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EXPERIMENTAL ARTICLES

Peculiarities of Exogenous Dormancy of *Aspergillus niger* Conidia

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Abstract—*Aspergillus niger* conidia are characterized by exogenous dormancy: the first stage of their germination is accomplished in twice-distilled water. However, germ tube formation requires the availability of carbon and nitrogen sources. Exogenous dormancy in *A. niger* conidia exhibits the following peculiar features: (i) nitrogen-containing substances are active stimulators of germination; (ii) temperature-dependent changes in the lipid bilayer and in the neutral lipid composition of conidia are virtually identical to those occurring in growing mycelium under temperature stress; and (iii) the spore viability threshold does not exceed 45°C; i.e., the spores are more heat-resistant than the mycelium, but they are less heat-resistant than the spores that are in the state of endogenous dormancy. According to the current classification of the types of cell metabolism arrest, the exogenous dormancy of *A. niger* conidia resembles the pattern of metabolism characteristic of vegetative cells during the idiophase.

Key words: Aspergillus niger, conidia, dormancy, stress, carbohydrates, lipids.

According to the traditional views, all organisms are assumed to undergo changes in the level of metabolic activities and to decelerate life-sustaining processes under the influence of deleterious factors [1]. Depending on the stress intensity, this phenomenon can take the form of *apparent death*, a peculiar state also referred to as *anabiosis*. Anabiosis is characteristic of many invertebrates (rotifers, nematodes, etc.), and it is accompanied by a virtually complete long-term arrest of life-sustaining activities.

Like the seeds of a large number of plant species, fungal spores are characterized by a different state (referred to as *dormancy*): their metabolic activities are decreased but not arrested; they retain some respiratory activity and use some functional links of the metabolic chain. Hence, a number of researchers denote the fungal spore as a dormant cell.

Fungal cells display two types of dormancy: it can be exogenous (superficial) and endogenous (constitutive) [2]. Constitutive dormancy requires a specialized cell barrier (restricting the entry of nutrients) and the presence of autoinhibitory compounds. This dormancy state is under the cell's control, and it is subject to regulation at the cytoplasm level. The other type, exogenous dormancy (also referred to as dormancy dependent on environmental factors). Once the influence of a deleterious factor is eliminated, the spore starts germination.

Importantly, both dormancy types are stress-induced, although different types of stress factors can be involved. For example, in oomycetes, substances stimulating oospore formation are formed only under nitrogen deficiency [3, 4]. Water shortage is the most widespread stress factor responsible for the sporulation in ascomycetes, whereas in mucorous fungi (*Blakeslea trispora*), stylospores form upon elevation of the temperature [5].

From a theoretical perspective, research on spore dormancy types and on the activation of spore germination is closely related to important subfields of biology dealing with cytodifferentiation, ontogeny, sensitivity to deleterious factors, etc. Elucidation of the factors promoting active germination of spore inoculum is one of the main goals of biotechnological studies. Most spores used as inoculum are in the state of exogenous dormancy. The sooner the spores wake up, the more economical is the biotechnological process. The fermentation efficiency is also conditional on the synchronicity of the germination process and on the number of germination tubes formed by one spore.

Therefore, the goals of this work were (i) to determine the dormancy type in *A. niger* conidia; (ii) to establish the conditions promoting the activation of dormant conidia; (iii) to characterize the physical and chemical properties of conidia (thermostability and the changes in the lipid and carbohydrate composition induced by thermal shock); and (iv) to compare the metabolic activities of dormant spores and vegetative cells under stress (using our own findings and the data available in the literature).

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MATERIALS AND METHODS

This work used *A. niger* VKPM F-790 spores (conidia) employed as inoculum for the production of citric acid at the Citrobel plant in Belgorod. The spores were grown on malt agar and harvested in a vacuum. They were desiccated to a humidity of 6–7% and stored at a temperature of 15–20°C.

In order to investigate the dormancy type of *A. niger* spores, they were placed into a liquid medium prepared on twice-distilled water. It was expedient to avoid the use of solid medium, because the addition of agar could enrich the medium with additional nutrients that it contains. Cultivation was carried out in 250-ml flasks with 30 ml of medium. The spore suspension (SS) used as inoculum was prepared as follows: 20 mg of spores were soaked in 0.05 ml of ethanol and supplemented with 10 ml of twice distilled water. SS was added to a final concentration of $(1–5) \times 10^6$ spores per 1 ml of medium. Cultivation was carried out in an orbital shaker (200 rpm) for 17 h at 32°C.

The influence of the temperature, pH, and carbon and nitrogen sources on the process of spore germination was investigated using an express method. Two ml of melted agarized medium was layered onto a slide; 1 drop of SS was applied on top of the medium after its solidification and the spores were evenly distributed over the medium with a glass rod. The slide was thereupon placed in a Petri dish on filter paper moistened with 0.2 ml of water. The dishes were incubated at 32°C for 8–17 h. In this system, spores germinated 2–3 h earlier than in submerged culture; in addition, our method made it easier to count germinated spores.

The effects of medium acidity were investigated in an agarized medium based on phosphate–citrate buffer with pH values ranging from 2.2 to 8. The buffer was supplemented with 0.2% glucose and 0.2% peptone. The same method was used in the studies on the influence of carbon and nitrogen sources; however, sugars were added to a concentration of 0.5%, and nitrogen-containing substances were added to a concentration corresponding, in terms of nitrogen content, to 0.5% $(NH_4)_2SO_4$. Spore thermostability was tested by heating SS for 20 min at 35–98°C and inoculating them thereupon onto glucose–peptone medium based on a buffer with pH 4.0–4.2.

Spore germination was monitored using a Jenaval light microscope (Germany) at a magnification of 250–900. The morphology of the spore surface was inspected with a Jeol JSM-T300 scanning electron microscope. Spores were attached with glue to the top of the column and stained with gold. No spore pretreatment was required, because the spores were hydrophobic, and the humidity value did not exceed 10%. An ocular grid placed on the surface of the solid medium was used for counting spores. The spores were counted in 6–10 squares (the total being about 600 spores). A spore was considered germinating if the length of its germ tube exceeded one half of the spore diameter.

To investigate the effect of temperature on the chemical composition of spores and their germination capacity, dry conidia were incubated at 10, 20, or 37°C for 30 and 60 days and at 2 and 50°C for 10 days.

Extraction of lipids from spores, analysis of their methyl esters, separation of lipids into the neutral, glyco-, and phospholipid fractions, and the investigation of the composition of fractions were performed in described earlier [5].

To determine the carbohydrate composition of the cytosol of fungal spores, sugars were extracted using a 4-step procedure (each step involved a 20 minute treatment with boiling water). Spores were separated by centrifugation. Proteins were removed from the resulting extract. The carbohydrate extract was further purified using a combined ion-exchange column with Dowex-1 (the acetate form) and Dowex 50W (H^+). The quantitative composition of sugars was determined by gas–liquid chromatography; for this purpose, trimethylsilyl sugar derivatives were obtained from the lyophilized extract [6]. Arabitol or α-methyl-D-mannoside (Merck) were used as internal standards. Chromatography was performed using a gas–liquid Model 3700 chromatographer equipped with a flame-ionization detector and a 2-m glass column packed with 5% SE-30 on Chromatone (70–90 mesh); the temperature was elevated at a rate of 5–6 deg/min from 130 to 270°C. Glucose, mannitol, arabitol, inositol, and trehalose (Merck) were used as standards.

RESULTS AND DISCUSSION

Under a light microscope, *A. niger* spores appeared as having a regular rounded shape (Fig. 1), whereas scanning microscopy revealed that the spores were wheel-shaped and displayed crest-like protrusions on their surface (Fig. 2). The spores of the strain studied formed unbranched chains (5–10 spores in each chain); the spores in the chain contact each other by their concave flattened sides, which is a characteristic feature of the genus *Aspergillus*.

Spore germination takes 6–8 h. The spheric pattern of spore growth, termed stage I, or swelling, occurs during the initial 4–5 h, resulting in a 3- to 4-fold increase in the spore diameter (Fig. 1). The following 3–4 h correspond to stage II of spore germination. It is characterized by the formation of a germ tube that displays an apical growth pattern, which is typical of fungi. The fungus studied typically forms one germ tube. Occasionally, two germ tubes form that are arranged on the same plane (Fig. 1). This arrangement is apparently due to the fact that a circular equatorial germ ring [2], not a germ pore, is characteristic of *Aspergillus* spp. spores. Importantly, the germination process is asynchronous in *A. niger*: from Fig. 2 it is evident that both stages may occur simultaneously.

In order to find out whether SS dormancy was exo- or endogenous, SS were inoculated into liquid media

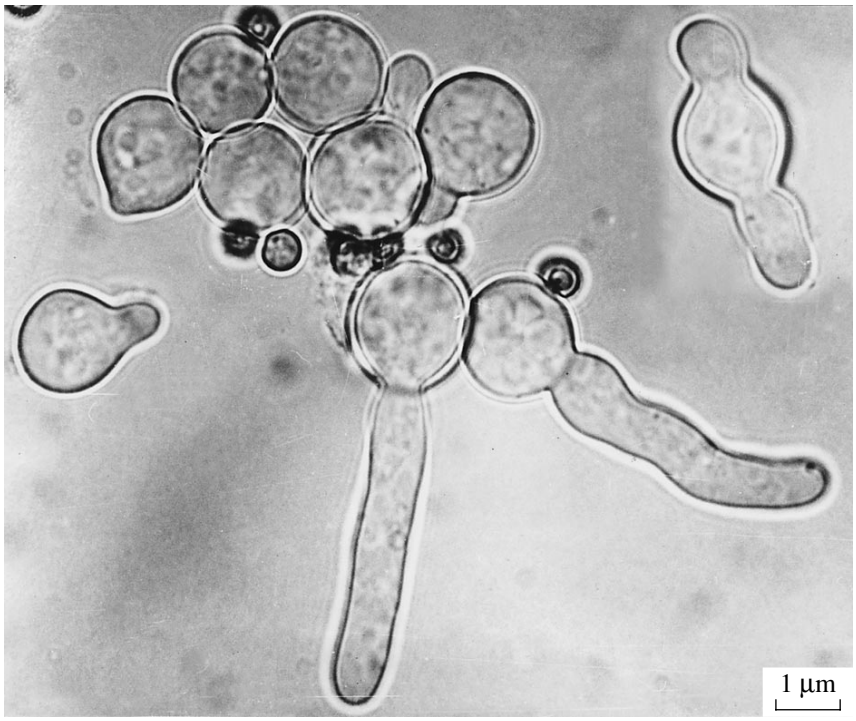

Fig. 1. Germination of *A. niger* conidia on the surface of glucose–peptone medium.

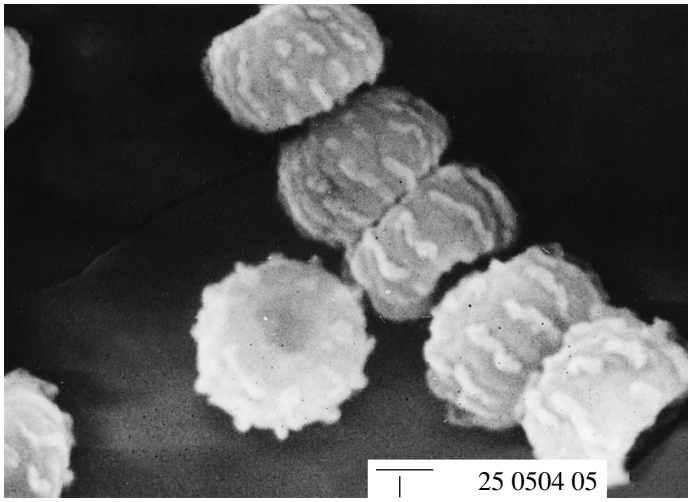

Fig. 2. Crest-like protrusions on the surface of conidia (7650×).

containing (1) distilled water; (2) 0.2% glucose; (3) 0.2% peptone; (4) 0.2% $(NH_4)_2SO_4$; (5) 0.2% glucose and 0.2 peptone; and (6) 0.2% glucose and 0.2% $(NH_4)_2SO_4$. After 2 days of cultivation, only swollen spores occurred in media 1 and 4, while media 2 and 3 contained spores with germ tubes of various length (i.e., the germination process had been completed in these systems). Branched mycelium formed only in media with nitrogen and carbon sources (5 and 6).

The above data indicate that it is water that triggers the activation of dormant *A. niger* conidia. Therefore, spore dormancy is exogenous in this system (this is characteristic of a large number of aspergilla).

In distilled water, mold fungi display a wide variety of developmental patterns, resulting in the formation of solitary germination tubes (e.g., in *A. niger* 24) or sporulating mycelia (in *A. niger* 472, *Fusarium moniliforme* 637, *Alternaria tenuis* 635, and *Trichothecium roseum* 531) [7]. Presumably, deuteromycete conidia are modified vegetative cells, since they can form conidiophores, conidia, and chlamydospores [8] like hyphae. Numerous representatives of these fungi (*A. niger*, *Pen-*

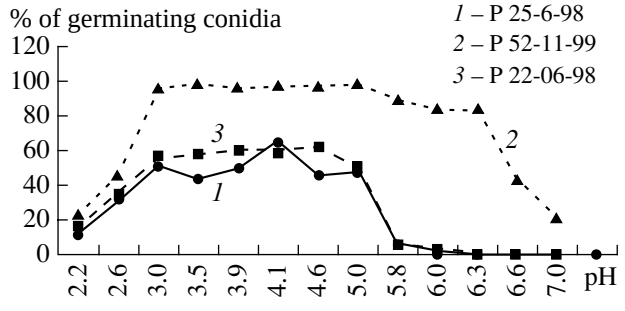

Fig. 3. Effect of pH on the germination of *A. niger* conidia.

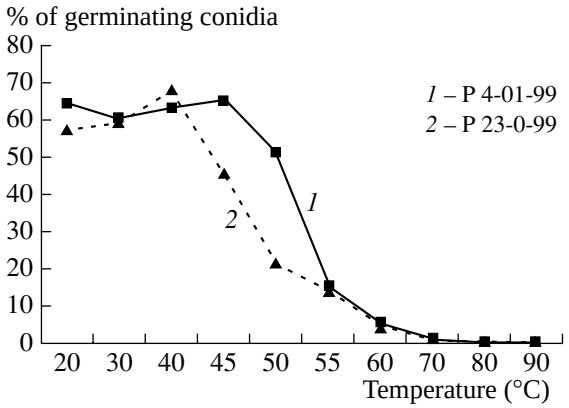

Fig. 4. Thermostability of *A. niger* conidia.

icillium digitatum, *Helminthosporium spiciferum* etc.) are characterized by the microcyclic pattern of conidium formation (i.e., the germ tube converts into a conidiophore with conidia).

Only germination stage I occurred if the *A. niger* strain studied was incubated in water. The transition to stage II requires a nitrogen or carbon source, and prerequisite for the formation of branched mycelium is the presence of sugars and nitrogen-containing compounds.

The developmental traits of the *A. niger* strain can be related to the chemical composition of dormant conidia. These data will be presented after discussing the following results.

1. Influence of Physical and Chemical Factors on Conidium Germination

1.1. Impact of medium acidity and spore storage time. The influence of the acidity of the medium on *A. niger* spore germination was investigated using three spore samples characterized by different storage periods. The spores from samples P 25-06-98 and P 22-06-98 were stored for 1.5 years, the maximum percentage of germinating spores was 60%, and the optimum pH for spore germination was 3–5. Interestingly, more freshly harvested spores (sample 52-11-99, stored for 3 months) germinated within a wider pH range (3–6.5, Fig. 3).

1.2. Temperature effect. The temperature optimum for spore germination was established by growing spores on glucose–peptone medium at 18–20, 26–28, 32–34, and 36–38°C. The germination rate was 1.3, 26, 57, and 51%, respectively. From these data it follows that the optimum temperature range was 32–34°C.

1.3. Spore thermostability. *A. niger* spores were stable within a narrow temperature range (up to 45°C). Half of the spores lost their germination capacity at 50°C (Fig. 4). Interestingly, a minor heat-resistant spore fraction (less than 0.5%) retained the germination capacity at 98°C. Of relevance in this context is a significant difference in terms of thermostability between dry and water-incubated spores. Equal amounts (12%) of spores capable of germination occurred after incubating dry spores for 20 days at 50°C and water-soaked spores for 20 min at 50°C. Importantly, heating water-suspended and dry spores at 121°C for 1 h in an autoclave did not result in a complete loss of their germination capacity; solitary spores still could germinate (after a significant lag period). These findings in conjunction with our data on a considerable (twofold) difference between the percentages of spores germinating at 26°C and 32°C point to a significant heterogeneity of the spores in terms of their thermostability.

1.4. Effect of the carbon and nitrogen sources. The data on the effect of carbon and nitrogen sources shown in Table 1 indicate that almost all sugars tested (except fructose and 2-deoxy-D-glucose) increased the amount of germinated spores by a factor of 1.5 to 2.5 in phosphate–citrate buffer, while only glucose, inositol, and D-glucosamine stimulated spore germination in distilled water (other sugars produce an inhibitory effect). 2-deoxy-D-glucose, a nonmetabolizable glucose analogue, completely inhibited the germination process on both media. Curiously enough, nitrogen-containing substances increased the amounts of germinated spores 3- to 5-fold on both media. This stimulatory effect was more pronounced in phosphate–citrate buffer, but it also occurred in twice-distilled water. The data obtained indicate that D-glucosamine, meso-inositol, L-proline, and peptone exert a stimulatory influence on spore germination. Interestingly, L-proline and D-glucosamine at concentrations of 0.1–2.0% (8.6×10^{-5}–1.7×10^{-3} M) were almost equally powerful stimulators of the germination of *A. niger* conidia.

Hence, a new type of exogenous spore dormancy was revealed in this work. Its distinctive feature is the active stimulation of germination by nitrogen-, not carbon-containing compounds. Almost all nitrogen sources tested increased the percentage of germinating *A. niger* spores by a factor of 3 to 5. This does not occur with spores of other fungi during exogenous dormancy.

2. Chemical (Lipid and Carbohydrate) Composition of Dormant Spores

During exogenous dormancy, *A. niger* conidia contained 7.0 to 8.9% of extractable lipids, including neutral lipids (NL, 70–75% of the total lipid fraction, the predominant fraction), glycolipids (GL, 17–19%), and phospholipids (PL, 7–10%). $C_{18:1}$ and $C_{18:2}$ (21.3 and 28.9%, respectively) dominated the unsaturated fatty acids in NL. $C_{16:0}$ (28.1%) was the main fatty acid in GL, which contained almost equal amounts (14–15%) of $C_{18:0}$, $C_{18:1}$, and $C_{18:2}$ (Table 2, see the control studies conducted at 20°C). The fatty acid composition of the phospholipid fraction was quite different. $C_{18:2}$ (about 60% of the total fatty acid fraction) was the predominant species, and these lipids were characterized by the highest desaturation degree (1.4). The composition of phospho- and neutral lipids is shown in Tables 3 and 4 (see the control studies conducted at 20°C). Triacylglycerols prevailed (40–50%) in neutral lipids, and phosphatidylcholine (over 60% of the total PL fraction) was the main component of the PL fraction.

Cytosol carbohydrates accounted for 10–11% of the dry weight of spores, and mannitol and trehalose (40–43 and 21–22%, respectively) were their predominant components (Table 5, see the control studies conducted at 20°C).

The analysis of the lipid and carbohydrate composition of *A. niger* conidia revealed that the level of the reserve (neutral) lipids was low (about 5%). As for the labile carbohydrates (such as trehalose) that are involved in the germination process, their content was as low as 2.3%. Comparing these data with those on the reserve compounds of other fungi, e.g., *Neurospora crassa* conidia (containing 18% of lipids) [10], enables us to conclude that *A. niger* conidia cannot reach the germination stage II without exogenous carbon and nitrogen sources primarily because of the deficiency of reserve compounds. In light of modern concepts, a spore is a resting (dormant) cell characterized by a very low level of metabolic activities. For instance, dormant spores do not exhibit any significant respiratory activity, even though they contain the cytochrome system [3]. Many types of spores can be activated by a specific trigger factor (water, temperature and pH changes, etc.). Presumably, the primary changes (physiological triggers) involved are cytoplasmic membrane modulations, particularly in the lipid bilayer [10].

No data on the effects of stress factors (e.g., temperature changes) on the lipid and carbohydrate composition of dormant spores and their germination capacity are available in the literature. However, such data, in conjunction with the data on vegetative cells, could provide additional information on dormancy.

Table 1. Effect of carbon and nitrogen sources on the germination of *A. niger* conidia (%)

Carbon and nitrogen sources	Twice distilled water	Phosphate–citrate buffer, pH 4.1
Control	14	14
Monosaccharides:		
Pentoses:		
D-ribose	12	28
D-fucose	5	30
L-arabinose	7	22
D-xylose	5	29
Hexoses:		
D-glucose	24	24
2-deoxy-*D*-glucose	0	0
D-fructose	13	13
D-galactose	8	20
D-mannose	6	21
Sugar alcohols:		
glycerol	9	31
erythritol	4	22
D-arabitol	9	32
D-mannitol	3	19
meso-inositol	28	36
Disaccharides:		
sucrose	18	22
trehalose	10	28
maltose	12	17
Nitrogen-containing compounds:		
peptone	64	90
$(NH_4)_2SO_4$	31	80
NH_4NO_3	–	51
L-asparagine	–	63
L-proline	77	81
L-threonine	20	53
L-cysteine	30	61
L-methionine	34	38
L-serine	45	73
D-glucosamine	40	49

3. Temperature Influence on the Chemical Composition of A. niger Conidia

It was established earlier that 20°C is the optimum temperature for storing air-dried spores (with a humidity of 6–8%). This temperature provides for the germination of about 90–95% of spores after 6 months of storage. Such a system was used in control studies. Storing conidia at a low temperature (2°C) for 60 days

Table 2. Effect of temperature on the fatty-acid composition of *A. niger* conidia lipids

Temperature, °C	Lipid class	Fatty acids, % of total											DD
		$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{17:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:1}$	
20	NL	Traces	Traces	21.7	1.7	Traces	Traces	22.6	21.3	28.9	3.8	Traces	0.92
	GL	Traces	3.6	28.1	5.0	10.3	3.8	14.4	14.4	14.6	Traces	3.6	0.58
	PL	Traces	Traces	13.9	1.7	2.9	Traces	2.9	18.9	59.7	Traces	Traces	1.40
2	NL	Traces	Traces	23.6	3.2	0.9	Traces	21.4	19.4	28.6	2.9	Traces	0.89
	GL	Traces	Traces	28.4	3.9	7.8	2.6	14.6	21.8	20.9	Traces	Traces	0.70
	PL	Traces	Traces	15.0	2.0	4.0	Traces	3.3	17.0	58.7	Traces	Traces	1.36
50	NL	Traces	Traces	22.8	2.6	Traces	Traces	17.7	20.4	33.2	3.3	Traces	0.99
	GL	Traces	Traces	26.8	4.0	12.8	4.0	14.6	14.6	23.2	Traces	Traces	0.69
	PL	Traces	Traces	15.8	1.6	1.1	Traces	4.4	22.0	54.0	1.1	Traces	1.35

Note: The table does not include the data on an unidentified fatty acid contained in the GL at 20°C. “Traces” means contents below 0.5%. “DD” is desaturation degree.

Table 3. Effect of temperature on the neutral lipid composition of *A. niger* conidia

Temperature, °C	Lipids, % of total NL					
	diglycerides	sterols	free fatty acids	triglycerides	sterol esters	hydrocarbons
20	3.7	7.7	5.1	42.6	26.5	13.6
10	7.6	8.2	4.5	41.8	22.0	14.1
37	8.4	14.5	5.4	49.3	Traces	22.2

Table 4. Effect of temperature on the phospholipid composition of *A. niger* conidia

Temperature, °C	Lipids, % of total PL				
	X	phosphatidylcholine	phosphatidylserine	phosphatidy-lethanolamine	cardiolipin
20	8.0	64.5	0.6	22.8	4.0
37	12.2	84.1	Traces	3.7	Traces
10	3.9	75.0	Traces	19.7	1.4

Note: *X* is an unidentified phospholipid.

Table 5. Effect of temperature on the carbohydrate composition of *A. niger* conidia

Temperature, °C	Cytosol carbohydrates, % of total	Carbohydrates, % of dry spores				
		glycerol	erythritol	arabitol	mannitol	trehalose
20	10.5	12.4	12.4	10.5	42.8	21.9
37	10.4	10.3	14.7	14.7	37.4	22.9
10	10.5	17.3	10.6	12.5	41.3	18.3

insignificantly decreased their germination capacity. Incubating conidia for the same period at 37°C lowered the number of germinating spores by one third, and only 10–15% of conidia remain viable after incubating them at 50°C for 20 days.

Changing the storage temperature produced virtually no effect on the lipid content of spores and the ratio between glyco-, phospho-, and neutral lipids therein. However, appreciable changes occurred in the composition of phospholipids and neutral lipids if spores were

incubated at high or low temperatures (Tables 3, 4). Of considerable interest is the significant increase in the sterol content that occurred at high temperatures. This pattern persisted if the spores were incubated at a high temperature for a long time (60 days), and this long-term incubation also resulted in a slight change in the triacylglycerol content. Studies within the 2–50°C temperature range revealed that low temperature-induced changes in neutral lipids primarily concerned the free fatty acid (FFA) content, which increased almost threefold under these conditions, while the sterol content virtually remained unchanged. Temperature-dependent changes are still more spectacular in PL (Table 4). High temperature caused an increase in the phosphatidylcholine (PC) content and a decrease in the phosphatidylethanolamine (PEA) level, and an analogous pattern persisted at a still higher temperature (50°C). As for the fatty acid composition of the lipids, the most substantial changes occurred in glycolipids. The desaturation degree of these phospholipids is altered at 2°C. It is the oleic and linoleic acid contents that undergo the most significant changes at 2°C. Curiously enough, the desaturation degree of phospholipids did not change at 50°C, whereas the $C_{18:2}$ content increased in glycolipids (Table 2).

The above data are of particular interest in the context of the research on the cell's state termed exogenous dormancy. The changes occurring in the lipid bilayer and in the NL composition are practically identical to those characteristic of growing, metabolically active fungal cells under temperature stress [9]. Changes in phospholipids involving the PC and PEA contents and in the sterol and FFA levels are particularly prominent [9, 10]. FFA and especially sterols are probably involved in the regulation of sporogenesis, and they promote the stability and stress resistance of the membrane [11–13].

The temperature also influenced the carbohydrate composition of the conidium cytosol. High temperatures inhibited glycerol and mannitol formation in *A. niger* conidia and increased the erythritol, arabitol, and trehalose levels. Conversely, low temperatures significantly increased the glycerol level and slightly decreased the erythritol and trehalose contents (Table 5). Interestingly, we have revealed analogous patterns in our studies on the effects of temperature stress on the mycelium of ascomycetes and basidiomycetes [14].

From the data obtained it is evident that conidia are capable of biochemical adaptation to thermal shock, even though they represent dormant cells formed in response to stress. In addition, the response of conidia to temperature stress is in principle the same as that of metabolically active cells.

The extensive data accumulated up to now suggest that cell ontogeny involves—at an early or a late stage—a deceleration of life-sustaining processes due to the effects of stress factors. Recent findings point to the existence of extremely diverse modes of life in nature. Based on the extent to which metabolic activities are inhibited, they can be classified as follows: anabiosis (a 100% arrest of life-sustaining processes)–dormancy (varying degrees of metabolism inhibition)–active (vegetative) lifestyles [15]. The state referred to as dormancy actually includes a particularly wide variety of lifestyles characterized by inhibited life-sustaining processes. They are typical of bacterial endospores, fungal memno- and xenospores, bacterial exospores and cysts, refractile cyst-like cells of *Bacillus cereus* and a number of non-spore-forming bacteria, etc. [16]. It should be noted that, in a nutrient-depleted medium, the vegetative submerged mycelium of fungi can form specialized hyphae with an elevated lipid content, thickened cell walls, and enhanced resistance to environmental factors [17]. In terms of their metabolic activities, the cells of this type seem to be particularly similar to *A. niger* conidia in the exogenous dormancy state.

The results obtained are of interest in respect to the evolution of protective cell mechanisms, which appear to be sufficiently uniform irrespective of the organizational level and state of cells using them. In all tested systems (see above), stress causes an inhibition of cell activities and the synthesis of new protective modules. These data nicely confirm the idea put forward by Engelhardt as early as in 1963 that "nature... staunchly adheres to... a principle that satisfies its needs particularly well and widely uses it in diverse, quite dissimilar systems" [18].

The above-described type of exogenous dormancy of *A. niger* conidia can be regarded as a peculiar intermediate mode of existence (in comparison to anabiosis and the active mode of life) that resembles the vegetative lifestyle in respect to the degree of metabolism inhibition. The existence of states with differing degrees of metabolism inhibition in nature is a manifestation of the dialectics of life. In order to secure their survival, living organisms use biochemical mechanisms of adaptation that cause a temporary arrest of life-sustaining processes (an apparent death) or result in a resting (dormant) state.

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