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EXPERIMENTAL ARTICLES

Effect of Sodium Chloride Concentration in the Medium on the Composition of the Membrane Lipids and Carbohydrates in the Cytosol of the Fungus *Fusarium* sp.

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Abstract—The fungus *Fusarium* sp. isolated from saline soil was identified by the ITS1-5.8S-ITS2 and the D1/D2 domains of LSU RNA as a member of the *Fusarium incarnatum-equiseti* species group. Its growth patterns on media with different NaCl concentrations indicated its adaptation as halotolerance. The mechanisms of halotolerance included accumulation of arabitol (a five-atom noncyclic polyol), a decreased sterols/phospholipids ratio, elevated level of phosphatidic acids in the phospholipids, and increased unsaturation of phospholipids, which was especially pronounced in the idiophase. The mechanisms of halotolerance of the mycelial fungus *Fusarium* sp. are discussed in comparison with yeasts and yeastlike fungi.

Keywords: Fusarium, halotolerance, lipids, carbohydrates

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Hypersaline habitats are common in nature. They include hypersaline lakes, saline soils (salt marshes, solonchaks etc.), and sites of salt recovery, where solar radiation causes evaporation of seawater. High concentrations of salts, especially of sodium chloride, low availability of water with its drastic and random fluctuations, and high insolation are the life-limiting factors in such environments.

Analysis of the biodiversity and ecophysiology of the fungi from saline habitats revealed the presence of halophilic, halotolerant, and salt-sensitive species [1, 2]. The fact that halophilic/halotolerant fungal species are also characterized by resistance to other stress factors, such as UV radiation, temperature difference, and extreme pH, may indicate the universal character of some adaptive mechanisms. Salt-resistant members of the *Dothideomycetes* exhibited significant phylogenetic similarity to the rock-inhabiting species from arid regions, from the polar zone to the subtropics [3]. Enhanced salinization may be considered as a two-factor stress, combining the osmotic and the toxic components, since elevated ion concentration in the environment results in dehydration and the loss of turgor pressure, as well as to elevated intracellular concentrations of the ions. The known strategies of halophilic fungi include accumulation of osmotically active compounds (osmolites or compatible solutes) in the cytoplasm in order to protect the cell from dehydration, and the rearrangement of the composition of the membrane lipids in order to maintain the dynamic state (viscosity) of the membranes and their functioning under varied environmental conditions [4–6]. Polyhydric alcohols glycerol, erythritol, arabitol, and mannitol were shown to be the most important osmolites in yeasts and mycelial fungi [7]. Trehalose is noted as a universal protector stabilizing the membranes during dehydration caused either by heat shock or by high concentrations of organic and inorganic compounds [8, 9].

The mechanisms maintaining the water and ion homeostasis have been investigated for yeasts and yeastlike dark-pigmented fungi, with the halophilic species *Hortaea werneckii* used as the model object in many works [10]. Unlike yeasts and yeastlike fungi, the mechanisms of resistance to ionic and osmotic stresses in mycelial fungi are less studied. *Fusarium* species (*F. solani, F. oxysporum, F. equiseti, F. chlamydosporum*, and *F. compactum*), which are widespread in natural habitats, including dry saline soils, and are known to be moderately halotolerant [11].

The goal of the present work was to investigate the dynamics of radial colony growth, as well as the composition of the soluble carbohydrates in the cytosol and the membrane lipids of a *Fusarium* sp. isolate from saline soil under cultivation at different concentrations of sodium chloride.

MATERIALS AND METHODS

The isolate *Fusarium* sp. BM42, a sterile (not forming spores) mycelial morphotype, was the subject of

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the work. It was identified based on the nucleotide sequences of the ITS1-5.8S-ITS2 region and the D1/D2 domains of LSU rDNA. The protocols for DNA extraction, amplification, and sequencing were described previously [12, 13]. The nucleotide sequences deposited in the NCBI GenBank database (www.ncbi.nih.gov) were used for phylogenetic analysis. For construction of the phylogenetic tree, the nucleotide sequences of the closely related species were taken from GenBank in accordance with the present investigation of the genus *Fusarium* [14]. Phylogenetic analysis was carried out using the MAFFT 6 [15] and MEGA4 software packages [16]. The maximal parsimony (MT) algorithm was used to construct the phylogenetic tree.

Fusarium sp. BM42 was isolated from soil samples with chloride type of salinization and pH 8.8. The samples of soil (upper 0–5 cm) with the chloride type of salinization were collected at the Black Sea coast close to Pomorie (Bulgaria), at the site of salt recovery.

The fungus was grown on media with different NaCl concentrations. Wort agar (WA, wort 1.8°B, agar 1.2%) was supplemented with NaCl to the final concentration of 1.0, 1.5, and 2.5 M. The block with the mycelium was placed in the center of the petri dish and incubated at 29 ± 1°C for 14 days. The colony diameter was measured throughout this period at three-day intervals. The experiment was carried out in five repeats. The data were treated using the Microsoft Office Excel 2003 software package in order to build a graph of the growth rates at different NaCl concentrations.

For biochemical investigation, three blocks with the fungal mycelium were plated per petri dish. Three types of agar media were used: WA and WA with 1 or 1.5 M NaCl. The fungus was grown in the dark at 21 ± 1°C for 7 (exponential growth phase) or 14 days (stationary phase). The biomass was collected with a scalpel, washed with distilled water, dried with filter paper, and stored at –70°C.

The lipids were extracted according to Nichols [17]. A sample of the mycelium (~1 g) was ground with isopropanol in order to inactivate lipases and incubated for 30 min at 70°C. The residue was extracted twice with isopropanol : chloroform mixtures (1 : 1 and 1 : 2) under the same conditions and dried in a rotor evaporator. The lipids were dissolved in chloroform : methanol (1 : 1) and water-soluble compounds were removed by adding NaCl solution (2.5%) to the extract. After vortexing, the chloroform layer was collected, dried by passing through anhydrous sodium sulfate, evaporated, and dried to constant mass under vacuum. The residue was dissolved in a small amount of chloroform : methanol (1 : 1) and stored at –21°C.

Neutral lipids (NL) were analyzed by ascending TLC on glass plates with silica gel 60 (Merck, Germany). NL separation was carried out using the hexane : dimethyl ether : acetic acid (85 : 15 : 1) solvent system [18]. Ergosterol (Sigma, United States) (5 and 10 μg) was used as the standard for quantitative determination of sterols. Phospholipids (PL) and glycolipids (GL) were separated by two-dimensional TLC [19]. The lipids (100–200 μg) where applied to the plate. The solvent systems I (chloroform : methanol : water 65 : 25 : 4) and II (chloroform : acetone : methanol : acetic acid : water 50 : 20 : 10 : 10 : 5) were used for chromatography in the first and second direction, respectively. The standards for sphingolipids and phospholipids (bull serum glycoceramides and phosphatidylcholine, respectively) were applied prior to chromatography in the second direction. The amounts of glycoceramides and phosphatidylcholine were 5 or 10 μg and 10 or 20 μg, respectively. The chromatograms were sprayed with 5% sulfuric acid in ethanol and heated at 180°C to reveal the spots. PL were identified using the individual markers and qualitative reactions with ninhydrin (for the amino group), Dragendorff reagent (for choline-containing PL), and α-naphthol (for glycolipids) [18]. The sphingolipid nature of glycolipids was confirmed by saponification [18]. Neutral lipids were identified using the individual markers: mono-, di-, and triacylglycerols, free fatty acids, sterols (ergosterol), and hydrocarbons (Sigma, United States). Quantitative analysis of the lipids was carried out using the Dens software package (Lenkhrom, Russia) in the mode of direct approximation according to the calibration curves for the standard solutions of phosphatidylcholine (Sigma, United States), glycoceramides (Larodan, Sweden), and ergosterol (Sigma, United States).

For analysis of the fatty acid composition of phospholipids, they were isolated by chromatography on two plates and eluted overnight in chloroform : methanol (1 : 1). The supernatant was decanted, evaporated, treated with 1 mL toluene and 2 mL 2.5% H_2SO_4 in methanol, and incubated for 2 h at 70°C. Fatty acid methyl ethers were extracted with hexane, dried, and analyzed on a Kristall 5000.1 gas–liquid chromatograph (Khromatek, Russia) with an Optima-240 capillary column (0.25 μm, 60 m, 0.25 mm) (Macherey-Nagel GmbH&Co, Germany). The temperature program from 130 to 240°C was used. The Supelco 37 Component FAME Mix (United States) of the markers of individual fatty acid methyl ethers was used for identification.

Sugar extraction for determination of the soluble carbohydrates was carried out with boiling water (four times for 20 min). Proteins were removed from the extract [20]. Further purification of the carbohydrates from charged compounds was carried out on a combined column with Dowex-1 (acetate form) and Dowex 50W (H+) ion exchange resins. The internal standard used was α-methyl-D-mannoside (Merck, United States). The carbohydrate composition was determined by gas–liquid chromatography of trimethylsilyl sugar derivatives obtained from the lyophilized extract [21]. Analysis was carried out on a Kristall 5000.1 gas–liquid chromatograph (Khro-

Table 1. Membrane lipids of *Fusarium* sp. in the trophophase and idiophase during surface growth in the presence of NaCl

Lipids	Membrane lipids, % of the total					
	WA, 7 days	WA + 1 M NaCl, 7 days	WA + 1.5 M NaCl, 7 days	WA, 14 days	WA + 1 M NaCl, 14 days	WA + 1.5 M NaCl, 14 days
PE	7.6	6.5	4.5	5.3	6.9	3.8
PC	14.0	11.2	15.3	6.4	9.4	16.0
CL	7.3	5.9	7.6	6.4	6.7	5.6
X	5.5	7.2	2.3	3.4	5.9	2.4
PA	21.4	19.0	31.7	17.5	21.7	29.6
SL	6.2	4.6	6.8	6.7	5.8	6.6
St	38.0	45.6	31.8	54.3	43.6	36.0
Σ lipids, μg/g dry biomass	36027.6	27376.3	24494.3	23331.6	16984.9	21573.7
Sterols/phospholipids	0.62	0.83	0.47	1.25	0.77	0.55

Designations: PE, phosphatidylethanolamines, PC, phosphatidylcholines, CL, cardiolipins, PA, phosphatidic acids, SL, sphingolipids, St, sterols, X, unidentified phospholipid.

matek, Russia) with a ZB-5 capillary column (30 m, 0.32 mm, 0.25 μm) (Phenomenex, United States) at temperature increasing from 130 to 270°C at 5–6°C/min. Glycerol, glucose, mannitol, arabitol, inositol, and trehalose (Sigma, United States) were used as markers.

All experiments were carried out in three replicates. The results of the typical experiments are presented. Dispersion of the results did not exceed 10%, with the major patterns being the same.

RESULTS

According to the results of phylogenetic analysis, the isolate belonged to the "*incarnatum-equiseti*" group of species within the genus *Fusarium* (Fig. 1). The strain was conspecific to *F. equiseti* strains (MAFF236434 and MAFF236723) *F. incarnatum* strain (MAFF236521) by the used set of ribosomal genes. Ribosomal genes alone are insufficient for species identification of the "*incarnatum-equiseti*" group and many other *Fusarium* species [14].

Analysis of the radial growth rate on solid media with different NaCl concentrations revealed the highest values at 1 M NaCl. The growth rate on WA without salt was 1.7 times lower than in the presence of 1 M NaCl (Fig. 2). Increased salinity (1.5 and 2.5 M) also resulted in suppression of growth (3.4 and 19 times, respectively).

Colony morphology of the isolate BM42 *Fusarium* sp. grown on WA and WA with 1 or 2.5 M NaCl differed significantly, while colony morphology did not change significantly with age. *Fusarium* sp. BM42 grown on WA formed prostrate colonies with uneven edges. The substrate mycelium was dense, forming a leathery interlacement. The aerial mycelium was fibrous, light-colored, and often yellow in the colony center, up to 5 mm high. Numerous chlamydospores were formed during the idiophase. On the medium with 1 M NaCl, the isolate grew actively, forming shaggy, white, loose aerial mycelium up to 3 mm high. Chlamydospores were not formed. On WA with 2.5 M NaCl, almost no aerial mycelium was formed on the colonies. The colonies were leathery, formed by the substrate mycelium, with poorly developed shaggy aerial mycelium at the edge of the colony. Chlamydospores were not formed.

On the medium without salt, mannitol (over 50% of the total sugars) and trehalose (~20%) were the major soluble carbohydrates of the cytosol during both the trophophase (7 days of growth) and the idiophase (14 days of growth) (Fig. 3). Glycerol, arabitol, and glucose were the minor components. During the idiophase, the amount of carbohydrates was 1.5 times higher than in the trophophase. Addition of NaCl (both 1 and 1.5 M) resulted in increased content of arabitol and decreased content of mannitol. In the presence of 1 M NaCl, the amount of carbohydrates did not differ significantly from that on the salt-free medium, while at 1.5 M NaCl the amount of carbohydrates increased noticeably, especially during the trophophase. Under hypersaline conditions (1.5 M NaCl), the content of trehalose also increased.

Considerable amounts of lipids were formed on salt-free medium (20 and 15% of the dry weight for the trophophase and idiophase, respectively). At elevated salinity the amount of lipids decreased in the trophophase and increased in the idiophase. The qualitative composition of the membrane lipids did not vary depending on the growth phase and salinity. The main components were sterols (St), phosphatidic acids

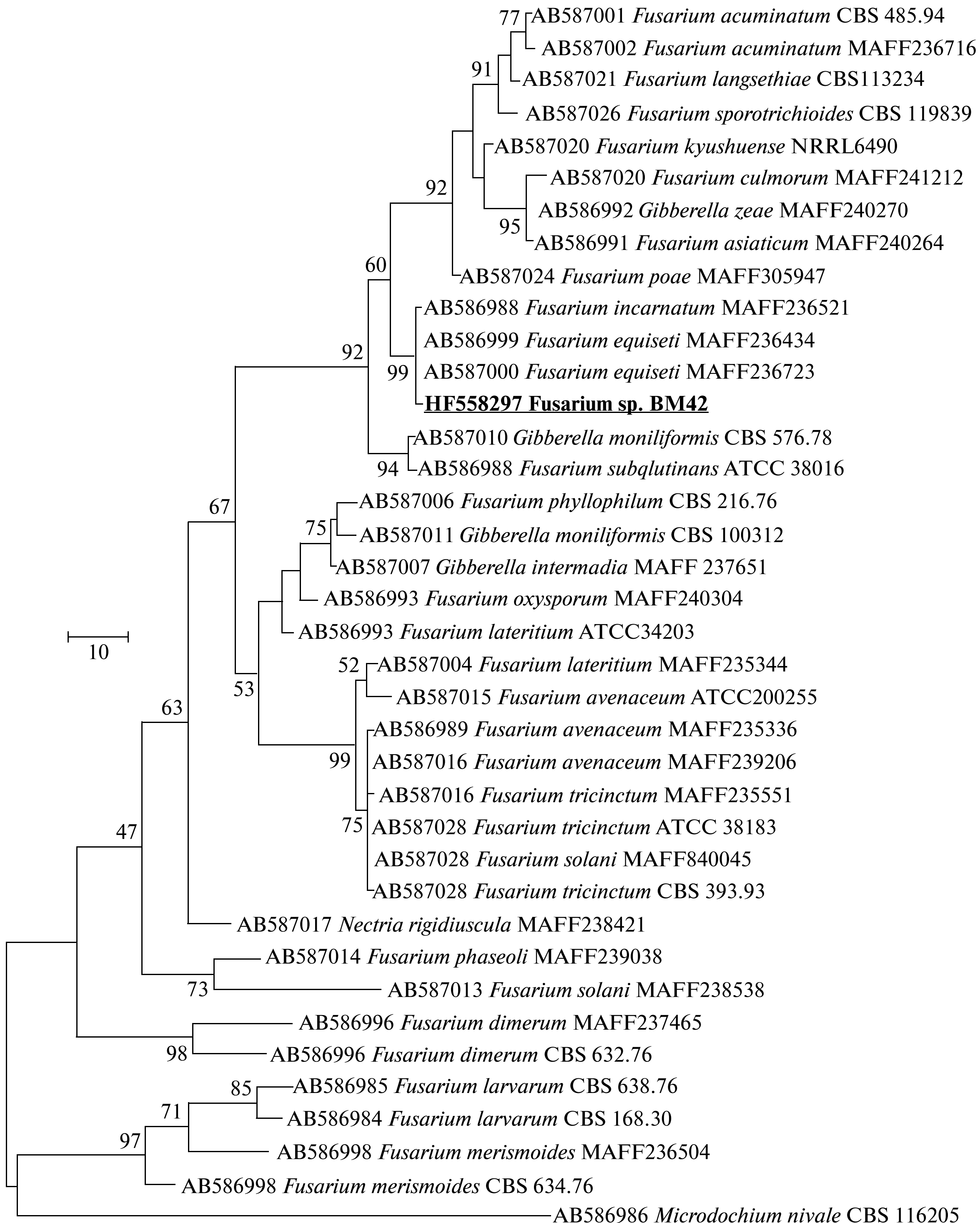

Fig. 1. Phylogenetic position of the isolate *Fusarium* sp. BM42.

(PA), and phosphatidylcholines (PC), comprising 70–80% of the total lipids (Table 1). Cardiolipin (CL), sphingolipids (SL), and phosphatidylethanolamine (PE) were the minor components. During the idiophase, the amount of the membrane lipids and the share of PC decreased, while the share of sterols increased. While the addition of 1 M NaCl had practically no effect on the composition of the membrane lipids, at 1.5 M NaCl the share of phosphatidic acids increased both in the trophophase and the idiophase.

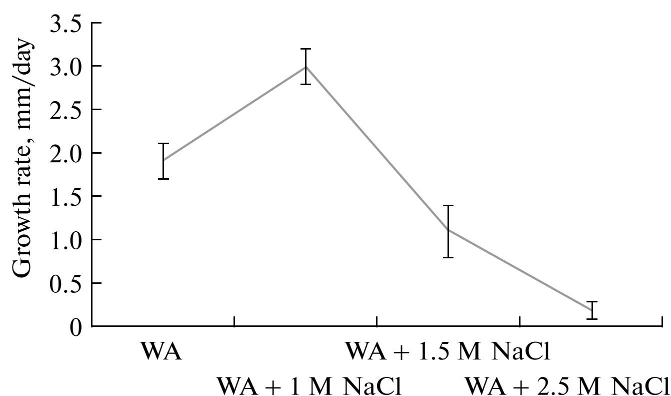

Fig. 2. Rates of colony growth (mm/day) for *Fusarium* sp. BM42 grown on agar media at different salinities.

Increase in salinity resulted in a 1.5-fold decrease in the amount of the membrane lipids in the trophophase, while no changes were observed in the idiophase.

In order to elucidate the mechanisms of tolerance to salinization, all phospholipids were isolated and their fatty acid composition was determined. Palmitic acid and the family of C_{18} acids—stearic ($C_{18:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), and linolenic ($C_{18:3}$) acids—were the main components (Tables 2, 3). PC was the most unsaturated phospholipid during the trophophase (Table 2). Increased salinity did not result in significant changes in the unsaturation degree (UD) of this phospholipid. In the case of the most massive phospholipid, PA, increase in salt concentration to 1 M resulted in higher UD, while UD in hypersaline conditions was the same as in the absence of salt. In the WA-grown mycelium, phosphatidylethanolamine (PE) was the most saturated phospholipid. At elevated salinity, independent on salt concentration, UD of the fatty acids of this phospholipid increased significantly due to increased content of the $C_{18:1}$–$C_{18:3}$ fatty acids. In WA-grown mycelium the degree of unsaturation of the PC, CL, and PA fatty acids during the idiophase was half of the value for the trophophase (Table 3). At 1 M salinity, UD of the CL and PA fatty acids increased, while further increase in salt concentration to 1.5 M resulted in higher UD in all phospholipids.

DISCUSSION

Analysis of linear growth of *Fusarium* sp. on media with different NaCl concentrations revealed that WA with 1 M NaCl, rather than the salt-free WA, was the optimal medium for growth. Thus, the organism may be considered a halotolerant fungus. By definition, halotolerant organisms are able to grow in a broad range of salt concentrations from zero to the saturated (4 M) NaCl solution, while halophiles grow within a more narrow NaCl range (1.0–1.5 M) and do not grow on salt-free media [22]. Highly diverse values of pH and salt concentrations differentiating between the "philic" and "tolerant" species may be found in the literature. Since the range of NaCl concentrations supporting prokaryotic growth is much broader than the range suitable for most eukaryotic organisms, different criteria are used for pro- and eukaryotes [23–25].

Eukaryotes react to the low availability of water resulting, in particular, from high salt concentrations, by accumulation of high amounts of compatible solutes, such as non-cyclic polyols, some amino acids

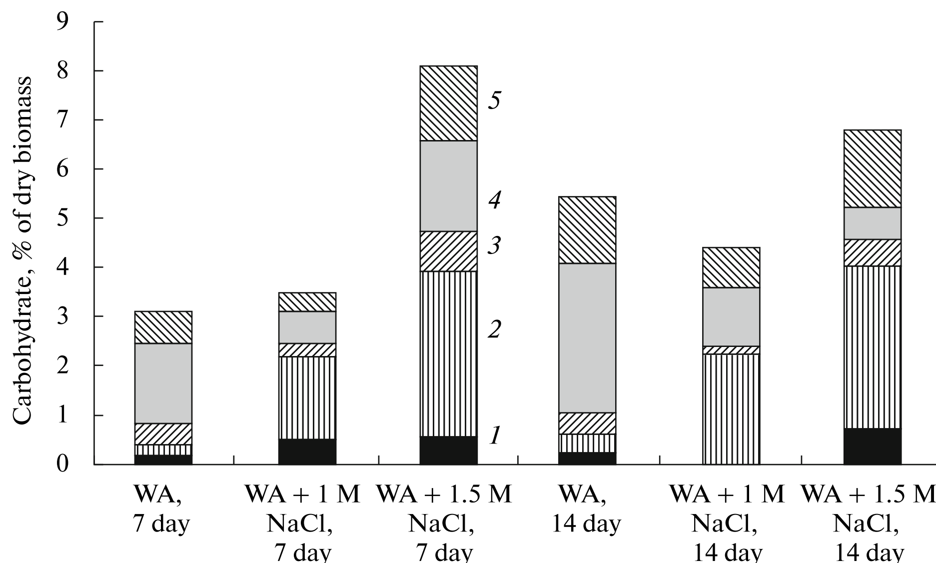

Fig. 3. Effect of salinity on the composition of the cytosol carbohydrates in the trophophase and idiophase of *Fusarium* sp. BM42: glycerol (*1*), arabitol (*2*), glucose (*3*), mannitol (*4*), and trehalose (*5*).

Table 2. Phospholipid fatty acids in the trophophase of *Fusarium* sp. depending on salinity of the medium (% of the total)

Fatty acids	WA, 7 days				WA + 1 M NaCl, 7 days				WA + 1.5 M NaCl, 7 days			
	PE	PC	CL	PA	PE	PC	CL	PA	PE	PC	CL	PA
$C_{14:0}$	–	–	–	–	–	–	–	–	18.6	–	–	–
$C_{14:1}$	–	–	–	–	–	–	–	–	–	5.5	53.1	–
$C_{15:0}$									22.3	7.0	4.0	14.0
$C_{16:0}$	56.2	27.5	26.8	34.4	35.1	25.5	48.3	35.1	13.7	26.5	15.4	36.9
$C_{16:1}$	–	–	–	–	–	–	–	–	–	–	14.2	–
$C_{18:0}$	26.9	15.3	20.3	18.8	19.9	15.6	26.1	14.5	9.7	17.9	5.6	13.7
$C_{18:1}$	11.6	19.1	31.6	27.1	22.2	33.1	12.0	18.2	11.2	8.7	1.6	10.3
$C_{18:2}$	5.3	14.0	18.6	19.7	14.0	14.8	13.6	19.3	12.7	11.1	2.8	18.0
$C_{18:3}$	–	24.1	2.7	–	8.8	11.0	–	12.9	11.8	23.3	3.3	7.1
CH	0.22	1.15	0.77	0.67	0.77	0.96	0.39	0.96	0.72	1.06	0.84	0.67

Table 3. Phospholipid fatty acids in the idiophase of *Fusarium* sp. in the presence of NaCl (% of the total)

Fatty acids	WA				WA + 1 M NaCl				WA + 1.5 M NaCl			
	PE	PC	CL	PA	PE	PC	CL	PA	PE	PC	CL	PA
$C_{14:0}$	3.0	–	–	–	–	–	–	–	–	–	6.2	–
$C_{14:1}$	23.5	19.0	–	–	–	–	–	–	–	–	–	–
$C_{15:0}$	4.5	–	–	–	–	–	–	–	12.4	1.1	9.4	26.3
$C_{16:0}$	37.3	34.0	41.9	49.8	51.4	33.6	41.6	32.8	30.9	20.1	29.7	9.9
$C_{16:1}$	–	–	–	–	–	–	–	–	–	–	–	–
$C_{17:0}$	–	–	–	–	–	–	–	–	7.6	–	–	–
$C_{17:1}$	–	–	–	–	–	–	–	–	–	–	–	31.2
$C_{18:0}$	17.0	24.6	43.2	28.4	35.1	31.7	26.6	17.8	16.0	17.1	19.2	6.8
$C_{18:1}$	11.2	13.5	10.0	12.5	9.9	20.6	23.1	20.0	6.7	25.2	9.9	4.2
$C_{18:2}$	3.5	8.9	4.9	9.3	3.6	14.1	8.7	29.4	7.5	31.1	10.1	5.1
$C_{18:3}$	–	–	–	–	–	–	–	–	18.9	5.4	15.5	16.5
CH	0.42	0.50	0.20	0.31	0.17	0.49	0.41	0.79	0.78	1.04	0.76	0.95

(proline), sugars, and their derivatives (trehalose) [26]. At up to 1 M concentrations, these osmoprotectors do not affect the activity of intracellular enzymes [27]. Glycerol is the major osmoprotector in xerophilic, halotolerant, and halophilic yeasts, in xerophilic mycelial fungi, and in halophilic algae [1, 5, 26]. While erythritol, arabitol, xylitol, mannitol, sorbitol, galactitol, etc. are known among the polyols produced by fungi, genetic confirmation of their role in protection from osmotic stress was not obtained [5].

In the present work, it was shown for the halotolerant mycelial fungus *Fusarium* sp. BM42 that growth in the presence of salt was accompanied by a drastic change in the composition of its cytosol carbohydrates. Arabitol became the major component, while the level of mannitol, which was predominant under salt-free conditions, decreased. In the presence of salt, the share of arabitol increased from 6 to 50% of the total. A positive correlation was found between arabitol content in the cell and salinity of the medium. Thus, in the presence of 1 M NaCl the total carbohydrate content was the same as in the salt-free control, but arabitol became the main component, while at 1.5 M NaCl the share of arabitol did not change, but the total amount of carbohydrates increased twofold. While positive correlation between the total amount of carbohydrates and salinity is known for yeasts and mycelial fungi, glycerol is considered the most impor-

tant polyol under conditions of high salt concentration, since it has low molecular mass and provides for the lowest water activity at a given molar concentration [8]. Efficiency of glycerol accumulation and retention in the cells is associated with melanization of the cell walls, since non-melanized cells of *H. werneckii* compensated for the low glycerol level by high contents of erythritol and arabitol [9]. It should be noted that while the relative content of mannitol and arabitol changed, the share of trehalose did not change noticeably. However, the total amount of carbohydrates, including trehalose, increased at elevated salinity (1.5 M) (Fig. 3). These data suggest that, unlike yeasts, arabitol and probably trehalose act as osmoprotectors in mycelial fungi. Interestingly, it was shown for the halotolerant *Debaryomyces hansenii* that under conditions of moderate salt stress more trehalose than glycerol was accumulated in the cytosol, while glycerol predominated at 2–3 M NaCl [1]. Accumulation of arabitol may result from the prevalence of the oxidative pentose phosphate pathway of carbohydrate metabolism in mycelial fungi, unlike yeasts, which utilize the glycolytic pathway, with glycerol synthesized from its metabolites. The capacity of arabitol for creation of osmotic pressure is not lower than that of glycerol [28]. Synergetic action of glycerol with trehalose was shown.

Apart from the osmolites, whose function has been replacement of water and protection of the cellular membranes and macromolecules from stressors, membrane lipids may play an important role in osmoprotection. The functional state of the membranes should be preserved at any stress impact. Hyperosmotic conditions, as well as cold, were hypothesized to result in increased membrane viscosity; while hypoosmotic and heat stresses, to cause its decrease [29]. The authors considered the degree of unsaturation of the phospholipid fatty acids and the sterol/phospholipid ratio to be the main factors affecting the viscosity of the protein bilayer, since sterols are considered to rigidify the membranes. Moreover, the alterations in the composition of the soluble cytosol carbohydrates, i.e., accumulation of low-molecular polyols under hyperosmotic conditions, similar to the cold shock conditions, may be an evidence for this hypothesis [30].

Analysis of the membrane lipids of the investigated fungal isolate revealed two characteristic features: high levels of phosphatidic acids and of sterols. Phosphatidic acids and phosphatidylcholines are the major cellular phospholipids. Since 1 M NaCl was the optimal concentration for growth, salt-free conditions and the presence of 1.5 M NaCl may be considered hypoosmotic and hyperosmotic conditions, respectively. From this point of view, no changes in the membrane lipids developed as a response to hypoosmotic conditions, while hyperosmotic conditions caused a 1.5-fold increase in the share of phosphatidic acids and phosphatidylcholine, as well as a decrease in the relative content of sterols. These processes were independent on the growth phase. In agreement with the above hypothesis, at both growth phases, the sterols/phospholipids ratio decreased under hyperosmotic conditions. Analysis of the fatty acid composition of phospholipids revealed a pattern for the idiophase: UD of the fatty acids of all phospholipids increased significantly under hyperosmotic conditions, while under hypoosmotic conditions UD decreased for the PA and CL fatty acids, did not change in PC ones, and increased in the minor PE. No clear pattern was observed for the trophophase. For example, UD of the PC fatty acids changed insignificantly depending on salinity, while UD of PA decreased under both hyper- and hypoosmotic conditions. On the contrary, a decrease in UD of the fatty acids was observed for CL under conditions of both decreased and increased salt concentrations. For PE, a drastic decrease in UD was observed under hypoosmotic conditions, while no changes occurred under hyperosmotic ones.

The analogy between osmotic stress and heat shock suggests that the ratio of "bilayer" and "non-bilayer" lipids in the membranes may play a certain role in protection of the membranes against hyperosmotic stress [4]. Bilayer lipids include PC, phosphatidylserines, and phosphatidylinositols; they have molecules of cylindrical shape and are able to form a lipid bilayer. Phospholipids with the conical or inverse conical shape of the molecules (PE, PA, CL) form micella. For example, fungi of various taxonomic groups, a zygomycete *Cunninghamella japonica,* an ascomycete *Aspergillus niger*, and a basidiomycete *Pleurotus ostreatus*, were shown to accumulate high amounts of PA under prolonged heat shock [31]. From this point of view, a noticeable increase in the share on non-bilayer PA under hypersaline conditions, independent on the growth phase, may indicate the role of these phospholipids in protection of the membranes against unfavorable factors. It is suggested that the domains of non-bilayer PA may be involved in bending of the membranes and in the processes of exo- and endocytosis [32, 33].

The literature data on variability of the composition of the membrane lipids under hyperosmotic conditions for yeasts and yeastlike fungi are contradictory. Moreover, they deal with the total phospholipid fraction, rather than its individual components. Differences in the experimental setup make comparison of these data still more difficult. It is unclear whether the halotolerant and halophilic fungi possess the same mechanisms of salt resistance. Thus, comparison of the membrane lipids in two black yeast species under salt stress revealed that the degree of unsaturation of the phospholipid fatty acids changed in different directions, increasing in *H. werneckii* and somewhat decreasing in *Phaeotheca triangularis* [5]. Both halophilic fungi had very low sterol/phospholipid ratios, while viscosity of the lipid bilayer was lower than in the

halotolerant *Aureobasidium pullulans* and osmic shock-sensitive *Saccharomyces cerevisiae*. No changes in the content of sterols was observed in *H. werneckii* at 3 M NaCl, and viscosity of the membranes decreased due to an increased ratio of linoleic acid ($C_{18:2}$) and decreased content of palmitic acid ($C_{16:0}$) [10]. In the halotolerant *Debaryomyces hansenii*, growth at 2 M NaCl resulted in an increased St/PL ratio and elevated UD of the phospholipid fatty acids, but had no effect on the membrane viscosity [6]. For another halotolerant fungus, *Zygosaccharomyces rouxii*, addition of 2.5 M NaCl resulted in decreased UD of the membrane lipids and the fivefold increased ergosterol/phospholipids ratio [34]. These literature data show the absence of a single mechanism of protection against salt stress.

We investigated the mechanisms of salt tolerance in a mycelial fungus *Fusarium* sp. of the *Fusarium incarnatum-equiseti* group. The positive correlation between the total amount of carbohydrates and salinity of the media, which was known for yeasts, was confirmed. Unlike yeasts and yeastlike fungi, the studied organism in the presence of 1 or 1.5 M NaCl accumulated the 5-carbon sugar alcohol arabitol, rather than glycerol. Under hyperosmotic conditions, the level of phosphatidic acids increased, while the sterols/phospholipids ratio decreased. Under these conditions, the degree of unsaturation of the fatty acids of all phospholipids was shown to increase during the idiophase. Thus, specific features of the protective mechanisms against salt stress were revealed for this organism, compared to yeasts.

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