and in mesophilic fungi exposed to the thermal stress (Glatz et al., 2016). According to the modern views, protection of cell macromolecules and membranes exposed do different stress factors is the main for this multifunctional disaccharide (Elbein et al., 2003; Crowe, 2007; Iturriaga et al., 2009; Feofilova et al., 2014; Tapia and Koshland, 2014; Glatz et al., 2016). Moreover, trehalose acts as a transport form of carbon in the

fungal mycelium, storage compound, antioxidant, and chemical chaperone. However, our previous studies demonstrated that trehalose is not a universal protector against all stress factors since trehalose content in *Aspergillus niger* was low under osmotic and oxidative stress conditions (Ianutsevich et al., 2016a). Mannitol was the main cytosol soluble carbohydrate, the osmolytic function of which was shown to be based on the compatibility of high concentrations of the polyols required to maintain osmotic balance in conditions of water deficiency (Hohman, 2002). Mannitol also possesses antioxidant activity and is involved in the quenching of free radicals including hydroxyl ions in fungi and plants both in vivo and in vitro (Patel and Williamson, 2016). Inactivation of mannitol-1-phosphate dehydrogenase in *A. niger* mycelium caused a 70% decrease in mannitol content in the spores and hypersensitivity to thermal and oxidative stresses, freezing, and lyophilization (Ruijter et al., 2003). It is interesting that glycerol, which decreases formation of reactive oxygen intermediates (Shen et al., 1999), is involved in the maintaining of redox balance in cells since NADH is used in its synthesis (Ansell et al., 1997), and is present in high concentrations in halophilic yeasts exposed to osmotic stress, was not detected in any of the studied fungi (Kogei et al., 2007; Gunde-Cimerman et al., 2009; Zajc et al., 2014). Arabitol function in alkaliphilic and alkalitolerant fungi has not yet been understood but it was revealed that this polyol is involved in the adaptation of the halophilic mycelial fungus *Fusarium* sp. to high sodium chloride concentration (Smolyanyuk et al., 2013) and is accumulated in *Cladosporium fulvum* and *Magnaporthe grisea* under osmotic shock conditions (Solomon et al., 2007). The data obtained in the present work demonstrated importance of arabitol in the adaptation to alkaline conditions both in alkalitolerant and in alkaliphilic fungi.

Changes in the membrane lipid composition at decreased pH also differed. In alkalitolerant fungi, proportion of one of the phospholipids increased, whereas in alkaliphiles, PC proportion decreased and PA fraction increased. In both cases, the proportion of sterols decreased.

Comparison of the data obtained for alkaliphiles in the present study and those for the alkaliphile *S. tronii* demonstrated the absence of common patterns of the changes in membrane lipid composition caused by the changes in ambient pH. In membrane lipid composition of *S. tronii*, proportions of sterols and sphingolipids increased and PC proportion decreased during growth on acidic and neutral media that suggested the involvement of rafts in the adaptation (Bondarenko et al., 2017). This effect was not found in the studied fungi *S. magadii* and *S. alkalinus*, whereas the proportion of the phosphatidic acids increased and PC fraction decreased. These changes in membrane lipid composition were previously observed in the neutrophilic fungus *A. niger* in response to thermal, osmotic,

cold, and oxidative stress (Tereshina et al., 2011; Ianutsevich et al., 2016a).

In two studied alkalitolerant fungi, we did not find changes in lipid composition caused by changes in pH that were typical for alkaliphilic fungi. The response was rather the opposite: the decrease in pH caused the increase in the proportion of phospholipids and decrease in sterol fraction. Proportions of sphingolipids and phosphatidic acids did not change. The sterol fraction decreased both in alkalitolerant and in alkaliphilic fungi under neutral and acidic conditions. Analysis of fatty acid composition of the main phospholipids demonstrated a significant increase in the unsaturation degree both in alkalitolerant and in alkaliphilic fungi in response to the decrease in pH, suggesting that this mechanism is involved in the adaptation to the external pH. TAG were predominant neutral storage lipids in all studied fungi. General patterns in response to the changes in the ambient pH were not revealed. In alkalitolerant fungi, the TAG fraction did not change. In both alkaliphiles, decreased pH caused a decrease in TAG proportion and an increase in FFA (in *S. magadii*) or DAG (in *S. alkalinus*) proportions.

Thus, comparison of the mechanisms of stress protection demonstrated that osmolytes are involved in the adaptation to growth under different pH values both in alkaliphiles and in alkalitolerant fungi. In alkaliphiles, trehalose plays an important role, whereas in alkalitolerant fungi, protective reactions include change in the ratio between the main osmolytes, arabinol and mannitol. Arabinol predominates under alkaline conditions, whereas mannitol is predominant under neutral and acidic conditions. These data suggest that the adaptation mechanisms differ in alkaliphilic and alkalitolerant fungi. Revealed changes in the composition of membrane lipids indirectly confirmed these differences. In alkaliphiles, PC proportion decreased and PA proportion increased, whereas this effect was not found in alkalitolerant fungi. Alkalitolerant fungi were characterized by an increase in the fraction of one of the phospholipids and a decrease in the sterol fraction. It should be noted that decrease in the sterol fraction under neutral and alkaline conditions is the common pattern for both alkaliphilic and alkalitolerant fungi. Increase in unsaturation degree of the fatty acids of the main membrane phospholipids at decreased pH is another common pattern suggesting that this mechanism is used to alter membrane viscosity for the adaptation to the ambient pH.

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