
EXPERIMENTAL
ARTICLES

The Effect of Hydrogen Peroxide on the Growth of Microscopic Mycelial Fungi Isolated from Habitats with Different Levels of Radioactive Contamination

A. E. Ivanova^{*1}, K. B. Aslanidi^{**}, Yu. V. Karpenko^{***}, and T. A. Belozerskaya^{****}

^{*}Faculty of Soil Science, Moscow State University,
Vorob'evy gory, Moscow, 119992 Russia

^{**}Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences,
Pushchino, Moscow oblast, 142290 Russia

^{***}Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine,
ul. Zabolotnogo 154, Kiev, 03143 Ukraine

^{****}Bach Institute of Biochemistry, Russian Academy of Sciences,
Leninskii pr. 33, Moscow, 119071 Russia

Received February 8, 2005; in final form, March 17, 2005

Abstract—The effect of hydrogen peroxide (10^{-9} – 10^{-1} M) on the mycelial growth of the fungi *Alternaria alternata*, *Cladosporium cladosporioides*, *Mucor hiemalis*, and *Paecilomyces lilacinus* has been studied. The growth of fungi isolated from habitats with a background level of radioactive contamination was stopped by H_2O_2 concentrations equal to 10^{-3} and 10^{-2} M, whereas the growth of fungi that were isolated from habitats with high levels of radioactive contamination was only arrested by 10^{-1} M H_2O_2 . The response of the different fungi to hydrogen peroxide was of three types: (1) a constant growth rate of fungal hyphae at H_2O_2 concentrations between 10^{-9} and 10^{-4} M and a decrease in this rate at 10^{-3} M H_2O_2 , (2) a gradual decrease in the growth rate as the H_2O_2 concentration was increased, and (3) an increase in the growth rate as the H_2O_2 concentration was increased from 10^{-6} to 10^{-5} M. The melanin-containing species *A. alternata* and *C. cladosporioides* exhibited all three types of growth response to hydrogen peroxide, whereas the light-pigmented species *M. hiemalis* and *P. lilacinus* showed only the first type of growth response. A concentration of hydrogen peroxide equal to 10^{-1} M was found to be lethal to all of the fungi studied. The most resistant to hydrogen peroxide was found to be the strain *A. alternata* 56, isolated from the exclusion zone of the Chernobyl Nuclear Power Plant.

Key words: microscopic fungi, growth, hydrogen peroxide.

It is well known that ionizing radiation induces the formation of reactive oxygen species (ROS), which provoke oxidative stress in cells and can eventually cause their death [1, 2]. In cells with an aerobic type of metabolism, hydrogen peroxide is the most long-lived transformation product of ROS. It is always present in such cells at low concentrations, being involved in intra- and intercellular signal transduction [3]. In fungi, hydrogen peroxide is a necessary component of the intracellular signalling system in such processes as cell differentiation and proliferation [4, 5].

Hydrogen peroxide is of great interest for simulating the effect of radiation on living objects, since up to 90% of the damage induced by ionizing radiation is due to water radiolysis products [1].

The aim of this work was to study the effect of a wide range of hydrogen peroxide concentrations on the

growth of microscopic fungi isolated from habitats with different levels of radioactive contamination.

MATERIALS AND METHODS

Fungal strains. Experiments were carried out with four strains of microscopic fungi isolated from the exclusion zone of the Chernobyl Nuclear Power Plant (ChNPP), which has a high level of radioactive contamination, and from habitats with a background level of radioactive contamination (see Table 1). The strains differed in relation to the structure of their mycelium (either septate or nonseptate), the presence of melanin pigments in the cell wall (dark- and light-pigmented strains), and in their growth rate (slow- and fast-growing strains).

Preparation of hyphal fragments. The strains were grown for 48 h (to the exponential growth phase) at 25°C in the dark on a cellophane film placed onto the surface of Czapek agar [8]. The peripheral zone of the

¹ Corresponding author; e-mail: tab@inbi.ras.ru

Table 1. Characteristics of the microscopic fungi under study

Species	<i>Alternaria alternata</i> (Fr. : Fr.) Keissler 1912		<i>Cladosporium cladosporioides</i> (Fres.) de Vries 1952		<i>Paecilomyces lilacinus</i> (Thom) Samson 1974		<i>Mucor hiemalis</i> Wehmer 1903		
Strain	56**	60	224	4	5	396	1941	10	111
Source	1	2	2	1	1	1	1	2	2
Habitat	ChNPP, inner wall of containment structure, 1998	Moscow region, Umbric Albeluvisols, 1989	Moscow region, Umbric Albeluvisols, 1989	ChNPP, soil in the Red Forest, 1986	ChNPP, reactor graphite surface, 1993	L'vov region, Umbric Albeluvisols An-thropic, 1957	ChNPP, 10-km zone, soil in the Rusty Forest, 1994	Novgorod region, Sappric Histosols, 2000	Moscow region, Umbric Albeluvisols, 1997
Radioactivity level	$\alpha = 500 \text{ Bq/cm}^2$, $\beta = 2 \times 10^4 \text{ Bq/cm}^2$, $\gamma = 700 \text{ mR/h}$ [6]	Background level*	Background level*	$3.6 \times 10^5 \text{ Bq/kg}$ [6]	$3.7 \times 10^9 \text{ Bq/kg}$ [6]	Background level*	$3.2 \times 10^4 \text{ Bq/kg}$ [6]	Background level*	Background level*
Type	Septate								
Mycelium	Dark-colored				Light-colored				
	Aggregation of hyphae	No	No	Aggregation	Aggregation	Aggregation	Aggregation	No	No
Elongation rate of hyphae	4.4 ± 0.3	1.8 ± 0.2	2.1 ± 0.2	0.9 ± 0.2	0.7 ± 0.2	1.4 ± 0.3	0.8 ± 0.2	0.5 ± 0.2	5.5 ± 0.5

Notes: **1** denotes strains from the collection of the Department of Physiology and Systematics of Micromycetes at the Zablotny Institute of Microbiology and Virology; **2** denotes strains from the test collection of microscopic fungi in the Department of Soil Biology at the Faculty of Soil Science, Moscow State University. The background level of radioactive contamination on the territory of the Russian Federation is 0.22 Bq/cm² (ca. 0.06 Ci/km²) of ¹³⁷Cs and 0.15 Bq/cm² (ca. 0.04 Ci/km²) of ⁹⁰Sr. Soils are considered to be uncontaminated if their radiocontamination level does not exceed ca. 3.7 Bq/cm² (1 Ci/km²) of ¹³⁷Cs. * The background level of γ -radiation dose rate on the territory of the Russian Federation is 9–17 $\mu\text{R/h}$ [7]. ** Strains that were isolated from habitats with a high level of radioactive contamination are given in bold.

colonies was cut off, together with a piece of cellophane, using a microscalpel and a binocular magnifying glass. The size of the cut-off zone of a colony was such that the length of the cut-off apical fragments of hyphae was sufficient for the growth of these fragments at the original rate [9]. The sufficient width for the cut-off piece of the colonies was determined experimentally and comprised 1.5–2 mm for the slow-growing species *C. cladosporioides* and *P. lilacinus* and 3–4 mm for the fast-growing species *A. alternata* and *M. hiemalis*. The cut piece of cellophane with apical fragments of fungal hyphae was put to a new place and kept for 15 min to allow the hyphal fragments to adapt to mechanical damage and to restore their original growth rate [9].

Preparation of hyphal specimens with certain concentrations of H₂O₂. Two milliliters of a liquid Czapek agar was poured onto the surface of a microscope slide to form an agar layer of 1.6–1.8 mm in thickness. Hydrogen peroxide solutions (10⁻⁷–10⁻¹ M in concentration) were evenly spread onto the agar surface. The solutions were prepared using 30% H₂O₂ (Sigma). The concentration of hydrogen peroxide in these solutions was controlled by measuring their optical density at 240 nm with a Specord UV-VIS spectrophotometer. The oxidation of H₂O₂ in the Czapek medium did not exceed 10% over 8 h [10]. After spreading the H₂O₂ solutions, the agar plates were kept for 15 min to allow the H₂O₂ to evenly diffuse over the agar surface. Then, the cellophane pieces with apical hyphal fragments, prepared as described above, were placed onto the surface of the agar plates. In the case of the control, the Czapek agar was used without adding H₂O₂. It should, however, be noted that aqueous solutions in equilibrium with the atmospheric air contain ~10⁻⁹ M H₂O₂, which is formed under the action of heat [11].

Observation of hyphal growth. The hyphal specimens were placed in a humid chamber at 25°C. The growth of the hyphal fragments was observed microscopically under an objective 25 × 0.5 and recorded with a CCD camera, initially, at 4-min intervals (during the first 20 min of incubation) and, then, at 20-min intervals (during the next 60 min of incubation). The hypha images were transferred to a PC with the aid of a Pixel View Station. The 0.788-mm-pixel images had a size of 258 × 193 μm.

Image processing. The digital images were processed with Adobe Photoshop 5 (Fig. 1). The length of the apical hyphal fragments was measured to the first side branch, since its position does not change in the process of growth [6]. The absolute value and the rate of hypha elongation were determined at regular intervals. The accuracy of the measurements was ±0.25 μm/min.

Data processing. The data obtained were statistically processed with the aid of the STATISTICA software package and variance analysis [12]. The effect of hydrogen peroxide was evaluated from the mean elonga-

tion rate of 20–30 hyphal fragments, which was determined for each of the H₂O₂ concentrations studied.

RESULTS

A comparative study of the growth of the microscopic fungi isolated from the radiocontaminated exclusion zone of the ChNPP and those isolated from habitats with a background level of radioactive contamination showed that, unlike the background isolates of *A. alternata* and *P. lilacinus*, exclusion-zone isolates *A. alternata* 56 and *P. lilacinus* 1941 exhibited hyphae aggregation (see Table 1 and Fig. 1). The growth rate of hyphae in the isolates with an aggregated mycelium was 1.5–2 higher than in the background isolates with an unaggregated mycelium (Table 1).

It should be noted that both the background and the exclusion-zone strains of *C. cladosporioides* had an aggregated mycelium. However, the growth rate of hyphae was lower in the case of strains 4 and 5, which were isolated from the exclusion zone of the ChNPP, than in the case of strain 396, which was isolated from a background habitat.

The study of the effect of hydrogen peroxide on the growth characteristics of the microscopic fungi isolated from the exclusion zone of the ChNPP and habitats with the background level of radioactive contamination showed that H₂O₂ influenced the growth of fungal hyphae within a wide range of concentrations (from 10⁻⁷ to 10⁻¹ M). As the concentration of H₂O₂ increased within this range, the elongation rate of the hyphae decreased to almost zero (see Fig. 2). At a concentration of 10⁻³ M, hydrogen peroxide partially inhibited the mycelial growth of all the strains under study and, at 10⁻¹ M, completely arrested growth (see Fig. 3).

The strains that were isolated from habitats with a background level of radioactive contamination were found to be more susceptible to high H₂O₂ concentrations than those from the radiocontaminated zone. Indeed, the growth of hyphae in the case of the background strains was partially inhibited by 10⁻³ M H₂O₂ (Fig. 2) and completely inhibited by 10⁻² M H₂O₂ (Fig. 3). In the presence of 10⁻³ M H₂O₂, the most H₂O₂-resistant background strain, *A. alternata* 60, retained its growth rate of hyphae at a level of 5–10% of the control (Fig. 3). In the presence of 10⁻² M H₂O₂, the growth of this strain was arrested by the 10th min of incubation (Fig. 2).

In general, the H₂O₂ susceptibility of the strains isolated from the radiocontaminated zone was an order of magnitude lower. Indeed, the elongation rate of the hyphae of these strains was partially inhibited by 10⁻² M H₂O₂ and completely inhibited by 10⁻¹ M H₂O₂ (Figs. 2, 3). Among the strains isolated from the radiocontaminated zone, the strain *A. alternata* 56 was found to be the most resistant to H₂O₂ (Figs. 2, 3). It should be noted that the H₂O₂ susceptibility of the strain *C. cladosporioides* 4, which was isolated from the exclusion

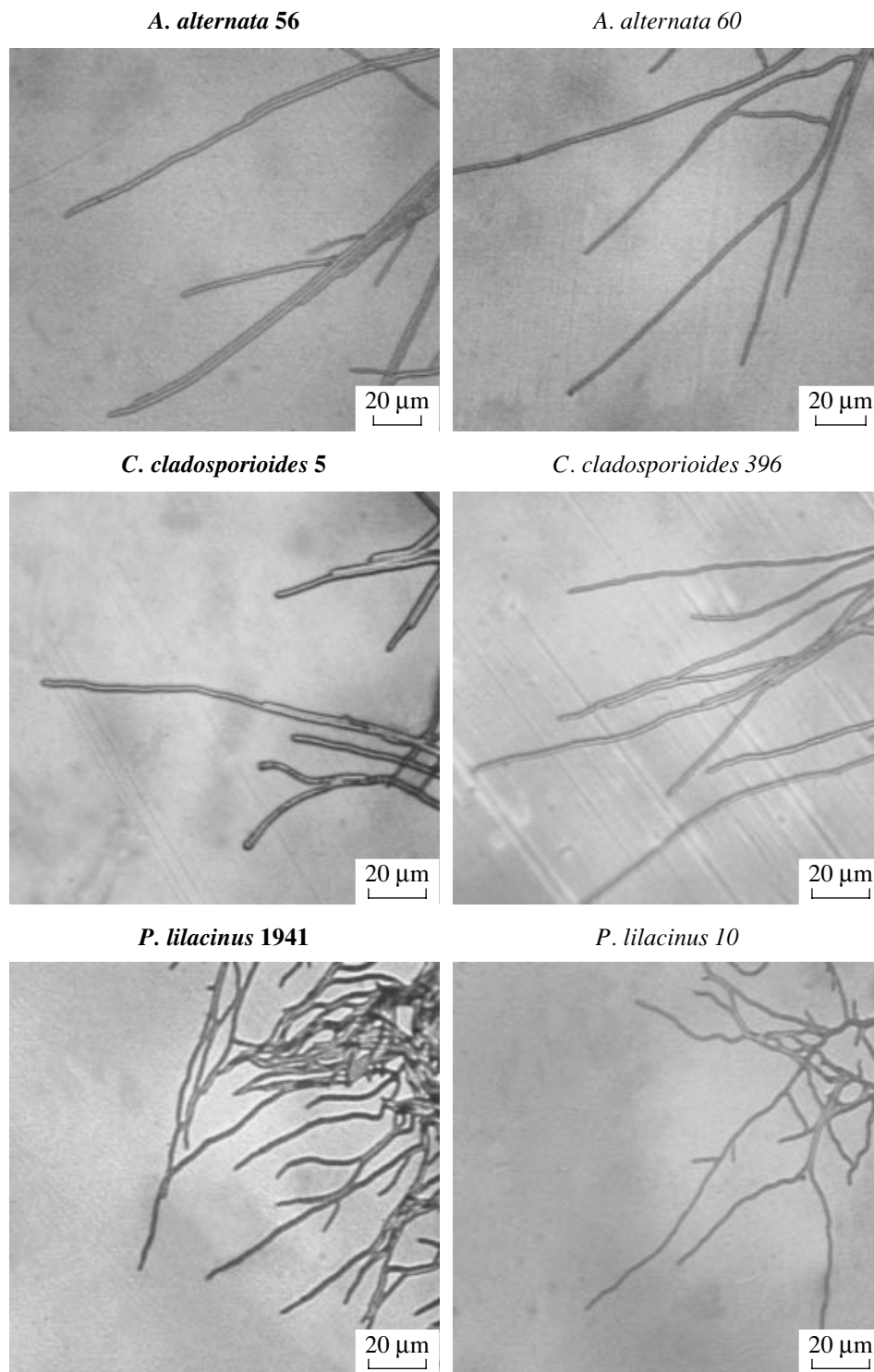


Fig. 1. Specific growth of microscopic fungi isolated from habitats with different levels of radioactive contamination. The figure shows hyphae growing on cellophane film placed onto the surface of Czapek agar containing no H_2O_2 . The names of the strains that were isolated from habitats with a high level of radioactive contamination are given in bold.

zone of the ChNPP but from a site with radioactive contamination 3 to 4 orders lower than the other exclusion-zone isolates (Table 1), was the same as in the case of the background strains (Figs. 2, 3).

Hydrogen peroxide at a concentration of 10^{-1} M completely arrested the growth of all the strains under study. The inhibition of growth was accompanied by leakage of the cytoplasm, cell damage and disruption of

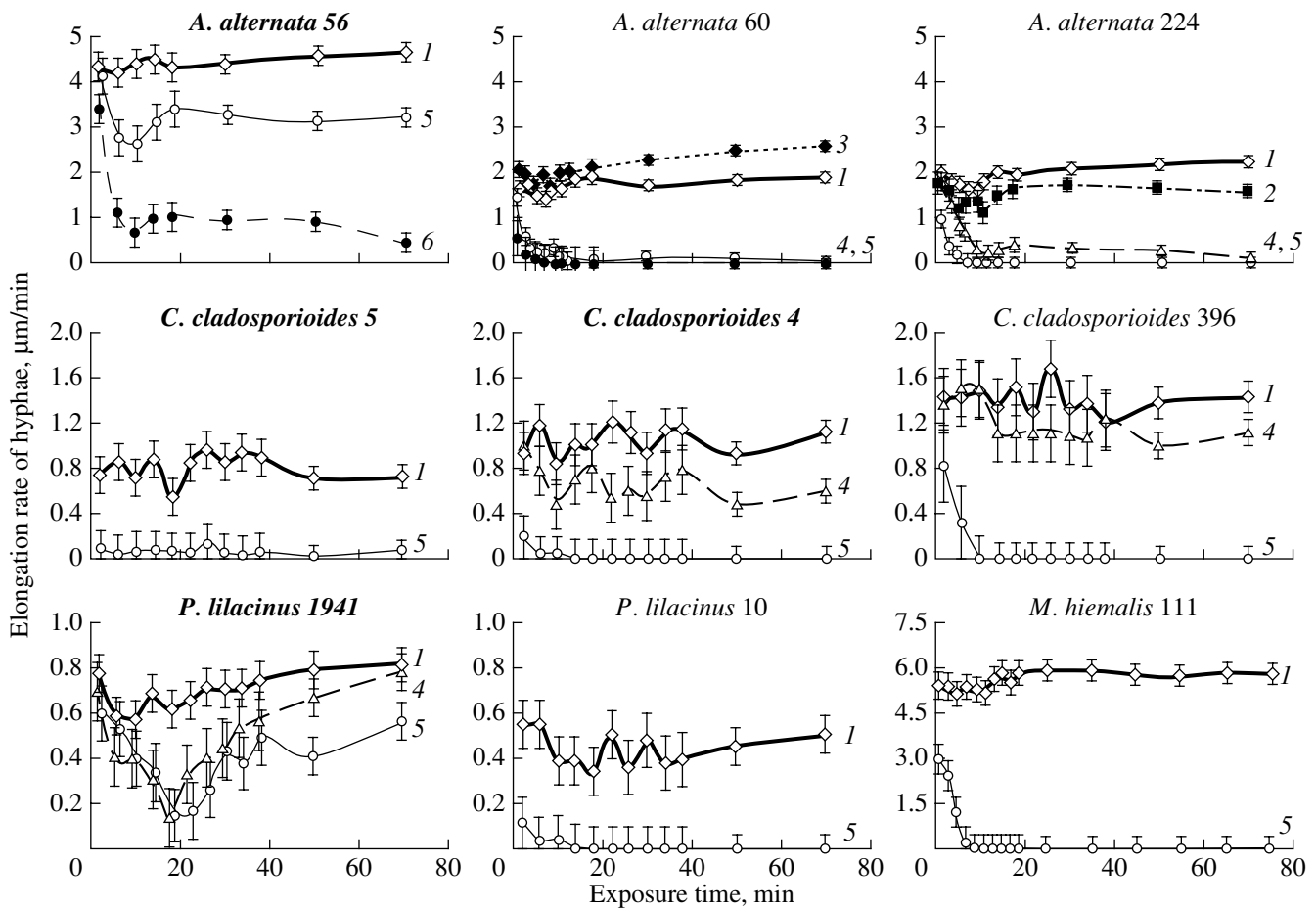


Fig. 2. Changes in the elongation rate of hyphae in the presence of different H_2O_2 concentrations (M): (1) 10^{-9} , (2) 10^{-6} , (3) 10^{-5} , (4) 10^{-4} , (5) 10^{-3} , and (6) 10^{-2} . The names of the strains that were isolated from habitats with a high level of radioactive contamination are given in bold.

the cell walls of the hyphae, and their depigmentation in the melanin-containing species *C. cladosporioides* and *A. alternata*.

Thus, the ability of the microscopic fungi to grow in the presence of various concentrations of H_2O_2 differed in different strains and depended on the level of radioactive contamination present at the site from which a particular strain was isolated.

Incubation of the fungi with H_2O_2 led to a temporal decrease in the elongation rate of their hyphae. The span of time during which this decrease occurred, as well as the new level of growth rate, were found to be dependent on the H_2O_2 concentration used (Fig. 2).

This adaptive reaction was different in different strains. For instance, raising the concentration of H_2O_2 from 10^{-6} to 10^{-4} M slowed down the growth of the hyphae of *A. alternata* 56, which was isolated from the exclusion zone of the ChNPP, to a level of 85–88% of the control value (after 16 min of incubation). At a concentration of 10^{-3} M, hydrogen peroxide decreased the elongation rate of the hyphae of this strain to a level of 60% of the control value (after 12 min of incubation).

On further incubation, the growth rate of the hyphae increased to 80% of the control. At 10^{-2} M, hydrogen peroxide diminished the growth rate of the hyphae to 70% of the control value after 4 min of incubation and to 15–20% of the control value after 8 min of incubation (Fig. 2).

As the H_2O_2 concentration increased, the growth rate of the hyphae became constant at lower values than in the case of the control. For instance, the elongation rate of the hyphae of background isolate *A. alternata* 224 stabilized at levels of 87, 75, 8, and 0% of the control at H_2O_2 concentrations equal to 10^{-7} , 10^{-6} – 10^{-5} , 10^{-4} , and 10^{-3} M, respectively (Fig. 3). The growth rate of the hyphae of exclusion-zone isolate *P. lilacinus* 1941 decreased to 25–30% of the control after 20 min of incubation in the presence of 10^{-5} , 10^{-4} , and 10^{-3} M H_2O_2 as compared to the control (Fig. 2). After 40 min of incubation in the presence of 10^{-5} and 10^{-4} M H_2O_2 , the elongation rate of the hyphae of this strain was restored to the control value. In the presence of 10^{-3} M H_2O_2 , the restoration comprised 80% of the control value.

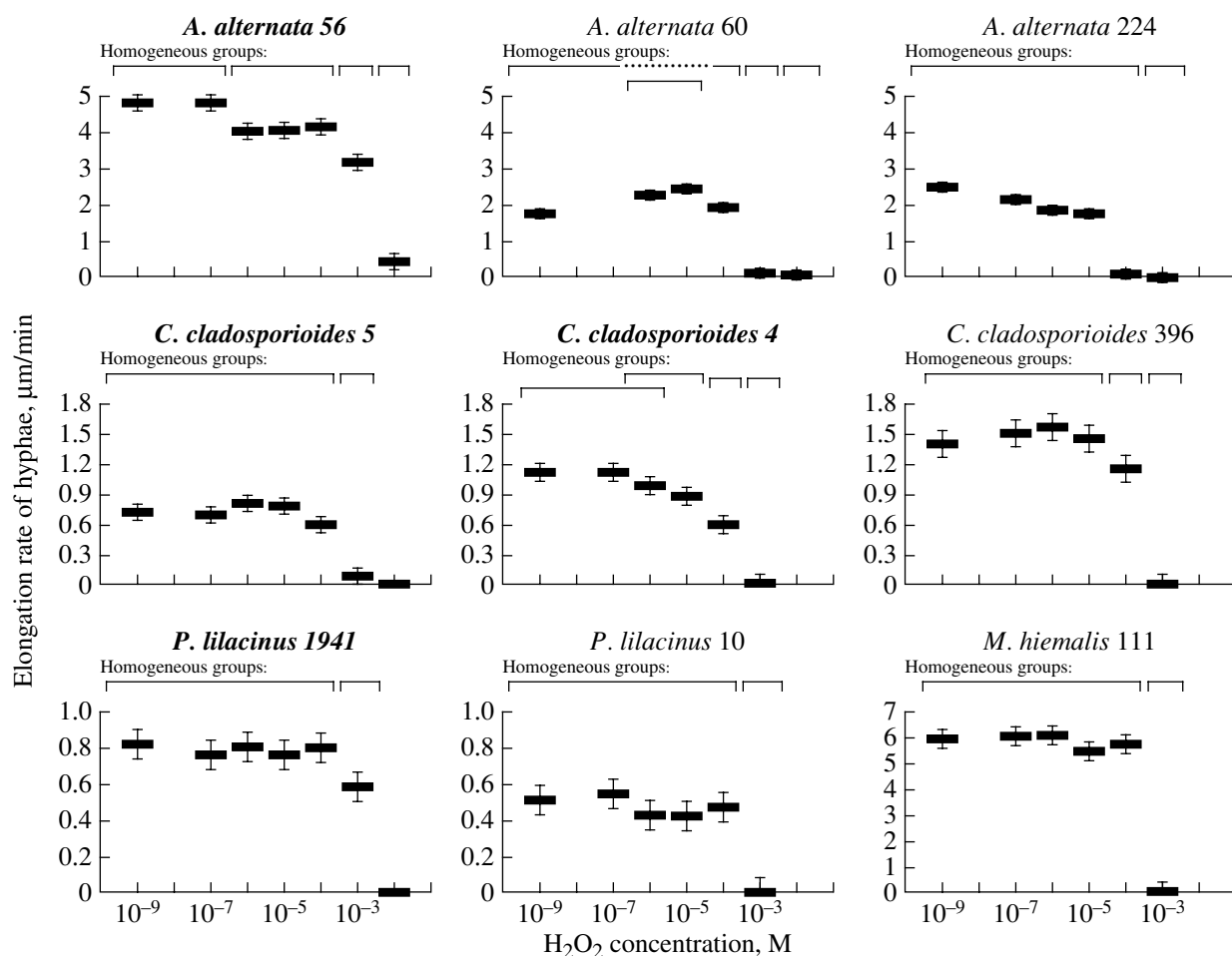


Fig. 3. Growth responses of various strains to different H_2O_2 concentrations as determined from the results of a variance analysis. Homogeneous groups comprise those H_2O_2 concentrations for which differences between the mean elongation rates of the hyphae are statistically insignificant. The names of the strains that were isolated from habitats with a high level of radioactive contamination are given in bold.

In general, the growth rate of the hyphae of the fast-growing species *A. alternata* and *M. hiemalis* reached a new constant level after 20 min of incubation in the presence of hydrogen peroxide, whereas, in the case of the slow-growing species *C. cladosporioides* and *P. lilacinus*, it reached a constant level after 40 min of incubation (see Fig. 2 and Table 2). The inhibitory H_2O_2 concentrations (10^{-3} and 10^{-2} M) suppressed the growth of the slow-growing strains over longer periods of time than in the case of the fast-growing strains.

After 20 min of incubation with hydrogen peroxide concentrations of 10^{-5} and 10^{-6} M, the elongation rate of the hyphae of the melanin-containing septate-mycelium strain *A. alternata* 60 gradually increased to reach 125–135% of the control value after 1 h of incubation (Fig. 2 and Table 2). Within 6–18 min of incubation in the presence of 10^{-4} M H_2O_2 , the hyphae of *M. hiemalis* showed a statistically significant increase in their elongation rate of 20–30% compared to the control. After 20 min of incubation, however, the growth rate of this fungus was restored (Table 2) and, then, did not differ

from the control (Figs. 2, 3). The other strains under study showed no increase in their growth rates in the presence of the investigated concentrations of H_2O_2 .

A variance analysis [12] of these data allowed us to reveal three types of growth responses of fungi to hydrogen peroxide. The first type was characterized by a constant growth rate of fungal hyphae at H_2O_2 concentrations between 10^{-9} and 10^{-4} M, a decrease in this rate at 10^{-3} M H_2O_2 , and the complete arrest of growth in the presence of 10^{-2} or 10^{-1} M H_2O_2 . The second type of growth response was characterized by a gradual decrease in the growth rate as the concentration of H_2O_2 was increased from 10^{-9} to 10^{-3} M and the complete arrest of growth in the presence of 10^{-2} or 10^{-1} M H_2O_2 . The third type was characterized by an increase in the growth rate of hyphae at H_2O_2 concentrations equal to 10^{-6} and 10^{-5} M and a slowing down (to the complete arrest) of growth at hydrogen peroxide concentrations equal to 10^{-2} or 10^{-1} M.

Table 2. Periods of time elapsed before the elongation rate of hyphae levels off to a plateau

Species	H ₂ O ₂ concentration						
	10 ⁻⁷	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴	10 ⁻³	10 ⁻²	10 ⁻¹
<i>A. alternata</i> 56*	–	18 ± 2 (↓)	18 ± 2 (↓)	18 ± 2 (↓)	14 ± 2 (↓)	8 ± 2 (↓)	0 ± 2 (0)
<i>A. alternata</i> 60	30 ± 10 (↑)	30 ± 10 (↑)	30 ± 10 (↑)	–	8 ± 2 (↓)	8 ± 2 (0)	0 ± 2 (0)
<i>A. alternata</i> 224	–	14 ± 2 (↓)	14 ± 2 (↓)	10 ± 2 (↓)	8 ± 2 (0)	0 ± 2 (0)	0 ± 2 (0)
<i>C. cladosporioides</i> 5*	–	–	–	–	2 ± 2 (↓)	0 ± 2 (0)	0 ± 2 (0)
<i>C. cladosporioides</i> 4*	–	–	–	50 ± 10 (↓)	10 ± 2 (0)	0 ± 2 (0)	0 ± 2 (0)
<i>C. cladosporioides</i> 396	–	–	–	50 ± 10 (↓)	10 ± 2 (0)	0 ± 2 (0)	0 ± 2 (0)
<i>P. lilacinus</i> 1941*	–	–	36 ± 4 (=)	36 ± 4 (=)	36 ± 4 (↓)	0 ± 2 (0)	0 ± 2 (0)
<i>P. lilacinus</i> 10	–	–	–	–	14 ± 2 (0)	0 ± 2 (0)	0 ± 2 (0)
<i>M. hiemalis</i> 111	–	–	–	18 ± 2 (=)	8 ± 2 (0)	0 ± 2 (0)	0 ± 2 (0)

* Strains that were isolated from habitats with a high level of radioactive contamination are given in bold. The symbols “–”, “↓”, “=”, “↑”, and “0” denote, respectively, the following changes in the elongation rate of hyphae as compared to the control: “no difference,” “decrease,” “restoration to the control value,” “increase,” and “decline to zero.”

The fungi that were isolated from habitats with a background level of radioactive contamination exhibited all three types of response to hydrogen peroxide: the first type was specific to *P. lilacinus* 10 and *M. hiemalis* 111, the second type was specific to *A. alternata* 224 and *C. cladosporioides* 396, and the third type was specific to *A. alternata* 60. At the same time, the fungi that were isolated from the radiocontaminated exclusion zone of the ChNPP showed only two types of response to hydrogen peroxide: the first type was specific to *C. cladosporioides* 5 and the second type was specific to *A. alternata* 56, *P. lilacinus* 1941, and *C. cladosporioides* 4.

Thus, the response of fungi to hydrogen peroxide probably depends on the level of radioactive contamination of the site from which a particular strain was isolated.

The character of the changes in the elongation rate of hyphae differed for the melanin-containing and light-pigmented fungal strains. The melanin-containing species *A. alternata* and *C. cladosporioides* exhibited all three types of growth response to hydrogen peroxide: constant growth rates of hyphae within a wide range of H₂O₂ concentrations (*C. cladosporioides* 5), gradual deceleration of growth as the H₂O₂ concentration was increased (*A. alternata* 56 and 224 and *C. cladosporioides* 4 and 396), and growth activation by low H₂O₂ concentrations (*A. alternata* 60). In contrast, the light-pigmented species *P. lilacinus* and *M. hiemalis* showed only the first type of growth response to hydrogen peroxide.

Thus, the effect of hydrogen peroxide on the growth of fungal mycelium depends on the species, the level of radioactive contamination of the site from which a particular strain was isolated, the nature of the pigment produced by this strain, and the growth rate of its hyphae.

DISCUSSION

All of the strains isolated from the exclusion zone of the ChNPP showed an aggregated growth of their hyphae, which is typical of fungi growing under unfavorable conditions [4, 5]. This aggregated growth also precedes their differentiation under the conditions of oxidative stress and, hence, like some other morphological characteristics (such as the formation of sclerotia), may be considered as a mechanism of adaptation to high concentrations of free radicals in the medium [4, 5]. In the fungi studied, the capacity for aggregated growth of hyphae has probably been acquired due to their growth under extreme environmental conditions. This suggestion is based on the fact that the strains *A. alternata* 56 and *P. lilacinus* 1941, isolated from the exclusion zone of the ChNPP, show aggregated growth, whereas the background isolates *A. alternata* 60 and 224 and *P. lilacinus* 10 do not. It should be noted that the aggregated growth of the first two strains (*P. lilacinus* 1941 and the most H₂O₂-resistant strain, *A. alternata* 56) occurs at high growth rates.

In the case of the slow-growing species *C. cladosporioides*, it is the capacity of the hyphae for aggregation, which diminishes the growth rate and metabolic activity of the exclusion-zone isolates, that is responsible for the survival and prevalence of this species in the radioactive exclusion zone [13].

The data presented in this paper show, for the first time, the growth dynamics of fungi under the action of H₂O₂. According to some authors [14], the specific growth rate is an important physiological indicator of microbial resistance to stresses such as starvation and hydrogen peroxide.

Even the small number of isolates investigated in this study (compared to the large number of species iso-

lated from the exclusion zone of the ChNPP [13]) was sufficient to reveal the specific growth responses of particular exclusion-zone species to H_2O_2 in comparison with the background isolates. These responses, particularly the specific reactions of strains and the temporal deceleration of their growth under the action of H_2O_2 followed by the stabilization of the growth rate at a new level, are probably universal responses of microbial cells to all stresses. Of interest is the fact that H_2O_2 can also stimulate the growth of background isolates, which show higher growth rates compared to the other strains studied (Table 1). The enhancement of growth and metabolic activity under the action of sublethal concentrations of stress agents, including H_2O_2 and low-activity γ radiation, is a typical response exhibited by not only microbial but also animal cells [15–17]. Bacteria are probably more resistant to H_2O_2 than microscopic fungi [14, 18].

The mycelial growth of most of the studied background isolates was arrested by 10^{-3} M H_2O_2 . This hydrogen peroxide concentration is probably crucial for many fungi occurring in the phase of active growth. For instance, this concentration of hydrogen peroxide causes an apoptosis-like phenotype and death of *Aspergillus fumigatus* cells [19] and diminishes the survival rate of *Fusarium decemcellulare* by 2–3 orders [20].

In general, the background isolates are less resistant and more diverse in their growth responses to hydrogen peroxide than the fungal strains isolated from radioactive habitats. Of great importance is the fact that these responses are persistent and preserved for up to 30 cell transfers to fresh media (Table 1), which suggests that the metabolic shifts of cells are genetically conserved.

The growth of the fungi isolated from the exclusion zone of the ChNPP is arrested by a concentration of H_2O_2 (10^{-2} M) that is an order of magnitude higher than in the case of the background isolates (Figs. 2, 3). Moreover, for the exclusion-zone isolates preliminarily exposed to 10^{-2} M H_2O_2 for 1.5 h, a subsequent 5- to 6-h incubation in an H_2O_2 -free medium restores the original elongation rate of their hyphae [6]. The only exceptions to these rules are the exclusion-zone isolate *C. cladosporioides* 4, whose growth is arrested by 10^{-3} M H_2O_2 , and the background isolate *A. alternata* 60, whose growth can be restored after exposure to the inhibitory concentration of H_2O_2 [6].

It should be noted that the adaptive response of temporal deceleration of growth under the action of sublethal concentrations of H_2O_2 (10^{-5} to 10^{-3} M) and subsequent stabilization of the growth rate at a new level took more time in the slow-growing than in the fast-growing species (Fig. 2 and Table 2). This response, which is typical of microorganisms subjected to the action of unfavorable factors, in particular, sublethal concentrations of heavy metals [15], may explain the enhanced H_2O_2 resistance of slow-growing fungi and their prevalence in the exclusion zone of the ChNPP and in the

interior of the containment structure of damaged Chernobyl Unit 4 [13].

The data obtained allow us to suggest that each strain under study is characterized by its own optimal concentration of endogenous H_2O_2 that regulates metabolic processes. In general, this concentration might be higher for dark-pigmented melanin-containing fungi than for light-pigmented ones. It is known that the resistance of cells to unfavorable environmental factors, including radiation, depends on their ability to scavenge free radicals and detoxify H_2O_2 [2, 4, 5]. A comparative study of protective antioxidant systems in the fungal strains isolated from the exclusion zone of the ChNPP and habitats with a background level of radioactive contamination may provide deeper insight into the mechanisms responsible for microbial resistance to extreme environmental conditions.

ACKNOWLEDGMENTS

We are grateful to N.N. Zhdanova for providing the strains isolated from the exclusion zone of the ChNPP and her valuable comments, L.A. Panchenko from the Department of General Ecology at the Faculty of Biology of Moscow State University for help with data processing, and to V.Yu. Sokolovskii, N.L. Rebrikova, A.G. Malygin, and A.A. Zamyatnin for their contribution to a valuable discussion of the manuscript.

REFERENCES

1. Korystov, Yu.N., Contributions of the Direct and Indirect Effects of Ionizing Radiation to Reproductive Cell Death, *Radiat. Res.*, 1992, vol. 129, no. 2, pp. 228–234.
2. Burlakova, E.B., Mikhailov, V.F., and Mazurik, V.K., System of Redox Homeostasis in the Radiation-induced Genome Instability, *Radiats. Biol. Radioekol.*, 2001, vol. 41, No. 5, pp. 489–499.
3. Gamalei, I.A. and Klyubin, N.N., Hydrogen Peroxide as a Signal Molecule, *Tsitologiya*, 1996, vol. 38, no. 12, pp. 1233–1247.
4. Hansberg, W. and Aguirre, J., Hyperoxidant States Cause Microbial Cell Differentiation by Cell Isolation from Dioxygen, *J. Theor. Biol.*, 1990, vol. 142, no. 2, pp. 287–293.
5. Sidery, M. and Georgiou, Ch.D., Differentiation and Hydrogen Peroxide Production in *Sclerotium rolfisii* Are Induced by the Oxidizing Growth Factors, Light, and Iron, *Mycologia*, 2000, vol. 92, no. 6, pp. 1033–1042.
6. Aslanidi, K.B., Ivanova, A.E., Blazheevskaya, Yu.V., Zhdanova, N.N., and Belozerskaya, T.A., Resistance of Microscopic Fungi to Oxidative Stress, *Dokl. Akad. Nauk*, 2003, vol. 392, no. 1, pp. 119–122.
7. Radiation Conditions, in *Report on the State of Natural Environment in the Russian Federation in 2000*, Moscow: Min. Prir. Res. RF, 2001, section 7, pp. 51–56.
8. *Metody pochvennoi mikrobiologii i biokhimii* (Methods in Soil Microbiology and Biochemistry), Moscow: Mosk. Gos. Univ., 1991, p. 304.

9. Aslanidi, K.B., Vachadze, D.M., Zamyatnin, A.A., Pozharskaya, T.R., Rochev, Yu.A., Selezneva, I.I., Tsyganov, M.A., and Chailakhyan, L.M., Compartmentation Determines Dynamics of Multicellular System's Growth, *Membrane Cell Biology*, 1996, vol. 10, no. 3, pp. 311–321.
10. Ichise, N., Morita, N., Hoshino, T., Kawasaki, K., Yumoto, I., and Okuyama, H., A Mechanism of Resistance to Hydrogen Peroxide in *Vibrio rumoiensis* S-1, *Appl. Environ. Microbiol.*, 1999, vol. 65, no. 1, pp. 73–79.
11. Bruskov, V.I., Masalimov, Zh.K., and Chernikov, A.V., Formation of Reactive Oxygen Species in Water under the Action of Heat, *Dokl. Akad. Nauk*, 2002, vol. 384, no. 6, pp. 821–824.
12. Variance Analysis, in *Komp'yuternaya biometrika* (Computer Biometrics), Moscow: Mosk. Gos. Univ., 1990, pp. 116–130.
13. Zhdanova, N.N., Zakharchenko, V.A., Vember, V.V., and Nakonechnaya, L.T., Fungi from Chernobyl: Mycobiota of the Inner Regions of the Containment Structure of the Damaged Nuclear Reactor, *Mycol. Res.*, 2000, vol. 104, no. 11, pp. 1421–1426.
14. Ostrowski, M., Cavicchioli, R., Blaauw, M., and Gottschalk, J.C., Specific Growth Rate Plays a Critical Role in Hydrogen Peroxide Resistance of the Marine Oligotrophic Ultramicrobacterium *Sphingomonas alaskensis* Strain RB2256, *Appl. Environ. Microbiol.*, 2001, vol. 67, no. 3, pp. 1292–1299.
15. Sentsova, O.Yu. and Maksimov, V.A., The Effect of Heavy Metals on Microorganisms, *Usp. Mikrobiol.*, 1985, no. 20, pp. 227–252.
16. Wagner, S., Hussain, M.Z., Hunt, T.K., Bacic, B., and Becker, H.D., Stimulation of Fibroblast Proliferation by Lactate-Mediated Oxidants, *World Repair Regeneration*, 2004, vol. 12, no. 3, pp. 368–373.
17. Eidus, L.Kh., *Membrannyi mekhanizm biologicheskogo deistviya malykh doz: Novyi vzglyad na problemu* (A Membrane Mechanism of the Biological Effect of Low Radiation Doses: A New Insight into the Problem), Moscow: Inst. Teoret. Eksperim. Biofiz., 2001, p. 82.
18. Samuilov, V.D., Bezryadnov, D.V., Gusev, M.V., Kitashhev, A.V., and Fedorenko, T.A., H₂O₂ Inhibits the Growth of Cyanobacteria, *Biokhimiya*, 1999, vol. 64, no. 1, pp. 60–67.
19. Mousavi, S.A. and Robson, G.D., Oxidative and Amphotericin B-Mediated Cell Death in the Opportunistic Pathogen *Aspergillus fumigatus* Is Associated with an Apoptotic-Like Phenotype, *Microbiology*, 2004, vol. 150, no. 6, pp. 1937–1945.
20. Medentsev, A.G., Arinbasarova, A.Yu., and Akimenko, V.K., Adaptation of the Phytopathogenic Fungus *Fusarium decemcellulare* to Oxidative Stress, *Mikrobiologiya*, 2001, vol. 70, no. 1, pp. 34–38.