Characteristics of Extremophylic Fungi from Chernobyl Nuclear Power Plant

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Fungi isolated from Chernobyl Nuclear Power Plant (ChNPP) where radiation dose was from 3 to 5 orders higher than the background radioactivity offered following properties: aggregation of hyphae; oligotrophy; high resistance to oxidative stress due to adaptation to low (0.2%) carbohydrate content in the medium; resistance to an order higher H₂O₂ concentration than fungi from habitats with background radiation; ability to resume growth after severe oxidative stress (10³ M H₂O₂); activation of antioxidant enzymes, and increased biosynthesis of melanin in Paecilomyces lilacinus possessing hyaline mycelium. Biochemical characteristics point that adaptation mechanisms to oxidative stress are strain specific. Ability to survive in hostile environment makes these micromycetes perspective models to study evolutionary process of extremophylic fungi.

Keywords Chernobyl fungi; strain specific properties; aggregation of hyphae; oligotrophy; high resistance to oxidative stress

1. Introduction

Of the major groups of soil microorganisms, fungi are of fundamental importance with primary roles in plant pathogenesis, symbiosis and the cycling of important nutrient elements, e.g. carbon, nitrogen, phosphorus, sulphur and many trace metals [1]. According to their trophic characteristics, the overwhelming majority of the saprophytic fungi belong to the group of decomposers, which actively destroy various vegetable and technogenic substrates [2]. Microfungi (anamorphs, producing conidiospores) represent an extensive group of organisms in soil. They have been shown to take up and translocate in the mycelium a waste array of naturally occurring as well as man-made radionuclides [3]. Dead mycelia (cell walls) made into filters have been used to adsorb heavy metals and radionuclides from industrial effluents [3].

Grassland soil saprotrophic fungi are capable of the transformation of radioactive particles with high specific activity to a soluble form. Unfortunately these soluble elements might be accumulated into food webs [4]. Absorption of radionuclides by fungi seems to be strain-specific [3]. Fungi are able to absorb not only ¹³⁷Cs, ¹²⁵Sr and ¹⁵²Eu, but also such radioisotopes as ²³⁹Pu and ²⁴¹Am [4, 5]. This suggests that fungi could be long-term retainers of radionuclides in the environment [3].

To cope such environment these fungi worked out resistance mechanisms such as asexuality, synthesis of melanin-like pigments, flexible morphology, and growth under limited nutrient content in the habitat.

Recent work from in and around the remains of the ChNPP demonstrates that soil microfungal communities have been altered by the intense radiation fallout, leading to simpler community structure and a dominance of melanin-containing (pigmented) fungal species [6]. During the last 15 years, about 2000 strains of 200 species of 98 genera of fungi have been isolated around the Chernobyl Atomic Energy Station. Some of these microfungi show formerly unknown adaptive features, such as directed growth of fungi to sources of ionizing radiation [7, 8].

The mechanisms whereby fungi arrive at sources of radionuclides in the environment and the processes that occur during the decomposition/modification of those sources are ecologically important in regulating radionuclide movement and for potential site remediation. Capacity to acquire radionuclides in micromycetes from Chernobyl appears to be an interaction between the physical nature of the radioactive source, fungal species and, presumably, their enzymatic and pigment potential.

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 [9, 10]. (Fig. 1). 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 [11]. In fungi, hydrogen peroxide is a necessary component of the intracellular signaling system in such processes as cell differentiation and proliferation [12, 13]. 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 [9].
The aim of the present work was to reveal some morphological, physiological and biochemical peculiarities of Chernobyl's microfungi allowing for adaptation to the extreme environment hostile to most eukaryotes. The individual parameters (characteristic values) of Chernobyl's fungi were also studied under oxidative stress via $H_2O_2$ treatment.

### 2. Fungal strains under investigation

Experiments were carried out with four species of microscopic fungi isolated from the exclusion zone of the 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 the structure of their mycelium (either septate or nonseptate), the presence of melanin pigments in the cell wall (dark-colored and hyaline mycelia), and in their growth rate (slow- and fast-growing strains).

#### Table 1  Fungal isolates studied

<table>
<thead>
<tr>
<th>Species, No strain</th>
<th>Isolates habitats</th>
<th>Level of radioactive pollution, Bk/kg</th>
<th>Mycelial structure</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alternaria alternata</em> (Fr.:Fr.) Keissler 1912</td>
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<tr>
<td>56 ChNPP territory. Wall of the 4th block. Isolated 1998.</td>
<td>$\alpha=500$ Bq/cm$^2$; $\beta=2\times10^4$ Bq/cm$^2$; $\gamma=700$ mR/h</td>
<td>Melanin-containing, septate</td>
<td></td>
</tr>
<tr>
<td>60 Moscow region. Umbric Albeluvisols. Isolated 1989.</td>
<td>Background</td>
<td>-**-</td>
<td></td>
</tr>
<tr>
<td><em>Cladosporium cladosporioides</em> (Fres.) de Vries 1952</td>
<td></td>
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<tr>
<td>4 ChNPP territory. Soil of Red Forest. Isolated 1986.</td>
<td>$3.6 \times 10^7$</td>
<td>Melanin-containing, septate</td>
<td></td>
</tr>
<tr>
<td>5 ChNPP territory. Surface of carbon radioactive debris from the reactor. Isolated 1993.</td>
<td>$3.7 \times 10^9$</td>
<td>-**-</td>
<td></td>
</tr>
<tr>
<td>396 Liov region. Chernozems Anthropic. Isolated 1957.</td>
<td>Background *</td>
<td>-**-</td>
<td></td>
</tr>
<tr>
<td><em>Mucor hiemalis</em> Wehmer 1903</td>
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<tr>
<td><em>Paecilomyces lilacinus</em> (Thom) Samson 1974</td>
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<tr>
<td>1941 ChNPP territory. Soil of Red Forest. Isolated 1994.</td>
<td>$5.9 \times 10^7$</td>
<td>hyaline, septate</td>
<td></td>
</tr>
<tr>
<td>1492 ChNPP territory. Soil of Red Forest. Isolated 1992.</td>
<td>$2.7 \times 10^7$</td>
<td>-**-</td>
<td></td>
</tr>
<tr>
<td>744 ChNPP territory. Soil of Western Track. Isolated 1992.</td>
<td>$1.3 \times 10^7$</td>
<td>-**-</td>
<td></td>
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<tr>
<td>1786 ChNPP territory. Soil of Red Forest. Isolated 1993.</td>
<td>$1.2 \times 10^2$</td>
<td>-**-</td>
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</tbody>
</table>
The second part of the work was devoted to investigation of *P. lilacinus* strains widely distributed all around the world. The genus *Paecilomyces* is a cosmopolitan filamentous fungus, and the strain *P. lilacinus* has been isolated from a wide range of habitats including cultivated and uncultivated soil as well as from soil with anthropogenic effects. In Chernobyl there is a trend of change in dominance of fungal species with radiation level. At present *P. lilacinus* is one of the indicators of high levels of radionuclide soil contamination (3.7 x 10^6–3.7 x 10^8 Bq/kg) there [3].

All of the strains isolated from around ChNPP showed an aggregated growth of their hyphae (Fig 2)

![Aggregated hyphae of Alternaria alternata 56 isolated from ChNPP, inner wall of contaminated structure. 1998. Radioactivity level is shown in Table 1. 40 min growth on agar Chapek media.](image)

The hyphal aggregation 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.

3. Olygotrophic growth

It has been shown previously that some species of Chernobyl habitat can grow as olygotrophs [16]. Olygotrophs are microorganisms that can grow in the presence of low concentrations of nutrients, or even nutrients appear to be nonexistent. The term olygotrophy is generally used to describe the strategy used by microorganisms to grow on low concentration of organic carbon, more strictly referred to as olygocarbotrophy [15]. Oligotrophic microorganisms cause a number of problems associated with biocontamination and biodeterioration, e.g. contaminate bottled drinking water and colonize solid surfaces such as glass, metals and electronic components.

On investigation of growth peculiarities of *P. lilacinus* strains it was found that in all the strains tested radial growth rates were higher under glucose level lower than 0,5% (Fig. 2). Strains of *P. lilacinus* from around Chernobyl showed higher radial growth rates under lowest glucose concentrations (0,02%-0,2%) in comparison with the strains from habitats with background radioactivity.

The data presented confirm previous investigations [6] and allow referring to *P. lilacinus* Chernobyl strains studied as olygocarbotrophs for their ability to show maximal growth rate under low glucose content.
Future investigations will unravel how these fungi could provide life energy: through CO₂ fixation, acceptance of volatile compounds or using melanins as energy source [15-18].

4. Oxidative stress and fungal growth under various glucose concentrations

According to some authors the specific growth rate is an important physiological indicator of microbial resistance to stresses such as starvation and hydrogen peroxide [19]. The data presented in this paper show that H₂O₂ increase in the medium (10⁻⁹ - 10⁻³M) influenced elongation rate of fungal hyphae (Fig. 4). The growth of fungi isolated from habitats with a background level of radioactive contamination was stopped by 10⁻⁴ - 10⁻³M H₂O₂, whereas the growth of fungi that were isolated from habitats with high levels of radioactive contamination was arrested only by 10⁻² - 10⁻¹M H₂O₂.

Adaptation slowdown reaction has been found as a result of the first 10–30 min H₂O₂ treatment most profound in the ChNPP P. lilacinus 1941 strain. Under 10⁻² M H₂O₂ growth of 1941 resumed with 20% loss in growth rate after such a slowdown. Cessation of growth of the control strain 10 (Table 1) was observed under these conditions [20].

One of the important physiological criteria of resistance to oxidative stress is the ability to re-establish hyphal growth after H₂O₂ (10⁻²M, 90 min) treatment. All the fungi from Chernobyl habitat were able to regenerate after 10⁻³M H₂O₂ for 90 min. Cessation of growth in all the fungi from background habitats except for A. alternata 60 (40% resumed growth) was observed [21].

Thus Chernobyl fungi were more resistant to oxidative stress. They could cope with severe oxidative stress: an order higher H₂O₂ concentration than isolates from habitats with background radioactivity.

Among strains investigated, those isolated from “background” habitats (A. alternata 60, A. alternata 224, C. cladosporioides 396, and P. lilacinus 10) [Table 1] exhibited three types of responses to H₂O₂ (Fig.4 A).

Type 1. An initial increase in the elongation rate of hyphae upon an increase of H₂O₂ concentration from 10⁻⁶ to 10⁻⁵ M, followed by a decrease of hyphal elongation and complete growth arrest at 10⁻²–10⁻¹ M (Fig.4 A, strain 2).

Type 2. An initial decrease of hyphal elongation and complete growth arrest at 10⁻³ M (Fig.4 A, strain 1).

Type 3. A slowdown of hyphal elongation at 10⁻² M, whereas the growth of fungi from background habitats stopped at 10⁻¹ M (Fig.4 A, strain 3).

Fig. 4 Scheme illustrating different types of hyphal growth of the soil fungi isolated from habitats with background radiation (A) and the strains isolated in the Chernobyl zone (B) under various H₂O₂ concentrations. Dependences of relative rates of leading hyphal elongation (V/V₀, %) for different species and strains of filamentous fungi on the H₂O₂ concentration (C, M), in an agar nutrient medium are shown. The rates were normalized to the corresponding values of elongation rates, obtained in the hydrogen peroxide-free medium (10⁻⁸ M H₂O₂) [22]. Designations: (1) M. hiemalis; (2) A. alternata 60; (3) A. alternata 224; (4) C. cladosporioides 396; (5) P. lilacinus 10; (6) A. alternata 56; (7) C. cladosporioides 5; (8) C. cladosporioides 4; (9) P. lilacinus 1941.
Type 2. A gradual decrease of the hyphal elongation rate as a result of increasing H$_2$O$_2$ concentration. Growth was completely inhibited at $10^{-3}$–$10^{-2}$ M of H$_2$O$_2$ (Fig. 4 A, strain 3).

Type 3. A constant rate of hyphal growth observed at $10^{-9}$–$10^{-4}$ M H$_2$O$_2$, a growth rate decrease at $10^{-3}$ M H$_2$O$_2$, and growth arrest at $10^{-2}$ M H$_2$O$_2$ (Fig. 4 A, strain 5).

Strains isolated from the radioactively contaminated zones (A. alternata 56, C. cladosporioides 4, 5, P. lilacinus 1941) [Table 1] showed only second and third types of response (Fig. 4 B). The broadest H$_2$O$_2$ concentration activity range allowing hyphal growth ($10^{-2}$–$10^{-1}$ M) was described in A. alternata 56 isolated from the wall of the 4th block of the ChNPP (Table 1). In all the studied strains of filamentous fungi elongation rates of the leading hyphae determined on H$_2$O$_2$-free media ($10^{-6}$ M) [22], fell within a range from 0.5±0.2 to 5.5±0.5 µm/min [14].

These different types of growth of microscopic fungi under varying H$_2$O$_2$ concentrations (especially A and B) were basis for creation of a mathematical model of fungal growth in a gradient of H$_2$O$_2$ [23]. As it was mentioned earlier, upon interaction with water, all types of radiation sources generate reactive oxygen species (ROS), primarily H$_2$O$_2$, as the most stable ROS. These isolated radiation sources create local gradients of H$_2$O$_2$ in moist substrates that affect the growth rates of microscopic fungus hyphae. Unfortunately only a few studies have been devoted to the directional growth of hyphae towards these sources of ionizing radiation (hot particles, or carbon-based radioactive graphite from the 4th block ChNPP 1-100nm size) [7, 8].

Analysis of the model worked out revealed that H$_2$O$_2$ action on fungal growth apparently determines the radioactivity level that enables overgrowth of the hot particles by fungi [23].

Upon comparative study of fungi from Chernobyl zone and fungi from habitats with background radiation the question arises what fungal species are more stressed growing under various glucose concentrations? To answer the question protein carbonyls of P. lilacinus strains from different habitats grown under various glucose concentrations have been studied.

It was revealed that P. lilacinus 1941 from Chernobyl and the background strain 10 were more stressed than the radioactive strain 1492 and background strain SM upon growth on all the glucose concentrations tested (Fig. 5). However H$_2$O$_2$ treatment revealed that the Chernobyl fungi growing under optimal growth conditions (0,2% glucose) were the most resistant ones. SM strain from the cleanest soil was stressed severely under all the glucose concentrations tested.

5. Antioxidant resistance systems of Chernobyl strains

The following work was devoted to enzymatic antioxidant defense systems: superoxide dismutase (SOD) and catalase, the main antioxidant (AO) defense mechanisms in animal cells, and melanins – the prevailing pigments of Chernobyl fungi previously not detected in Paecilomyces spp. (Fig. 6A, B).

Profound SOD and catalase activities were determined only in the background strain SM under all the glucose concentrations tested. SOD activity was high in SM as well as 1492 from ChNPP where it increased under H$_2$O$_2$ on 2% glucose only (Fig. 6A). Catalase activity was high in SM and increased with arising glucose concentration (Fig. 6B). Thus activity of AO enzymes appeared to be an individual property of tested strains irrespectively to their habitat. It is of interest that melanin amount in 1941 was two times higher than in 1492.
Melanin pigments are found in all biological kingdoms. Melanins are complex molecules with a variety of properties able to absorb all types of electromagnetic radiation [25]. They are capable of both: energy transduction and shielding. It has been proposed recently that melanins have functions analogue to other energy harvesting pigments such as chlorophylls [17, 18]. Antioxidant properties of fungal melanins have been studied long ago. Antioxidant capacity of fungal melanins is similar to AO activity of macrophages [26]. Modern investigations of Ascomycetes and Fungi Imperfecti have demonstrated convincingly that melanins of these fungi mainly originate from 1,8-dihydroxynaphthalene [16, 27].

Melanins in P. lilacinus were detected by ESR method (Fig. 7). Electron spin resonance spectroscopy (ESR) of dried mycelia of each P. lilacinus spp. showed the presence of stable free radical population, distinguishing of melanin [28]. This population varies among Paecilomyces strains. The highest numbers of stable free radicals were revealed in Chernobyl strain 1941 (4,6x10^{16} spins/g) and lowest in the strain 10 (1,4x10^{16} spins/g).

Dominance of melanin containing (pigmented) fungal species with higher radionuclide absorption capacity has been observed in around Chernobyl lately [3]. Thus finding the melanins in P. lilacinus strains indicators of high radioactive contamination at present confirms the pivotal role of these pigments in adaptation to extreme conditions of ChNPP.

6. Conclusion

Fungi from around ChNPP show aggregation of hyphae, increased resistance to oxidative stress, certain growth peculiarities under H_{2}O_{2} treatment, increased growth rate under low glucose concentrations. Adaptation to low glucose content in the medium (0.2%) is coupled with an increased resistance to oxidative stress. These fungi demonstrate strain-specific mechanisms of antioxidant defense.

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References


