

1 **Fungi in PAH-contaminated marine sediments: cultivable diversity and tolerance**
2 **capacity towards PAH**

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16

17 **Abstract**

18 The cultivable fungal diversity from PAH-contaminated sediments was examined for the tolerance
19 to polycyclic aromatic hydrocarbon (PAH). The 85 fungal strains, isolated in non-selective media,
20 revealed a large diversity by ribosomal internal transcribed spacer (ITS) sequencing, even including
21 possible new species. Most strains (64%) exhibited PAH-tolerance, indicating that sediments retain
22 diverse cultivable PAH-tolerant fungi. The PAH-tolerance was linked neither to a specific taxon nor
23 to the peroxidase genes (LiP, MnP and Lac). Examining the PAH-removal (degradation and/or
24 sorption), *Alternaria destruens* F10.81 showed the best capacity with above 80% removal for
25 phenanthrene, pyrene and fluoranthene, and around 65% for benzo[*a*]pyrene. *A. destruens* F10.81
26 internalized pyrene homogenously into the hyphae that contrasted with *Fusarium pseudogamai*
27 F5.76 in which PAH-vacuoles were observed but PAH removal was below 20%. Thus, our study
28 paves the way for the exploitation of fungi in remediation strategies to mitigate the effect of PAH
29 in coastal marine sediments.

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33 **Keywords:** peroxidase genes, fungal ITS, PAH-uptake, *Alternaria*, PAH-contamination

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36 **Introduction**

37 Polycyclic aromatic hydrocarbons (PAHs) are important pollutants threatening the marine
38 environment due to their toxicity (Duran and Cravo-Laureau, 2016). Although the more
39 spectacular input of PAHs in marine environments is due to accidental oil spills, the main source
40 remains on natural oil seeps (Duran and Cravo-Laureau, 2016). PAHs accumulate in sediments
41 because of their hydrophobicity constituting a chronic contamination (Rothermich et al., 2002).
42 Their fate in the environment depends on biotic and abiotic factors (Duran and Cravo-Laureau,
43 2016).

44 Many microorganisms including archaea, bacteria, algae and fungi are able to degrade PAHs
45 (Duran and Cravo-Laureau, 2016; Bordenave et al., 2008; Germouche M'rassi et al., 2015; Haritash
46 and Kaushik, 2009). In the last years, the interest on PAHs removal and biodegradation by fungi
47 has increased (Mineki et al., 2015; Morales et al., 2017). The fungal removal of PAHs consists in
48 three main processes: two oxidation processes involving extracellular peroxidases (lignin
49 peroxidase, manganese peroxidase and laccase; Chen et al., 2001; Scheel et al., 2000), and
50 membrane attached monooxygenases (cytochrome P450; Črešnar and Petrič, 2011; Syed et al.,
51 2010), and absorption and storage of PAHs in lipid vacuoles (Verdin et al., 2005). The ability of
52 fungi to use PAHs as sole carbon and energy sources has been described (Rafin et al., 2000).
53 However, it has been reported that most of fungi require co-metabolism with another carbon
54 source for PAH degradation (Cerniglia et al., 1986).

55 Fungi have been found in all marine habitats (Orsi et al., 2013), revealing their high diversity
56 (Jones, 2000). Ascomycota and Basidiomycota are the main fungal phyla found in marine
57 environments as described for soil ecosystems (Clemente et al., 2001; Field et al., 1992; Godoy et
58 al., 2016; Li et al., 2008; Mineki et al., 2015; Potin et al., 2004; Valentín et al., 2006). Although
59 fungi of terrestrial origin have been isolated from marine ecosystems (Li and Wang, 2009), recent
60 molecular analysis revealed specific fungal sequences suggesting the existence of novel species of
61 marine fungi (Amend et al., 2019; Grossart and Rojas-Jimenez, 2016). Fungi isolated from marine
62 habitats exhibit similar morphological characteristics to their terrestrial counterparts (Méjanelle et
63 al., 2000). However, they might possess particular properties to survive in marine environments
64 (Amend et al., 2019), particularly in PAHs contaminated sediments (Greco et al., 2018). Such
65 properties, as salinity tolerance and the capacity to degrade and accumulate PAHs, less
66 bioavailable due to adsorption solid materials, remain to be explored (Bonugli-Santos et al., 2015;
67 Bugni and Ireland, 2004; Trincone, 2010).

68 This study aimed to explore the cultivable marine fungi, recovered from oil-contaminated saline
69 sediments, for their PAH-tolerance capacity. For this purpose fungal strains were isolated from
70 various marine coastal environments, characterized and identified by ITS sequence analysis, and
71 their features explaining the PAH tolerance examined.

72

73 **Material and Methods**

74 ***Culture media***

75 The culture media used in this study were based on the seawater minimal medium (swMM; Brito
76 et al., 2006), which composition was as follow: KCl 0.75 g/L, CaCl₂·2H₂O 1.47 g/L, NH₄Cl 1.5 g/L,
77 MgSO₄·7H₂O 6.64 g/L, NaCl 20 g/L, Na₂CO₃ 0.265 g/L, 1 mL of trace elements solution (H₃BO₃ 300
78 mg/L, FeSO₄·7H₂O 1.1 g/L, CoCl₂·6H₂O 190 mg/L, MnCl₂·2H₂O 50 mg/L, ZnCl₂ 42 mg/L, NiCl₂·6H₂O
79 24 mg/L, Na₂MoO₄·2H₂O 2mg/L), 1mL of vitamin solution (biotine 2 mg/L, p-aminobenzoate 10
80 mg/L, thiamine 10 mg/L, pantothenate 5 mg/L, pyridoxamine 50 mg/L, vitamin B₁₂ 20 mg/L,
81 nicotinate 20 mg/L), and 100 µL of phosphate buffer 50 mM. The pH was adjusted with HCl to 6.5.
82 Chemicals were purchased from Sigma Aldrich (Germany).

83 The malt dextrose agar (MDA) and malt dextrose (MD) media, in which distilled water was
84 exchanged by swMM (MDAsw and MDsw respectively) to keep salinity conditions, were used for
85 the isolation and for maintaining fungal strains.

86 ***Selection and conservation of fungal strains***

87 Oil polluted sediment collected from different coastal areas were used as inoculum for the
88 isolation of fungal strains with the ability to degrade PAHs. Each sample was inoculated directly in
89 MDAsw and incubated for 5 days. Also, dilutions at 10⁻¹, 10⁻² and 10⁻³ were performed taking 100
90 mg of each source.

91 The isolated fungal strains were conserved as conidia and mycelia in glycerol at -70°C. Fungi were
92 inoculated in MDsw grown until conidia overwhelmed cultures. Mycelia and conidia were
93 recovered from the flask and then dispatched in at least 3 Eppendorf tubes (100 mg of biomass
94 each) for each strain. After addition of 1 mL glycerol (30% solution), the tubes were frozen and
95 kept at -70 °C until use. In order to check viability one tube with mycelia was tested after 7 days of
96 storage by inoculating MDsw culture.

97 ***Fungi identification sequencing and phylogenetic analysis***

98 Fungi were harvested from MDAsw cultures from 10 days of incubation and DNA was extracted
99 using the QUIAGEN DNeasy® UltraClean® Microbial Kit (Cat. No. 12224-40) following the
100 manufacturer instructions. The identification was based on ITS sequences, which were amplified
101 using the primers ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS4 (TCCTCCGCTTATTGATATGC) that
102 amplify the ITS1, 5.8S and ITS2 region of the rRNA genes operon. The amplified region allows the
103 identification at the species level and even at the subspecies level (Fajarningsih, 2016). The PCR
104 reaction mix was prepared with 1 µL of extracted DNA in 9.5 µL of DEPC-treated water, 1 µL of
105 each primer (20 µM), 12.5 µL AmpliTaq Gold 360 Master Mix 2X (Thermo Fisher Scientific, USA).
106 The amplification was performed through 35 cycles of 95 °C (30 s), 55 °C (30 s) and 72 °C (1 min),
107 with a previous activation start of 95 °C (10 min) and final extension step at 72 °C (10 min). ITS
108 amplified fragments were sequenced at the Eurofins platform (France).

109 Sequence data were edited using Chromas Pro version 1.34. For identification, fungal ITS rRNA
110 sequences were compared with NCBI (National Centre for Biotechnology Information;
111 <http://www.ncbi.nlm.nih.gov>) database as previously described (Giloteaux et al., 2010). Fungal ITS
112 sequences in this study and reference sequences from GenBank were edited and aligned using
113 CLUSTAL-W (Thompson et al., 2003) as described (Bruneel et al., 2008). The aligned sequences
114 were imported into MEGA 3.1 (Kumar, 2004) for creating Neighbour-joining (NJ) trees based on
115 pairwise genetic distances. The quality of the branching patterns for NJ was assessed by bootstrap
116 resampling of the data sets with 1,000 replications and rooted to *Rhizopus oryzae* CBS 112.07^T (NR
117 103595.1) and *Trametes versicolor* CFMR FP-135156-Sp^T (NR 154494.1). The sequences
118 determined in this study have been submitted to the ITS NCBI database and assigned Accession
119 nos. MT889820 to MT889904.

120 ***Fungal tolerance to PAHs***

121 The tolerance to hydrocarbons was tested by inoculating and cultivating the fungi in swMM
122 supplemented with 25 mg/L of each fluoranthene, phenanthrene, pyrene and 5 mg/L of
123 benzo[*a*]pyrene as only carbon source. The analytical grade PAHs (Aldrich Chemical Co) were
124 added to the media as solution in acetone. Fungal strains were inoculated in the plates and
125 incubated at 20 °C in darkness during 15 days in order to maintain culture condition closer to that
126 observed in the environment. The capacity of fungi to grow and develop conidia was considered as
127 tolerance while in absence of development the strain was classified as no-tolerant.

128 ***Fungal PAHs removal rates***

129 Between 80-100 mg of mycelia and conidia were recovered of MDAsw plates and inoculated in 80
130 mL flasks with 30 mL of MDsw (1% MD). Fluoranthene, phenanthrene, pyrene and benzo[a]pyrene
131 were then added from a stock solution prepared in acetone that contain 20 mg/L of each
132 hydrocarbon. Samples were set for 1 hour before incubation to let acetone evaporate. An un-
133 inoculated flask was used as abiotic control and PAHs concentration reference. Cultures were
134 incubated in darkness for 20 days at 20 °C with gentle shaking at 80 rpm, in order to maintain
135 culture condition closer to that observed in the environment. Hydrocarbons were extracted after
136 incubation adding 30 mL of ethyl acetate and shacked for 15 min at 600 rpm. The recovery yield
137 was estimated to be about 98% of the initial concentration using the abiotic controls as reference.
138 Chrysene was used as internal standard during extraction in a concentration of 10 mg/L. Two
139 milliliters of organic phase was pulled in a glass vial for its analysis in Gas Chromatography
140 equipped with Flame Ionization Detector (GC-FID) (Agilent Technologies®, Network 6850 GC
141 System) with a capillarity C18 reverse column (30 m*0.25 mm*0.25 µm). For the analysis, 1 µL was
142 injected with a split ration of 1/50 using helium as carrier gas. Column temperature ramp settle
143 from 200 to 240 °C with stepped temperature increase of 5 °C/min and held during 1 min at 240°C.
144 Flame ionization detector was settled at 290 °C.

145 The removal capacities (degradation and/or sorption) for selected strains (*Alternaria destruens*
146 F10.81 and *Fusarium pseudonygamai* F5.76 strains exhibiting the highest and the lowest removal
147 capacities, respectively) was determined in triplicate with an incubation period of 15 days at with
148 gentle shaking at 80 rpm in order to maintain culture condition closer to that observed in the
149 environment. PAHs extraction was performed as above described. *Phanerochaete chrysosporium*
150 strain was used as reference for PAH-removal capacity, which often serves as reference for the
151 comparison of PAH-removal capacities even between strains from different phyla, as it is the fungi
152 the most studied in PAH-degradation (Cao et al., 2020). A one-way of analysis of variance (ANOVA)
153 was used to assess the significance of PAH-removal differences between samples with a
154 significance level of $p < 0.05$.

155 ***PCR detection of peroxidase and laccase genes***

156 The presence of genes encoding for enzymes known to be related to PAHs degradation: laccase
157 (lac), manganese peroxidase (MnP1, MnP2, MnP3) and lignin peroxidase (LiP1, LiP2, LiP3, LiP4,
158 LiP5, LiP6) was checked by PCR amplification. *Phanerochaete chrysosporium*, an effective PAH

159 degrader (Bamforth and Singleton, 2005; May et al., 1997), was used as positive control for the
160 presence of the peroxidase genes. The sequences of the primers and the T_m for the amplification
161 of the different genes are presented in Table 1. The reaction mix was prepared with 1 µL of
162 extracted DNA in 9.5 µL of DEPC-treated water, 1 µL of each primer (20 µM), 12.5 µL AmpliTaq
163 Gold 360 Master Mix 2X (Thermo Fisher Scientific, USA). The amplification was performed through
164 35 cycles of 95 °C (30 s), T_m (Table1, 45 s) and 72 °C (45 s), with a previous activation start of 95 °C
165 (10 min) and final extension step at 72 °C (10 min). Peroxidase genes amplified fragments were
166 sequenced at the Eurofins platform (France).

167 ***PAHs internalization and transport***

168 The capacity to internalize and transport PAHs through hyphae was examined for selected strains
169 (*F. pseudonygamai* F5.76 and *A. destruens* F10.81). The experimental setup consisted on an empty
170 petri dish with two MDAsw cubes over a crystal slide with a separation of 6 mm between them.
171 One of the cubes contained pyrene at 30 mg/L while the other no. The fungi were inoculated in
172 the cube with pyrene and incubated for 7 days in darkness at 20°C. The transport of PAHs was
173 evaluated inside the mycelia that reach the cube without pyrene using an epifluorescence
174 microscope (Nikon, Eclipse E600) with DAPI light filter (excitation 345 nm, emission 485 nm) for
175 PAH detection (fluorescence wavelengths range from 210-380 nm) (Verdin et al., 2005).

176

177 **Results and discussion**

178 ***Identification of fungal strains isolated from coastal sediments***

179 In total, 85 fungal strains were isolated from PAHs contaminated coastal sediments in seawater
180 media containing malt dextrose agar (swMDA). The strains were identified with the complete ITS
181 sequence (including ITS1, 5.8S rRNA gene, and ITS2 regions), which provide accurate identification
182 of fungi species even at the subspecies level (Fajarningsih, 2016). The phylogenetic analysis
183 showed that 83 strains belong to the Ascomycota Phylum and two strains belong to the
184 Basidiomycota Phylum (Fig. 1 and 2). Such result was not surprising since fungi belonging to
185 Ascomycota have been found prevalent in marine sediments (Babu et al., 2010; Birolli et al., 2018;
186 Ravelet et al., 2000) and other environments (Reyes-César et al., 2014). The 85 fungal strains fall
187 into six different Orders: Capnodiales (59 strains), Eurotiales (14 strains), Trichosphaeriales (1

188 strain), Hypocreales (2 strains), Pleosporales (7 strains) and Polyporales that belong to
189 Basidiomycota Phylum (2 strains).

190 The isolated strains affiliated to Eurotiales included strains belonging to *Talaromyces* (*T. helicus*),
191 *Aspergillus* (*A. fumigatus* and *A. chevalieri*), and *Penicillium* (*P. glandicola*, *P. crustosum*, and *P.*
192 *bialowiezense*) genera (Fig. 1). *Talaromyces* and *Aspergillus* genera are known for their ability of
193 PAH degradation in soil (Fayeulle et al., 2019), while *Aspergillus* genera, especially *A. fumigatus*,
194 has been detected in oil-contaminated mangrove sediments (Ghizelini et al., 2019). The isolated
195 strains related to the *Nigrospora* genus (Trichosphaeriales), *N. rubi* and *N. gorlenkoana*, are
196 described for the first time in marine sediments. The presence of these strains in the sediments
197 might be explained by plant material entering into the sea by air transportation or runoff, as they
198 are known to be associated with plants (Hao et al., 2020). Similarly, the strains affiliated to the
199 Hypocreales, *Fusarium pseudonygamae* (plant pathogen), *Lecanicillium longisporum* and
200 *Akanthomyces muscarius* (entomopathogens) have been described only in soil so far (Ansari and
201 Butt, 2012; Bashyal et al., 2016; Danilovich et al., 2020). Regarding the Pleosporales, the strain
202 F1.72, closely related to *Neosulcatispora strelitziae* and *Phaeosphaeria podocarpi*, recently
203 described fungal species (Crous et al., 2014, 2016), represents probably also a novel fungal
204 species. However, further analysis, including multi-locus based phylogeny, is required to
205 characterize the strain. Two other strains were closely related to species within the Pleosporales,
206 *Alternaria destruens* and *Epicoccum poae*, which have been isolated from plants (Kumar and
207 Kaushik, 2013; Chen et al., 2017). So far, these strains have not been shown to exhibit
208 hydrocarbon degradation capacity. The strains affiliated to the Polyporales were related to
209 *Trametes versicolor* and *Bjerkandera adusta* that are known to be able to degrade hydrocarbon
210 (Lladó et al., 2012; Andriani et al., 2016).

211 All the Capnodiales were affiliated to two complexes of the *Cladosporium* genus (Fig. 2) defined by
212 a multi-locus phylogeny (Schubert et al., 2007). Among the Cladosporioides complex, the isolated
213 strains were affiliated to species known to be associated with human and animals diseases such as
214 *C. crousii*, *C. welwitschiicola*, *C. austroafricanum*, *C. pini-ponderosae*, and *C. puyae* (Sandoval-Denis
215 et al., 2016), and with marine organisms such as *C. colombiae* (Ravi Theja and Chandra, 2020).
216 Similarly, the isolated strains belonging to the Herbarum complex, *C. rhusicola*, *C. subcinereum*, *C.*
217 *angustiherbarum* have been described involved in human and animals infections (Sandoval-Denis
218 et al., 2016), while *C. allicinum* was found associated with marine organisms (Poli et al., 2020;

219 Bovio et al., 2019) and several strains related to *C. herbarum* have been described for their ability
220 to degrade PAH in marine sediment (Marco-Urrea et al., 2015; Xiao et al., 2020). Noteworthy, the
221 strain D16.68 is the more distant from *Cladosporium* species (Fig. 2) suggesting that it might
222 represent a novel species within the *Cladosporium* genus, but further phylogenetic analysis based
223 on multi-locus are required to elucidate the taxonomic position. Although *Cladosporium* has been
224 already reported in saline environments (Zalar et al., 2007), in hydrocarbon contaminated
225 sediments (Ravelet et al., 2000) showing as well resistance to metals (Shao and Sun, 2007), it was
226 surprising to obtain mainly strains of this genus. It is likely that members of the *Cladosporium*
227 genus are well adapted to the culture conditions imposed during the screening procedure. The
228 cultural approach owns some limitations. Indeed, the development of conidia is controlled by
229 different factors (Tan et al., 1995), such as the presence of PAHs (Zafra et al., 2015), influencing
230 the selection of cultivable strains. In order to overcome such limitations, the application of
231 different culture conditions will enlarge the diversity of isolated strains.

232 Despite the limitations inherent of the cultivable approach, a large diversity of cultivable fungi was
233 obtained from hydrocarbon-contaminated marine sediments, spanning 11 fungal genera. The
234 isolated strains included not only strains affiliated to Orders which members were isolated from
235 marine sediments (Mouton et al., 2012; Ravelet et al., 2000) showing the capacity to degrade
236 PAHs (Fedorak et al., 1984; Simister et al., 2015), but also some isolated strains yet not described
237 in marine sediments, nor for their tolerance to the presence of PAHs. Thus, our study shows that a
238 large fungal diversity remains hidden in marine sediments, which represent a metabolic potential
239 for the development of remediation strategies for the mitigation of the effect of PAHs.

240

241 ***PAHs tolerance and removal capacities of the fungal isolated strains***

242 Most of the isolated fungal strains (54 strains, 64%) were able to grow in the presence of at least
243 the presence of one PAH showing their tolerance to hydrocarbons (Fig. 3). Among them, 61%
244 tolerate the presence of benzo[*a*]pyrene, 52% pyrene and 45% fluoranthene. Few fungal strains
245 were able to develop in presence of phenanthrene either alone (19%) or in mixture with other
246 PAHs (14%). Similar results showing high tolerance of fungal strains to pyrene, and low tolerance
247 to phenanthrene and PAHs mixture have been reported in the same range of concentrations (Lee
248 et al., 2014). Toxic effects on fungal growth have been observed with phenanthrene (Lisowska,
249 2004) and metabolites produced from PAHs mixture (Lundstedt et al., 2003). Interestingly, the

250 tolerance capacity is consistent with the phylogeny (Fig. 3), the members of the same Order
251 showing similar tolerance patterns. Noteworthy, the two groups Cladosporioides and Herbarum
252 within the Capnodiales Order showed distinct tolerance capacities, which further support the
253 classification into two distinct groups.

254 In order to assess the PAHs removal capacity (degradation and/or sorption) of fungal isolated
255 strains, maltose and dextrose were added as extra carbon source, since fungi have been shown to
256 have low ability to use PAHs as sole carbon source (Harrison, 2009). In these conditions, fungal
257 strains belonging to the Pleosporales Order showed the most efficient removal capacities while
258 strains affiliated to the Hypocreales Order showed the lowest removal capacity (Fig. 3). In the
259 Pleosporales Order, 85% of the strains showed removal capacity above 70%. The Cladosporioides
260 group of the Capnodiales Order exhibited the less number of strains with removal capacities above
261 70%. The comparison of the removal capacities of the isolated strains showed two main clusters
262 (Fig. 4) separating the strains with high removal capacities from those with low removal capacities.
263 Interestingly, members of the same species showed divergent removal capacities. Such
264 discrepancies have been described (Lee et al., 2014), strains from the same species showing
265 different metabolic capacities.

266 The analysis also showed that pyrene and fluoranthene (4 rings PAHs) clustered together, further
267 confirmed by strong correlation between pyrene and fluorentene removal capacities (Pearson
268 coefficient: 0.996, R^2 : 0.993), indicating that they were removed by almost a similar pattern of
269 fungal strains (Fig. 3). The benzo[*a*]pyrene (5 rings PAH) and phenanthrene (3 rings PAH) were
270 apart indicating that the patterns of fungal strains able to remove them were different. Such
271 observations highlighted that the removal capacity depends also on the PAH structure as
272 previously suggested (Ghosal et al., 2016).

273 In order to further characterize the genetic PAH degradation potential of the isolated fungal
274 strains, the presence of genes encoding manganese peroxidase (MnP), lignin peroxidase (LiP) and
275 laccase (Lac), known to participate in the degradation of PAHs (Ghosal et al., 2016), was examined
276 in their genomes by PCR (Fig. 4). All strains posses at least one of these genes, the LiP2 being the
277 most distributed (82/85 strains, 96%) among the isolated fungal strains (Fig. 4). Noteworthy, when
278 the LiP2 gene was not present, the strain possessed the MnP2 gene. Almost all strains (80/85
279 strains, 94%) possessed at least a manganese peroxidase gene, MnP2 gene being the most
280 detected (75/85 strains, 88%). Such results were not surprising since most of the peroxidase

281 enzymes are known to be produced in marine environment (Bonugli-Santos et al., 2015).
282 Surprisingly, the Lac gene, found in many marine fungal species (Ben Ali et al., 2020; D'Souza-Ticlo
283 et al., 2009), was detected in only 4 strains, which exhibited the most genetic potential possessing
284 more than 5 of the targeted genes. However, since various types of Lac genes have been described
285 in fungi (Moreno et al., 2017; Yang et al., 2016), the primers used to detect the presence of Lac
286 genes are probably not well suited for recovering the entire Lac gene diversity. The use of primers
287 targeting broader Lac gene diversity or targeting at least Lac gene detected within the Ascomycota
288 phyla (the major phyla of the isolated strains) is required to better define the presence of Lac
289 genes in the isolated strains. Similar observations can be drawn for the LiP and MnP genes
290 indicating that further efforts are needed for in depth characterization of the genetic potential of
291 the isolated strains.

292 Interestingly, the genetic potential of *Alternaria destruens* F10.81, exhibiting the highest PAH
293 removal capacity, was different to that of *Fusarium pseudonygamai* F5.76, showing the lowest
294 removal capacity, by just the presence of the LiP1 gene. Although it cannot be excluded that the
295 expression of the genes might be controlled by different regulation mechanisms in both strains,
296 such observation suggested that the presence of the LiP, MnP and Lac genes was not linked with
297 the PAH-removal capacity. In the same way, Pearson correlation analysis was unable to establish
298 correlation between the genetic profiles (LiP, MnP and Lac) and PAH-removal capacity of the
299 isolated fungal strains, but confirmation by determining the activity of the enzymes would be
300 required in order to conclude on the involvement of the enzymes in PAH-removal. Anyway,
301 considering the observed genetic potential and despite the inherent bias of the molecular tools
302 used in our study, these results suggested that other mechanisms are probably involved in PAH
303 removal. Thus, further studies are required for elucidating whether the PAH-removal potential of
304 the isolated strains involves degradation and/or sorption mechanisms. The mechanisms described
305 so far involve monooxygenase genes (Cerniglia, 1997; Cerniglia and Sutherland, 2010), particularly
306 the intracellular P450 monooxygenase gene that implies the internalization of PAH into fungal cells
307 (Cerniglia, 1997). In order to determine whether the internalization of PAH and the hyphae PAH
308 transport are mechanisms involved in PAH removal, the *Alternaria destruens* F10.81 and *Fusarium*
309 *pseudonygamai* F5.76 strains were selected, because they exhibited the highest and lowest PAH
310 removal capacities respectively, for further characterization.

311

312 ***PAH removal characterization of Alternaria destruens F10.81 and Fusarium pseudonygamai***
313 ***F5.76***

314 The removal capacity of *Alternaria destruens* F10.81 and *Fusarium pseudonygamai* F576 was
315 compared with that of *Phanerochaete chrysosporium*, which is the most studied fungi for PAH-
316 degradation (Cao et al., 2020). It serve often as control fungi even for comparing PAH-removal
317 capacity from strains belonging to different phyla (Cao et al., 2020), because it exhibit the capacity
318 to degrade a broad range of organic compounds (Deschler et al., 1998; Duran et al., 2002),
319 including several PAHs (Pointing, 2001). Under our conditions *P. chrysosporium* presented low
320 rates of PAHs removal (< 30%), just above to that exhibited by *F. pseudoygamai* F5.76 and around
321 3 times less to that observed for *A. destruens* F10.81 (Fig. 5). In fungi, gene regulation involves
322 complex control mechanisms as those observed for peroxidases genes. It is known that in most
323 fungal strains the LiP, MnP and Lac genes are expressed during the idiophase, the fungal
324 secondary phase, when nitrogen is limited and under the control of complex regulation signals
325 (Junghanns et al., 2005; Kamitsuji et al., 2004; Knop et al., 2015; Duran et al., 2002; Solé et al.,
326 2012), although the expression of MnP genes have been observed under high nitrogen content in
327 fungal genera such as *Pleurotus* and *Trametes* (Kaal et al., 1995; Janusz et al., 2013; Stajić et al.,
328 2006). Thus, the differences observed in removal capacities between the fungal strains are
329 probably due to the medium composition and culture conditions.

330 It is likely that the seawater medium with high nitrogen content as well as the culture conditions
331 used in our study limited the removal capacities of *P. chrysosporium* (Singh and Chen, 2008) and *F.*
332 *pseudoygamai* F5.76. In contrast, *A. destruens* F10.81 exhibited removal rates above 80% for all
333 PAHs except for benzo[*a*]pyrene (65% removal; Fig. 5). Such higher PAH removal capacity of *A.*
334 *destruens* F10.81 suggested that either its genes involved in PAH removal respond to different
335 regulation signals than the other two strains or the PAH removal was performed by other
336 mechanisms. For example, the expression of LiP, MnP and Lac genes has been observed under
337 high nitrogen content in some fungal species (i.e. *Pleurotus ostreatus* and *Trametes trogii*) and
338 even under both high and low nitrogen content for *Dichomitus squalens*, while for other fungal
339 species, such as *P. chrysosporium*, the peroxidase genes are expressed under nitrogen limitation
340 (Janusz et al., 2013; Stajić et al., 2006). The expression of genes involved in PAH-removal even in
341 high nitrogen content might be an asset for the fungal saprotrophic life-style in marine

342 environments where secreted enzymes, such as peroxidases, are likely to be lost by rapid diffusion
343 in the aquatic environment (Richards et al., 2012).

344 Possible PAH removal has been described through biosorption mechanisms, which include
345 adsorption onto cell surface (Raghukumar et al., 2006) and absorption into the cell (Verdin et al.,
346 2005; Yang et al., 2013). Several studies have demonstrated the capacity of fungi to uptake PAHs
347 (Deng et al., 2010; Wu et al., 2009) and also to transport them along the fungal hyphae (Furuno et
348 al., 2012; Schamfuß et al., 2013). Both strains, *Fusarium pseudoygamae* F5.76 and *Alternaria*
349 *destruens* F10.81, were able to uptake and transport pyrene (Fig. 6). Clear pyrene containing
350 vacuoles were observed in *F. pseudoygamae* F5.76 (Fig. 6c,d) while pyrene was homogeneously
351 distributed in *A. destruens* F10.81 (Fig. 6e,f) suggesting that the fungal strains have developed
352 different strategies for PAH uptake. It has been demonstrated that the vacuoles serves for possible
353 storage of PAHs as carbon source and for PAH transport along the hyphae allowing the distribution
354 of PAH within the mycelia network (Darrah et al., 2006; Furuno et al., 2012). Consistently, pyrene
355 was also accumulated into conidia in *F. pseudoygamae* F5.76 (Fig. 6c,d), which represents carbon
356 source reserve for the development of conidia as previously reported (Allaway et al., 1997; Bago et
357 al., 2002). In contrast, the homogenous pyrene distribution in *A. destruens* F10.81 (Fig. 6e,f)
358 suggested a diffusion mechanism. Such different pyrene uptake mechanism probably explains the
359 highest removal capacities of *A. destruens* F10.81 in comparison to *F. pseudoygamae* F5.76 (Fig. 5).
360 However, further studies are required to determine whether higher pyrene absorption or internal
361 degradation by monooxygenase (i.e. cytochrome P450) are the underlying physiological
362 mechanisms of PAH removal in *A. destruens* F10.81.

363

364 **Conclusion**

365 The exploration of the cultivable fungal diversity of hydrocarbon-contaminated coastal sediments
366 revealed that coastal sediment hide fungal diversity yet unexplored for their metabolic potential,
367 especially regarding PAH removal capacity. A large proportion of the isolated strains (48%),
368 dispatched within 6 fungal genera, exhibited PAH-tolerance with a removal capacity (degradation
369 and/or sorption) above 60%. Such diversity in PAH-removal capacity represents a functional
370 potential for ecosystem recovery exploitable for bioremediation treatments (Harms et al., 2011).
371 However, the mechanism underlying the PAH-removal capacity (degradation and/or sorption) is
372 unclear because it is probably not related to the presence of extracellular peroxidase genes (LiP,

373 MnP and Lac) and it is strain specific. The comparison of two isolated strains exhibiting contrasted
374 removal capacities showed different PAH-uptake behaviour suggesting that the mechanisms by
375 which fungi perform PAH-uptake might determine the efficiency of PAH-removal. *Alternaria*
376 *destruens* F10.81, the most efficient PAH-remover (above 80%) was able to internalize pyrene
377 homogenously into the hyphae that contrasted with the behaviour of *Fusarium pseudogamai*
378 F5.76 in which PAH-vacuoles were observed but exhibiting a PAH-removal capacity below 20%. It
379 is likely that *Alternaria destruens* F10.81 owns features well adapted to PAH-contaminated coastal
380 sediments, which represent potential for the development of a bioremediation process. However,
381 further studies are required to understand the PAH-removal mechanism in order to manage fungal
382 resources to mitigate the effects of PAH contamination.

383

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391

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785

786 **Figure captions**

787 **Fig. 1: Phylogenetic tree, based on ITS sequences, showing the positions of fungal strains**
788 **isolated from oil contaminated coastal marine sediments.** The Neighbour joining phylogenetic
789 tree was rooted with the ITS sequence of *Rhizopus oryzae* CBS 112.07^T (NR 103595.1). The scale
790 bar corresponds to 0.05 substitutions per nucleotide position. Percentages of 1,000 bootstrap re-
791 sampling that supported the branching orders in each analysis are shown above or near the
792 relevant nodes. For the isolates, the number of isolated strains is indicated in parenthesis. For the
793 type strains, the accession number is indicated in parenthesis.

794 **Fig. 2: Phylogenetic tree, based on ITS sequences, showing the positions of fungal strains**
795 **isolated from oil contaminated coastal marine sediments within the *Cladosporium* genus.** The
796 Neighbour joining phylogenetic tree was rooted with the ITS sequence of *Trametes versicolor*
797 CFMR FP-135156-Sp^T (NR 154494.1). The scale bar corresponds to 0.05 substitutions per
798 nucleotide position. Percentages of 1,000 bootstrap re-sampling that supported the branching
799 orders in each analysis are shown above or near the relevant nodes. For the isolates, the number
800 of isolated strains is indicated in parenthesis. For the type strains, the accession number is
801 indicated in parenthesis.

802 **Fig. 3: PAHs tolerance and removal capacities of isolated fungal strains.** PAHs tolerance
803 corresponds to the capacity of the fungal strains to grow (green) or not (red) on solid seawater
804 minimal medium in the presence of different PAHs and PAHs mixture. PAHs removal capacity,
805 determined in liquid cultures containing a mixture of PAHs, corresponds to the percentage of PAHs
806 eliminated after 20 days of fungal growth. The color gradient follows to the removal capacity from
807 low (red) to high (green). Phe, phenanthrene; Flu, fluoranthene; Pyr, pyrene; BaP, benzo[*a*]pyrene;
808 Mix, mixture of the 4 PAHs.

809 **Fig. 4: Comparison of PAHs removal capacity of the isolated fungal strains and their genetic**
810 **potential.** The heatmap is based on similarity index calculated from removal capacity data shown
811 in Fig 3. Phe, phenanthrene; Flu, fluoranthene; Pyr, pyrene; BaP, benzo[*a*]pyrene. The genetic
812 potential corresponds to the presence (dark blue) of peroxidase genes assessed by PCR targeting
813 manganese peroxidase (MnP1-3), lignin peroxidase (LiP1-6) and laccase (Lac). The absence of the
814 genes is indicated in pale blue. The fungal strains selected for further analysis, corresponding to
815 the highest and lowest removal capacities, are highlighted in red.

816 **Fig. 5: PAHs removal capacities of *Alternaria destruens* F10.81 and *Fusarium pseudonygamai***
817 **F5.76 compared to that of the reference strain *Phanerochaete chrysosporium*.** Means of tree
818 replicates are presented. The bar indicates SD. In each hydrocarbon removal test, mean followed
819 by the same letter do not differ statistically by the Turkey test at 5%.

820 **Fig. 6: PAHs internalization in the hyphae of *Alternaria destruens* F10.81 and *Fusarium***
821 ***pseudonygamai* F5.76.** (a) Experimental schema for the detection of internal transport of PAHs
822 along the mycelia. Fungi were inoculated in a 1 cm³ cube of solid seawater minimal media with
823 10% LB and 20 mg/L of pyrene. The red arrow indicates the direction of the hyphae growth. (b)
824 Macroscopic observation showing the colonization of *Fusarium pseudonygamai* F5.76 of a piece of
825 media from the other. The red arrow indicates the hyphae forming bridges between the two
826 pieces of media. Observation of *Fusarium pseudonygamai* F5.76 hyphae after colonization by (c)
827 light microscopy and by (d) fluorescence after exposing to DAPI light. The red arrows show the
828 storage of PAHs into conidia. Observation of *Alternaria destruens* F10.81 hyphae after colonization
829 by (e) light microscopy and by (f) fluorescence after exposing to DAPI light. The red arrows show
830 the homogeneous distribution of PAHs into the hyphae. The microscopic observations were
831 performed at a magnification of 160X.

832

833 **Table 1. Primers used for the amplification of fungal peroxidases genes**

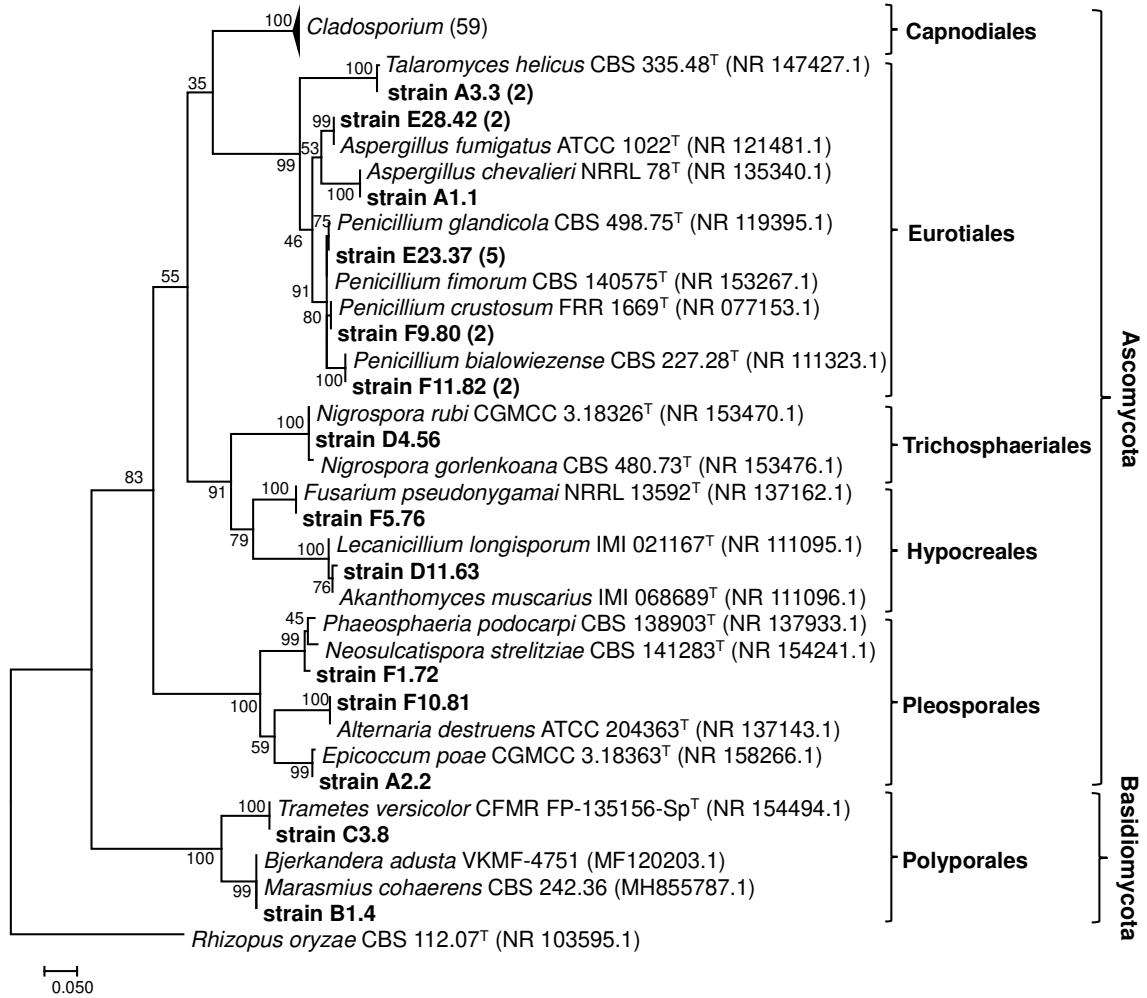
Gene*	Sequence (5'-3')**	Tm (°C)	Size (nt)	Reference
LiP1	LIG1u (F): GCCGCAATTTCTCTTGCTCTTTCCA	57	179/126	Broda et al., 1995
	LIG1d (R): TACATCGAACCACGCGCACGAGATT			
LiP2	LIG2u (F): CATCGCAATTTCCGCCCGCCATGGAGGA	57	222/179	Broda et al., 1995
	LIG2d (R): ACCTTCTGAACGAATGGCTTCTGGAGC			
LiP3	LIG3u (F): TATTGCCATCTCTCCTGCTATGGAGGCC	57	179/126	Broda et al., 1995
	LIG3d (R): ATGTTAGGGTGGAAGTTGGGCTCGATG			
LiP4	LIG4u (F): GTGCGCCTGGTTCCCCATTCTGCAG	57	350/222	Broda et al., 1995
	LIG4d (R): AATTGGTCTCGATAGTATCGAAGAC			
LiP5	LIG5u (F): GGTCTCGATCGAGGAGAAGGTAATGATC	57	350/222	Broda et al., 1995
	LIG5d (R): TTGCCCCGACGGCGTGCACAC			
LiP6	LIG6u (F): GACCTGCTCGAACGGCAAGGTCGTCC	57	350/222	Broda et al., 1995
	LIG6d (R): CATGATAGAACCATCGGCGCCTCGC			
MnP1	mnp1-f (F): CAGACGGTACCCGCGTCACC	60	246/123	Bogan et al., 1996
	mnp1-r (R): AGTGGGAGCGGCGACATCAC			
MnP2	mnp2-f (F): CCGACGGCACCCGCGTCAGC	60	≈900	Bogan et al., 1996
	mnp2-r (R): CGAGCGGGAGCGGCGACGCC			
MnP3	mnp3-f (F): CCGACGGTACCAAGGTCAAC	60	≈900	Bogan et al., 1996
	mnp3-r (R): AGCGGCAGCGGCGACGCGAC			
Lac	Lac (F): CACTGGCACGGNTTCTTCCA	52	246/123	D'Souza et al., 1996
	Lac (R): GTGACTATGATAACCAGAANGT			

834 *LiP, lignine peroxidase; MnP, manganese peroxidase; Lac, laccase. **(F), forward; (R), reverse.

835

836 Fig. 1

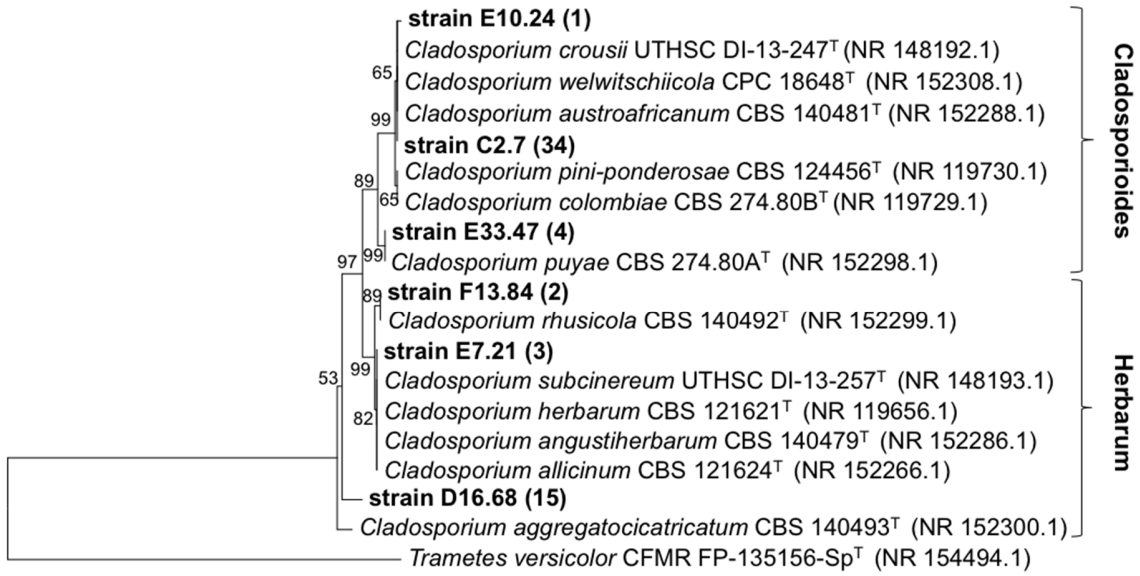
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842 Fig. 2

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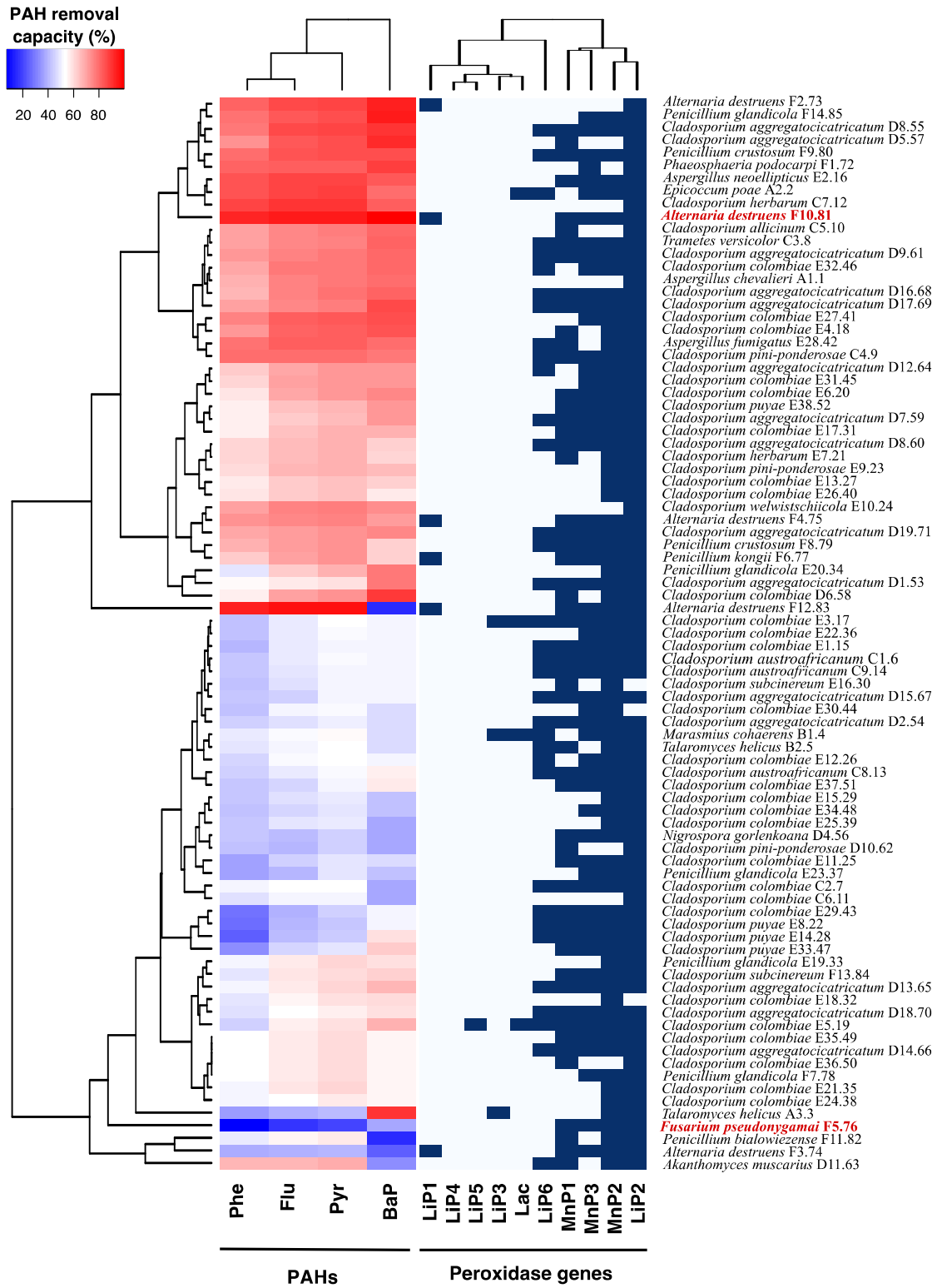


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849 Fig. 3

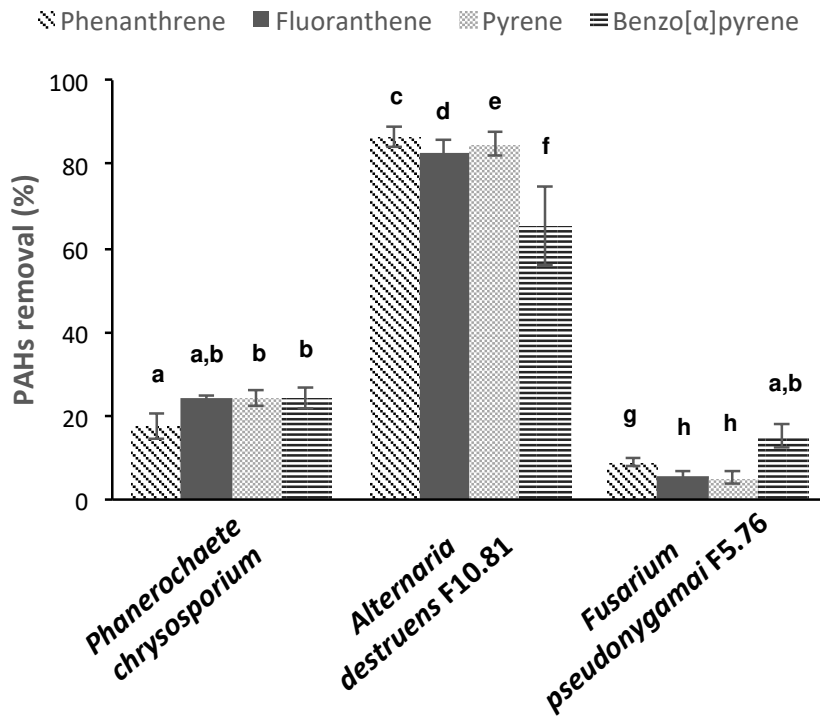
	Fungal strain	PAH tolerance					PAH removal capacity (%)				
		Phe	Flu	Pyr	BaP	Mix	Phe	Flu	Pyr	BaP	
Capnodiales Cladospoides	<i>Cladosporium austroafricanum</i> C1.6	Green	Green	Green	Green	Green	43.2	49.7	52.6	51.1	
	<i>Cladosporium austroafricanum</i> C8.13	Green	Green	Green	Green	Green	44.6	49.7	52.6	56.4	
	<i>Cladosporium austroafricanum</i> C9.14	Green	Green	Green	Green	Green	43.2	48.5	51.5	51.1	
	<i>Cladosporium welwitschiicola</i> E10.24	Green	Green	Green	Green	Green	71.0	76.6	76.9	74.2	
	<i>Cladosporium pini-ponderosae</i> C4.9	Green	Green	Green	Green	Green	80.2	80.1	80.2	77.8	
	<i>Cladosporium pini-ponderosae</i> E9.23	Green	Green	Green	Green	Green	60.4	67.3	68.0	65.8	
	<i>Cladosporium pini-ponderosae</i> D10.62	Green	Green	Green	Green	Green	41.9	40.4	44.9	37.8	
	<i>Cladosporium colombiae</i> C2.7	Green	Green	Green	Green	Green	51.2	53.2	53.7	37.8	
	<i>Cladosporium colombiae</i> C6.11	Green	Green	Green	Green	Green	47.2	50.9	51.5	37.8	
	<i>Cladosporium colombiae</i> E1.15	Green	Green	Green	Green	Green	40.6	49.7	51.5	51.1	
	<i>Cladosporium colombiae</i> E3.17	Green	Green	Green	Green	Green	41.9	49.7	53.7	51.1	
	<i>Cladosporium colombiae</i> E4.18	Green	Green	Green	Green	Green	72.3	81.3	82.4	84.4	
	<i>Cladosporium colombiae</i> E5.19	Green	Green	Green	Green	Green	44.6	56.7	59.2	67.6	
	<i>Cladosporium colombiae</i> E6.20	Green	Green	Green	Green	Green	59.1	69.6	72.5	75.6	
	<i>Cladosporium colombiae</i> E11.25	Green	Green	Green	Green	Green	36.6	45.0	48.2	46.7	
	<i>Cladosporium colombiae</i> E12.26	Green	Green	Green	Green	Green	45.9	52.0	53.7	51.1	
	<i>Cladosporium colombiae</i> E13.27	Green	Green	Green	Green	Green	57.8	63.7	64.7	62.7	
	<i>Cladosporium colombiae</i> E15.29	Green	Green	Green	Green	Green	43.2	47.4	49.3	42.2	
	<i>Cladosporium colombiae</i> E17.31	Green	Green	Green	Green	Green	56.4	64.9	68.0	67.6	
	<i>Cladosporium colombiae</i> E18.32	Green	Green	Green	Green	Green	48.5	55.6	59.2	60.9	
	<i>Cladosporium colombiae</i> E21.35	Green	Green	Green	Green	Green	51.2	59.1	61.4	55.6	
	<i>Cladosporium colombiae</i> E22.36	Green	Green	Green	Green	Green	41.9	49.7	52.6	51.1	
	<i>Cladosporium colombiae</i> E24.38	Green	Green	Green	Green	Green	51.2	54.4	58.1	55.6	
	<i>Cladosporium colombiae</i> E25.39	Green	Green	Green	Green	Green	43.2	48.5	49.3	37.8	
	<i>Cladosporium colombiae</i> E26.40	Green	Green	Green	Green	Green	59.1	63.7	64.7	57.8	
	<i>Cladosporium colombiae</i> E27.41	Green	Green	Green	Green	Green	76.2	82.5	83.5	85.8	
	<i>Cladosporium colombiae</i> E29.43	Green	Green	Green	Green	Green	28.7	39.2	44.9	51.1	
	<i>Cladosporium colombiae</i> E30.44	Green	Green	Green	Green	Green	41.9	50.9	52.6	46.7	
	<i>Cladosporium colombiae</i> E31.45	Green	Green	Green	Green	Green	61.7	70.8	72.5	72.9	
	<i>Cladosporium colombiae</i> E32.46	Green	Green	Green	Green	Green	69.6	77.8	78.0	80.4	
	<i>Cladosporium colombiae</i> E34.48	Green	Green	Green	Green	Green	41.9	46.2	48.2	42.2	
	<i>Cladosporium colombiae</i> E35.49	Green	Green	Green	Green	Green	53.8	57.9	60.3	55.6	
	<i>Cladosporium colombiae</i> E36.50	Green	Green	Green	Green	Green	53.8	57.9	60.3	55.6	
	<i>Cladosporium colombiae</i> E37.51	Green	Green	Green	Green	Green	43.2	46.2	51.5	58.2	
	<i>Cladosporium colombiae</i> D6.58	Green	Green	Green	Green	Green	56.4	70.8	73.6	88.9	
	<i>Cladosporium puyae</i> E8.22	Green	Green	Green	Green	Green	27.4	40.4	42.7	51.1	
	<i>Cladosporium puyae</i> E14.28	Green	Green	Green	Green	Green	24.8	41.5	43.8	60.0	
	<i>Cladosporium puyae</i> E33.47	Green	Green	Green	Green	Green	32.7	46.2	48.2	63.6	
	<i>Cladosporium puyae</i> E38.52	Green	Green	Green	Green	Green	56.4	64.9	66.9	72.9	
	Capnodiales Herbarium	<i>Cladosporium aggregatocitricatum</i> D1.53	Green	Green	Green	Green	Green	55.1	57.9	59.2	78.2
		<i>Cladosporium aggregatocitricatum</i> D2.54	Green	Green	Green	Green	Green	44.6	47.4	50.4	46.7
		<i>Cladosporium aggregatocitricatum</i> D3.55	Green	Green	Green	Green	Green	77.6	86.0	86.8	90.2
		<i>Cladosporium aggregatocitricatum</i> D5.57	Green	Green	Green	Green	Green	73.6	83.6	85.7	92.0
		<i>Cladosporium aggregatocitricatum</i> D7.59	Green	Green	Green	Green	Green	56.4	63.7	65.8	72.9
		<i>Cladosporium aggregatocitricatum</i> D8.60	Green	Green	Green	Green	Green	61.7	66.1	68.0	62.7
		<i>Cladosporium aggregatocitricatum</i> D9.61	Green	Green	Green	Green	Green	72.3	76.6	78.0	80.9
		<i>Cladosporium aggregatocitricatum</i> D12.64	Green	Green	Green	Green	Green	63.0	69.6	72.5	72.9
<i>Cladosporium aggregatocitricatum</i> D13.65		Green	Green	Green	Green	Green	51.2	57.9	61.4	66.7	
<i>Cladosporium aggregatocitricatum</i> D14.66		Green	Green	Green	Green	Green	53.8	57.9	60.3	55.6	
<i>Cladosporium aggregatocitricatum</i> D15.67		Green	Green	Green	Green	Green	43.2	45.0	51.5	51.1	
<i>Cladosporium aggregatocitricatum</i> D16.68		Green	Green	Green	Green	Green	67.0	76.6	79.1	81.3	
<i>Cladosporium aggregatocitricatum</i> D17.69		Green	Green	Green	Green	Green	71.0	75.4	76.9	86.2	
<i>Cladosporium aggregatocitricatum</i> D18.70		Green	Green	Green	Green	Green	47.2	53.2	57.0	60.0	
<i>Cladosporium aggregatocitricatum</i> D19.71		Green	Green	Green	Green	Green	69.6	71.9	73.6	75.1	
<i>Cladosporium allicinum</i> C5.10		Green	Green	Green	Green	Green	71.0	75.4	76.9	80.9	
<i>Cladosporium herbarum</i> C7.12		Green	Green	Green	Green	Green	87.2	89.5	90.0	82.2	
<i>Cladosporium herbarum</i> E7.21		Green	Green	Green	Green	Green	61.7	66.1	68.0	61.8	
<i>Cladosporium subcinereum</i> E16.30		Green	Green	Green	Green	Green	41.9	47.4	51.5	51.1	
<i>Cladosporium subcinereum</i> F13.84	Green	Green	Green	Green	Green	48.5	59.1	60.3	62.2		
Eurotiales	<i>Talaromyces helicus</i> A3.3	Green	Green	Green	Green	Green	35.3	39.2	41.6	89.8	
	<i>Talaromyces helicus</i> B2.5	Green	Green	Green	Green	Green	48.5	50.9	53.7	46.7	
	<i>Aspergillus chevalieri</i> A1.1	Green	Green	Green	Green	Green	68.3	76.6	78.0	80.0	
	<i>Aspergillus neoellipticus</i> E2.16	Green	Green	Green	Green	Green	84.2	87.1	86.8	83.6	
	<i>Aspergillus fumitragus</i> E28.42	Green	Green	Green	Green	Green	78.9	82.5	82.4	80.0	
	<i>Penicillium glandicola</i> E19.33	Green	Green	Green	Green	Green	51.2	57.9	61.4	60.0	
	<i>Penicillium glandicola</i> E20.34	Green	Green	Green	Green	Green	48.5	63.7	68.0	77.8	
	<i>Penicillium glandicola</i> E23.37	Green	Green	Green	Green	Green	36.6	40.4	48.2	42.2	
	<i>Penicillium kongii</i> F6.77	Green	Green	Green	Green	Green	63.0	70.8	73.6	62.7	
	<i>Penicillium gladicola</i> F7.78	Green	Green	Green	Green	Green	53.8	57.9	60.3	55.6	
	<i>Penicillium crustosum</i> F8.79	Green	Green	Green	Green	Green	68.3	71.9	73.6	62.7	
<i>Penicillium crustosum</i> F9.80	Green	Green	Green	Green	Green	80.2	84.8	85.7	84.9		
<i>Penicillium bialowiezense</i> F11.82	Green	Green	Green	Green	Green	49.8	55.6	58.1	15.6		
<i>Penicillium gladicola</i> F14.85	Green	Green	Green	Green	Green	78.9	83.6	85.7	94.7		
Tricosphaeriales	<i>Nigrospora gorlenkoana</i> D4.56	Green	Green	Green	Green	Green	43.2	41.5	44.9	37.8	
	<i>Fusarium pseudonygamai</i> F5.76	Green	Green	Green	Green	Green	07.6	17.0	19.6	37.8	
Hypocreales	<i>Akanthomyces muscarius</i> D11.63	Green	Green	Green	Green	Green	67.0	67.3	69.1	33.3	
	<i>Phaenopharia podocarpi</i> F1.72	Green	Green	Green	Green	Green	81.5	82.5	82.4	88.4	
Pleosporales	<i>Alternaria destruens</i> F2.73	Green	Green	Green	Green	Green	81.5	86.0	86.8	93.3	
	<i>Alternaria destruens</i> F3.74	Green	Green	Green	Green	Green	38.0	39.2	40.5	24.4	
	<i>Alternaria destruens</i> F4.75	Green	Green	Green	Green	Green	73.6	75.4	75.8	71.1	
	<i>Alternaria destruens</i> F10.81	Green	Green	Green	Green	Green	93.0	94.6	95.0	99.6	
	<i>Alternaria destruens</i> F12.83	Green	Green	Green	Green	Green	93.9	96.3	96.4	15.6	
<i>Epicoccum poae</i> A2.2	Green	Green	Green	Green	Green	84.2	87.1	87.9	80.0		
Polyporales	<i>Trametes versicolor</i> C3.8	Green	Green	Green	Green	Green	71.0	75.4	75.8	81.8	
	<i>Marasmius cohaerens</i> B1.4	Green	Green	Green	Green	Green	49.8	52.0	54.8	46.7	

851 Fig. 4



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854 Fig. 5

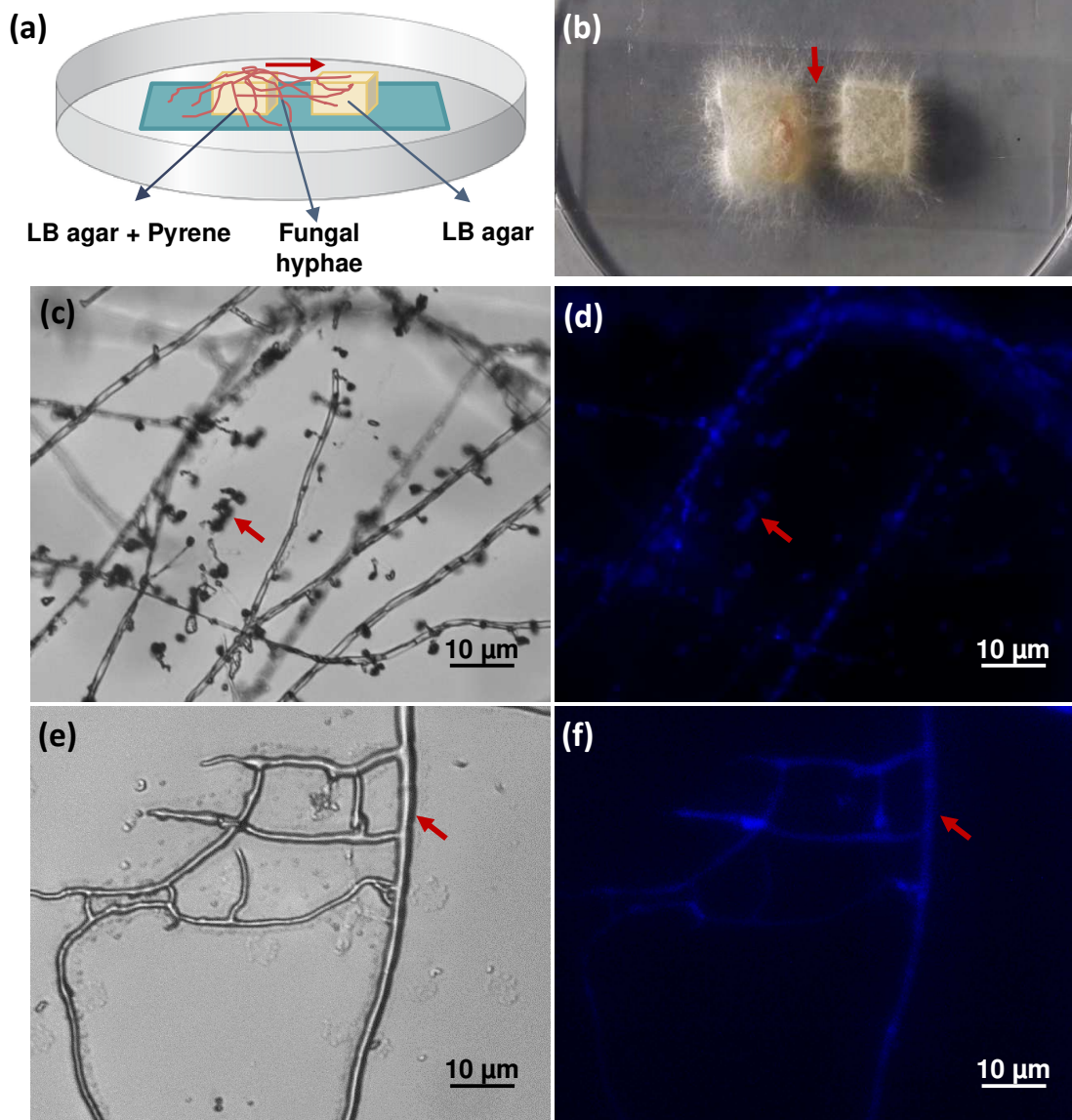


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858 Fig. 6

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Cultivable diversity of PAH-removing fungi

