

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

**Degradative properties of two newly isolated strains of the ascomycetes *Fusarium oxysporum* and *Lecanicillium aphanocladii***

**This is the author's manuscript**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/1691291> since 2019-02-08T14:30:12Z

*Published version:*

DOI:10.1007/s10123-018-0032-z

*Terms of use:*

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

1                   **Degradative Properties of Two Newly Isolated Strains of the Ascomycetes**

2                   *Fusarium oxysporum* and *Lecanicillium aphanocladii*

3  
4                   Natalia N. Pozdnyakova<sup>1</sup>, Giovanna C. Varese<sup>2</sup>, Valeria Prigione<sup>2</sup>,

5                   Ekaterina V. Dubrovskaya<sup>1</sup>, Svetlana A. Balandina<sup>1</sup>, Olga V. Turkovskaya<sup>1</sup>

6                   <sup>1</sup>*Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, Russia*

7                   <sup>2</sup>*Department of Life Sciences and Systems Biology, University of Turin, Turin, Italy*

8  
9                   **Corresponding author**

10                  Name: Natalia Pozdnyakova

11                  Postal address: IBPPM RAS, 13 Prospekt Entuziastov, Saratov 410049, Russia

12                  E-mail: [pozdnyakova\\_n@ibppm.ru](mailto:pozdnyakova_n@ibppm.ru) Phone: +78452970403

13

14

## 15 **Abstract**

16 Two ascomycete strains were isolated from creosote-contaminated railway sleeper wood. By using a polyphasic  
17 approach combining morpho-physiological observations of colonies with molecular tools, the strains were  
18 identified as *Fusarium oxysporum* Schltdl. (IBPPM 543, MUT 4558; GenBank accession no. MG593980) and  
19 *Lecanicillium aphanocladii* Zare & W. Gams (IBPPM 542, MUT 242; GenBank accession no. MG593981).  
20 Both strains degraded hazardous pollutants, including polycyclic aromatic hydrocarbons, anthraquinone-type  
21 dyes, and oil. Oil was better degraded by *F. oxysporum*, but the aromatic compounds were better degraded by *L.*  
22 *aphanocladii*. With both strains, the degradation products of anthracene, phenanthrene, and fluorene were 9,10-  
23 anthraquinone, 9,10-phenanthrenequinone, and 9-fluorenone, respectively. During pollutant degradation, *F.*  
24 *oxysporum* and *L. aphanocladii* produced an emulsifying compound(s). Both fungi produced extracellular Mn-  
25 peroxidases, enzymes possibly involved in the fungal degradation of the pollutants. This is the first report on the  
26 ability of *L. aphanocladii* to degrade four-ring PAHs, anthraquinone-type dyes, and oil, with the simultaneous  
27 production of an extracellular Mn-peroxidase.

28

29 **Keywords** *Fusarium oxysporum* • *Lecanicillium aphanocladii* • degradation • pollutants • Mn-peroxidase

30

## 31 **Introduction**

32 Ascomycetes are a large group of higher fungi that are widely distributed in the world. They inhabit various  
33 ecological niches, are highly adaptable, and have different types of relationships with other organisms – from  
34 mutualism to parasitism. Many ascomycetes are both useful and harmful to humans, which makes them  
35 important for research and use in biotechnology. The degradative properties of ascomycetes are well-known also  
36 (Harms 2011; Aranda 2016).

37 *Fusarium* fungi are diverse and are omnipresent in nature. Except the well-known *Fusarium* pathogens,  
38 most species and strains are saprotrophic and live in soil, utilizing lignin, complex carbohydrates, and plant litter  
39 as their carbon sources. Some strains form a symbiosis with plants, protect them from diseases (Gordon et al.  
40 1989; Lemanceau et al. 1993), and even promote host growth, increasing plant biomass and indirectly increasing  
41 the efficiency of phytoextraction (Zhang et al. 2012).

42 *Fusarium* fungi are also implicated in the degradation of pollutants. For example, *F. oxysporum*  
43 significantly reduced the concentration of oil (Fariba et al. 2010) and degraded and mineralized anthracene,  
44 phenanthrene, and pyrene (Jacques et al. 2008). Two *F. solani* strains (H30 and H50) and one *F. oxysporum*

45 strain (H80) converted glyphosate to unidentified metabolites (Krzysko-Lupicka and Sudol 2008). In  
46 combination with *Rhodococcus erythropolis*, *F. solani* effectively degraded benzo[ $\alpha$ ]pyrene, toluene, and  
47 formaldehyde (Morales et al. 2017); and in combination with *Arthrobacter oxydans* (included in microcosms  
48 with alfalfa), *F. solani* significantly reduced the concentrations of phenanthrene, pyrene, and  
49 dibenz[*a,h*]anthracene (Thion et al. 2013).

50         Whereas *Fusarium* fungi have been studied for more than 100 years, the genus *Lecanicillium* was  
51 introduced relatively recently (Zare and Gams 2001). The study of newly isolated *Lecanicillium* strains relates  
52 primarily to their entomopathogenic properties (Ali et al. 2017). However, some *Lecanicillium* species are  
53 biotechnologically beneficial. For example, *L. muscarium* is a potent producer of extracellular cold-resistant,  
54 chitin-hydrolyzing enzymes (Fenice 2016); and *L. aphanocladii* produces the pigments osporine, orivactaine,  
55 and dihydricricodimerol (Souza et al. 2016). *L. aphanocladii* was formerly known commonly as *Verticillium*  
56 *lecanii* (Zimmerman) Viegas, a producer of verticillin (Gingina et al. 1990). The degradative properties of  
57 *Lecanicillium* have been poorly studied. There are data that *L. saksenae* is a good decomposer of the pesticide  
58 pendimethalin (Pinto et al. 2012) and that *V. lecanii* is a decomposer of 2,4-dichlorophenol and 2,4-  
59 dichlorophenoxyacetic acid (Vroumsia et al. 1999) and of anthracene (Krivobok et al. 1998).

60         The degradative activities of Ascomycota are often attributed to their extracellular enzyme systems.  
61 These can be similar to the ligninolytic systems of basidiomycetes, which include lignin peroxidase, Mn-  
62 peroxidase, and laccase. These enzymes catalyze the key steps of the degradation of lignin and a wide range of  
63 aromatic pollutants (Wong 2009; Kadri et al. 2017). The ascomycete enzymes have received much study.  
64 *Fusarium* species have ligninolytic enzymes such as Mn-peroxidase, lignin peroxidase, and laccase, which are  
65 involved in stress reactions and in the degradation of lignocelluloses (Obruca et al. 2012). The laccases of  
66 ascomycetes have been well described. The role of laccase in fungal pathogenesis has been confirmed in  
67 numerous studies (Kwiatos et al. 2015), and the laccase genes of *F. oxysporum* were functionally analyzed  
68 (Cañero and Roncero 2008). However, no data seem available on the production of similar enzymes by  
69 *Lecanicillium*.

70         Thus, degradative soil-inhabiting ascomycetes that produce extracellular ligninolytic enzymes can  
71 greatly contribute to the degradation of hazardous pollutants in the environment. Searching for new species and  
72 strains of ascomycetes with degradative properties and studying their physiology, biochemistry, and ecology  
73 may promote the understanding of the role these fungi play in the self-regeneration of natural ecosystems and  
74 may promote their use in biotechnology.

75           The aims of this work were as follows: (1) to examine the degradative activity of two newly isolated  
76 ascomycete strains, *Fusarium oxysporum* Schltdl. (IBPPM 543, MUT 4558) and *Lecanicillium aphanocladii*  
77 Zare & W. Gams (IBPPM 542, MUT 242), toward a range of hazardous pollutants; and (2) to detect ligninolytic  
78 enzymes in these two fungi.

79

## 80 **Materials and methods**

### 81 Organism isolation and identification

82 The fungi had been collected from the grounds of the Saratov oil refinery. The *Fusarium* strain had been isolated  
83 from creosote-contaminated railway sleeper wood, and the *Lecanicillium* strain had been isolated from a  
84 sporocarp of *Stropharia* sp., which had been collected from oiled litter. Samples were placed in petri dishes  
85 containing an agarized basidiomycetes rich medium (Bezalel et al. 1997) with our modifications (g/L):  
86 NH<sub>4</sub>NO<sub>3</sub>, 0.724; KH<sub>2</sub>PO<sub>4</sub>, 1.0; MgSO<sub>4</sub>×7H<sub>2</sub>O, 1.0; KCl, 0.5; yeast extract, 0.5; FeSO<sub>4</sub>×7H<sub>2</sub>O, 0.001;  
87 ZnSO<sub>4</sub>×7H<sub>2</sub>O, 0.0028; CaCl<sub>2</sub>×2H<sub>2</sub>O, 0.033; D-glucose, 10.0; peptone, 10.0. The dishes were maintained in the  
88 dark at 24–26°C for 4–6 days. The visually clear part of the mycelium was collected and placed in new petri  
89 dishes containing the same medium.

90           The strains were identified as *Fusarium oxysporum* Schltdl. (IBPPM 543, MUT 4558) and  
91 *Lecanicillium aphanocladii* Zare & W. Gams (IBPPM 542, MUT 242). A polyphasic approach was used that  
92 combined morpho-physiological observations of colonies with molecular tools. DNA was extracted with a  
93 NucleoSpin kit (Macherey Nagel GmbH, Duren, DE, USA) and was amplified by PCR with specific primers for  
94 the ITS1–5.8S–ITS2 region. PCR products were purified and sequenced at the MacroGen Europe Laboratory  
95 (Amsterdam, The Netherlands). The resulting sequences were compared with the reference sequences in the  
96 online databases provided by the CBS-KNAW Collection (Westerdijk Fungal Biodiversity Institute, The  
97 Netherlands) and by the National Center for Biotechnology Information (USA). Both fungi are maintained at the  
98 IBPPM RAS Collection of Rhizospheric Microorganisms (Saratov, Russia) and at the Mycotheca Universitatis  
99 Taurinensis (Turin, Italy). The accession numbers of the sequences deposited in GenBank are MG593980 for *F.*  
100 *oxysporum* Schltdl. (IBPPM 543, MUT 4558) and MG593981 for *L. aphanocladii* Zare & W. Gams (IBPPM  
101 542, MUT 242).

102

103 Examination of degradative properties

104 The fungi were grown submerged at 24–26°C in the basidiomycetes rich medium, or they were grown at the  
105 same temperature in Kirk's medium (Kirk et al. 1986) with our modifications (g/L):  $\text{KH}_2\text{PO}_4$ , 0.2;  
106  $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ , 0.05;  $\text{CaCl}_2$ , 0.01; thiamine, 0.0025;  $\text{NH}_4\text{NO}_3$ , 0.724. The pH of Kirk's medium was maintained  
107 with 25 mM phosphate buffer (pH 6.0), and 1% maltose was the carbon and energy source. Ligninolytic enzyme  
108 production was increased by supplementing Kirk's medium with 0.1% Tween 80 (Jager et al. 1985). The fungi  
109 were grown in 250-mL Erlenmeyer flasks, each containing 100 mL of either basidiomycetes rich medium or  
110 Kirk's medium. Growth was in the dark at 24–26 °C for 14 days with orbital shaking (120 rpm).

111 Pollutants were added on day 2 of fungal growth to final concentrations of 0.05 mg/L [PAHs  
112 (anthracene, phenanthrene, fluorene, pyrene, or fluoranthene) and anthraquinone dyes (Acid Blue 62 and  
113 Reactive Blue)], and 5 g/L [oil containing alkanes (47.4%), naphthene (22.3%), low-molecular-weight aromatic  
114 hydrocarbons (4.4%), high-molecular-weight aromatic hydrocarbons (5.4%), tars (3.9%), and pyrobitumen  
115 (16.6%)]. Anthracene, fluorene, phenanthrene, pyrene, fluoranthene, Acid Blue 62, and Reactive Blue were from  
116 Fluka (Switzerland); all the other compounds were from Reachim Co. (Russia). The PAHs were added as a  
117 chloroform stock solution (5 mg per 100  $\mu\text{l}$  of chloroform). The control treatments contained 100  $\mu\text{l}$  of  
118 chloroform, which too was added on day 2 of fungal growth. At intervals, the mycelium was separated from the  
119 growth medium by filtration. The increase in mycelium production (mg of dry biomass) was found by weighing.  
120 Residual PAHs and oil were extracted from the bulk of the flasks (without separation of the growth medium  
121 from mycelia) with chloroform (5 mL, three times). The resulting extracts were evaporated and were analyzed as  
122 described below.

123

#### 124 Pollutant measurements

125 PAHs and their metabolites were analyzed by gas chromatography with a Shimadzu 2010 instrument equipped  
126 with a flame photometric detector. Compounds were separated with an HP5 column (Agilent), and helium was  
127 the carrier gas. The column temperature was kept at 200°C for 3 min, then programmed to increase to 270°C at a  
128 rate of 15°C  $\text{min}^{-1}$ , and finally kept at 270°C for 2 min. PAH oxidation products were identified with commercial  
129 9,10-anthraquinone, phenanthrene-9,10-quinone, and 9-fluorenone (retention times, 4.51, 5.04, and 4.33 min,  
130 respectively) as the marker compounds. The gas chromatography analysis was carried out at the Simbioz Center  
131 for the Collective Use of Research Equipment in the Field of Physical–Chemical Biology and  
132 Nanobiotechnology (Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy  
133 of Sciences, Saratov).

134 Oil disappearance was measured by adsorption chromatography with gravimetric termination. Oil was  
135 extracted three times with 5 mL of chloroform, and the extracts were evaporated and applied to a column of  
136  $\text{Al}_2\text{O}_3$  (10 mL). After the oil was eluted with chloroform and dried to the complete evaporation of the solvent,  
137 the amount of oil was found gravimetrically (Polunina and Kushik 1977).

138 Because the basidiomycetes rich medium is optically opaque, the decolorization of dyes was studied on  
139 Kirk's medium at pH 6.0. Aliquots (2 mL) were taken from the flasks at intervals, and dye disappearance was  
140 monitored spectrophotometrically by the change in the absorption spectra at 590 nm (Pozdnyakova et al. 2015).

141

#### 142 Enzyme activity measurements

143 Laccase activity was measured by the oxidation rate of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic  
144 acid)diammonium salt (ABTS) at 436 nm ( $\epsilon=29300 \text{ M}^{-1}\text{cm}^{-1}$ ; Niku-Paavola et al. 1988) and by the oxidation rate  
145 of syringaldazine at 525 nm ( $\epsilon=65000 \text{ M}^{-1}\text{cm}^{-1}$ ; Leonowicz and Grzywnowicz 1981). Mn-peroxidase activity  
146 was measured by the oxidation rate of 2,6-dimethoxyphenol (DMP) with  $\text{H}_2\text{O}_2$  and  $\text{Mn}^{2+}$  at 468 nm ( $\epsilon=14800 \text{ M}^{-1}\text{cm}^{-1}$ ;  
147 Heinfling et al. 1998) and by the oxidation rate of 2,7-diaminofluorene (DAF) at 600 nm ( $\epsilon=51000 \text{ M}^{-1}\text{cm}^{-1}$ ;  
148 Criquet et al. 2000) under the same conditions. Peroxidase activity was calculated as the difference between  
149 the values for DMP (or DAF) oxidation with and without  $\text{H}_2\text{O}_2$ . Lignin peroxidase was measured by the  
150 formation of the oxidation product of veratryl alcohol at 310 nm ( $\epsilon=9300 \text{ M}^{-1}\text{cm}^{-1}$ ; Tien and Kirk 1984). One  
151 unit of enzyme activity (U/mL) is defined as the amount of enzyme that oxidizes 1  $\mu\text{M}$  of substrate per min.

152

#### 153 Emulsifying activity measurements

154 The emulsifying activity of the culture medium was measured according to Cooper and Goldenberg (1987).  
155 After fungal growth (with and without the pollutants) was completed, the growth medium was mixed 2:3 with  
156 kerosene, shaken for 20 min, and left to stand at room temperature for 48 h. The emulsifying activity ( $E_{48}$ ) was  
157 calculated as the ratio of emulsion volume to total liquid volume and expressed as a percentage.

158

#### 159 Statistics

160 All experiments were run in triplicate, each having been repeated at least three times. Standard statistical  
161 methods were used to calculate the mean values and standard deviations (SD). Data were processed with  
162 Microsoft Excel 2003.

163

## 164 Results and Discussion

165 Ascomycetes have a strong degradative potential toward natural compounds and pollutants. *L. aphanocladii*  
166 IBPPM 542 and *F. oxysporum* IBPPM 543 were isolated from the same source (creosote-contaminated sleepers)  
167 in a search for new fungal strains with degradative properties. The isolated strains were checked for their ability  
168 to degrade PAHs from creosote used to preserve railway sleepers. PAHs are priority environmental pollutants,  
169 and their degradation and transformation are well known (Kadri et al. 2017).

170 In our experiments, *F. oxysporum* had weak degradative activity toward PAHs. The least recalcitrant  
171 was the three-ring fluorene, with a removal value of 42% (Fig. 1A). Possibly, the low ionization potential (7.88  
172 eV) and the relatively high solubility (1.98 mg/L) of fluorene make it accessible for fungal degradation. A  
173 metabolite from fluorene was found whose retention time (4.33 min) corresponded to that of commercial 9-  
174 fluorenone. Phenanthrene and fluoranthene were degraded by 21% and 26%, respectively. Finally, anthracene  
175 and pyrene were not degraded by this fungus.

176 Data are different on the degradation of PAHs by *Fusarium* strains. The ability to degrade anthracene is  
177 species specific: for example, *F. moniliforme* degraded about 77% of anthracene from 100 mg/L, while *F. solani*  
178 did not degrade anthracene at all (Krivobok et al. 1998). In some cases anthracene cannot be a single source of  
179 carbon and energy for *Fusarium* fungi, and the need for cometabolism was often reported.

180 The other fungus tested was *L. aphanocladii*. The reason for our interest in it is related to its typical  
181 ecological niche: *L. aphanocladii* is usually reported to be an entomopathogenic fungus. In this work, *L.*  
182 *aphanocladii* was isolated only with the traditional basidiomycetes rich medium containing peptone and yeast  
183 extract; therefore, it can be considered a saprotroph.

184 *L. aphanocladii* IBPPM 542 intensely degraded all the PAHs, removing 40 to 80% for three-ring PAHs  
185 (anthracene, phenanthrene, and fluorene) and about 50% of those with four condensed rings (pyrene,  
186 fluoranthene) (Fig. 1A). Some degradation metabolites were identified. These included 9,10-anthraquinone  
187 (retention time, 4.51 min), 9,10-phenanthrenequinone (retention time, 5.04 min), and 9-fluorenone (retention  
188 time, 4.33 min), the products of degradation of anthracene, phenanthrene, and fluorene, respectively.

189 The appearance of the degradation products and the disappearance of the substrate were checked only  
190 after 14 days of growth. In separate experiments, in which the growth time of both fungi was extended to 28  
191 days, the degradation products disappeared and phthalic acid was simultaneously formed (data not presented).

192 Oil and oil products are ubiquitous and the most abundant environmental pollutants. Undoubtedly,  
193 ascomycetes, which are a large part of the soil microbiota, participate in the degradation and detoxification of



194 oil hydrocarbons. Both ascomycetes used in this study were active oil degraders, with 60% of oil transformed  
195 with *L. aphanocladii* and 84% with *F. oxysporum* (Fig. 1A).

196 The degradation by both fungi of all pollutants used in this study was accompanied by the production  
197 of an emulsifying compound(s). Emulsifying activity of the medium ( $E_{48}$ ) varied from 6.2 to 41%, depending  
198 on the fungal species and on pollutant solubility (Fig. 1B). No emulsifying activity was detected in the control  
199 (pollutant-free) treatments. The production of a biosurfactant that increases the solubility of hydrophobic  
200 compounds was found earlier in the basidiomycetes *Coriolus versicolor* and *Pleurotus ostreatus* (Arun et al.  
201 2008; Nikiforova et al. 2009). Biosurfactant production by ascomycetes was described, too (Bhardwaj et al.  
202 2013). However, this report is the first to describe the production of an emulsifying compound(s) by *Fusarium*  
203 and *Lecanicillium* in response to the presence of hydrophobic compounds in their growth medium.

204 As said above, 9,10-anthraquinone was a metabolite from PAH degradation by the fungi tested. The  
205 three-ring structure of anthraquinone occupies the centers of the molecules of synthetic anthraquinone-type dyes,  
206 which are highly resistant to biodegradation (Eichlerova et al. 2007). Synthetic dyes are also hazardous  
207 pollutants, entering the environment through runoff from paint-and-varnish and textile facilities.

208 Of the wide range of anthraquinone dyes available, those used most often as models are Poly R-478 and  
209 Remazol Brilliant Blue SN4R (Liu et al. 2004). Dyes of this group can be used to screen fungi for the ability to  
210 produce extracellular ligninolytic peroxidases and laccases.

211 In our experiments, both fungi decolorized the two anthraquinone-type dyes used, Acid Blue 62 and  
212 Reactive Blue 4. As expected, *L. aphanocladii*, an active degrader of aromatic compounds, intensely decolorized  
213 the dyes (to a level as high as 70%; Fig. 2).

214 Data on the decolorization activity of *F. oxysporum* and *L. aphanocladii* may indirectly point to the  
215 production of ligninolytic enzymes by these fungi. Therefore, we next measured the activities of the main  
216 ligninolytic enzymes (lignin peroxidase, Mn-peroxidase, and laccase) during pollutant degradation and in the  
217 control (pollutant-free) treatments.

218 In the controls, no ligninolytic enzyme activity was found. In polluted environments, both fungi  
219 produced one type of ligninolytic enzyme. The enzyme was active in the presence of  $H_2O_2$ , and 0.5 mM  $Mn^{2+}$   
220 produced a 2- to 3-times increase in its activity. The enzyme did not oxidize veratryl alcohol and  
221 syringaldazine, the test substrates for lignin peroxidase and laccase, respectively. From these data, the enzyme  
222 was tentatively identified as an Mn-peroxidase.

223 Fig. 3A1 presents the time course of Mn-peroxidase production by *F. oxysporum* (A) and *L.*  
224 *aphanocladii* (B) during PAH degradation. The most active inducers of *F. oxysporum* peroxidase were  
225 phenanthrene, fluoranthene, and/or their degradation products. Although anthracene and pyrene were not  
226 actively degraded by *Fusarium*, they too induced peroxidase activity (Fig. 3A1). Fig. 3A2 shows the data on  
227 polyacrylamide gel electrophoresis (PAGE) under nondenaturing conditions. The enzyme was detected only  
228 after the gels had been stained with *o*-dianizidine in the presence of H<sub>2</sub>O<sub>2</sub>. Throughout growth, *F. oxysporum*  
229 produced only one isoform of Mn-peroxidase, regardless of the PAH used.

230 *L. aphanocladii*, too, produced Mn-peroxidase in response to the presence of pollutants in the medium  
231 (Fig. 3B1). Best peroxidase induction was achieved with anthracene and/or its degradation products. Only one  
232 enzyme was detected by nondenaturing PAGE after the gels had been stained with *o*-dianizidine and H<sub>2</sub>O<sub>2</sub> (Fig.  
233 3B2). Throughout growth, *L. aphanocladii* produced only one isoform of Mn-peroxidase, regardless of PAH  
234 used.

235 For crude enzyme preparations, the fungi were grown until peroxidase production was maximal, after  
236 which the mycelium was separated from the culture medium by filtration and the culture liquid was concentrated  
237 by ultrafiltration on Amicon PM10 and was used as a source of crude peroxidases. Some catalytic properties of  
238 these peroxidases have been investigated. Both enzymes oxidized ABTS, DAF, and DMP only in the presence of  
239 H<sub>2</sub>O<sub>2</sub>. The reaction was largely stimulated by Mn<sup>2+</sup> – by 3 and 2 times for the peroxidases of *F. oxysporum* and  
240 *L. aphanocladii*, respectively. These properties make these enzymes similar to the Mn-peroxidases of  
241 ligninolytic basidiomycetes (Wong 2009).

242 The Ascomycota have been mainly studied for laccase production, and several reviews have  
243 summarized laccase production and use on an industrial scale for the processing of paper pulp and for the  
244 discoloration of dyes. Ascomycete laccases have been comprehensively studied and some have been sequenced  
245 (Fernaud et al. 2006; Dekker et al. 2007; Castilho et al. 2009). *F. solani* produced three ligninolytic enzymes in  
246 the presence of different inducers (Obruca et al. 2012). Because H<sub>2</sub>O<sub>2</sub> significantly increased the activities of  
247 Mn-peroxidase, lignin peroxidase, and laccase, the authors suggested that these enzymes can be involved in  
248 stress response to H<sub>2</sub>O<sub>2</sub> (Obruca et al. 2012). The participation of *Fusarium* enzymes in the degradation of PAHs  
249 is still a matter of discussion. For example, Wua et al. (2010b), using *F. solani* to treat mangrove deposits  
250 contaminated by PAHs, showed that laccase is involved in the degradation of anthracene and  
251 benzo[a]anthracene, whereas lignin- and Mn- peroxidase were not detected. The laccase was isolated, purified,  
252 and characterized (Wua et al. 2010a).

253 In *F. oxysporum* cultures used to transform the aromatic components of the dry residue from an olive  
254 mill, Sampedro et al. (2007) detected the activities of Mn-peroxidase and Mn-independent peroxidase but did not  
255 detect laccase activity. No information on Mn-peroxidase production by *Lecanicillium* seems available.

256

### 257 **Conclusions**

258 Both fungi were degradative toward a range of hazardous pollutants, including PAHs, anthraquinone-  
259 type dyes, and oil. Both produced peroxidase (presumably Mn-peroxidase) and an emulsifying compound(s) in  
260 response to the presence of the pollutants in the growth medium. These properties may be necessary for the  
261 survival of the fungi in polluted environments and for the degradation of pollutants. The physiological  
262 peculiarities of *Fusarium* and *Lecanicillium*, including the functions of the peroxidases and an emulsifying  
263 compound(s), and their participation in the natural cleansing of contaminated environments will be the subject of  
264 further work.

265

266 **Acknowledgements.** This research was supported by a grant from the Russian Science Foundation  
267 (project no. 16-14-00081). We are grateful to Dr. M.P. Chernyshova (IBPPM RAS) for gas chromatographic  
268 analysis. We are also grateful to Dmitry N. Tychinin for his assistance in preparation of the English text of this  
269 paper.

270

### 271 **References**

272 Ali S, Zhang C, Wang Z, Wang X, Wu J, Cuthbertson A, Shao Z, Qiu B (2017) Toxicological and biochemical  
273 basis of synergism between the entomopathogenic fungus *Lecanicillium muscarium* and the insecticide  
274 matrine against *Bemisia tabaci* (Gennadius). *Sci Rep* 20: 46558

275 Aranda E (2016) Promising approaches towards biotransformation of polycyclic aromatic hydrocarbons with  
276 Ascomycota fungi. *Curr Opin Biotechnol* 38:1-8

277 Arun A, Raja P, Arthi R, Ananthi M, Kumar K, Eyini M (2008) Polycyclic aromatic hydrocarbons (PAHs)  
278 biodegradation by basidiomycetes fungi, *Pseudomonas* isolate, and their cocultures: Comparative in vivo and  
279 *in silico* approach. *Appl Biochem Biotechnol* 151:132-142

280 Bezalel L, Hadar Y, Cerniglia C (1997) Enzymatic mechanisms involved in phenanthrene degradation by the  
281 white rot fungus *Pleurotus ostreatus*. *Appl Environ Microbiol* 63:2495-2501

- 282 Bhardwaj G, Cameotra S, Chopra H (2013) Biosurfactant from fungi: A Review. *J Pet Environ Biotechnol*  
283 4:doi.10.4172/2157-7463.1000160
- 284 Cañero D, Roncero M (2008) Functional analyses of laccase genes from *Fusarium oxysporum*. *The American*  
285 *Phytopathological Society*. 98:509-518
- 286 Castilho F, Torres R, Barbosa A, Dekker R, Garcia J (2009) On the diversity of the laccase gene: A  
287 phylogenetic perspective from *Botryosphaeria rhodina* (Ascomycota: Fungi) and other related taxa. *Biochem*  
288 *Genet* 47:80-91
- 289 Cooper D, Goldenberg B (1987) Surface-active agents from two *Bacillus* species. *Appl Environ Microbiol*  
290 53:224-229
- 291 Criquet S, Joner E, Leyval C (2001) 2,7-Diaminofluorene is a sensitive substrate for detection and  
292 characterization of plant root peroxidase activities. *Plant Science* 161:1063-1066
- 293 Dekker R, Barbosa A, Giese E, Godoy S, Covizzi L (2007) Influence of nutrients on enhancing laccase  
294 production by *Botryosphaeria rhodina* MAMB-05. *Int Microbiol* 10:177-185
- 295 Eichlerova I, Homolka L, Benada O, Kofronova O, Hubalek T, Nerud F (2007) Decolorization of Orange G and  
296 Remazol Brilliant Blue R by the white rot fungus *Dichomitus squalens*: Toxicological evaluation and  
297 morphological study. *Chemosphere* 69:795-802
- 298 Fariba M, Simin N, Alireza M, Ramin N, Doustmorad Z, Gholam K, Abdolkarim C (2010) Phytoremediation of  
299 petroleum-polluted soils: Application of *Polygonum aviculare* and its root-associated (penetrated) fungal  
300 strains for bioremediation of petroleum-polluted soils. *Ecotoxicol Environ Safety* 73:613-619
- 301 Fenice M (2016) The psychrotolerant antarctic fungus *Lecanicillium muscarium* CCFEE 5003: A powerful  
302 producer of cold-tolerant chitinolytic enzymes. *Molecules* 21:447
- 303 Fernaud J, Marina A, González K, Vázquez J, Falcón M (2006) Production, partial characterization and mass  
304 spectrometric studies of the extracellular laccase activity from *Fusarium proliferatum*. *Appl Microbiol*  
305 *Biotechnol* 70:212-221
- 306 Gingina GM, Mitina GV, Pavlushin VA (1990) Toxigenicity of *Verticillium lecanii* (Zimmermann) viegas  
307 natural isolates. *Mycology and Phytophatology* 24:576-582 (in Russian)
- 308 Gordon T, Okamoto D, Jacobson D (1989) Colonization of muskmelon and non-susceptible crops by *Fusarium*  
309 *oxysporum* F. sp. melonis and other species of *Fusarium*. *Phytopathology* 79:1095-1100
- 310 Harms H (2011) Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. *Nature*  
311 *Reviews. Microbiology* 9:177-192

- 312 Heinfling A, Martinez M, Martinez A, Bergbauer M, Szewzyk U (1998) Purification and characterization of  
313 peroxidases from dye-decolorizing fungus *Bjerkandera adusta*. FEMS Microbiol Lett 165:43-50
- 314 Jacques R, Okeke B, Bento F, Teixeira A, Peralba M, Camargo F (2008) Microbial consortium bioaugmentation  
315 of a polycyclic aromatic hydrocarbons contaminated soil. Bioresour Technol 99:2637-2643
- 316 Jager A, Croan S, Kirk T (1985) Production of ligninases and degradation of lignin in agitated submerged  
317 cultures of *Phanerochaete chrysosporium*. Appl Environ Microbiol 50:1274-1278
- 318 Kadri T, Rouissi T, Brar SK, Cledon M, Sarma S, Verma M (2017) Biodegradation of polycyclic aromatic  
319 hydrocarbons (PAHs) by fungal enzymes: A review. J Environ Sci 51:52-74
- 320 Kirk T, Croan S, Tien M, Murtagh K, Farrell R (1986) Production of multiple ligninases by *Phanerochaete*  
321 *chrysosporium* effect of selected growth condition and use mutant strain. Enzyme Microbial Technol 8:27-32
- 322 Krivobok S, Miriouchkine E, Seigle-Murandi F, Benoit-Guyod J-L (1998) Biodegradation of anthracene by soil  
323 fungi. Chemosphere 37:523-530
- 324 Krzysko-Lupicka T, Sudol T (2008) Interactions between glyphosate and autochthonous soil fungi surviving in  
325 aqueous solution of glyphosate. Chemosphere 71:1386-1391
- 326 Kwiatos N, Ryngajłło M, Bielecki S (2015) Diversity of laccase-coding genes in *Fusarium oxysporum* genomes.  
327 Frontiers in Microbiology 6:933
- 328 Lemanceau P, Bakker P, DeKogel W, Alabouvette C, Schippers B (1993) Antagonistic effect of nonpathogenic  
329 *Fusarium oxysporum* Fo47 and pseudobactin 358 upon pathogen *Fusarium oxysporum* F. sp. Dianthi. Appl  
330 Environ Microbiol 59:74-82
- 331 Leonowicz A, Grzywnowicz K (1981) Quantitative estimation of laccase forms in some white-rot fungi using  
332 syringaldazine as a substrate. Enzyme Microbial Technol 3:55-58
- 333 Liu W, Chao Y, Yang X, Bao H, Qian S (2004) Biodecolourization of azo, anthraquinonic and  
334 triphenylmethane dyes by white rot fungi and laccase-secreting engineered strain. J Ind Microbiol Biotechnol  
335 31:127-132
- 336 Morales P, Cáceres M, Scott F, Díaz-Robles L, Aroca G, Vergara-Fernández A (2017) Biodegradation of  
337 benzo[ $\alpha$ ]pyrene, toluene, and formaldehyde from the gas phase by a consortium of *Rhodococcus erythropolis*  
338 and *Fusarium solani*. Appl Microbiol Biotechnol 101:6765-6777
- 339 Nikiforova SV, Pozdnyakova NN, Turkovskaya OV (2009) Emulsifying agent production during PAHs  
340 degradation by the white rot fungus *Pleurotus ostreatus* D1. Curr Microbiol 58:554-558

- 341 Niku-Paavola M, Karhunen E, Salola P, Raunio V (1988) Ligninolytic enzymes of the white rot fungus *Phlebia*  
342 *radiata*. *Biochem J* 254:877-884
- 343 Obruca S, Marova I, Matouskova P, Haronikova A, Lichnova A (2012) Production of lignocellulose-degrading  
344 enzymes employing *Fusarium solani* F-552. *Folia Microbiol* 57:221-227
- 345 Pinto A, Serrano C, Pires T, Mestrinho E, Dias L, Teixeira D, Caldeira A (2012) Degradation of terbuthylazine,  
346 difenoconazole and pendimethalin pesticides by selected fungi cultures. *Sci Total Environ* 435-436:402-410
- 347 Polunina AG, Kushik GI (1977) Metody analiza organicheskogo veshchestva porod, nefiti i gaza (Methods of  
348 Analyslis of Organic Matter in Rocks, Oil, and Gas). In: Ryl'kov AV (ed) Tyumen': Tr. Zap.-Sib. NIGNI,  
349 122 (in Russian).
- 350 Pozdnyakova NN, Jarosz-Wilkolazka A, Polak J, Graz M, Turkovskaya OV (2015) Decolourisation of  
351 anthraquinone-and anthracene-type dyes by versatile peroxidases from *Bjerkandera fumosa* and *Pleurotus*  
352 *ostreatus* D1. *Biocatal Biotransform* 33:69-80
- 353 Sampedro I, D'Annibale A, Ocampo J, Stazi S, García-Romera I (2007) Solid-state cultures of *Fusarium*  
354 *oxysporum* transform aromatic components of olive-mill dry residue and reduce its phytotoxicity.  
355 *Bioresource Technol* 98:3547-3555
- 356 Souza P, Grigoletto T, de Moraes L, Abreu L, Guimarães L, Santos C, Galvão L, Cardoso P (2016)  
357 Production and chemical characterization of pigments in filamentous fungi. *Microbiology* 162:12-22
- 358 Thion C, Cebren A, Beguiristain T, Leyval C (2013) Inoculation of PAH-degrading strains of *Fusarium solani*  
359 and *Arthrobacter oxydans* in rhizospheric sand and soil microcosms: microbial interactions and PAH  
360 dissipation. *Biodegradation* 24:569-581
- 361 Tien M, Kirk K (1984) Lignin-degrading enzyme from *Phanerochaete chrysosporium*: purification,  
362 characterization, and catalytic properties of a unique H<sub>2</sub>O<sub>2</sub>-requiring oxygenase. *Proc Nat Acad Sci USA*  
363 81:2280-2284
- 364 Vroumsia T, Steiman R, Seigle-Murandi F, Benoit-Guyod J-L (1999) Effects of culture parameters on the  
365 degradation of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4-dichlorophenol (2,4-DCP) by selected fungi.  
366 *Chemosphere* 39:1397-1405
- 367 Wong DWS (2009) Structure and action mechanism of ligninolytic enzymes. *Appl Biochem Biotechnol*  
368 157:174-209
- 369 Wua Y-R, Luo Z-H, Chow R, Vrijmoed L (2010a) Purification and characterization of an extracellular laccase  
370 from the anthracene-degrading fungus *Fusarium solani* MAS2. *Bioresource Technol* 101:9772-9777

371 Wua Y-R, Luo Z-H, Vrijmoed L (2010b) Biodegradation of anthracene and benzo[a]anthracene by two  
372 *Fusarium solani* strains isolated from mangrove sediments. Bioresource Technol 101:9666-9672

373 Zare R, Gams W (2001) A revision of *Verticillium* section Prostrata. IV. The genera *Lecanicillium* and  
374 *Simplicillium* gen. nov. Nova Hedwig 73:1-50

375 Zhang X, Lin L, Chen M, Zhu Z, Yang W, Chen B, Yang X, An Q (2012) A nonpathogenic *Fusarium*  
376 *oxysporum* strain enhances phytoextraction of heavy metals by the hyperaccumulator *Sedum alfredii* Hance.  
377 J Hazard Mater 229-230:361-370

378

379

380

381

382

383

384

385

386

387

388

389

390

391

392

393

394

395

396

397

398

399

400

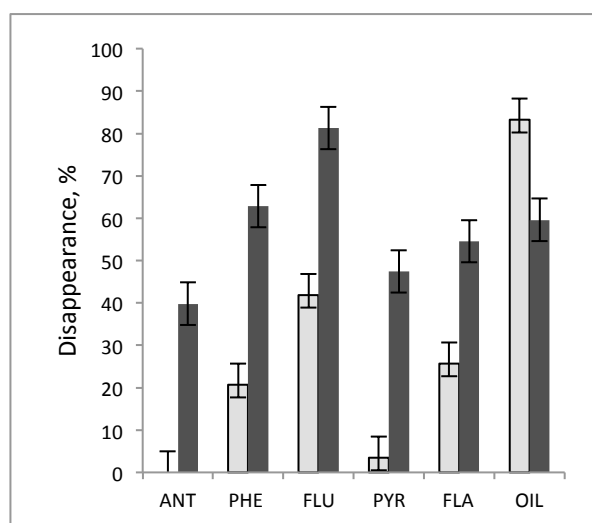
401 **Table 1** Catalytic properties of *F. oxysporum* and *L. aphanocladii* peroxidases  
402

Fungus	Activity, U/mL					
	-Mn <sup>2+</sup>			+Mn <sup>2+</sup>		
	ABTS	DMP	DAF	ABTS	DMP	DAF
<i>F. oxysporum</i>	2.8±0.7	4.1±0.5	3.6±0.4	9.5±1.6	14.0±2.4	12.2±0.8
<i>L. aphanocladii</i>	2.4±0.8	5.3±0.8	4.8±0.6	5.5±1.2	12.4±1.8	11.0±0.9

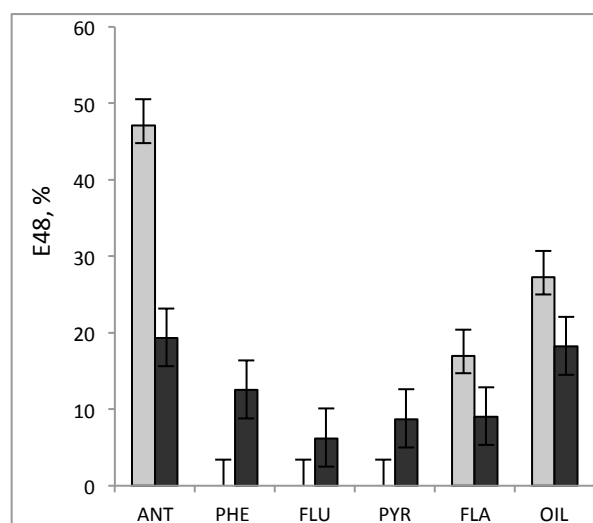
403  
404  
405  
406  
407  
408  
409  
410  
411  
412  
413  
414  
415  
416  
417  
418  
419  
420  
421



A



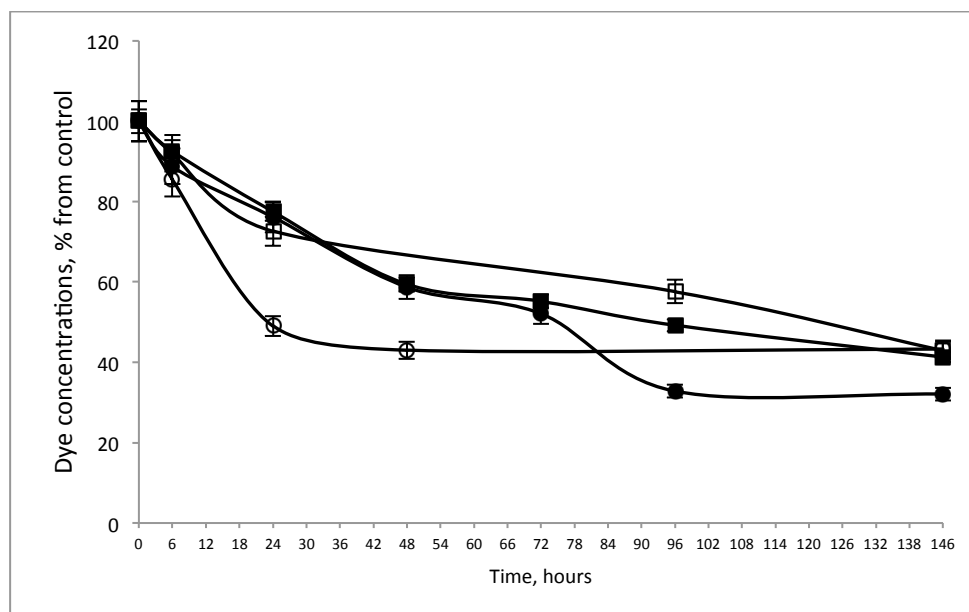
B



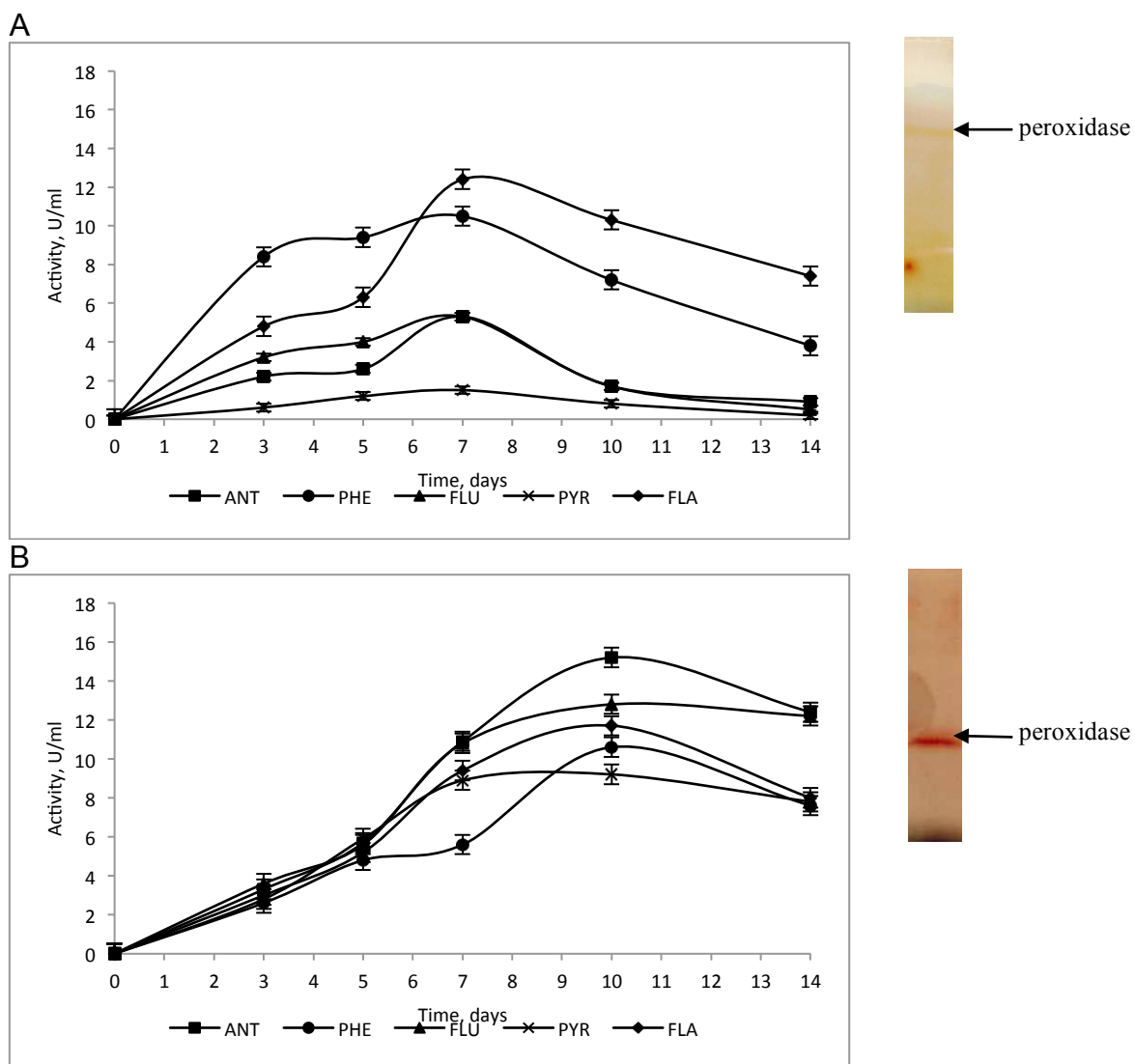
422 **Fig.1** Pollutant disappearance (A) and emulsifying activity production (B) during submerged cultivation of *F.*  
 423 *oxysporum* (□) and *L. aphanocladii* (■): ANT, anthracene; PHE, phenanthrene; FLU, fluorene; PYR, pyrene;  
 424 FLA, fluoranthene. Data always represent means  $\pm$  standard deviations from triplicate cultures,  $P \leq 0.05$ .

425

426



427 **Fig. 2** Decolorization of anthraquinone-type dyes by *F. oxysporum* (■, □) and *L. aphanocladii* (●○): Acid Blue  
 428 62 (■, ●) and Reactive Blue 4 (□, ○). Data always represent means  $\pm$  standard deviations from triplicate cultures,  
 429  $P \leq 0.05$ .  
 430



431 **Fig. 3** Time course of Mn-peroxidase production by *F. oxysporum* (A1) and *L. aphanocladii* (B1) and  
 432 nondenaturing PAGE of the detected enzymes (A2, B2). Data always represent means  $\pm$  standard deviations  
 433 from triplicate cultures,  $P \leq 0.05$ .

434

435

436

437

438