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EXPERIMENTAL ARTICLES

Effect of Trehalose on the Viability of Sporangiospores of the Mucorous Fungus *Blakeslea trispora*

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Abstract—Sporangiospores of *Blakeslea trispora* are in a state of exogenous dormancy, and water is the key factor controlling their germination. A wide range of carbohydrates, ammonium salts, and yeast extract had a weak stimulating effect (less than 50%) on spore germination, whereas amino acids could significantly inhibit this process. Cultivation of *B. trispora* on sporogenous sucrose- and trehalose-containing media (S and T spores, respectively) resulted in significant changes in spore formation, as well as in the chemical composition of spores and their viability. In the presence of trehalose, the amount of spores increased twofold; spore viability during storage increased as well. All changes in the carbohydrate composition of the cytosol and in the composition of the spore membrane lipids indicated that the dormancy of T spores was deeper than that of S spores, which has a favorable effect on their viability.

Keywords: Blakeslea trispora, spores, dormancy, viability, trehalose, phospholipids.

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The heterothallic mucorous fungus *Blakeslea trispora* is an overproducer of the carotenoids β-carotene and lycopene [1]. A complex culture consisting of the (+) and (–) strains of *B. trispora* is used in biotechnological processes, since sexual interactions result in the production of a sex hormone (trisporic acids) involved in the stimulation of carotenogenesis, which, in the case of the (–) strain, is more pronounced, indicating that this strain plays a key role in carotene production. *B. trispora* spores are used as inoculum; however, spores of the (–) strain, unlike those of the (+) strain, rapidly lose their germinating capacity (over the course of one month). This phenomenon has attracted our attention to the process of spore germination, as well as to possibilities to enhance spore viability. It is well known that spores are able to accumulate trehalose, which acts as a protective, rather than storing, compound, which protects the membranes and macromolecules against various stress factors [2–4]. It has been previously demonstrated [5] that spores of the T strain (–) of *B. trispora* contain approximately 2% (wt/wt) of trehalose, whereas (+) spores contain up to 6% of trehalose. It is well-known that the addition of trehalose to the sporogenous medium results in an increase in the trehalose content in conidia of the ascomycete fungus *Aspergillus niger*. [6].

The goal of this work was to study the germination conditions of the (–) sporangiospores of *B. trispora*, and the effect of trehalose on their chemical composition and viability.

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MATERIALS AND METHODS

The subject of the study was the mucorous fungus *Blakeslea trispora* Thaxter T (–) obtained from the strain collection of the Winogradsky Institute of Microbiology, Russian Academy of Sciences.

Sporangiospores of the fungus were obtained on potato–carrot agar slants incubated at 26–27°C for 5–7 days. Only freshly harvested spores (stored for less than 7 days) were used in the experiment. The spores were washed off from the agar surface with cold water (0–4°C), concentrated by centrifugation, and washed with water once more.

To study the effects of pH, temperature, and carbon and nitrogen sources on spore germination, an express method was used: melted agarized medium (2 ml) was spread in a thin layer on glass slides; then, after solidification of the medium, one drop of the spore suspension was placed on the agar surface and spread uniformly with a spatula. The slides were placed in petri dishes on filter paper wetted with 0.2 ml of water in order to maintain the required moisture level.

To determine the temperature optimum for spore germination, Goodwin's agarized medium [7] was used. The spores were incubated at 26, 28, 30, 32, and 34°C for 4h. To determine the pH optimum for spore germination, fungal spores were grown on Goodwin's agarized salt-free medium prepared using 0.5 M citrate–phosphate buffer at pH 4.6, 5.18, 5.68, 5.59, 6.25, 6.50, 6.76, 7.05, 7.43, and 7.84 at 28–30°C for 4 h.

To study the effect of carbon and nitrogen sources, the agar medium prepared using distilled water (pH 5.3–5.5) was used; sugars were added to a concentration of 0.5%; nitrogen-containing compounds were added to a concentration equal to the nitrogen content of 0.5% $(NH_4)_2SO_4$. The cultivation was carried out at 29.0–29.5°C for 3 h.

The observation of spore germination and calculation of germinated spores were carried out under a Jenaval light microscope (Germany) at 400× magnification. Calculation of the spores was carried out using an eyepiece grid which was placed on the surface of solid medium. Calculation of at least 600 spores was performed. For calculation of germinated spores, those with germ tubes longer than one half of the spore diameter were counted.

To study the intensity of spore formation and the loss of spore germination during storage, the spores grown on the potato–carrot agar medium containing 0.5% trehalose or 0.5% sucrose were used. To obtain sporangiospores, the cultures were grown at 26–27°C and stored at room temperature for two months. The numbers of germinated spores after cultivation for 5 or 24 h were determined every 10 days. The intensity of spore formation (the number of spores per unit area) was determined by washing off the spores from a known area and counting them in a Goryaev count chamber.

To study the effect of stimulators on spore germination, they were added to the following concentrations (mM): putrescine, 0.1–200 mM; spermidine, 0.025–100 mM; and cAMP, 0.0075–1.0 mM(Sigma, United States). The spore suspensions were supplemented with solutions of these compounds to the required concentrations and incubated in a thermostat at 29.0–29.5°C for 10 min. Then, one drop of each suspension was applied to a glass slide covered with the minimal medium, as described earlier. The inoculated petri dishes were incubated in a thermostat at 29.0–29.5°C for 3–5 h.

For chemical analyses, the spores were washed off with cold water (0–4°C), concentrated by centrifugation, washed with water once more, and lyophilized. When determining the lipid composition, weighed portions of spores were ground with isopropanol and incubated at 70°C for 30 min in order to inactivate lipases [8]. Then, the residue was extracted twice with isopropanol mixed with chloroform (1 : 1) at 70°C and evaporated in a rotary evaporator; the lipids were dissolved in chloroform mixed with methanol (1 : 1). To remove the water-soluble compounds, the extract was supplemented first with water and then with a 5% solution of sodium chloride. After the mixture was separated on a Vortex, the chloroform layer was dried over anhydrous sodium sulfate, evaporated, and dried under vacuum to a constant weight. The obtained residue was dissolved in a small amount of the chloroform–methanol mixture (1 : 1) and stored at –21°C.

The composition of neutral lipids (NL) was determined by ascending thin-layer chromatography on glass plates covered with 60 silica gel (Merck, Germany). For the separation of the NLs, a hexane–ethyl ether–acetic acid (85 :15 :1) solvent system was used [9]. Phospholipids (PL) and glycolipids (GL) were separated by two-dimensional thin layer chromatography, according to Benning [10]. A 150–200 μg lipid sample was applied to a plate. The chromatograms were treated with 5% sulfuric acid in ethanol, and then heated at 180°C until the spots appeared. To identify the phospholipids present, we used individual standards. The qualitative reactions with ninhydrin (for lipids containing free amino groups), Dragendorff reagent (for choline-containing lipids), and α-naphthol (for glycolipids) were carried out [9]. The neutral lipids were identified using the individual standards: mono-, di-, and triacylglycerides, free fatty acids, sterols (ergosterol), and carbohydrates (Sigma, United States). To determine the sphingolipid origin of the glycolipids, saponification was performed [9]. The quantitative analysis of the lipids was carried out using the Dens software package (Lenchrom, Russia). Phosphatidylcholine (Sigma, United States), a mixture of glycoceramides (Larodan, Sweden), and stigmasterol (Sigma, United States) were used as standards for phospholipids, sphingolipids, and sterols, respectively.

The fatty acid composition of the lipids was determined on a Kristall 5000.1 gas-liquid chromatograph (ZAO Chromatec, Russia) equipped with a 60-m Optima-240-0.25 μm capillary column with an inner diameter of 0.25 mm (Macherey-Nagel GmbH&Co, Germany). To obtain the methyl esters of fatty acids, the lipids were treated with 2.5% H_2SO_4 at 80°C for 1 h. The temperature was programmed to rise from 130 to 240°C. Identification was performed by using a standard mixture of fatty acid methyl esters (Supelco 37 Component FAME Mix, United States), as well as by gas liquid chromatography–mass spectrometry.

To determine the carbohydrate composition of the mycelium, sugars were extracted with boiling water four times for 20 min. Proteins were removed from the obtained extract [11]. The subsequent removal of charged compounds from the carbohydrate extract was performed using a combined column with Dowex -1 (acetate form) and Dowex 50W (H+) ion-exchange resins. The carbohydrate composition was determined by gas-liquid chromatography by obtaining trimethylsilyl sugar derivatives from the lyophilized extract [12]. α-methyl-D-mannoside (Merck) was used as an internal standard. The chromatography was performed on a Kristall 5000.1 gas-liquid chromatograph (ZAO Chromatec, Russia) equipped with a 30-m ZB-5-0.25 μm capillary column with an inner diameter of 0.32 mm (Phenomenex, United States). The temperature was programmed to rise from 130 to 270°C at a rate of 5–6°C/min. Glucose, mannitol, arabitol,

inositol, and trehalose (Sigma, United States) were used as standards.

Each measurement was performed in triplicate. The data presented are the results of a typical experiment.

RESULTS AND DISCUSSION

Spores from the *Blakeslea trispora* sporangioles are polymorphic and differ in size. The majority of the spores are ellipsoid-shaped; however, spherical, nearly-triangular, and bean- and lancet-shaped spores were detected. The absence of the pronounced stage of spherical growth is a unique property of spore germination. The spore size increases insignificantly, whereas its shape remains the same. In most cases, the fungus forms one germ tube, although sometimes it forms two or three. The process of spore germination on the minimal medium takes approximately 3 h and is asynchronous. During the first hour, protrusions ("beaks") emerge; in the following 2 h, some spores form germ tubes the length of which exceeds one half of the spore diameter. By the third hour of germination, almost all spores form germ tubes attached to different parts of the spores (in most cases, to spore poles, but in rare cases, to the middle part).

To study the spore viability during storage, the optimal conditions for spore germination should be determined. Due to the lack of these data in the literature, our first goal was to study the spore germination conditions and the type of their dormancy.

It was demonstrated that the optimal temperature for spore germination was 28–32°C; after 3-h cultivation at this temperature, the amount of germinated spores reached 58–62%, whereas the amount of spores germinated at 26 and 34°C was about half as high. To determine the optimal pH, the experiments were carried out using citrate–phosphate buffer, as well as distilled (pH 5.5) and double-distilled (pH 7.0) water. The optimum pH values ranged from 5.2 to 6.0; however, it should be noted that the amount of spores germinated in the variant with distilled water was higher than that in the variant with the citrate–phosphate buffer at the optimum pH.

The study of the effect of soluble carbohydrates on spore germination indicated that, at this stage, *B. trispora* was capable of utilizing a wide range of sugars, including pentoses, hexoses, disaccharides, sugar alcohols, and amino sugars (Fig. 1). However, the level of stimulation of spore germination did not exceed 50%. The ketosugar fructose had a slight inhibiting effect. The poorly-metabolized carbohydrate 2-deoxy-D-glucose completely inhibited spore germination.

Unlike carbohydrates, nitrogen-containing compounds had different effects on spore germination. For example, only yeast extract, ammonium sulfate, and ammonium nitrate had a weak stimulating effect (15–20%) on spore germination, whereas amino acids, peptone, and urea could inhibit this process to different extent (Fig. 2). Threonine, serine, glutamate, and aspartate inhibited spore germination almost completely. The addition of 0.5% sucrose to nitrogen-containing compounds did not alter their inhibitory effect, and the additive effect was observed only when slightly stimulating nitrogen-containing compounds (ammonium sulfate and nitrate) were used (Fig. 3). Some nitrogen-containing compounds changed the spore germination pattern. For example, in the presence of arginine in the medium, most spores produced an additional germ tube; in the presence of peptone, the spores became rounded and twice as large, but the growth of germ tubes was considerably inhibited. In the presence of yeast extract in the medium, spores with two or three germ tubes were detected.

The effect of polyamines and cAMP on spore germination was studied, since they stimulate this process in some fungi. However, these compounds had no such effect on *B. trispora*. Putrescine (0.1–200 mM), spermidine (0.025–100 mM), and cAMP (0.0075–1.0 mM) had no stimulating effect on spore germination.

Trehalose (T spores) or sucrose (S spores) added to the sporogenous medium (potato–carrot agar) had different effects on the intensity of spore formation. The number of spores obtained in the presence of trehalose was 1.5–2 times higher than that obtained in the presence of sucrose. After 14-day storage at room temperature on agar slants, the germination ability of S spores was 2 times lower; after 30-day storage, they lost this ability almost completely. Spore germination was not observed both on the minimal medium and Goodwin's agarized medium even when the time of incubation was increased up to 24 h. This indicates that the spores lost their germination ability almost completely. The addition of trehalose to the solid medium increased the intensity of spore formation and had a favorable effect on spore viability. For example, after 2-week storage, the amount of the T spores germinated on the minimal medium during 3-h incubation was 2.5–3 times higher as compared to S spores; however, after one-month storage, this amount decreased to 5–7%. However, unlike S spores, T spores retained their germination ability. Prolonged incubation resulted in almost complete germination (95%) both on the minimal medium and Goodwin's nutrient-rich agarized medium, which indicates that spore germination was slowed down, while the spores retained their viability. A similar pattern was observed after 66-day storage. It should be noted that we failed to detect any differences between the germination dynamics of freshly harvested S and T spores.

The second part of this work dealt with the changes in the chemical composition of S and T spores occurring during storage. The study of the lipid composition of *B. trispora* spores demonstrated that their membrane lipids were represented by sterols, as well as by phospo- and sphingolipids (Table 1). Interestingly, the

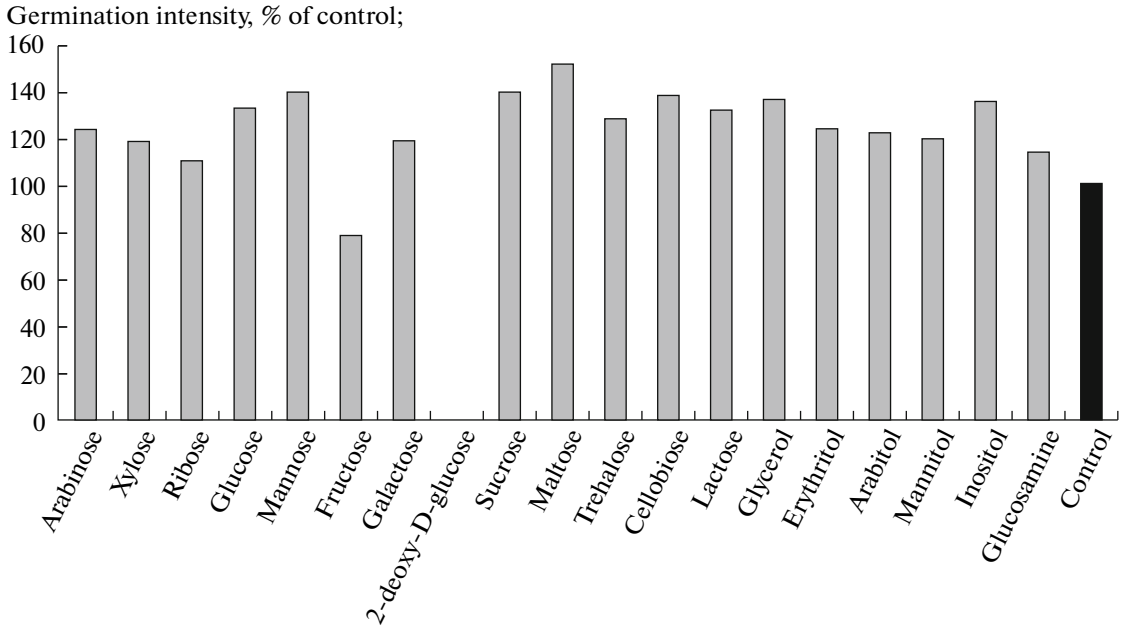

Fig. 1. Effect of carbohydrates on the intensity of spore germination.

lipid composition of spore membranes differed considerably in these two variants. When grown on sucrose, phosphatidylcholine and sterols were the main membrane lipids. In the variant with trehalose, the main membrane lipids were sphingolipids and sterols. Moreover, in the spores grown in the presence of trehalose, the concentration of phosphatidylethanolamine (PEA) was extremely low and cardiolipin (CL) was absent. The S spores stored for one month lost their germination ability, whereas the germination ability of the T spores slowed down. The contents of phosphatidylethanolamine and phosphatidylcholine (PC) in the membrane lipids of S spores increased, whereas the proportion of sterols decreased. In T spores, the proportions of phosphatidylethanolamine and phosphatidylcholine increased as well, but the contents of both sterols and sphingolipids decreased. Interestingly, cardiolipin was not detected during the storage of T spores.

The study of the fatty acid composition of the main phospholipids, phosphatidylcholine and phosphatidylethanolamine, did not reveal any differences for phosphatidylcholine; however, it was demonstrated that the unsaturation degree of the fatty acids of the phosphatidylethanolamine of T spores was higher than that of S spores due to a higher concentration of linoleic acid (Table 2).

Neutral lipids were represented by sterols and sterol esters, di- and triacylglycerides (DAG and TAG, respectively), free fatty acids (FFA), carotenoids, and carbohydrates. The differences in the proportions of the neutral lipids of freshly harvested spores were restricted to the proportions of TAG and DAG. After one-month storage, similar changes were detected in both S and T spores: the proportion of DAG decreased, whereas the proportion of free fatty acids increased, especially in S spores. A decrease in the content of TAG was detected only in T spores (Table 3).

Some differences in the carotenoid composition were detected in the spores grown in the presence of sucrose or trehalose (Table 4). The spores contained γ-carotene, β-carotene, and lycopene. The main difference of T spores was the relatively high content of lycopene and the low content of γ-carotene.

Analysis of the composition of soluble carbohydrates revealed that the total content of carbohydrates, particularly of trehalose, was higher in S spores; however, the concentration of trehalose (about 80%) was lower, and, in addition to this disaccharide, glycerol, arabitol, and glucose were detected (Table 5). By contrast, in freshly harvested T spores, only glucose was detected, apart from trehalose (about 90% of total). After one-month storage, the amount of the soluble carbohydrates of the cytosol of both T and S spores decreased by 10–15%; the composition of soluble carbohydrates changed as well. For example, a fivefold increase in the concentration of glycerol was detected. At the same time, the concentrations of arabitol and glucose decreased. In T spores, the concentration of arabitol increased, whereas glucose was not detected at all.

Hence, the chemical composition of spores grown on sucrose- and trehalose-containing media differed significantly. The main purpose of this study was to compare changes in the composition of S and T spores occurring during storage, as well as to gain insights into these changes in order to use fungal spores for

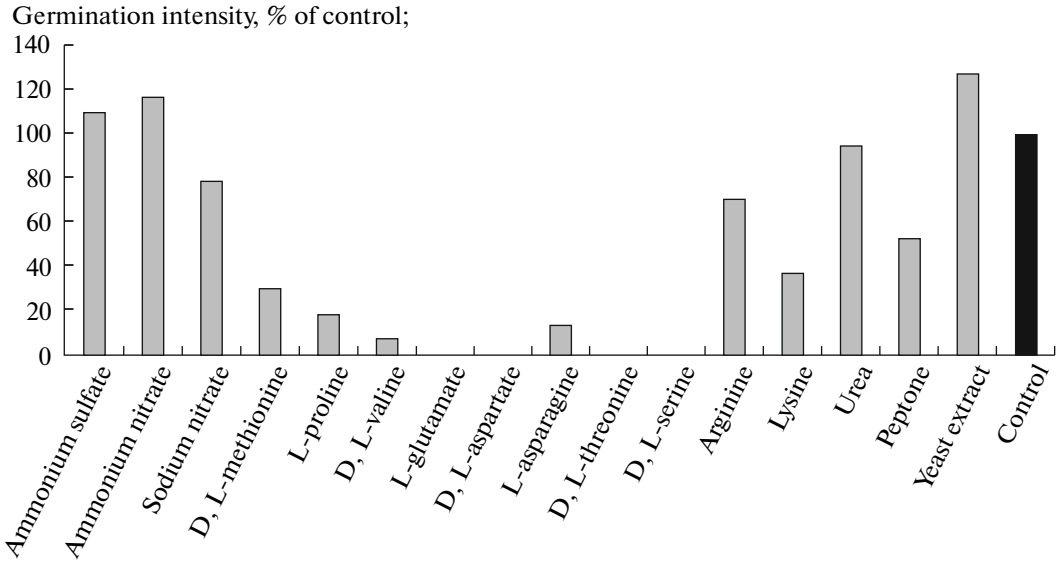

Fig. 2. Effect of nitrogen-containing compounds on the intensity of spore germination.

inoculation in the biotechnology for the production of carotenoids (including lycopene). We demonstrated that both variants were similarly relative to the productivity of the fungal mycelium (Table 6). This makes it possible to use T spores both for storage and inoculation.

During asexual spore formation, the fungus *B. trispora* produces two types of sporangia, small sporangioles (containing three spores) and large stylosporangia (containing higher amounts of spores). The type of spore formation depends on the temperature conditions. It has been previously demonstrated [13] that spores obtained from the sporangioles grown at 26–27°C should be used in the fermentation processes developed for carotenoid production. The (–) strain is responsible for carotenogenesis mediated by the complex culture consisting of the (+) and (–) strains; however, its viability is much lower than that of the (+) strain. Therefore, an attempt was made to study the process of spore germination of the (–) strain and to increase the viability of its spores by addition of the protective disaccharide trehalose, which is accumulated in fungal spores.

There are two types of fungal spore dormancy: endogenous, when spore germination is induced by stress factors, and exogenous, which depends on the favorable conditions for spore germination [14]. The study of the germination conditions of *B. trispora* spores revealed that they were in the state of exogenous dormancy, since the presence of water and the optimal temperature conditions induced spore germination. The effect of a wide spectrum of carbohydrates on the intensity of spore germination was insignificant (less than 50%), as compared to other fungi. It is unusual that the stimulating effect of glucose on spore germination was insignificant, unlike other fungi [15–16]. Interestingly, many nitrogen-containing compounds inhibited spore germination. We failed to eliminate this effect, even by addition of a carbon source, sucrose. Proline, which specifically accelerates the process of spore germination of many fungi, had no stimulating effect. For example, in the case of other mucor fungus, *Cunninghamella japonica*, the presence of glucose and proline induced a two- to threefold increase in spore germination [17]; in the case of *Aspergillus niger* a three- to fivefold increase in spore germination was induced by nitrogen-containing compounds [15]. It should be noted that the optimal conditions of spore germination for *B. trispora* differed from those for other fungi with an exogenous type of dormancy. For example, the presence of carbon sources is required for swelling of *Rhizopus oligosporus* sporangiospores, and additional nitrogen sources are required for the formation of germ tubes [16]. In the case of *B. trispora*, the swelling stage was almost absent, and the presence of water was the only requirement for the emergence of germ tubes.

It has been previously demonstrated that potato–carrot agar is the most sporogenous medium for *B. trispora* [5]; however, the content of intracellular carbohydrates in the strain (–) spores grown on this medium was about 2%, whereas, according to present-day concepts, 4–5% of trehalose is required for protection of the cell macromolecules and membranes. In order to increase the trehalose concentration, the medium was additionally supplemented with 0.5% sucrose or trehalose. As a result, the content of intracellular carbohydrates, including trehalose (5–6% wt/wt), in both variants increased significantly. However, their composition differed considerably. The presence of glycerol and arabitol in the freshly-harvested S spores indicated that the carbohydrate metab-

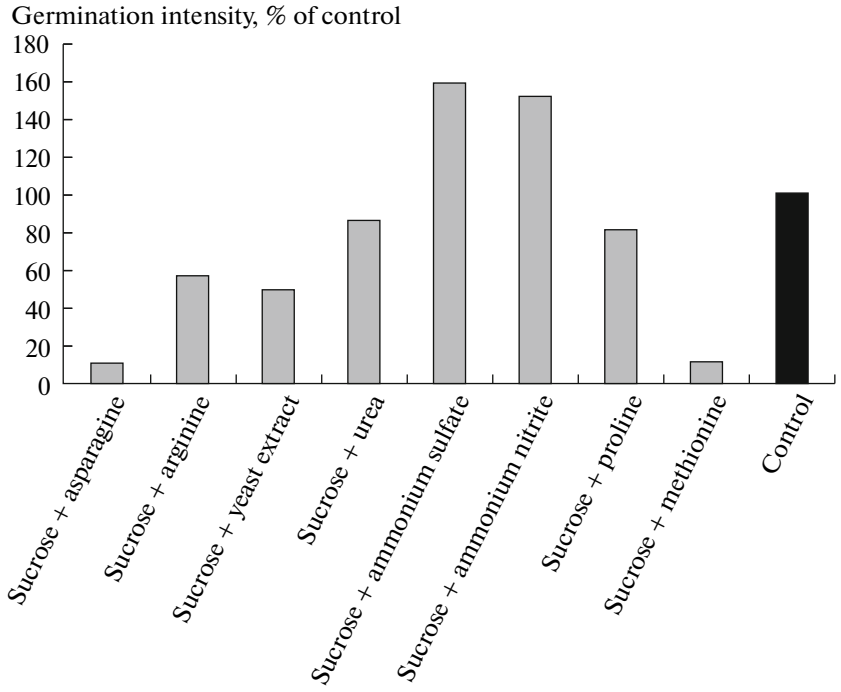

Fig. 3. Effect of nitrogen-containing compounds in the presence of sucrose on the intensity of spore germination.

olism was still intense enough, since arabitol and glycerol were synthesized via the pentose-phosphate and glycolytic pathways of carbohydrate metabolism, respectively [18]. The results obtained correspond with the data on the composition of phospholipids: in S spores, unlike T spores, the content of the main phospholipid of mitochondria, cardiolipin, as well as of the phospholipid of vegetative fungal cells, phosphatidylethanolamine, was considerably higher. In addition, the content of sphingolipids, having a membrane-stabilizing effect [19], and lycopene, a carotenoid with high antioxidant activity [1], was much lower in S spores. All these results demonstrate that the protection of T spores against unfavorable conditions is more efficient than that of S spores. The dormancy of T spores is deeper as well. This conclusion was confirmed by the data on spore germination during storage. For example, almost all T spores retained their viability during 66-day storage, whereas S spores lost their viability after 30-day storage. Moreover, the results of our investigation demonstrated that both high concentrations of trehalose and low metabolic activity were required for the maintenance of spore viability. These results correspond with the data on *Botrytis cinerea*, a fungus deficient in trehalose synthetase (Δ*tps1*), whose conidia lost their viability at room temperature much faster than the conidia of the wild strain [20]. In addition, it has been previously demonstrated that *A. niger* conidia retained their viability for 7 months at the trehalose concentration above 4%, rather than at high trehalose concentrations in freshly harvested conidia [6]. Interestingly, during

Table 1. Membrane lipids of spores during storage

Lipids	S spores		T spores	
	Initial	30 days	Initial	30 days
	% of total	% of total	% of total	% of total
Cardiolipi	6.0	4.7	–	–
Phosphatidylethanolamine	12.4	20.1	1.2	8.9
Phosphatidylethanolamine	34.8	40.5	17.0	31.7
Sphingolipids	11.8	11.8	40.2	31.2
Sterols	35.0	22.8	41.6	28.2
Total lipids, μg/g dry biomass	11599.9	11158.1	6580.9	11054.3

Table 2. Fatty acid composition of the main phospholipids of *B. trispora* spores (% of total)

	Phosphatidylethanolamine		Phosphatidylcholine	
Fatty acids	S spores	T spores	S spores	T spores
$C_{16:0}$	19.7	19.3	20.6	23.0
$C_{17:0}$	36.8	17.3	20.3	21.3
$C_{18:0}$	4.8	4.5	8.4	5.0
$C_{18:1n9c}$	13.7	20.1	15.3	18.0
$C_{18:2n6c}$	24.4	38.0	34.6	32.0
UD*	0.6	0.8	0.8	0.7

* UD denotes unsaturation degree.

Table 3. Neutral lipids of *B. trispora* spores during storage (% of total)

Lipids	S spores		T spores	
	Initial	30 days	Initial	30 days
Diacylglycerols	19.7	4.5	17.2	3.3
Sterols	20.7	20.6	17.4	14.7
Free fatty acids	13.7	22.2	15.4	18.3
Triacylglycerols	11.7	11.5	17.2	11.7
Carotenoids, sterol esters, and hydrocarbons	24.1	39.5	32.8	46.9
Unidentified	10.1	1.7	–	5.1

Table 4. Carotenoid composition of S and T spores

Carotenoids	S spores, % of total	T spores, % of total
Lycopene	11.5	34.0
γ-carotene	64.3	45.8
β-carotene	24.2	20.2
Sum, % of dry biomass	0.041	0.049

storage, sharp fluctuations in the content of carbohydrates in conidia were observed, which indicates that the main carbohydrates, trehalose and mannitol, were probably resynthesized. In the sporangiospores of the studied fungus, only a decrease in the content of carbohydrates was observed, which demonstrates that they had no any mechanism responsible for the regulation of carbohydrate metabolism.

Due to the diversity of the dormancy types, the mechanisms of transduction of the signal-activating spore germination are extremely diverse and poorly understood. The following pathways have been described: (1) Ca^{2+}/calmodulin-dependent protein kinase pathway (in *Magnaporthe grizea* and *Colletotrichum trifolii* conidia); (2) Ras/mitogen-activated protein kinase pathway (in *Colletotrichum lagenarium* conidia); (3) cAMP-dependent protein kinase pathway (in *M. grizea*), and (4) Ras/cAMP-dependent protein kinase pathway (regulates the isotropic growth of *Aspergillus nidulans* and *Neurospora crassa* conidia) [21]. Moreover, it was demonstrated that polyamines could be involved in the process of spore germination [22].

The results obtained demonstrate that exogenous nutrients and regulators of spore germination (cAMP, which is known to control the involvement of storage compounds in the germination of *Botrytis cinerea* spores [20], and polyamines) are not required for spore germination in the case of *B. trispora*. This indicates that the mechanism responsible for the regulation of the germination of *B. trispora* spores is still unknown. However, the fact that the poorly metabolized sugar 2-deoxy-D-glucose completely inhibited germination indicates that intracellular carbohydrates must be involved in spore germination. It is possible that trehalose-6-phosphate, a trehalose precursor, is the regulator controlling the involvement of storage carbohydrates in spore germination, as well as the regulator controlling the carbohydrate metabolism and utilization of nitrogen sources at two levels, enzymatic and genetic [23].

Hence, the main outcome of this study is the enhancement of spore viability during storage by addition of trehalose into the sporogenous medium result-

Table 5. Changes in the composition of the soluble carbohydrates of the spore cytosol during storage

Sugars	S spores				T spores			
	Initial		30 days		Initial		30 days	
	% dry biomass	% of total	% dry biomass	% of total	% dry biomass	% of total	% dry biomass	% of total
Glycerol	0.08	1.0	0.38	5.6	Trace amounts	–	Trace amounts	–
Arabitol	0.79	10.1	0.49	7.2	Trace amounts	–	0.65	13.7
Glucose	0.70	8.9	0.51	7.5	0.53	9.8	Trace amounts	–
Trehalose	6.26	80.0	5.42	79.7	4.88	90.2	4.11	86.3
Total	7.83	100.0	6.80	100.0	5.41	100.0	4.76	100.0

Table 6. Effect of S and T spores on lycopene production

Variant	Dry biomass, g/l	β-carotene		Lycopene	
		% dry biomass	g/l	% dry biomass	g/l
S spores, control	48.1	0.80	0.39	–	–
S spores, MAP	39.3	0.16	0.06	1.83	0.72
T spores, control	39.5	1.29	0.51	–	–
T spores, MAP	48.4	0.47	0.23	1.62	0.78

Designations: MAP is 6-methyl-2-aminopyridine, a stimulator of lycopene production.

ing in the stimulation of spore formation, and the attainment of a deeper state of dormancy as compared to S spores.

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